URINARY PHARMACOKINETIC METHODOLOGY TO DETERMINE THE RELATIVE LUNG BIOAVAILABILITY OF INHALED BECLOMETASONE

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Abstract

Urinary pharmacokinetic methods have been introduced to identify the relative lung and systemic availability of inhaled drugs but have not been extended to corticosteroids. The main aims were to validate the urinary pharmacokinetic methodology when applied to inhaled beclometasone dipropionate (BDP), demonstrate the usefulness of the method and compare its indices to the *in-vitro* characteristics of the emitted dose.

A simple and sensitive LC-MS method for quantifying BDP and its metabolites in methanol (for *in-vitro* studies) and urine samples was identified and validated in accordance with the FDA and ICH guidelines. The accuracy, precision, and recovery of the method were within acceptable limits (±15%).

Twelve healthy volunteers completed the *in-vivo* urinary pharmacokinetic validation of the methodology to determine the relative lung bioavailability of inhaled beclometasone following inhalation. Twelve healthy volunteers received randomised doses, separated by >7 days, of 2000µg BDP solution with (OralC) and without (Oral) 5g oral charcoal, ten 100µg inhalations from a Qvar® Easi breathe metered dose inhaler (pMDI) with (QvarC) and without (Qvar) oral charcoal and eight 250µg inhalations from a Clenil® pMDI (Clenil). Subjects provided urine samples at 0, 0.5, 1, 2, 3, 5, 8, 12, and 24 hours post study dose. Urinary concentrations of BDP and its metabolites, 17-beclometasone monopropionate (BMP) and beclometasone (BOH) were measured. No BDP, BMP, or BOH was detected in any samples post OralC dosing. Post oral dosing, no BDP was detected in any of the urine samples and no BMP or BOH was excreted in the first 30 minutes. Significantly more ($p<0.001$) BDP, BMP and BOH was excreted in the first 30 minutes and cumulative 24 urinary excretions post Qvar and Clenil compared to Oral. Using 30 minute urinary excretion the mean ratio (90% confidence interval) for Qvar compared to Clenil was 231.4 (209.6, 255.7). The results confirm that the relative lung and systemic bioavailability can be identified from urinary excretion of BDP and its metabolites over the first 30 minutes and 24 hours respectively. The 2-fold difference between Qvar and Clenil is consistent with related clinical and pharmacokinetic studies. The low inter and intra-subject variability of the study confirms the reproducibility of this method. When compared to the in-vitro aerodynamics characteristics of the emitted dose, using standard compendial methods, the in-vivo indices showed a relationship to the fine particle dose (FPD) and the emitted dose (ED), respectively.

The application of this urinary pharmacokinetic method was demonstrated in further studies to compare the effect of different spacers and different washing methods on the *in-vivo* drug delivery post inhalation from Clenil and Qvar inhalers in healthy volunteers. In addition, the in-vitro aerodynamic particle size distribution of the same inhalation methods has been investigated using the Andersen Cascade Impactor according to the standard compendial methodology. Urinary excretion, using 24 hour excretion, revealed that relative bioavailability to the body was reduced with spacers for both inhalers. There was no increase in the relative lung bioavailability when Qvar was used with spacers. When Clenil was attached to a spacer (either AeroChamber or Volumatic) the relative lung bioavailability was significantly greater only if the spacers were not rinsed after washing with detergents. Consistent with the above study there were correlations between the in-vivo urinary indices and the in-vitro characteristics of the emitted dose.

The thesis highlights the extension of the urinary pharmacokinetic method to inhaled beclometasone dipropionate and provides further evidence of *in-vitro in-vivo* correlations between the urinary methodology and the aerodynamic characteristics of the emitted dose.

Keys words: beclometasone dipropionate, metabolites, urinary excretion, metered dose inhalers, spacers, relative lung bioavailability, and in-vitro dose emission.

This work is dedicated to my parents, my husband, and my daughter.

List of Publication

Sections of this thesis have been published in the following form:

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List of Abbreviations

ACI	Anderson Cascade Impactor
AHR	Airway hyperresponsiveness
ANOVA	Analysis of Variance
APLUS	Aerochamber Plus
APSD	Aerodynamic Particle Size Distribution
BDP	Beclometasone dipropionate
17-BMP	17-Beclometasone monopropionate
BOH	Beclometasone
BP	British Pharmacopoeia
BTS	British Thoracic Society
CFC	Chlorofluorocarbon
CI	Confidence intervals
CITDAS	Copley Inhaler Testing Data Analysis Software
COPD	Chronic Obstructive Pulmonary Disease
CV	Coefficient of Variation
DPI	Dry Powder Inhaler
DSU	Dose Sampling Unit
EB	Easi-Breathe
EP	European Pharmacopoeia
ESC	Electrostatic Charge
ESI	Electrospray ionisation
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in one second
FP	Fluticasone propionate
FPD	Fine Particle Dose
FPF	Fine Particle Fraction
FVC	Forced Vital Capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GINA	Global Initiative for Asthma
GSD	Geometric Standard Deviation
HFA	Hydrofluoroalkane

ICH	International Committee of Harmonisation
ICRP	International Commission on Radiological Protection
ICS	Inhaled corticosteroid
IP	Induction Port
IS	Internal standard
LC-MS	Liquid Chromatographic Mass Spectrometric
$Lmin^{-1}$	Litre per minute
LOQ	Limit of Quantification
LOD	Limit of Detection
MDI	Metered Dose Inhaler
MHRA	Medicines and Healthcare Products Regulatory agency
MMAD	Mass Median Aerodynamic Diameter
MSLI	Multistage Liquid Impinger
NAEPP	National Asthma Education and Prevention Program
NGI	New Generation Impactor
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
OPT	Optimiser
P	Probability
PD	Pharmacodynamic
PET	Positron emission tomography
PK	Pharmacokinetic
R^2	Correlation Coefficient
RSD	Relative Standard Deviation
SD	Standard Deviation
SIGN	Scottish Intercollegiate Guidelines
SPC	Summary of Product characteristics
SPE	Solid Phase Extraction
SPECT	Single Photon Emission Computed Tomography
TED	Total Emitted dose
USP	United State Pharmacopoeia
UV	Ultraviolet
VOL	Volumatic

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Chapter 1: Introduction

1.1. Introduction

Pulmonary delivery has long been recognized as the most efficient route of drug administration for asthma and other diseases of the respiratory tract (Everard, 2001). Drugs are delivered directly to the site of action, where they exert a local effect and thus have a fast onset of action. The inhalation of drugs produces high local concentrations in the lungs, avoiding the high systemic concentrations that would result from equipotent oral and parental doses. Hence the lower doses used translate to a much lower incidence of systemic side effects which is particularly important in the case of inhaled corticosteroids (ICS) treatment (Chrystyn, 1994; Pedersen et al., 2010).

Inhaled corticosteroids are the most effective anti-inflammatory drugs available to clinicians for the control of inflammation in asthma. Inhaled corticosteroids effectively suppress the inflammatory processes in the airways of most asthmatics. Their clinical benefits include; decreased asthma symptoms, decreased airway hyperresponsiveness, improved pulmonary function, fewer exacerbations, fewer hospitalisations, and fewer asthma related deaths (Suissa et al., 2000; Adams et al., 2005; Sobande and Kercsmar, 2008). Among various ICS available in the market, beclometasone dipropionate was the first one introduced in 1972 in a pressurized metered dose inhaler and later in a dry powder inhaler and an aqueous nasal spray (Daley-Yates et al., 2001). Beclometasone dipropionate (BDP) is a powerful topically active inhaled corticosteroid that is used in treatment of asthma. It is actually a prodrug that is metabolised by esterases in the human lung to three different metabolites; 17-beclometasone monopropionate (17-BMP), 21-beclometasone monopropionate (21-BMP), and beclometasone (BOH). 17-beclometasone monopropionate (17-BMP) is the active metabolite whereas BOH and 21-BMP have a very low binding affinity to the glucocorticoid receptor (Wurthwein and Rohdewald, 1990; Derendorf et al., 2006). Since the early introduction of beclometasone

in the mid 1970s, a great number of new formulations, propellants, and inhaler devices were developed.

Worldwide, MDIs are the most widely used inhalation devices for the treatment of asthma as it is relatively inexpensive, widely available and portable devices that use a propellant under pressure to deliver a metered aerosol dose through an atomisation nozzle (Smyth, 2003). Although correct use of a MDI looks simple, several studies have reported failure of a large proportion of patients to use it properly. Adding a spacer device to MDIs or using a breath-actuated device such as the Autohaler or the Easi-Breathe device helps to solve the problem of poor hand breath coordination (Newman et al., 1991c). Furthermore, the use of spacers enables the aerosol cloud produced from the MDI to slow down and the propellant to evaporate, thus increasing drug delivery to the lung. Spacers also have a size selective function and retain the larger non-respirable particles on spacer walls, thus limiting oropharyngeal deposition. However, the presence of electrostatic charge on spacer surfaces may markedly interfere with drug delivery. Therefore, spacers should be washed with detergent and allowed to drip dry at least each month to limit electrostatic charge effect (Chrystyn and Price, 2009; Pedersen et al., 2010; Vincken et al., 2010).

Pressurized MDIs were routinely formulated with Chlorofluorocarbon (CFC) propellants for several decades, but due to their ozone depleting potential, they have been phased out and replaced by the more environmentally safer hydrofluoroalkane (HFA) alternatives. Nevertheless, switching to HFA propellants in MDIs was not straightforward due to their different physico-chemical properties as well as incompatibility with the conventional surfactants used in CFC-MDIs. These challenges forced everyone to consider new approaches and develop better ways to accommodate the new propellants and deliver inhaled medications. Two approaches were used in the reformulation of HFA-MDIs. The

first approach was to match the new HFA formulations to their chlorofluorocarbon counterparts on a microgram for microgram dose; therefore, no dosage modification was required on switching from the HFA-MDI to the CFC-MDI. An example of this approach is the development of Clenil Modulite® (Chiesi, Italy) which is the first CFC free BDP metered-dose inhaler directly interchangeable with CFC-BDP containing inhalers. It has a mass mean aerodynamic diameter of 2.9μm and its particle size distribution more closely matches that of CFC containing MDIs (Ganderton et al., 2002). The second approach has focused on tailoring the particle size distribution of the aerosol generated to produce extra fine particles for more efficient lung targeting (Ganderton et al., 2002; Lewis et al., 2005). An example of the second approach is the development of Qvar® (Teva Pharmaceuticals, UK) inhalers that has a mass median aerodynamic diameter of 1.1μm. The smaller median particle size of Qvar® (HFA-BDP) has been shown to improve drug delivery and produce equivalent asthma control to chlorofluorocarbon-based BDP inhalers, at approximately half the daily dose in both adults and children (Leach et al., 1998a; Leach et al., 2002; Janssens et al., 2003). Despite the improved lung deposition of Qvar®, it has a favourable safety profile (systemic and overall) compared with other inhaled corticosteroids (Thompson et al., 1998; Ayres et al., 1999).

After inhalation, up to 20% of the dose is delivered to the lungs whilst the majority is swallowed (Chrystyn, 1997). The proportion of the dose that enters the lung is either cleared from the body, either by mucociliary clearance (Borgstrom et al., 1992) then swallowed or by absorption through the airway wall into the systemic circulation. It is the latter delivered by the pulmonary route that has the potential to exert a therapeutic effect; this is termed the effective lung dose.

The amount of drug that deposits in different regions of the respiratory tract can be determined by *in-vivo* methods such as gamma scintigraphy, using radioactive tagged

aerosol particles (Leach et al., 1998a; Leach et al., 1998b), pharmacokinetic methods using plasma (Clark et al., 1996) or urine samples (Hindle and Chrystyn, 1992), and *in-vitro* methods mostly using the Andersen Cascade Impactor.

In-vitro methods are used as a quality assurance procedure to identify the total emitted dose and dose uniformity. In addition, they measure the aerodynamic particle size distribution of the aerosol cloud generated by the product yielding information about the mass fraction that has the potential to enter the deeper part of the lung. Various studies have shown that the aerodynamic particle size distribution of aerosols generated by inhalation products correlates with the amount of drug deposited in the lungs (Seale and Harrison, 1998; Silkstone et al., 2002).

Pharmacokinetic methods using plasma or urine samples can be used to identify the relative lung deposition of the drug and total systemic delivery. Borgstrom and Nilsson (1990) developed a charcoal block method to identify the relative lung deposition. They reported that the concurrent oral administration of activated charcoal blocked all absorption of the drug from the gastrointestinal tract. In this case, the amount of drug eliminated in the urine gives an absolute value for the total lung dose. However, because this method uses oral charcoal it would be unethical to extend it to patient studies due to their concomitant oral therapy (Chrystyn, 2001).

Hindle and Chrystyn (1992) first reported a urinary pharmacokinetic method to determine the relative bioavailability of salbutamol to the lung and to the body following an inhalation. Drugs delivered to the lungs are very rapidly absorbed into the body whereas there is a lag time after oral administration before its delivery to the systemic circulation. The body starts eliminating drugs as soon as they are delivered to the body. Using this principle, Hindle and Chrystyn (1992) found that the amount of salbutamol excreted in the urine over the first 30 minutes post inhalation was significantly greater

than the amount eliminated following oral administration. They have validated how this index represents the amount of the inhaled dose deposited in the lungs. This measurement represents the effective lung dose because it measures the drug delivered to the body following passage through the airway wall. Hindle and Chrystyn (1992) also reported that the amount of salbutamol and its metabolites excreted in urine over the 24 hours period post inhalation is an index of systemic delivery. This index is the relative bioavailability to the body following inhalation.

1.2. Aim and objectives

1.2.1. Aim

The aim of this research work is to:

- Develop and validate a urine pharmacokinetic methodology to identify the relative lung and systemic bioavailability of beclometasone following inhalation.
- Investigate the pharmacokinetics and *in-vitro* performance of beclometasone dipropionate inhaled from two different HFA-BDP formulations with or without spacer devices.

1.2.2. Objectives

1. To develop and validate a sensitive, robust and reliable LC-MS assay for the determination of beclometasone dipropionate and its metabolites in methanol samples for *in-vitro* testing of inhaled products and human urine samples following oral and inhaled administrations to subjects.
2. To identify and validate the Hindle and Chrystyn urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of beclometasone following inhalation.
3. To determine the aerodynamic characteristics of the emitted dose of beclometasone dipropionate obtained from two different HFA-BDP formulations (Clenil Modulite®

MDI and Qvar® inhalers) with and without different spacers. In addition, to test the effect of different spacers' handling methods on the aerodynamic particle size distribution of the studied aerosols.

4. To determine the effect of increasing the inspiratory flow rate on the aerodynamic characteristics of the emitted dose of beclometasone dipropionate from Clenil Modulite® MDI and Qvar® inhalers when used alone without spacers.
5. To demonstrate the application of the previously validated urinary pharmacokinetic method of beclometasone to investigate the effect of different spacers on the lung and systemic bioavailability following inhalation from either Clenil Modulite® MDI, Qvar® EB or Qvar® MDI with and without spacers. In addition, to test the effect of different spacers' handling methods on the *in-vivo* drug delivery.

1.3. Thesis structure

The work in this thesis as follows:

Chapter 1: a general introduction with a brief summary of work.

Chapter 2: an overview of literature related to the areas of study.

Chapter3: describes the validation of a sensitive, simple LC-MS assay for the determination of beclometasone dipropionate (BDP) and its metabolites 17-beclometasone monopropionate (17-BMP) and beclometasone (BOH) in methanol and urine samples for subsequent *in-vitro* and *in-vivo* studies, respectively. Fluticasone propionate was used as the internal standard. The parent compound, metabolites, and the internal standard were extracted from urine samples using a solid phase extraction method. The intra-day and inter-day accuracy, precision, limit of detection, and limit of quantification of BDP, 17-BMP, and BOH by the extraction method and the LC-MS assay have been determined. In addition, this chapter describes a method developed for

hydrolysis of beclometasone dipropionate via an esterase enzyme with identification and separation of its metabolites.

Chapter 4: describes the validation of the urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of beclometasone following oral, oral with charcoal, inhaled and inhaled with charcoal administration. Furthermore, the intra- and inter-subject variability of the 30 minutes and the 24hr urinary excretion post inhalation was investigated.

Chapter 5: it is divided into two sections:

(a) *In-vitro* study to characterise the dose emitted from Clenil Modulite® MDI alone and when attached to different spacers. In addition, determination of the aerodynamic particle size distribution obtained from Clenil Modulite® MDI alone at different flow rates.
(b) Application of the urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of beclometasone following inhalation from Clenil Modulite® MDI alone and when attached to different spacers.

Chapter 6: it is divided into two sections:

(a) *In-vitro* study to characterise the dose emitted from Qvar® MDI and Qvar® EB alone and when attached to different spacers. In addition, determination of the aerodynamic particle size distribution obtained from Qvar® MDI and Qvar® EB alone at different flow rates.
(b) Application of the urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of beclometasone following inhalation from Qvar® MDI and Qvar® EB alone and when attached to different spacers.

Chapter 7: it is divided into two sections:

(a) Comparison of the *in-vitro* emitted dose and aerodynamic particle size distribution of beclometasone dipropionate emitted from Qvar® EB, Qvar® MDI, and Clenil® inhalers with and without spacers by using the previously illustrated results in chapter 5.2 and 6.2 of this thesis.

(b) Comparison of the relative lung and systemic bioavailability of beclometasone dipropionate post-inhalation from Qvar® EB, Qvar® MDI, and Clenil® inhalers with spacers by using the previous results illustrated in chapter 5.3 and 6.3 of this thesis.

Chapter 8: describes a general conclusion from these studies and suggestions for future work.

Chapter 2: Literature Review

2.1. The respiratory system

The respiratory system may be defined as the organs and tissues through which air is passed into and out of the body to allow the necessary gaseous exchange to take place between the circulatory system and the outside world. When you breathe in or inhale, your body receives oxygen that is essential to the body to produce energy, perform its metabolic functions, and sustain life. When you breathe out or exhale, your body is cleared from carbon dioxide, a waste gas produced as result of chemical reactions within the cells, which must be continuously eliminated, as excessive amounts of carbon dioxide are toxic. The human respiratory system can be divided into two functional regions: upper respiratory tract (nasal passages, pharynx, and the larynx) and lower respiratory tract (the conducting airways and lungs). The lower respiratory tract structures are contained within the thoracic cavity. The upper respiratory tract passageways are lined with respiratory ciliated epithelium, which secretes mucus. These cilia prevent inhaled particles from reaching the lungs and help to propel secretion to the pharynx where they can be swallowed or coughed up (Waldron, 2008; Rogers, 2011).

2.1.1. The conducting airways

Atmospheric air is delivered in and out regularly into the respiratory portions of the lung through a system of airways called the conducting airways. They form a very complex branching tree of tubes, which become narrower, shorter and more numerous as they penetrate deeper into the lung. As illustrated in figure 2.1A, air is carried to and from the lungs by the trachea that extends from the larynx to the middle of the thorax where it divides into the right and left main bronchi, each of which feed air to one of the lungs. The trachea (windpipe) is a muscular tube supported by C-shaped cartilage rings that help to protect it and prevent it from collapse. The bronchial tubes subdivide and with each subdivision, their walls get thinner. After about 16 levels of branching, the airways

become the respiratory zone where gaseous exchange occurs. The airways from the trachea through and including the terminal bronchioles down to the 16th branch are known as conducting airways. This region actually contains no alveoli, so no gas exchange takes place in this area and it is often referred to as anatomical dead space.

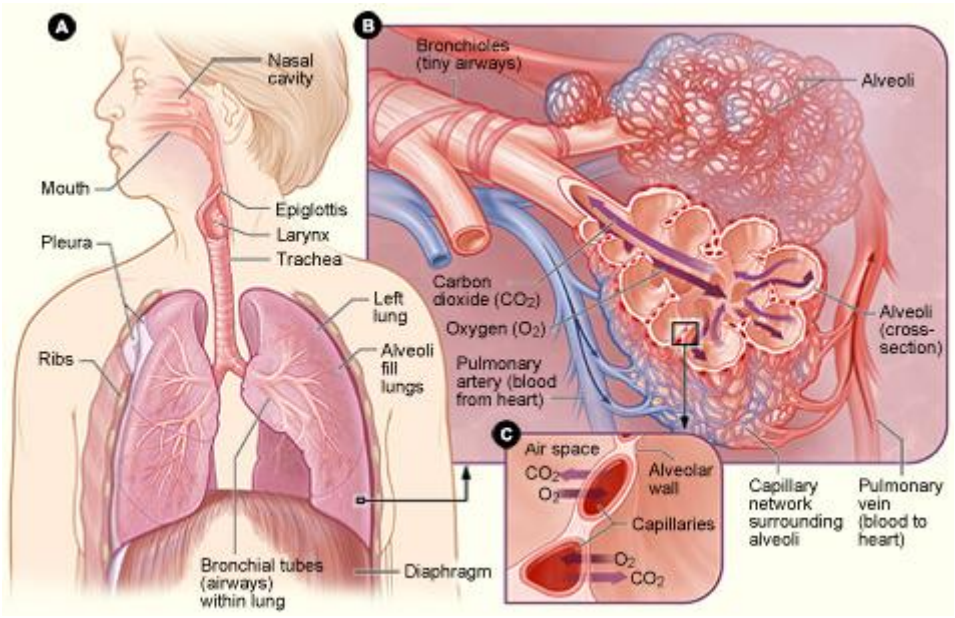


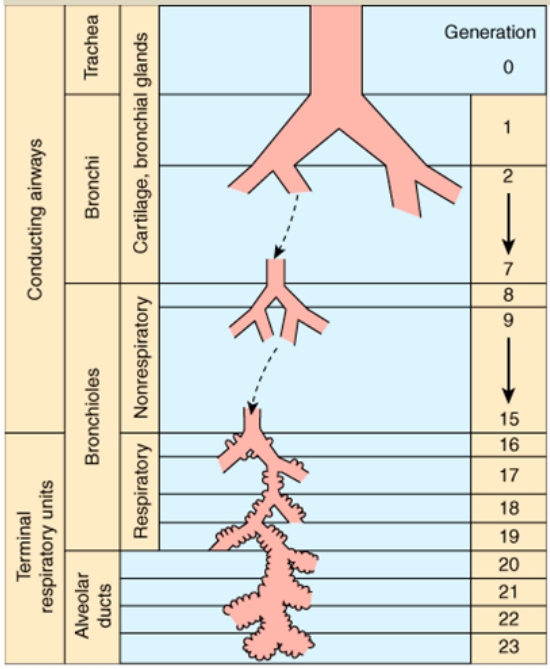
Figure 2.1: Lung structures (A) respiratory organs (B) respiratory bronchioles and alveoli (C) gaseous exchange. From (http://www.nhlbi.nih.gov/health/dci/Diseases/hlw/hlw_respsys.html).

The conducting airways have two major functions. First is to lead the inspired air to the more distal gas-exchange regions of the lung and second is to warm and humidify the air to avoid any damage to the delicate structure of the alveoli by excessive exposure to dry, cold air. The structures distal to the terminal bronchioles branch more into the respiratory bronchioles, these tiny respiratory bronchioles eventually become alveolar ducts, which terminate into groups of thin walled sacs called alveoli. This is the site where respiratory gas exchange takes place. The region, from the respiratory bronchioles through the alveoli, is known as the respiratory zone (Kelly, 2003; Ethier and Simmons, 2007; Whittemore, 2009).

As shown in figure 2.1 (B), the pulmonary circulation functions to bring blood into close contact with the alveoli (air sacs), where gas exchange takes place at the blood-gas interface. A dense network of blood vessels called pulmonary capillaries in the lung surrounds alveoli. The blood in these capillaries picks up oxygen from the alveoli to be transferred round the body, and transport carbon dioxide back to the alveoli to be excreted. The very small distance of 1µm and in some cases 0.1µm between the blood in an alveolar capillary and the air inside the alveolus is the reason behind the quick and efficient gas transfer between the blood and the lungs. Oxygen and carbon dioxide move between air and blood by simple diffusion, that is from an area of high concentration to an area of low concentration as illustrated in figure 2.1 (C). Fick's Law of diffusion states that, the amount of gas that moves across a sheet of tissue is proportional to the area of the sheet (A) but inversely proportional to its thickness (T). It is expressed by the following equation V_{Gas} α A.D (P1 – P2)/T, where V_{Gas}= gas flow, A= area, T= thickness, D= Diffusivity, and P_1-P_2 = partial pressure gradient (West, 2008).

Figure 2.2, shows that the airway branching system of the lung undergoes 23 bifurcations and the surface area of the alveoli is enormous compared to airways surface area, which allows the efficient gas exchange. Several studies in human adults have shown that the surface area of the airways averaged 2.5m^2 (Mercer et al., 1994; Patton, 1996; Leach et al., 2002), while the total surface area of the alveolar walls has been estimated to be as large as 140$m^{2,}$ which is about 75 times the body's external surface area. This is attributed to the fact that the human lung has about 500 million alveoli and the walls of each alveolus are completely lined with an enormous number of capillaries; there are 280 billion pulmonary capillaries or almost 1000 capillaries per alveolus, resulting in a massive surface area available for gas diffusion inside the limited thoracic cavity.

In addition, the extreme thinness of the blood gas interface facilitates gas exchange by diffusion and therefore, it is very well suited to the gas exchange function. The combination of an enormous alveolar surface area and the very thin tissue layer between blood and air makes the lung a very effective mass transfer device. Carbon dioxide moves into the alveolus, as the concentration is much lower than in the blood. Oxygen moves out of the alveolus due to the continuous flow of blood through the capillaries that prevents saturation of the blood with oxygen and thereby allowing maximal transfer across the membrane; this is known as gas exchange process or respiration (Kelly, 2003; Ethier and Simmons, 2007; West, 2007; Whittemore, 2009).



Airduct	Gen	Diameter (mm)	Number per gen	Total cross sectional area (cm^2)
Trachea	0	18	1	2.54
Main bronchus	1	13	2	2.33
Lobar bronchi	2-3	8.3	4	2.13
Segment bronchi	4	4	16	2.48
Terminal bronchi	5-11	1.1	2000	3.11
Term. bronchioles	12-16	0.6	66,000	180
Resp. bronchioles	17-19	0.5	260,000	10^3
Alveolar duct	20-22	0.3	2×10^6	---
Alveolar sac	23	0.3	8×10^6	10^4

Figure 2.2: Schematic design of airway branching in the human lung. Reproduced from (Weibel, 1963).

2.1.2. The lungs

The lungs are spongy, cone shaped structures. The left lung has two lobes and is slightly smaller than the right lung, which has three lobes. The base of the lungs rest on the

diaphragm (the main muscle of respiration) and the top, which is called the apex, starts at the root of the neck. The two lungs are each enclosed within a double membrane known as the pleura. The visceral pleura is the membrane adhered to the external surface of the lungs and the parietal pleura lines the wall of the thoracic cavity. The space in between the two layers, the pleural space is normally filled with the intra-pleural fluid. This fluid lubricates the membranes and reduces friction between the layers as they slide over each other during breathing. The elasticity or capacity of the lung to stretch is due to the presence of elastic fibres and collagen in lung tissue, which gives the lung the ability to inflate and deflate during breathing (Ward et al., 2006; Waldron, 2008; Whittemore, 2009). As shown in figure 2.3, contraction and relaxation of the muscles of the chest and the diaphragm are responsible for inspiration and expiration. During inspiration (inhalation), the diaphragm contracts, flattens, and moves downward and the inter-costal muscles between the ribs contract, pulling the ribcage upwards and outwards. Thereby, increasing the volume of the thoracic cavity and air is drawn into the lung by a negative intra-thoracic pressure.

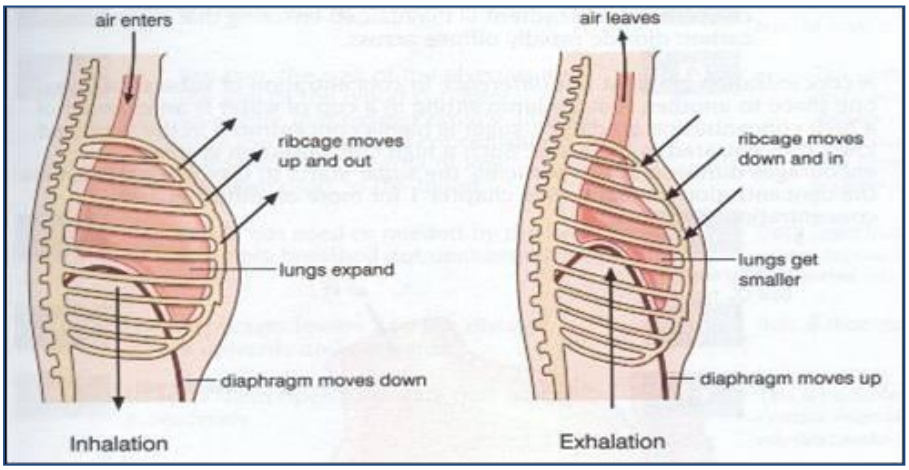


Figure 2.3: Mechanisms of respiration.

Expiration (exhalation) is a passive process that depends on the natural tendency of the lungs to collapse. The inter-costal muscles relax and the diaphragm falls back to its

original position, pulling the ribcage down and contracting the lungs. This reduces the volume of the chest and forces the air out of the lungs (Kelly, 2003; Ethier and Simmons, 2007; West, 2007; Waldron, 2008).

2.2. Diseases of the respiratory system

The respiratory system is susceptible to a number of diseases, caused by genetic factors, infections, and pollutants. The most common problems of the respiratory system are asthma and chronic obstructive pulmonary disease (COPD).

2.2.1. Asthma

Asthma is one of the most common chronic pathological conditions throughout the world. It has been estimated that asthma affects around 300 million people worldwide, a total that is expected to rise by an additional 100 million mainly in children over the next 20 years. In the UK, it is estimated that 5.2 million people are currently receiving asthma treatment, which is costing the National Health Service (NHS) over £889 million a year in terms of emergency room visits and hospitalisations (Masoli et al., 2004a; Adcock et al., 2008b; Waldron, 2008).

The international consensus report for the management and diagnosis of asthma defined it as a chronic inflammatory disorder of the airways in susceptible individuals, in which many cells and cellular elements play a role. This chronic inflammation is usually associated with widespread but variable airflow obstruction and an increase in airway response to a variety of stimuli. Obstruction is often reversible, either spontaneously or with treatment (GINA, 2010; Rees et al., 2010). A detailed explanation of asthma pathogenesis is provided in APPENDIX A.1 (refer to the enclosed DVD).

2.2.1.1. Asthma management and treatment

Asthma is more accurately thought as a multi-factorial overlapping syndrome rather than a single disease. Thus it is usually difficult to find a cure for asthma; hence the goals of

optimum asthma control according to GINA (2010) guidelines is the avoidance and removal of stimulus that induces airway constriction, control the symptoms, minimise the use of rescue medication, prevent asthma exacerbations and achieve best possible normal level of daily activity and lung function.

Asthma is a disease of two main components, inflammation, and bronchoconstriction, so treatment regimens that address both issues provide the most efficacious asthma treatment. Asthma medications fall into one of two groups: relievers (the mainstay therapy for bronchoconstriction) including inhaled short acting beta agonists (e.g. salbutamol, terbutaline), anti-cholinergics (e.g. ipratropium), and preventers (the main stay therapy for inflammation) mainly inhaled corticosteroids (ICS). In addition, controllers namely long acting beta agonists (e.g. salmeterol, formoterol) used in conjunction with ICS have been shown to provide extra benefits in the control of asthma symptoms (Barnes et al., 1998). Other agents used are inhaled anti-allergic non-steroidal agents (e.g. cromoglycate and nedocromil), leukotriene inhibitors, Xanthines (e.g. theophylline). However, current guidelines have pointed out that inhaled corticosteroids are the gold standard of control therapy for asthma (Suissa et al., 2000; Pauwels et al., 2003; Murphy, 2007; GINA, 2010).

The quick relievers group are best represented by the inhaled short-acting beta agonists or SABAs, which are effective bronchodilators with a rapid onset of action (Volovitz, 2008). Short-acting beta agonists (SABAs) should be used on an as-needed basis and are commonly prescribed to relieve acute asthmatic episodes, by relaxing the airway smooth muscle, inhibit mediators release from mast cells and reduce vascular permeability (Kassianos et al., 2005).

The British Thoracic Society's guidelines (BTS) (BTS/SIGN, 2008) in its stepwise approach, as illustrated in figure 2.4, recommends that inhaled short-acting $ß_2$ agonists

are the first line treatment in case of mild intermittent asthma and inhaled corticosteroids are the cornerstone of asthma management. Using β_2-agonists on as required basis was proven to be as good as regular administration (Dennis et al., 2000; Walters et al., 2003; BTS/SIGN, 2008).

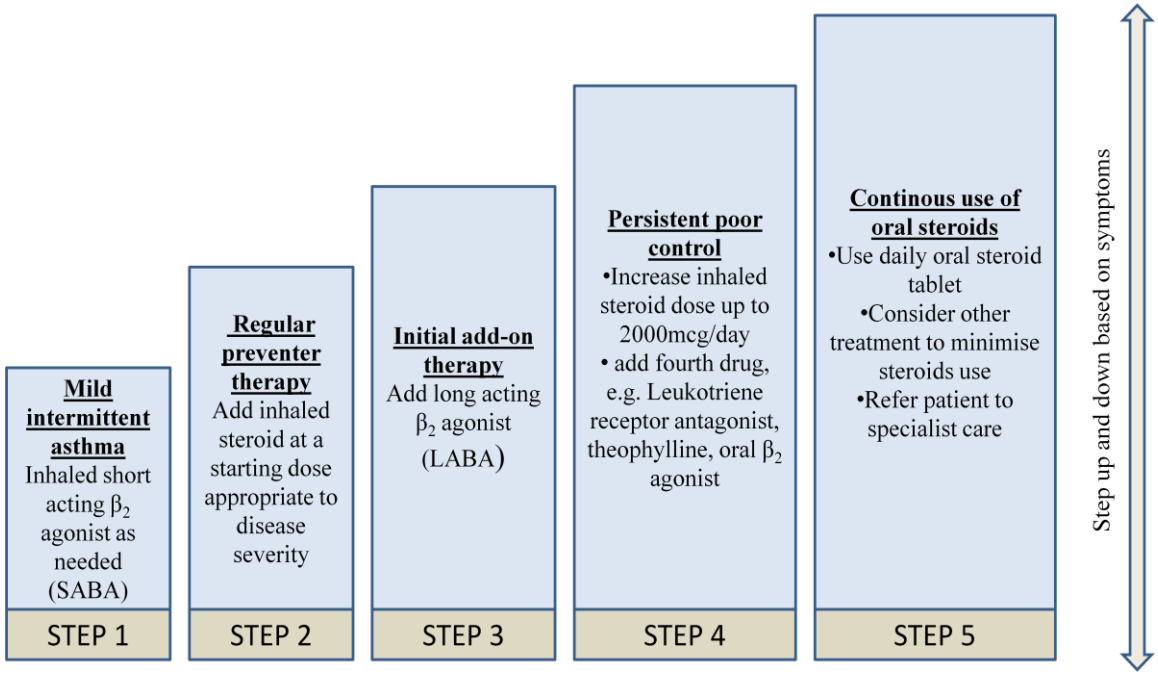


Figure 2.4: Summary of the stepwise management of asthma according to the most recent proposed BTS/SIGN guidelines (BTS/SIGN, 2008).

Asthma is a dynamic as well as chronic condition, this is why the treatment plan should include both a step up and a step down approach, in which the number and frequency of medications are increased or decreased according to the symptoms. The concept of self-management in asthma therapy is very important and has been shown to reduce morbidity and health care resource utilisation, so the patient initiates changes in therapy, according to the degree of symptoms, ß$_2$-agonist use (Lahdensuo et al., 1998; Miller-Larsson and Selroos, 2006; Bernstein, 2008). The increased use of short-acting β_2-agonist by asthmatic patients should be used as an index of worsening asthma control mandating the addition of an anti-inflammatory therapy (Holgate and Polosa, 2006). It is very

important to reduce treatment as asthma comes under control, so that the patient is on the minimal therapy required.

Inflammation is an early and persistent feature of asthma and many studies suggest that the early introduction of ICS anti-inflammatory treatment leads to a better improved asthma and less additional asthma medication use (Haahtela, 1995; Selroos et al., 1995; Bernstein, 2008; Busse et al., 2008; Corrigan et al., 2009). These studies also support current national and international asthma treatment guidelines which emphasize the importance of this early intervention with inhaled corticosteroids (ICS) as an initial anti-inflammatory treatment for asthma (NAEPP, 2007; BTS/SIGN, 2008).

Inhaled corticosteroids were found to be very effective in reducing the severity of symptoms, diminishing airway hyperresponsiveness, preventing exacerbations, improving asthma control, and quality of life (Pauwels et al., 2003; Adams et al., 2005; Adams et al., 2008; Reddel et al., 2008; GINA, 2010). Also, inhaled corticosteroids were beneficial in decreasing the need for hospitalizations (van Ganse et al., 1997), and deaths due to severe asthma (Suissa et al., 2000; Kips and Pauwels, 2001b; Neffen et al., 2006). ICS doses should be adjusted according to the level of control obtained and the dose should be titrated to the minimum dose required to achieve asthma control, thus reducing the potential for side effects (BTS/SIGN, 2008).

Nevertheless, a substantial proportion of asthmatic patients with more severe disease are insufficiently controlled with a low to moderate dose of ICS. For these patients several therapeutic options exist as recommended by the current guidelines, the first option is to add another form of controller medication to an unchanged dose of ICS such as long acting beta agonists or LABAs (e.g. salmeterol, formoterol); the second option is to increase the ICS dose (Kips and Pauwels, 2001a; BTS/SIGN, 2008).

Different clinical trials have found that ICS and LABA in a combination inhaler are superior to increasing the dose of ICS (Shepherd et al., 2008). ICS/LABA combination

therapy complements each other by working on two different components of the disease: inflammation and bronchoconstriction. They provide greater improvement in lung function, better symptoms control, and lower exacerbations compared with ICS alone, even at much higher doses of ICS (Shapiro et al., 2000; Masoli et al., 2005; Fabbri et al., 2008; Shepherd et al., 2008; Ducharme Francine et al., 2010). Long-acting β_2-agonists are believed to interact synergistically with inhaled corticosteroids and permit lower dosing of corticosteroid, but they should never be used as a mono-therapy but only as an additional therapy (Pauwels et al., 1997b; Kips and Pauwels, 2001a; Naedele-Risha et al., 2001; Miller-Larsson and Selroos, 2006). Corticosteroids have been shown to up-regulate the β-$_2$ receptor in the human airways, leading to more receptors available for β_2-agonist activation. On the other hand, LABA was shown to facilitate the entry of the glucocorticoid receptor ligand complex into the nucleus, hence enhancing its anti-inflammatory effects (Mak et al., 1995; Schmidt et al., 2001). A recent Cochrane review concluded that the use of LABA allows up to 57% reduction of inhaled corticosteroids use (Gibson et al., 2005). Importantly, no safety issues have been identified with this combination in patients with asthma and COPD (Miller-Larsson and Selroos, 2006).

Alternative to the addition of LABA therapy, leukotriene receptor antagonists (Currie et al., 2005) or theophylline (Ukena et al., 1997; Tee et al., 2007) can be added to the combination therapy with ICS for patients with persistent asthma. However, these combinations are less effective than ICS/LABA dual therapy, which is the preferred therapy (Busse et al., 1999a; Nelson et al., 2000; Meltzer, 2003).

Nevertheless, other patients with severe persistent, uncontrolled asthma will need to use oral corticosteroids at the lowest possible dose as adjunct to SABAs to speed recovery and prevent recurrence of exacerbations. Patients at this stage should be referred for specialist care (BTS/SIGN, 2008; Waldron, 2008).

2.2.2. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a common respiratory disorder and a huge health problem that causes considerable morbidity, patient suffering, and mortality throughout the world. Chronic obstructive pulmonary disease is the fifth leading cause of death in the UK and fourth worldwide and set to become the third leading cause of death worldwide by the year 2020 (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009; NICE, 2010). Approximately 835,000 people in England have been diagnosed with COPD only in 2008-9 (NHS Information Centre for Health and Social Care, 2009).Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterised by a slow progressive airways limitation that is not fully reversible. It is caused by an abnormal inflammatory response of the lungs to chronic inhalation of noxious particles, often cigarette smoke. It may also be associated with significant extra pulmonary effects that may contribute to its severity (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009). Information about the pathogenesis of COPD is provided in APPENDIX A.2 (refer to the enclosed DVD).

2.2.2.1. Management and treatment

Patients suspected of having COPD should undergo complete pulmonary function testing to confirm airway obstruction, quantify its severity, reversibility and to distinguish COPD from other diseases. The primary diagnostic test is the forced expiratory volume (FEV_1), which is the volume of air, expired during the first second after a full breath; forced vital capacity (FVC) which is the volume of air exhaled with maximum effort and speed after a full inspiration. As COPD progresses with increasing airway wall thickening, loss of alveolar attachments, and loss of lung elastic recoil, both FEV_1 and FVC decrease. Reductions of FEV_1, FVC and the ratio of FEV_1/FVC are markers for airway obstruction (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009).

The aim of chronic treatment of COPD is to improve the symptoms, exercise tolerance, and the quality of life by slowing down the progression of the disease, i.e. by improving FEV_1 or reduce the decline in FEV_1. Many studies have examined the efficacy of certain drugs in COPD by determining their ability to improve FEV_1(Lopez-Encuentra et al., 2005). Another further aim of COPD management and treatment is to reduce exacerbations and increase life expectancy. As shown in figure 2.5, the current global initiative for chronic obstructive pulmonary disease (GOLD) guidelines for the treatment of stable COPD disease, suggested simple classification of the disease severity, according to FEV_1, into four stages and a step wise management as the patient's airflow limitation and symptoms worsen (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009).

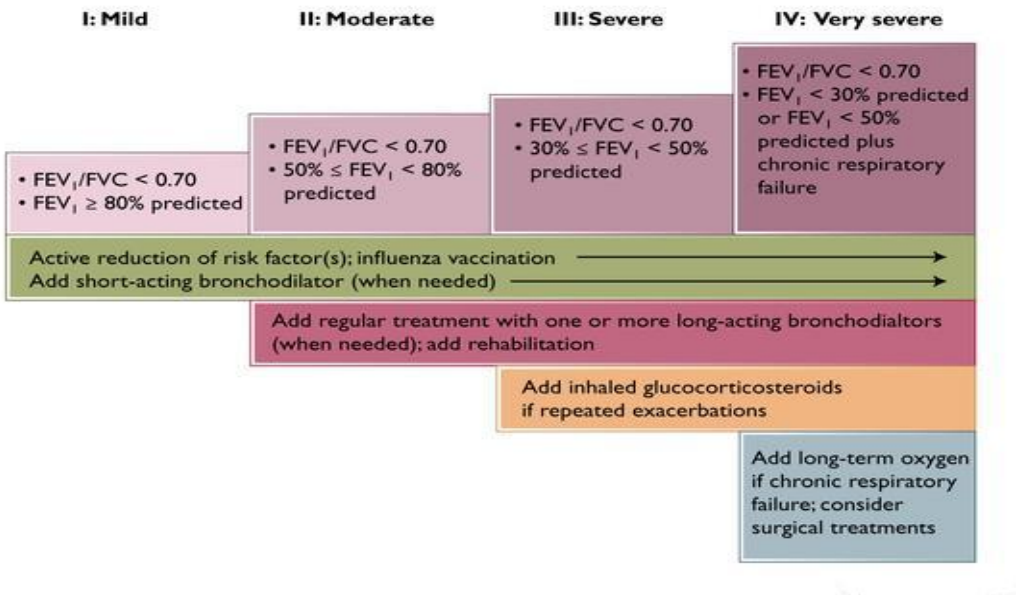


Figure 2.5: Diagrammatic representation of GOLD guidelines for the treatment of stable COPD disease (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009).

The first step in the proper management of COPD is the avoidance of risk factors; smoking cessation is a very effective intervention procedure to stop the progressive worsening of COPD and significantly influences the long-term evolution of the disease (Pauwels, 2000; Wise et al., 2003). Smoking cessation was found to decrease the

accelerated decline in FEV_1 characteristic of this disease (Anthonisen et al., 1994) and even decrease lung cancer mortality in COPD patients (Anthonisen et al., 2005).

The most recent comprehensive guidelines (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2009; NICE, 2010) recommend that the next important consideration for long term management of COPD is the introduction of inhaled bronchodilators. Bronchodilators such as anti-cholinergic agents, theophylline, and β_2-agonists are considered the cornerstone in symptomatic management of COPD. Despite the substantial differences in their sites of action within the cell, the most important consequence of bronchodilator therapy appears to be airway smooth muscle relaxation and improved emptying during tidal breathing; they improve the symptoms, exercise tolerance, and partially reverse the airflow limitation. Short-acting agents are best used for the rescue of symptoms; whereas long-acting agents are best used for maintenance therapy. The choice between different bronchodilators should depend on the patient symptomatic response. A systematic review showed that regular use of short-acting β_2-agonists in COPD was associated with an improvement in lung function and dyspnoea (Sestini et al., 2002). Many studies highlighted that long acting inhaled bronchodilators are at least as effective as the short acting ones and even more convenient (Mahler et al., 1999; Littner et al., 2000; Barnes et al., 2001; Hanania et al., 2005; Berger and Nadel, 2008). Furthermore, other studies have indicated the superiority of treatment of COPD with long acting bronchodilators compared to short acting ones, recommending them as a first line option for treatment of stable COPD (van Noord et al., 2000; Cazzola and Matera, 2004; Tashkin and Cooper, 2004).

Combination of more than one class of bronchodilators was found to be more beneficial than the use of single agents. The rationale behind that is not only due to the additional benefits of their different pharmacological action but also to avoid side effects of using

higher doses of single agents (Cazzola et al., 2004; van Noord et al., 2005; Di Marco et al., 2006; Kerstjens et al., 2007; Vogelmeier et al., 2008).

The addition of inhaled corticosteroids to maintenance treatment with long-acting β_2-agonists led to a more significant reduction in respiratory symptoms, exacerbation rates, improvement in FEV_1, and statistically significant improvements in health related quality of life compared to those provided with either treatment alone (Mahler et al., 2002; Calverley et al., 2003; Hanania et al., 2003; Barnes et al., 2006; Bourbeau et al., 2007; Puhan et al., 2009). Calverley et al (2003) reported that the budesonide/formoterol combination in a single inhaler were more effective than either component drug alone or placebo in stabilizing lung functions and decreasing exacerbations. The TORCH study was the first interventional study in COPD with mortality as a primary outcome measure. This study showed that mortality was significantly better in the combination therapy compared to fluticasone propionate therapy. Thus, it appears that the addition of salmeterol to fluticasone significantly modified the therapeutic effects of the ICS (Calverley et al., 2007; Seemungal et al., 2009). There is evidence from systematic reviews that suggests that an ICS in combination with a LABA appears to modestly reduce the risk of exacerbations, when compared to LABAs, by approximately 20%–25% (Nannini Luis et al., 2007). These previous findings suggest that treatment of both inflammation and bronchoconstriction with COPD patients may actually achieve clinically important effects.

The ability of corticosteroids to effectively suppress airway inflammation in asthma has led to this treatment becoming the cornerstone of asthma therapy whilst in COPD the role of corticosteroids is more controversial. A detailed explanation of the role of inhaled corticosteroids in COPD is presented in APPENDIX A.3 (refer to the enclosed DVD).

2.2.3. Summary

The above information about the guidelines highlight that inhaled therapy is the mainstay for the management of both asthma and COPD. It is important that the emitted dose is able to deposit in the airways during an inhalation.

2.3. Pulmonary drug delivery

The pulmonary route has several advantages, which makes it an attractive option for local drug delivery as illustrated in figure 2.6. There are many local lung diseases that are considered as prime candidates for inhalation therapy, such as asthma and chronic obstructive pulmonary disease (COPD). This type of topical application of the drug to the lung epithelium spares the patient the potential side effects caused by the high systemic concentrations typical of conventional delivery methods, maximises pulmonary specificity with a rapid onset of action and can reduce costs because smaller doses can be used (Chrystyn, 1994; Chrystyn, 2007; Vincken et al., 2010).

The decreased incidence of side effects is especially important for inhaled corticosteroids, where asthma control can be achieved at doses far lower than those required to cause adrenal suppression. However, some suppression does occur when high inhaled doses are used. The inhaled route also offers a better efficacy to safety ratio compared to systemic therapy and allows the use of drugs, which are not absorbed in the gastrointestinal tract (e.g. cromones). Therefore, this route of drug delivery has become the preferred route of administration in the therapy of a number of respiratory disorders (Smola et al., 2008; Virchow et al., 2008; Broeders et al., 2009).

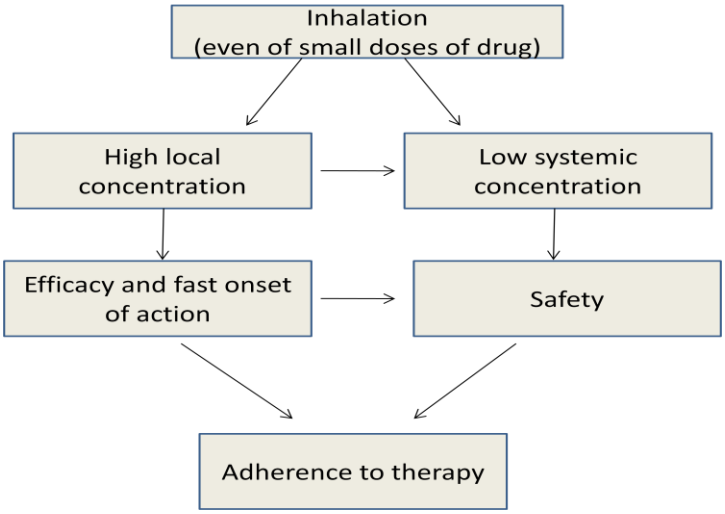


Figure 2.6: Advantages of the inhaled route of drug administration (Vincken et al., 2010).

2.3.1. Mechanisms of pulmonary particle deposition

Deposition means the event of a particle to adhere to the surface. There are three major mechanisms, by which inhaled particles deposit in the human respiratory tract: inertial impaction, gravitational sedimentation, and Brownian diffusion. Other deposition mechanisms include interception and electrostatic precipitation. These mechanisms are shown in figure 2.7 and described below.

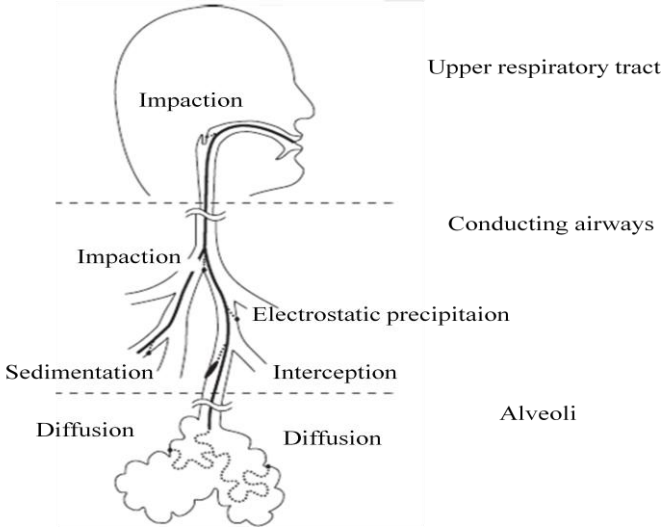


Figure 2.7: Mechanisms of deposition of inhaled particles in the respiratory tract from http://scribd.com/doc/28978774/Particle-Depsoition-in-the-Lung.

2.3.1.1. Inertial impaction

A particle with a large diameter and high density that is travelling in the airstream at high velocity will be very liable to impact because it will be unable to follow the changing direction of the airways. Deposition of particles by impaction occurs at airway bifurcations when a particle, owing to its momentum and the aerodynamic forces exerted on it by the air stream in which it is carried, fails to make the turn into either of the daughter branches and impacts at the branching junction. Impaction accounts for the majority of particle deposition on a mass basis; that is particle size and density, and it depends on the particle travelling velocity, which is determined by the inspiratory flow velocity prevailing in the airways. Deposition of particles by impaction becomes significant for particles > 2μm and it is most likely to occur in extra thoracic and large conducting airways in which there is a high flow velocity, short residence time of particles and rapid changes in airflow direction often take place (Schulz, 1998). Deposition by impaction increases with the branching angle and is independent on airway length. Rapid and shallow breathing increases impaction in the large airways producing a centralised particle deposition pattern (Rom and Markowitz, 2007; Adcock et al., 2008a). As, the gas velocity decreases due to the splitting of the airflow, impaction is expected to cease to be an important mechanism of deposition in small airways.

2.3.1.2. Sedimentation

Gravitational sedimentation is the settling of particles onto airway surfaces under the force of gravity. Particles reach their terminal settling velocity when the gravitational force equals the opposing resistive forces of the air. It occurs primarily for smaller particles (0.5-5μm) that do not impact and are carried by the inspired air into the lower parts of the airways where they settle under the effect of gravity when the airstream velocity becomes slow, e.g. the bronchioles and the alveolar region. The low air velocity in these regions gives enough time available for particles to settle within the airways. It is

important that a breath hold is included after an inhalation to allow this process to occur. Deposition by sedimentation increases with airway length and is independent on the branching angle. Therefore, slow, deep breathing enhances sedimentation and lead to relatively uniform distribution of particles throughout the airways (Rom and Markowitz, 2007; Adcock et al., 2008a).

2.3.1.3. Brownian diffusion

Unlike impaction and sedimentation, which increase with increasing particle size, deposition by Brownian diffusion increases with decreasing particle size. As the diameter of a suspended particle become smaller than 0.5 μm, they are more affected by the random thermal kinetic bombardment of the gas molecules in the air around them. Collision of gas molecules with these small particles lead to their irregular random wiggling motion called Brownian motion. Consequently, the small airway dimensions of the lung periphery, favour deposition by diffusion due to very low or absent airflow (i.e. alveoli), a short particle travelling distance before hitting an airway and long residence time giving particles enough time to diffuse to the surrounding surfaces (Rom and Markowitz, 2007; Adcock et al., 2008a). Similar to sedimentation a breath hold after each inhalation facilitates deposition by this mechanism.

All these mechanisms act simultaneously. Inertial impaction and gravitational sedimentation are most important for deposition of large particles (1-10μm), whereas Brownian diffusion is the main deposition determinant of smaller submicron particles (<1μm). Secondary, less important deposition mechanisms that occur within the respiratory tract such as interception and electrostatic precipitation do not significantly contribute to the deposition of inhaled therapeutic medical aerosols (Martonen and Katz, 1993; Adcock et al., 2008a).

2.3.2. Factors affecting pulmonary particle deposition

Aerosol drug deposition in the lung is not a simple matter, since the respiratory tract can be considered as a filter that efficiently removes particles from the inspired air. The effectiveness of this filter largely affect the resulting aerosol deposition pattern and is governed by several factors including; the physical characteristics of aerosol inhaled e.g. particle size, density and shape or the patient variables including; the individual breathing pattern and lung morphology (Heyder, 2004).

2.3.2.1. Aerosol physical properties

Considering the previously described deposition mechanisms, it becomes evident that particle size is one of the major variables influencing not only the extent but also the site of inhaled drug deposition within the airways (Heyder and Gehr, 2000; Usmani et al., 2003). Aerosol particles are often characterised by their mass mean aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) of their median aerodynamic diameter. The MMAD is the diameter around which the mass of particles are equally distributed. The GSD is a measure of dispersion of particle diameters in the aerosol (Schulz, 1998).

The respiratory anatomy has evolved in such a complex way to actively prevent inhalation of airborne particulate matter. Both, the upper airways and the branching anatomy of the trachea-bronchial tree act as a series of filters for inhaled particles. Particles between 2 and 10µm in aerodynamic diameter correspond to the inhalable particles capable to be deposited, in the upper respiratory tract. Aerosol drug particles with a MMAD larger than 8µm tend to impact on the throat and the first few airway generations, whereas sub-micrometer particles with MMAD less than 0.5µm diameter penetrate the lung deeply, but have a high tendency to be exhaled without deposition and thus contribute little to the therapeutic effect. However, some studies have found that

breath holding can minimize expiration of such small particles (Suarez and Hickey, 2000; Usmani et al., 2003; Haughney et al., 2010; Carvalho et al., 2011).

It is generally accepted that lung deposition is greater with particles in the size range 2-5 μm, particularly in obstructive lung disease, where the airways are narrowed and an aerosol will penetrate less deeply. Whilst particles with MMAD less than 2 μm will tend to deposit in the alveoli (Suarez and Hickey, 2000; Gradon and Marijnissen, 2003; Usmani et al., 2003; Pedersen et al., 2010).

Clay & Clarke (1987) investigated whether the size distribution of aerosols released from a jet nebuliser affects the amount of aerosol delivered to the lungs and reported that small nebulised aerosols (MMAD < 2 um) deliver a larger dose to the lungs and should be used to maximise lung deposition. They also indicated that utilising the optimum nebulised aerosol size is very beneficial not only to maximise lung deposition but also to use smaller doses to achieve the same therapeutic effect, thus patients would not be given unnecessarily large amounts of drugs (Clay and Clarke, 1987). Therefore, decreasing and increasing the particle size shifts the site of deposition from distal to proximal.

Targeting particles to deposit in a specific site within the respiratory tract may be desirable for pharmaceutical aerosols whereby effective treatment is only possible if therapeutic aerosols can reach the desired site (Asgharian et al., 2006). The ideal lung regions for optimal drug deposition differ with the class of drugs used and largely depend on selection of the appropriate particle size for target air space. For bronchodilators, e.g. β_2-agonists and anti-cholinergic agents, it is more beneficial to target drug deposition in the larger conducting airway regions to achieve a more effective therapy. As, although β_2-receptors are highly concentrated in the alveolar region, the airway muscles are relatively sparse, being predominantly located in the conducting airway region. In addition, the receptor sites for anti-cholinergic agents lie predominantly in the larger

airways (Newman, 1985; Martonen and Katz, 1993; Usmani et al., 2005; Haughney et al., 2010). The larger particle size aerosol (>3-6μm) is more preferred for bronchodilator therapy to avoid the penetration of the smaller sized particles (<2.5μm diameter) to the alveoli where they exert no pharmacodynamic effect and are rapidly absorbed and increase the risk of systemic adverse effects (Usmani et al., 2005; Virchow et al., 2008). For inhaled corticosteroids, a uniform lung distribution is preferred to reach the smaller airways, which are important and significant sites of airway inflammation (Hamid et al., 2003; Hamid and Tulic, 2007).

The optimum aerosol cloud should contain particles that are neither too small (often exhaled), nor too large (these mainly deposit in the upper airways, mouth and throat). Therefore, in order to target the lower respiratory tract, the aerosol aerodynamic diameter for an inhalation formulation should be between 2 to 5μm (Usmani et al., 2003; Usmani et al., 2005; Patton and Byron, 2007). Using the optimum drug particle size would in turn have a profound effect on the drug dose required to achieve a given clinical response (Adcock et al., 2008a).

2.3.2.2. Patient variables

Differences in particle deposition patterns between human lungs may be attributed to factors related to the patient itself. These factors are primarily differences in their breathing pattern and airway morphology.

2.3.2.2.1. Breathing pattern

The difference in the inhalation flow rate can substantially affect the regional deposition of aerosol particles in human subjects. An increase in tidal volume, while keeping the flow rate constant, will transport particles by convection deeper into the lung and increase their mean residence time. Hence, more particles reach, peripheral lung structures and more time is available for gravitational and diffusional particle transport

(Heyder and Gehr, 2000; Musante et al., 2002). However, keeping the tidal volume constant while increasing inhalation flow rate will increase the efficiency of the velocity dependent deposition mechanism (impaction) and decrease that of the time dependent deposition mechanisms (sedimentation and diffusion). The high flow rate will enhance deposition of particles larger than 2µm by impaction in the extra-thoracic and large conducting airways, but particles transport by diffusion and sedimentation are decreased because of the shorter time available for deposition. When inhaling aerosol particles, patients should inhale slowly over 3–4 s (to minimise impaction in the upper airways) and hold their breath (to maximise sedimentation and deposition in the peripheral areas of the lung). The optimum aerosol deposition in the lung was achieved during inhalation from a pressurized aerosol with 10 seconds breath hold after each puff (Newman et al., 1982b). The importance of the breath holding technique in optimising lung deposition was also illustrated by Hindle et al (1993) who recommended that this technique should be universally adopted.

In another study by Heyder et al (2004), the slow inhalation of aerosol with monodisperse particles of 1µm in conjunction with breath holding was found to be a very effective means of targeting drug to the lung periphery for the topical treatment of peripheral respiratory disease. Heyder et al (2004) also reported that breath holding not only allows targeting sub regions of the alveolar regions but also increases the dose delivered to these regions under consideration. Furthermore, the significance of the inhalation speed differs with the particle size. While small particles (1.5µm) were found to have a comparable effect on the forced expiratory volume in one second (FEV_1) regardless of the inhalation speed, the slow flow inhalation led to greater bronchodilator activity of larger aerosol particles (3 - 6µm) (Usmani et al., 2005; Virchow et al., 2008).

Generally speaking, lung deposition increases with the duration of breathing cycle; that is deposition is inversely related to the inspiratory flow rate (except for particle sizes >10μm) (Martonen and Katz, 1993). This slow airflow with breath holding will lead to substantial increase in the particles residence time and drug penetration index in the conducting airways regardless of the drug particle size, so that increased particle deposition by both sedimentation and diffusion in the trachea-bronchial region and alveoli (Pavia et al., 1977; Schulz, 1998; Stockley, 2005; Virchow et al., 2008). The smaller airways deposited fraction can be increased by 70% using a slow flow rate compared to inhalation with a normal flow rate even for drug particles as large as 6μm (Svartengren et al., 1996). This enhanced deposition of therapeutic aerosols in the peripheral airways would be of value, particularly with regard to treatment with inhaled corticosteroids for targeting the significant small airways inflammation in both asthma and COPD diseases (Tanaka et al., 2004; Corren, 2008; Hogg, 2008). In addition, this slow breathing is essential in decreasing the variation in lung deposition leading to a uniform drug deposition pattern (Newhouse and Ruffin, 1978; Häkkinen et al., 1999). For this reason, slow deep inhalation with breath holding is generally recommended after inhaling a medical aerosol and is incorporated in the patient instruction leaflet for many inhaled drug products. These principles apply when using MDIs and MDIs with spacers but when using dry powder inhalers (DPIs), patients must break these rules and inhale rapidly and forcefully, because unlike MDIs, DPIs do not dispense a gas, but a dry powder. These inhalers require users to increase their inspiratory flow to provide the energy required to disaggregate the drug powder formulation into small respirable particles that have the potential for lung deposition (Everard, 2001; Everard, 2003; Chrystyn, 2007; Haughney et al., 2010).

In summary, particles deposit more in the proximal airways with an increase in particle size and breathing rates, whereas enhanced pulmonary deposition take place with small sized particles and slow breathing rates (Kim and Hu, 1998).

2.3.2.2.2. Anatomy of the respiratory tract

Natural variation in airway geometry from one individual to another (e.g. airway length, airway diameter, branching angles and alveolar size) is an important determinant for the aerosol deposition pattern. Even in healthy subjects, inhaling the same aerosol with the same inhalation manoeuvre provides a wide inter-subject variation in aerosol deposition patterns, which represents the effect of random variation in airway anatomy (Heyder et al., 1982; Asgharian et al., 2001; Stockley, 2005).

Furthermore, structural changes caused by the presence of respiratory disease may considerably affect both the amount and location of particle deposition in the lung (Lipworth and Clark, 1997). Obstructive lung diseases, such as asthma and COPD, increase particle deposition in the central zones of the lungs and decreases particle penetration to the peripheral airways (Lipworth, 1996; Anderson, 1997; Kim and Hu, 1998; Dolovich and Dhand, 2011). The increased airway narrowing due to oedema, increased secretions, or smooth muscle constrictions led to lower total lung deposition and more central deposition post inhalation from MDI, MDI+ spacer and DPI for asthmatic subjects compared to normal subjects (Melchor et al., 1993; Dolovich and Dhand, 2011). Several other studies have reported lower systemic availability of salbutamol (Lipworth and Clark, 1997) and fluticasone propionate (Harrison et al., 2001; Harrison and Tattersfield, 2003) in asthmatic patients compared to healthy volunteers. These findings can be attributed to reduced lung deposition with more central deposition coupled with greater mucociliary elimination in patients than in healthy individuals (Weiner et al., 1999; Edsbäcker and Johansson, 2006).

2.4. Inhalation devices

The availability of an efficient aerosol delivery system or inhaler is very critical to the success of the inhaled treatment (Pedersen et al., 2010; Vincken et al., 2010). As illustrated in figure 2.8 there are several criteria that characterise an ideal inhaler including; the generation of aerosols with the optimum particle size ideally in the range 0.5-5µm for deep lung penetration post inhalation, accurate and uniform drug dosing, easy to use, preferable by patients, robust, portable and inexpensive (O'Connor, 2004; Brand, 2005; Chrystyn, 2007).

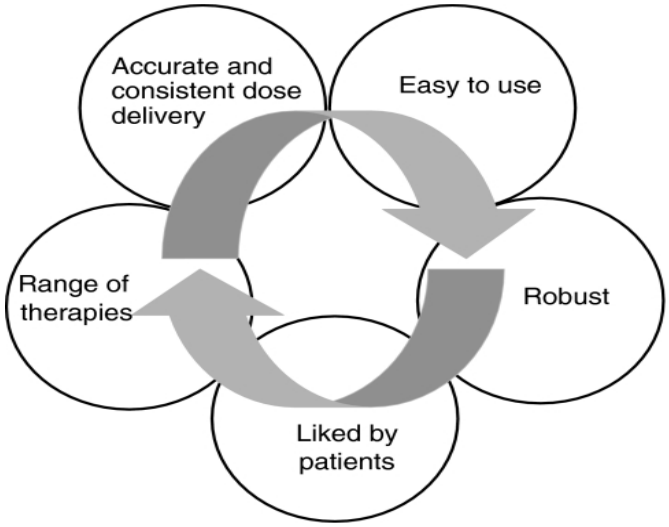


Figure 2.8: Criteria for an ideal inhaler (Chrystyn, 2007).

There are three principal types of devices that are widely used in aerosol administration; metered dose inhalers (MDIs), dry powder inhalers (DPIs), and nebulisers. Several clinical studies reported that these devices can be equally efficacious (Brocklebank et al., 2001; Dolovich et al., 2005; Cates et al., 2006). However we must bear in mind when selecting an aerosol delivery device for asthmatic and COPD patients, that the most efficacious device will be the one that is preferable by the patient and used correctly and consistently (Barry and O'Callaghan, 2003; Berger, 2009).

2.4.1. Metered dose inhalers

2.4.1.1. Conventional pressurised metered dose inhaler

Pressurised metered-dose inhalers (pMDIs) have been the dominant means of delivery of drug to the lungs since the early 1950s, and world-wide, they still constitute more than 80% of the global market (O'Connor, 2004). The reason behind its great popularity is that they are cheap, simple to manufacture and available with a wide range of drugs (Chrystyn, 2007). Pressurised metered-dose inhalers (pMDIs) are used to administer bronchodilators, anti-cholinergics, anti-inflammatory agents, and steroids and if properly used, they are at least as effective as other systems of aerosol drug delivery systems (Fink, 2000).

Metered dose inhalers (MDIs) are pressurized self-propelled aerosol devices that use propellants to administer the therapeutic agent. As shown in figure 2.9, the MDI consists of two major components: the canister and an actuator with a mouthpiece. The canister contains a metering dose valve with an actuating stem. The formulation resides within the canister and contains a liquid propellant with the drug either in solution or as a suspension of micronized particles. Actuation of the device triggers the release of a single metered dose of liquid propellant that contains the medication. The release of these contents under pressure combined with the low boiling point of the propellants rapidly evaporates the liquid mixture into an inhaled aerosol cloud of medication, enabling subsequent deposition within the lungs during an inhalation (Adams, 2007; Hickey, 2007; Mitchell et al., 2007b; Patterson et al., 2009).

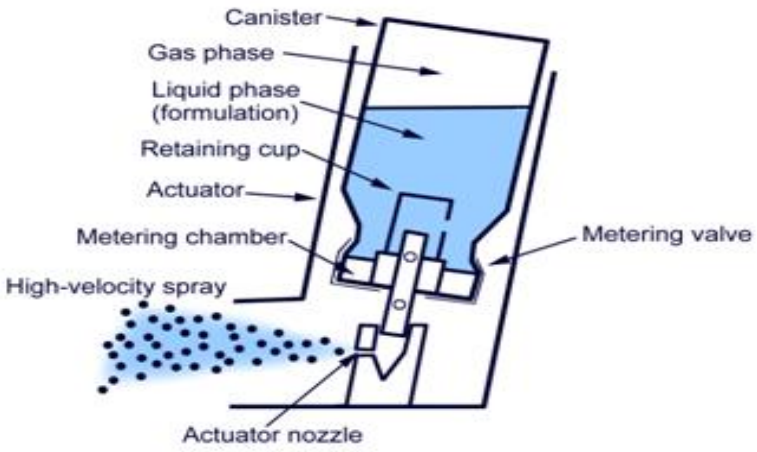


Figure 2.9: Schematic diagram of a metered dose inhaler from http://emedicine.medscape.com/article/1413366-treatment.

Although, MDIs are cheap, small, portable, quick to use and can deliver a precise unit dose giving a reproducible lung deposition, they are far from being perfect. Over the years, a number of deficiencies have been identified to MDIs in terms of both effectiveness and usability. Poor patient inhalation technique remains the most concern in clinical applications and has been reported in up to 94% of patients (Brocklebank et al., 2001; Crompton et al., 2006; Lavorini et al., 2008b; Rootmensen et al., 2010). Furthermore, many patients even after training are still unable to operate the device properly (Epstein et al., 1979; Kamps et al., 2000; Burkhart et al., 2005; Al-Showair et al., 2007b). The inability of many patients to synchronise aerosol actuation with inspiration is a very common problem (Crompton, 1982; Zeng et al., 2000; Crompton, 2004) and poor coordination can result in medication being released either two early or too late in the inspiratory cycle (Crompton, 1982; McFadden, 1995; Donnell, 2001). Although good coordination is required for MDIs and many patients have problems with this step, the most important problem of a MDI inhalation technique is failure to initiate a slow inhalation technique with insufficient breath-hold duration (Newman et al., 1982b; Everard et al., 1995; Tomlinson et al., 2005; Al-Showair et al., 2007a; Chrystyn and

Price, 2009; Haughney et al., 2010). However breath holding only improves lung deposition if preceded by slow deep inhalation (Newman et al., 1981b).

Moreover, the inhaler technique in MDIs can be complicated by what is known as the cold Freon effect. The cold Freon effect refers to the phenomenon caused by the forceful blast of high velocity cold liquid propellant impacting on the back of the throat, stopping the patient from inhalation or causing nose inhalation instead of mouth inhalation (Crompton, 1982; Broeders et al., 2009). The cold Freon effect is uncomfortable for the user and can cause inconsistent or non-existent drug delivery to the lung. It occurs particularly with CFC-containing inhaler devices. This problem is less important with hydrofluoroalkane (HFA) propellant aerosols due to their higher boiling points, which means a slower delivery speed and lower throat deposition (Gabrio et al., 1999).

Effective use of a MDI is technique-dependent and the inability to use the inhaler correctly may result in failure to get the intended dose of medication to the airways and hence poorer asthma control (Giraud and Roche, 2002; Chrystyn and Price, 2009). Fink and Rubin (2005) have stated that, "Management of chronic airways disease is 10% medication and 90% education." Therefore, adequate patient education about the proper inhalation technique is one of the keystones of successful inhalation therapy. Several studies have shown that correct inhalation can dramatically improve the lung delivery of inhaled medications as well as the clinical and economical outcome measures (Kamps et al., 2003; Fink and Rubin, 2005; Al-Showair et al., 2007a; Lavorini et al., 2010; Rootmensen et al., 2010). Lenney et al (2000) studied 100 adults naive to inhaler devices and found that only 21% could use the MDI efficiently after reading the instruction leaflet, while 52% could do so after expert training. Similar findings were also reported by Al-Showair et al (2007) who found that post-training of the proper inhalation technique reduced the flow rate markedly in both mild and severe COPD patients when

using their MDIs. Other studies even suggested that inhalation instructions should be given repeatedly to achieve and maintain the proper inhalation technique as patients readily fall into a habit of using an incorrect technique post-training (Crompton, 1990; Kamps et al., 2000; Kamps et al., 2002; Brand, 2005; Crompton et al., 2006; Deerojanawong et al., 2009). Another study highlighted the importance of a 2Tone Trainer (Canday Medical, UK) to maintain a trained slow inhalation flow and help patients to maintain the recommended MDI technique post-training (Al-Showair et al., 2007a).

Even with correct inhalation technique, most MDIs are inefficient due to their relatively high throat deposition. The combination of the high propellant velocity (>30m/sec) and initially large sized aerosol particles increase the likelihood of drug deposition in the oropharynx immediately following MDI actuation (Donnell, 2001; Newman, 2005). Typically, they deliver only about 1/3 of the amount of drug delivered to the lung compared to the newer dry powder inhalers (Newman et al., 1981c; Newman, 1985; Newman et al., 2000a; O'Connor, 2004; Virchow et al., 2008).

The growing awareness of the patients' limitations of MDI administration (hand-breath coordination problems, cold Freon effect, and high oropharyngeal deposition) has led to further development of devices that overcome these problems such as the addition of spacers and breath-actuated MDIs that will be discussed in the following sections. The formulation of some MDIs with HFA propellants as solution aerosols with the emission of extrafine particles has helped with the problems of inefficient lung deposition of inhalation techniques; this will also be discussed later.

2.4.1.2. Pressurized metered dose inhaler with a spacer device

Metered dose inhalers are sometimes used with add-on devices referred to as spacers, which are tubes attached to the inhaler that act as a reservoir or holding chamber. As shown in figure 2.10, there are several types of spacers available.

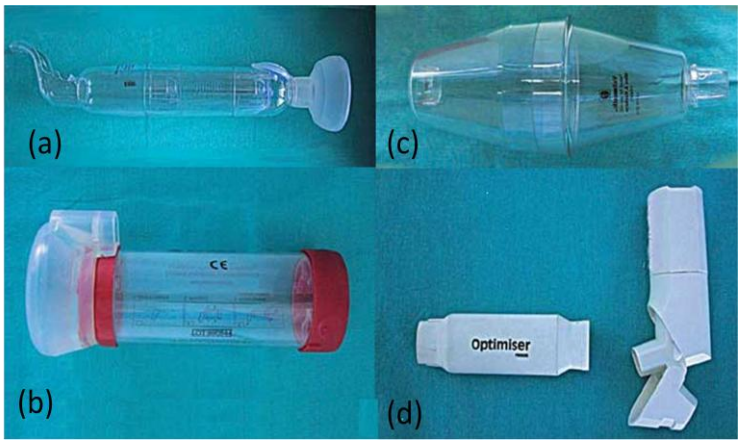


Figure 2.10: Example of spacer devices, (a) Babyhaler (b) Aerochamber with mask (c) Volumatic and (d) Optimiser.

Spacers are cheap, easy to use and place less demand on a patient's inhaler technique. They overcome problems of poor technique in both adults and children, which occurs when using MDIs alone (Newman, 2004; Dolovich et al., 2005; Rubin and Fink, 2005). The recent British guidelines on asthma management have supported the wider use of spacer devices (BTS/SIGN, 2008).

The attachment of a spacer device to MDIs compels the patient to inhale at some distance from the actuator to the mouth, consequently allowing time for the aerosol speed to slow down and propellant to evaporate with a reduction in particle size. Larger particles are entrapped on the spacer walls and more of the therapeutically beneficially respirable fraction is delivered to the lung. This makes it easier to use the inhaler and helps to ensure that more medication gets into the lungs instead of just into the mouth or air (Broeders et al., 2009; Nair et al., 2009).

As shown in figure 2.11, the proper use of spacers, increases lung deposition of the drug, limits oropharyngeal impaction and systemic absorption. Besides, it reduces drug loss that occurs with poor patient coordination, eliminates the cold Freon effect, and makes an inhaler somewhat more effective in delivering medicine (Crompton, 1982; Hindle and Chrystyn, 1994; Clark and Lipworth, 1996a; O'Callaghan and Barry, 2000). Hindle & Chrystyn (1994), reported that based on the 30 minutes urinary salbutamol excretion, the mean percentage increase for the relative bioavailability of salbutamol to the lung compared with the MDI alone were 19%, 23.5%, and 53.4% for the Volumatic, Bricanyl spacer, and Nebuhaler, respectively. However, the issue of electrostatic charge effect on drug output from spacers were not known at this time of study. Subsequently, Silkstone et al (2002) showed that the Volumatic spacer attached to salbutamol MDI was 2.3 times more efficient for lung delivery with less systemic concentrations than the same dose of the MDI alone used with a highly trained technique. In addition, Aswania & Chrystyn (2001) reported that a metered dose inhaler attached to a large volume spacer delivers an eight-fold improvement in the relative amounts deposited in the lung compared to the MDI without spacer. Another study that investigated the effect of spacers' attachment on aerosol deposition from a pressurised metered dose inhaler reported a reduction in the oropharyngeal deposition from 81% to 17% and an increase the lung deposition from 8.7% to 21% with 56% of the emitted dose deposited in the spacer (Newman et al., 1984). The previous results and many similar findings suggest that the size of the spacer may affect the amount of drug available for inhalation, and this will vary with the drug prescribed (Barry and O'Callaghan, 1996).

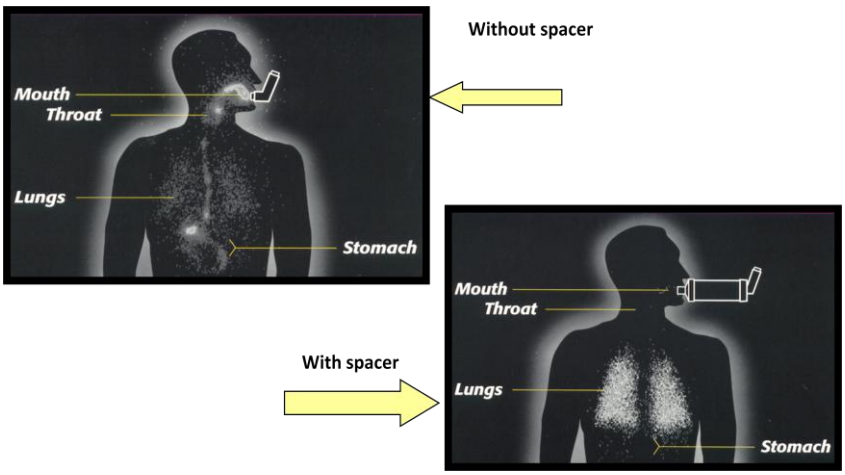


Figure 2.11: A diagram showing improved lung delivery and reduced oropharyngeal deposition with spacers use from http://www.clinicasubiza.com/Dispositivos/Optichamber/tabid/222/language/es-ES/Default.aspx.

Spacers can be especially helpful to adults and children who find a regular metered dose inhaler difficult to use (Pedersen, 1996). Furthermore, the MDI + spacer has proven to be a practical lower cost alternative to the use of nebuliser therapy in the management of severe acute asthma or chronic obstructive pulmonary disease (Newman, 2004; Cates et al., 2006; Fayaz et al., 2009; Lavorini and Fontana, 2009). Although the use of spacers did not always increase lung deposition, they always reduced oropharyngeal deposition (Newman and Newhouse, 1996). Thus patients who use corticosteroid inhalers should use a spacer to limit oropharyngeal deposition, minimise drug reaching the gastrointestinal tract, thus helping to prevent both systemic (adrenal suppression) and local oropharyngeal side effects (thrush and dysphonia) (Dolovich et al., 2005; Hickey, 2007; Pedersen et al., 2010). Their popularity has led to a rapid increase in the number of different spacer types available with considerable variations in the proportion of drug that reaches the airways (Barry and O'Callaghan, 1996; O'Callaghan and Barry, 1997; O'Callaghan and Barry, 2000). However, since the amount of drug that deposits in the airways is critical and traditionally considered to reflect lung dose, thereby each unit of

spacer-MDI combination can elicit profound effects on aerosol cloud characteristics (Berg et al., 1998).

The drug delivery from spacer devices may be affected by different factors, such as spacer volume, electrostatic charge, type of valve, dead space between inlet and outlet, mode of inhalation breathing and the drug/spacer combination. Furthermore, electrostatic charge is a commonly reported cause of inconsistent medication delivery from plastic spacers (Bisgaard, 1999; Dolovich, 1999; Dubus et al., 2001).

Most spacers are made from non-conducting plastic materials and hence, can easily accumulate electrostatic charge on their surface and negatively affect dose delivery. The net effect of these electrostatic charges is enhancing the attraction and deposition of charged drug particles on spacer walls upon dose aerolisation from the metered dose inhaler (O'Callaghan et al., 1993; Dewsbury et al., 1996; Wildhaber et al., 1996b; Pierart et al., 1999; Wildhaber et al., 2000b). The higher the electrostatic charge, the higher the amount of aerosolised drug attracted and retained within the spacer device, leading to a significant reduction in the drug aerosol available for inhalation (Dubus et al., 2003). Moreover, electrostatic charge causes significant dose variation due to different patient handling of the spacer (Kenyon et al., 1998; Janssens et al., 1999; Wildhaber et al., 2000a; Wildhaber et al., 2000b).

Several studies have shown that the level of electrostatic charge on a plastic spacer depends on the treatment of the spacer (Barry and O'Callaghan, 1995; Dewsbury et al., 1996; Wildhaber et al., 1996b; Wildhaber et al., 1998). *In-vitro* studies have shown that drug delivery is enhanced by more than two fold when using an antistatic lining (O'Callaghan et al., 1993; Barry and O'Callaghan, 1995) or when using non-electrostatic spacers (steel spacer) (Bisgaard, 1995; Nair et al., 2009). Steel is a conducting material that holds no electrostatic charge no matter how it is handled, and requires no chemical

treatment. Therefore, steel spacers readily solve the problem of reduced drug delivery due to electrostatic charge but issues of cost and availability retain simple plastic spacer as the device of choice worldwide (Bisgaard, 1995; Kenyon et al., 1998). Also a metallic walled spacer will not enable patients to see the formation of the aerosol plume that gives them the confidence that the medication was delivered (Mitchell et al., 2007b).

Another more practical widely used method that effectively overcomes electrostatic charge and significantly improves *in-vitro* (Barry and O'Callaghan, 1996; Dewsbury et al., 1996; Wildhaber et al., 1996a; Wildhaber et al., 1996b; Berg et al., 1998; Kwok et al., 2006) and *in-vivo* (Pierart et al., 1999; Wildhaber et al., 2000a; Wildhaber et al., 2000b) drug delivery is by simply washing plastic spacer devices in a detergent without subsequent rinsing and allowing them to air dry. This washing procedure was found effective to reduce or even eliminate electrostatic charge and increase total drug output through the spacer in both small (Wildhaber et al., 1996a) and large volume plastic spacers (Wildhaber et al., 1996b). Several Scintigraphic studies, providing better measurements of lung deposition, with labelled salbutamol and budesonide have confirmed the previous *in-vitro* work. These studies have reported that the reduction in the electrostatic charge of the plastic spacer devices provides a 10–35% increase in lung deposition, in both adult and asthmatic children due to an increase in the fine particle mass (Janssens et al., 1999; Pierart et al., 1999; Wildhaber et al., 2000a; Wildhaber et al., 2000b). Furthermore, there was a 10% increase in pulmonary function obtained with less drug when using treated rather than untreated Volumatic® spacers (Wildhaber et al., 2000b). Other pharmacokinetic studies have shown that the electrostatic charge in plastic spacers decreases the delivery of salbutamol to the lungs with an approximate twofold reduction in lung bioavailability with the Volumatic® in adults (Clark and Lipworth, 1996a) and the Babyhaler® in children (Anhoj et al., 1999). To limit static effects, it is recommended that plastic spacers should be soaked in a dilute solution of

household detergent and then allowed to drip-dry without water rinsing. It is very important not to wash the spacer in water post treatment with detergent or to dry the plastic with a cloth, since this immediately recharges the spacer (Bisgaard, 1999; Pierart et al., 1999; Mitchell et al., 2007b).

In summary, previous findings leave no doubt that the building of electrostatic charge on plastic spacers negatively affect lung deposition of inhaled drugs and may lead to significant under-dosing. By simply reducing electrostatic charge, the dose deposited in the lungs can be greatly increased with markedly reduced variability. Bearing in mind, the attention to the details of washing spacers, can effectively allow for a greater and more predictable drug delivery to the airways, and thus, may indicate the potential for dose reduction of inhaled medications while retaining similar therapeutic effect (Kenyon et al., 1998; Pierart et al., 1999; Mitchell and Nagel, 2007).

2.4.1.3. Breath actuated pressurized metered dose inhaler

In view of the difficulty some patients have in coordinating MDI actuation with inspiration, great interest has been devoted to the development of breath actuated metered dose inhaler for example, the Autohaler (Teva, UK) and the Easi-Breathe (Teva, UK) devices. As shown in figure 2.12 they contain a conventional pressurised canister with a flow-triggered system driven by a spring which automatically actuate the MDI and release the dose with patient inhalation (Newman et al., 1991c; Broeders et al., 2009; Patterson et al., 2009).

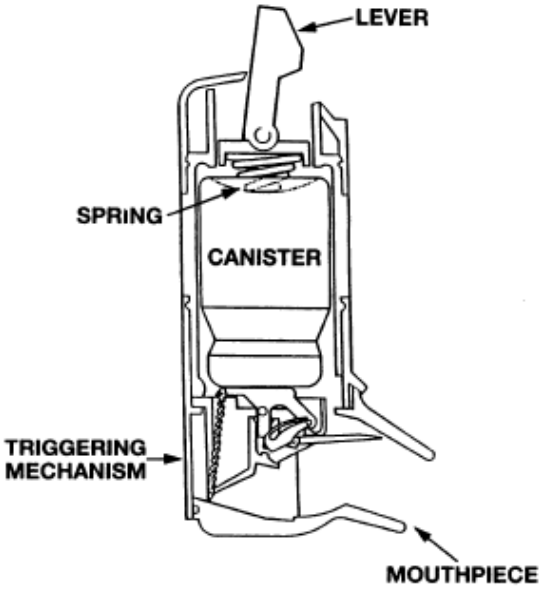


Figure 2.12: Cross-sectional diagram of a breath-actuated metered dose inhaler (Newman et al., 1991c).

The breath-actuated mechanism of these inhalers is designed to aid coordination by being actuated early in the inspiratory cycle by low inhalation flow rates (approximately 20L/min and 30L/min for the Easi-Breathe and Autohaler respectively). These inhalation flows are readily achievable by most patients even those with severe airflow obstruction and dose delivery does not change with increasing inspiratory effort (Hardy et al., 1996; Terzano, 2001). The audible click on actuation and the taste of the propellant in the dose of a breath-actuated MDI serves to reassure the patient that the dose has been dispensed, hence improving patient confidence, and compliance (Newman et al., 1991c; Donnell, 2001).

Despite the fact that these devices are of little additional benefit to patients with good inhalation techniques (Newman et al., 1991c; Soria et al., 2002), they greatly improved lung deposition in patients with poor coordination (Newman et al., 1991c; Schecker et al., 1993; Marshik et al., 1995). As shown in figure 2.13, the mean (SD) lung deposition was only 7.2% (3.4%) in subjects with poor coordination compared to 18.6% (2.9%) in

those with good coordination. This compares to a mean (SD) lung deposition of 20.8 % (1.7%) when the poor coordinators used a breath actuated metered dose inhaler (Newman et al., 1991c).

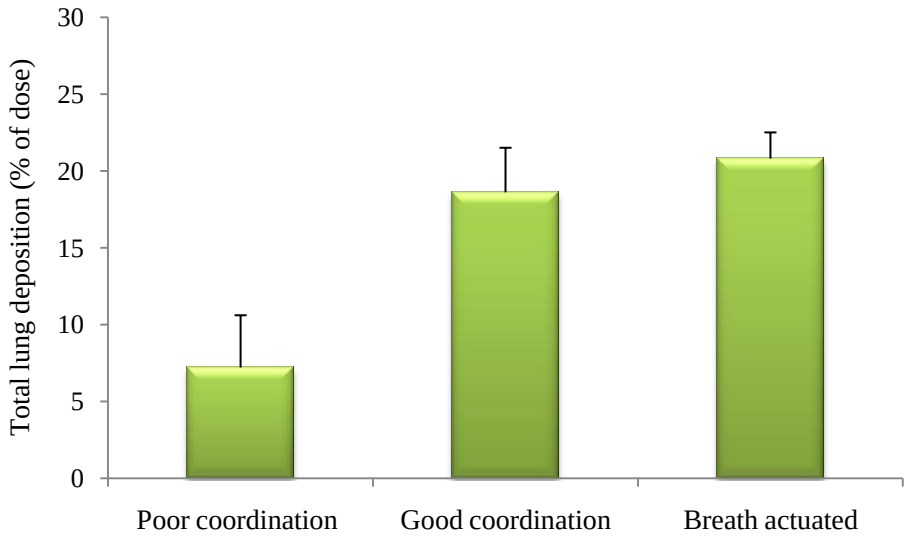


Figure 2.13: Mean (SD) lung deposition in good and poor coordinators and when the poor coordinators used a breath actuated device (Newman et al., 1991c).

Several studies have suggested that breath actuated MDIs are useful alternatives to MDIs due to their simple operation, they are easier to use, easier to teach and preferable by patients (Newman et al., 1991c; Chapman et al., 1993; Lenney et al., 2000). Many elderly patients have demonstrated a more efficient use of breath actuated MDI compared to either conventional MDIs or the Rotahaler® DPI (Diggory et al., 1991; Chapman et al., 1993). Also, a group of asthmatic children aged between 4 and 12 years old with acute exacerbations showed more successful Autohaler actuations (99%) compared to actuations from a dry powder device (74%) (Ruggins et al., 1993). Moreover, more cost savings were associated with the use of the Autohaler device as opposed to the conventional press and breathe MDI (Langley, 1999). This reduced cost associated with Breath actuated MDI can be attributed to their easy use and more optimal therapy in patients, with fewer emergency room visits and hospitalizations. In addition, the

Autohaler was reported to decrease drug usage by 23% compared to a conventional MDI (Kelloway and Wyatt, 1997). Patients using breath actuated MDIs were prescribed 25% less short-acting β_2-agonist, 64% less oral steroid, and 44% less antibiotics, than their counterparts using traditional MDIs. Consequently, these breath-actuated inhalers may result in clinically and economically important outcomes in real-world practice due to improved patient compliance and improved lung deposition (Price et al., 2003; Chrystyn and Price, 2009).

2.5. Transition of chlorofluorocarbon (CFC) propellants to hydrofluoroalkane (HFA) propellants in MDIs

Ozone in the stratosphere is a layer above the earth surface that absorbs the harmful high-energy ultraviolet (UV) radiations emitted from the sun, thus protecting the earth surface. The Nobel Prize winners M. Molina and S. Rowland were the first to find that the man made chlorofluorocarbon (CFCs) had been added to the environment in steadily increasing amounts and had caused an accelerated depletion of ozone in the Earth's stratosphere as shown in figure 2.14 (Molina and Rowland, 1974).

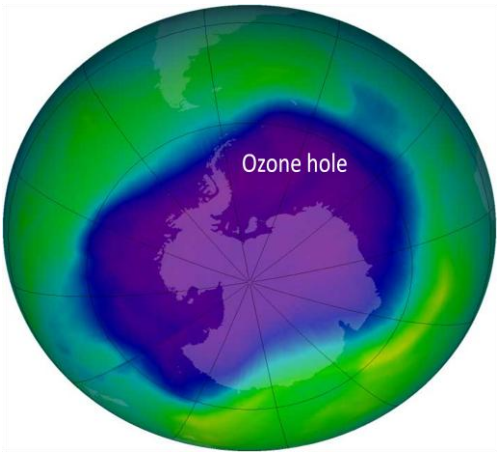


Figure 2.14: shows the ozone hole size.

Chlorofluorocarbons (CFCs) were developed in the early 1930s and were widely used in refrigerators, air conditioners, solvents, fire suppressants and as propellants for aerosols.

Chlorofluorocarbons (CFCs) are remarkably simple molecules with great stability and are characterised by being non-toxic, non-flammable, and non-reactive with other chemical compounds. These desirable safety and stability characteristics make them ideal to be used as aerosol propellants (Ariyananda et al., 1996). Ironically, this very high stability is also behind its damage to the ozone layer. In fact, the CFCs are so stable that they can reach the stratosphere intact and cause the release of chlorine fragments under the effect of sunlight, which is responsible for their ozone depleting potential (Manzer, 1990; Leach, 2005). As shown in figure 2.15 chlorine radicals catalyse the breakdown of ozone to molecular oxygen. One chlorine atom can be repeatedly recycled catalyzing thousand of reactions prior to formation of molecular chlorine. It has been estimated that one chlorine radical can destroy approximately 100.000 molecules of ozone (Leach, 2005), thus depleting the ozone concentration.

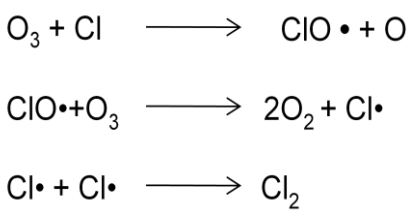


Figure 2.15: Proposed halogen disruption of stratospheric oxygen/ozone equilibrium (Noakes, 1995; McDonald and Martin, 2000).

Thinning of the ozone layer increases the levels of harmful UV radiations that reach the earth surface, consequently increasing levels of skin cancers, melanomas, cataracts, and causing important environmental damage (Partridge et al., 1998; Leach, 2005).

Subsequent to The Montreal protocol in 1987 which banned the use of CFC propellants (Montreal, 2000), a primary objective for researchers and the pharmaceutical industry in addressing this issue has been the replacement of chlorofluorocarbon (CFC) propellants by the more environmentally friendly hydrofluoroalkane (HFA) propellants. HFA-propellants have been found to be safe, have no ozone damaging potential , with much

less global warming effect than CFC-propellants and was considered to be suitable alternatives to CFCs used in the formulation of medicinal products (Partridge et al., 1998). In addition to HFA-propellant desirable safety characteristics, their higher plume temperature (5ºC) than that of CFC-BDP (-20ºC), were beneficial in reducing the undesirable cold Freon effect. Also, the necessary manual force to press down the HFA-BDP spray is three times smaller than that which is required for CFC-BDP sprays (Ibiapina et al., 2004).

However, the replacement of chlorofluorocarbon propellants in metered dose inhalers with hydrofluoroalkane propellants was simple on the surface but scientifically very challenging. The conventional surfactants used in CFC MDIs were not soluble in HFA MDIs. The insolubility of surfactants such as oleic acid and lecithin in HFA propellants necessitated the use of co-solvents such as ethanol to solubilise the surfactants to create a stable suspension formulation or to dissolve the drug substance for a solution formulation. Consequently, these differences mandated the development of new formulations, and manufacturing processes for HFA inhalers. Because of a major research and development effort, pharmaceutical companies have made good progress in the reformulation of existing corticosteroid compounds into two distinct classes of corticosteroid aerosols HFA suspensions and HFA solutions. The new HFA preparations of fluticasone propionate, triamcinolone acetonide, and mometasone furoate were formulated as suspensions that retained the same particle size, deposition, and efficacy profiles as their CFC counterparts. Whereas other drugs such as flunisolide, budesonide and beclometasone dipropionate necessitated a shift in design from suspension formulations to solutions due to formulation problems and stability issues. The development of MDI solution formulations has provided a way to manipulate the quality of the aerosol cloud generated by MDIs and obtain precise control of the delivered dose

with a chosen particle size by what is known as Modulite® technology (Ganderton et al., 2003; Acerbi et al., 2007).

2.5.1. Modulite® technology

Modulite® platform technology is an HFA-based aerosol solution that contains 12–15% (w/w) ethanol with up to a 1.3% (w/w) non-volatile excipient. Modulite solutions are capable of tailoring aerosol solution speed and particle size distribution to meet specific needs by controlling two interdependent variables, the addition of non-volatile component, and the actuator orifice geometry. Two other minor variables; change in vapour pressure of the propellant and the volume of the metered solution are also used to improve performance (Ganderton et al., 2002; Brambilla et al., 2011). Interestingly, an added non-volatile component decreases the system vapour pressure and increases the aerosol particle size upon propellant evaporation to values close to those of the CFC suspension formulations (Newman et al., 1982a; McDonald and Martin, 2000).

The spray characteristics of solution aerosols can also be manipulated by a reduction in actuator orifice diameter which is consistent with the widely known fact that a larger actuator orifice produced a coarser spray (Polli et al., 1969). Using finer apertures were not possible with suspension aerosol formulations due to the potential for clogging. Conversely, the solution technology used in Modulite® frees the formulation from this constraint and enables variations in aperture diameter to control the properties of the aerosol cloud. The smaller actuator orifices produce a finer spray and generate a slower moving aerosol cloud over longer dose emission periods (Brambilla et al., 2011). These functions combine to reduce oropharyngeal deposition. Studies comparing the plume profiles of CFC-MDIs and HFA-MDIs showed that despite similar plume geometries, a slower plume velocity with the HFA solution was observed, allowing the dose to be generated over a longer period (Acerbi et al., 2007).

Moreover, Modulite® solution technology ensures the stability and consistency of the formulation throughout the life of the MDI canister. The use of a co-solvent to dissolve the drug in the propellant precludes any phase separation and dose variation caused by differences in shaking, storage and handling of the canister that occur with suspension formulations (Cyr et al., 1991; McDonald and Martin, 2000; Ganderton et al., 2003).

The manipulation of these variables, by Modulite® technology, led to an aerosol technology that overcomes the problems of the coarse fast moving aerosol clouds usually associated with the conventional CFC-MDIs, which can interfere with optimal drug deposition in the lung. Using this Modulite® approach led to the successful seamless transition of a number of CFC-based aerosols, including, formoterol (Houghton et al., 2004) , budesonide (Vastagh et al., 2003) , and beclometasone dipropionate (Bousquet and Cantini, 2002; Ganderton et al., 2002) to HFA systems.

Modulite® technology was successfully used to reformulate HFA-BDP to match the CFC-BDP particle size, hence this allowed for a much faster and less expensive switch to new HFA inhalers (e.g., Clenil Modulite®; Chiesi, Italy). In addition, using HFA-solution technology made it possible for the first time to engineer the size and distribution of drug particles to produce an extrafine HFA-BDP formulation (1.1µm) for better targetting to different parts of the lung by values greater than 50% (e.g., Qvar,3M Pharmaceuticals) (Leach et al., 2009).

2.5.1.1. The extra-fine HFA formulation of beclometasone dipropionate (Qvar®, 3M Pharmaceuticals)

The reformulation of CFC-BDP with a non-CFC propellant, hydrofluoroalkane-134a (HFA-BDP) has provided the opportunity to tailor the size and distribution of particles to be targeted to different parts of the lung. The fact that BDP in the HFA-based formulation is in solution rather than in suspension, as in the case with CFC preparations

together with the improved inhaler technology led to the development of superfine particle HFA solution systems.

Qvar ® (Teva Pharmaceuticals, UK) is an example of a newly developed HFA-BDP formulation that is available as a press-and-breathe (PB) MDI, an Autohaler (AH) and an Easi-Breathe (EB) device. It was the first CFC-free MDI formulation for an inhaled corticosteroid. Qvar produces an extra-fine aerosol that has a MMAD of 1.1μm versus 3.5 μm for the CFC-propelled formulation and its fine respirable mass has a greater proportion of particles with a diameter less than 4.7 μm (approximately 60%) compared to the conventional CFC-BDP MDIs (approximately 30%) (Leach, 1998a; Donnell, 2000). In addition, HFA-BDP inhalers have a lower spray force, and a warmer temperature than CFC-BDP inhalers (Roller et al., 2007).

Consequently, these changes in HFA aerosol properties equate to better lung deposition (in both peripheral and central airways) together with 30% less oropharyngeal deposition (Leach et al., 2002; Agertoft et al., 2003; Leach et al., 2009), a decreased likelihood of experiencing the cold Freon effect due to decreased velocity of particles exiting the inhaler device (Gabrio et al., 1999), improved asthma control (Ederle, 2003) and better health related quality of life (Juniper et al., 2002). The increased efficiency of homogenous drug delivery to the lungs using the extrafine aerosol makes it ideal for use in both adults and children even when inhaled with a poor technique (Devadason et al., 2003; Lasserson et al., 2006; Roller et al., 2007). Many studies have confirmed that asthma control can be fully maintained when switching patients from CFC-BDP to Qvar inhalers, despite switching to a lower dose of BDP in the HFA-BDP inhalers (Davies et al., 1999; Szefler et al., 2002).

2.5.1.1.1. Effect of particle size

It is well documented that the particle size characteristics of the respired aerosol play a significant role in determining the total amount and the relative distribution of inhaled corticosteroid to the large and small airways (Leach et al., 1998a; Leach et al., 2002; Agertoft et al., 2003).

Theoretical mathematical models such as the ICRP model published by the Task group of the International Committee on Radiological Protection have been proposed to predict the fraction of inhaled particles deposited in each region of the respiratory tract as a function of particle size. According to this model presented in figure 2.16, there is a major increase in alveolar deposition of fine particles (0.1-1µm). This is attributed to their deposition predominantly by a diffusion mechanism that increases inversely with particle size and become negligible for larger particles.

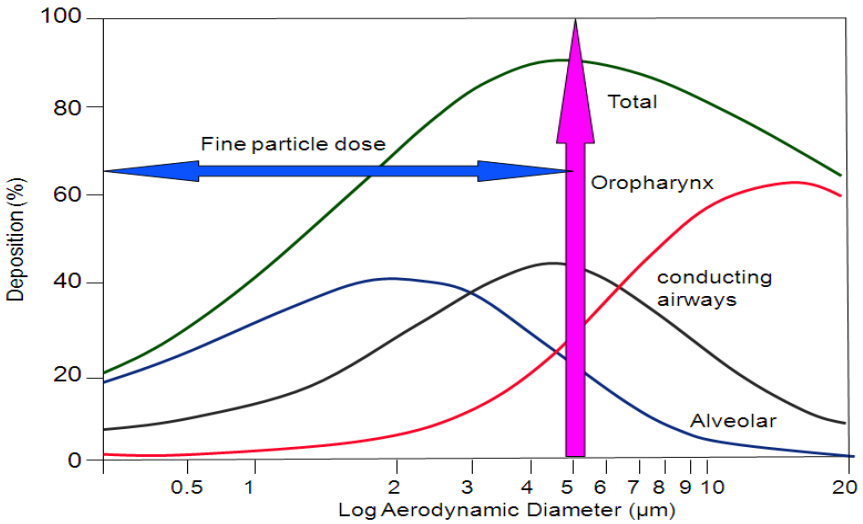


Figure 2.16 : The fate of inhaled particles depending on particle size reproduced from (ICRP, 1994; Köbrich et al., 1994).

On the other hand, particles that are 1-5µm in diameter are deposited mainly in the lower bronchial airways and alveoli due to sedimentation, whereas those larger than 5µm are deposited mainly in the large bronchial airways and the oropharynx due to inertial

impaction. The less total deposition seen for submicron particles may be due to a balance between a predominance of diffusion versus impaction/sedimentation mechanisms based on their particle size as these two modalities of particles deposition decrease with decreasing particle size (ICRP, 1994; Schulz, 1998).

A major advantage of the small particle ICS is their improved total lung deposition resulting in achieving effective asthma control at lower daily doses than the bigger particle ICS. As shown in figure 2.17, the site of particle deposition in the respiratory tract appears to be strongly related to the particle size of inhaler used, as the smaller particles of HFA-BDP Qvar product was associated with greater lung deposition and less oropharyngeal deposition than the bigger particles of CFC-BDP (Leach et al., 2002).

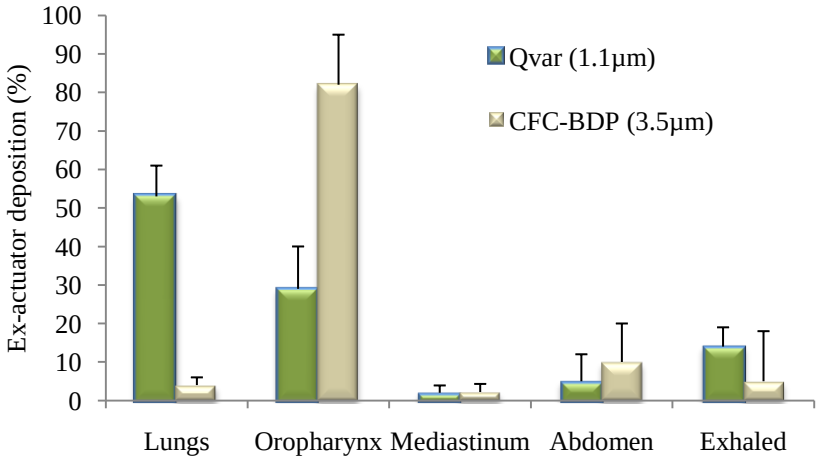


Figure 2.17: Distribution as percentage of ex-actuator dose of radiolabeled HFA-BDP and CFC-BDP to the lungs, oropharynx, mediastinum, abdomen, and expiratory filter. Reproduced from (Leach et al., 2002).

Despite that, experimental data and mathematical models predict an increased total lung deposition with increasing particle size from 0.5 to 10µm under tidal breath conditions, the use of slow deep inhalations followed by a breath hold increased particles residence time. This increased residence time together with the low inertial losses of small particles greatly favoured their total deposition and lung penetration to exceed that of larger

particles. The minimal upper airway aerosol losses for small particles are expected due to their greater ability to largely bypass the filtering mechanisms and abrupt airway geometry of the upper airways, which accounts for their less oropharyngeal deposition. Whereas the greater inertia of larger particles makes them more susceptible to leave the inspired, air stream during sudden changes in airflow direction and deposit mainly by impaction in the oropharynx and at airway bifurcations (Usmani et al., 2005; Asgharian et al., 2006).

The study by Usmani et al (2005) quantified lung deposition after 12 asthmatics inhaled radiolabeled monodisperse aerosols with a MMAD of 1.5, 3.0, and 6.0µm. They observed an increased lung deposition and penetration index values with decreasing particle size as shown in figure 2.18.

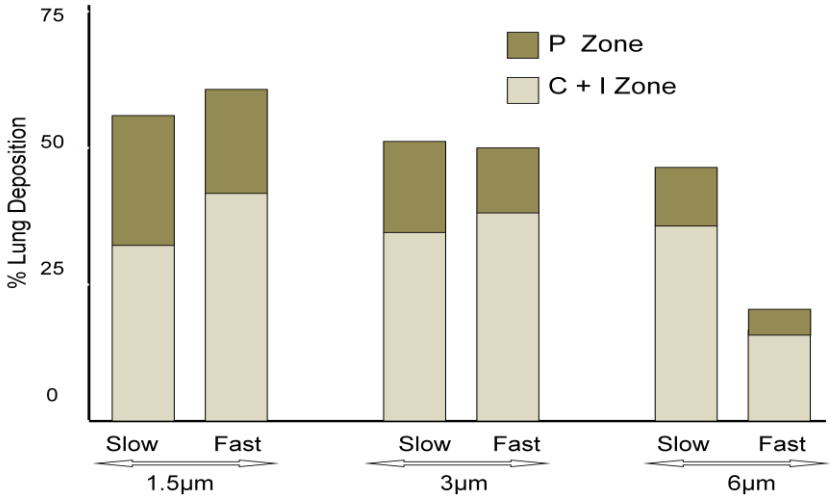


Figure 2.18: Effect of fast and slow inhalation rates on aerosol deposition in central (C), intermediate (I) and peripheral (P) regions of the lung. Reproduced from (Usmani et al., 2005).

This gamma scintigraphy study clearly confirmed the increased total and peripheral lung deposition of the 1.5µm particles than the 3 µm or 6 µm particles, whereas 6µm particles were more proximally distributed throughout the airways that present larger calibre. Moreover, oropharyngeal deposition increased with increasing particle size, whereas the

exhaled aerosol fraction was greatest with the 1.5 µm aerosols. Similarly, a more recent gamma scintigraphy study using an Aerolizer DPI device showed a more diffusive and greater deposition of small particles throughout the lung especially in the peripheral lung zone, alternatively, the deposition in the upper airways was significantly higher for bigger particles (>70%) (Glover et al., 2008)

Consequently, another advantage of the small particle ICS formulation is that they are more able to reach the small airways and consequently result in increased efficacy (Gentile and Skoner, 2010). The fact that both large and small airways are clearly involved in the pathophysiological processes of asthma together with the availability of the glucocorticoid receptors throughout the bronchial tree and especially in the alveolar walls (Adcock et al., 1996) provided the rationale for the need for small particles ICS therapy. The extra fine aerosol produced by HFA-BDP formulations (1.1µm) offered more even deposition throughout the airways with better targeting to small airways inflammation compared to the poor distal delivery offered by the larger particle size CFC-MDIs (3.5-4 µm) (Richards et al., 2001; Leach et al., 2002; Leach, 2005; Newman et al., 2006; Corren, 2008).

Moreover, the production of ultrafine particles MDI was found not only to improve lung deposition both peripherally and centrally, but it also produces similar deposition patterns when inhaled with a fast and a slow inhalation rate or without a breath hold (Janssens et al., 2003; Usmani et al., 2005). Leach et al (2005) compared drug delivery from HFA-BDP (Qvar® Autohaler) with proper and improper inhalation technique from Qvar® MDI. As shown in table 2.1, the breath activated Qvar® Autohaler and the proper Qvar® MDI technique provided optimal lung deposition of 60%, and 59% respectively. Furthermore, the smaller particle size and longer duration spray of Qvar® MDI resulted in patients receiving more than 30% lung deposition even under severe discoordination

of actuating before inhaling and as late as 2.5 seconds after the start of inhalation. A result that is extremely important to eliminate problems associated with patients' failure to achieve proper inhalation techniques when using MDIs.

Inhaler technique	Lungs	Oropharynx	Mediastinum	Abdomen	Exhaled
Autohaler (on time)	60 ± 7	31 ± 8	1 ± 1	0 ± 0	8 ± 4
P&B (on time)	59 ± 9	30 ± 8	2 ± 1	2 ± 1	7 ± 2
P&B (early)	37 ± 21	56 ± 22	1 ± 0	0 ± 1	5 ± 2
P&B (late)	50 ± 8	25 ± 7	1 ± 0	0 ± 0	24 ± 4

Table 2.1: Overall deposition of 99m Tc-HFA-BDP (Leach et al., 2005).

Comparison between the post treatment of Qvar and CFC-BDP subjects showed better ability of Qvar treated subject to reduce regional hyperinflation (Goldin et al., 1999) and effectively suppress the production of alveolar macrophages (Marshall et al., 2000), presumably because of better Qvar deposition in the peripheral airways and the alveoli. Hydrofluoroalkane (HFA-BDP) formulations provided more improvements in asthma outcomes due to their greater potential to effectively penetrate and suppress inflammation at the level of the small airways, which are the predominant site of obstruction in mild asthma (Goldin et al., 1999; Tanaka et al., 2004; Dolovich, 2009). A recent study showed that patients receiving Qvar therapy were more likely to achieve successful asthma control with less exacerbations (Kemp et al., 2009). In addition, these smaller particles largely bypass the filtering mechanisms of the upper airways, which accounts for their less oropharyngeal deposition (Usmani et al., 2005).

2.5.1.1.2. Lung deposition

Several lung deposition studies have demonstrated that the extra-fine HFA-BDP aerosol formulations were more effective than their CFC counterparts at equivalent doses due to their smaller aerosol particle size causing more efficient lung deposition of about 40% of nominal dose (Borgström, 1999) or 55-60% of the emitted dose (Leach et al., 1998a;

Leach, 1999). HFA-BDP formulations showed similar lung deposition patterns and equivalence to CFC-BDP formulations but at half the nominal dose (Busse et al., 1999b; Leach, 1999; Harrison, 2002).

Comparative deposition patterns of radiolabelled Qvar HFA-BDP and CFC-BDP as shown in figure 2.19 revealed that HFA-BDP was distributed in central, intermediate, and peripheral airways, whereas drug that reached the lungs from CFC-BDP was mostly in the central airways. Moreover, HFA-BDP delivered most of the drug to the lungs (55-60%) and less in the oropharynx (29-33%), with 9-14% being exhaled. Conversely, the majority of drug from CFC-BDP was deposited in the oropharynx (90-94%), and only (4-7%) reached the lung (Leach, 1998b; Leach et al., 1998a).

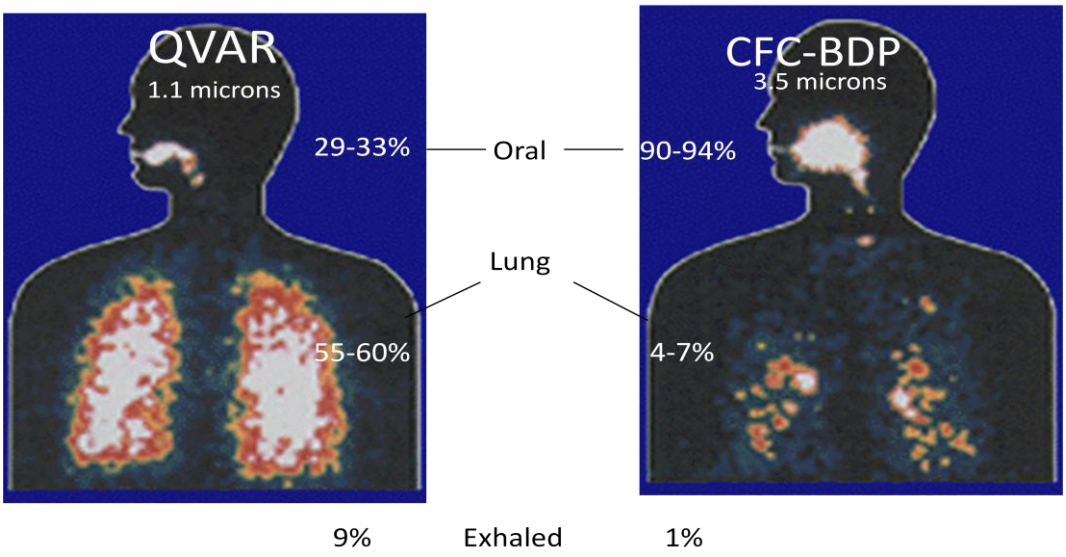


Figure 2.19: Comparative deposition pattern of radiolabelled BDP from Qvar (HFA-BDP) and CFC-BDP aerosol systems (Leach, 1998b; Donnell, 2000).

Since the HFA-BDP extrafine aerosol delivers most of the ICS dose directly to the lungs rather than to the oropharynx and gut, it should result in greater therapeutic benefit with a reduced incidence of oropharyngeal adverse events.

2.5.1.1.3. Pharmacokinetics and Pharmacodynamics

Several pharmacokinetic studies investigated the greater lung delivery of Qvar compared to CFC-BDP and reported that serum levels of beclometasone esters, as measured by AUC following HFA-BDP was approximately 2-2.5 times those obtained following CFC-BDP. In addition, the rate and extent of total beclometasone absorption increased with increasing the dose of HFA-BDP formulation (Harrison et al., 1997; Seale and Harrison, 1998; Harrison et al., 1999a; Harrison et al., 1999b; Agertoft et al., 2003). The greater efficiency and systemic drug delivery of the HFA formulation compared with the CFC formulation as shown in figure 2.20 can be attributed to greater swallowed and orally absorbed portion of CFC-BDP dose, whereas most of each inhalation from HFA-BDP is absorbed through the lungs due to its smaller particle size. However, time to maximum plasma concentration (T_{max}) was later with CFC-BDP than Qvar (2hours vs 0.6hours). This rapid T_{max} with Qvar is due to its rapid absorption from the lung compared to the slower absorption from the gut with CFC-BDP.

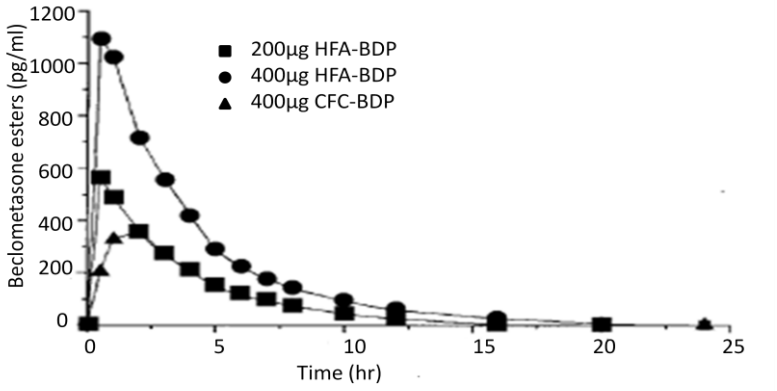


Figure 2.20: Mean serum concentration of beclometasone esters following 200µg HFA-BDP, 400µg HFA-BDP, and 400µg CFC-BDP from (Harrison et al., 1999b).

A clinical study by Busse et al (1999) demonstrated a dose response relationship for HFA-BDP and CFC-BDP and investigated the effect of treatment of multiple doses on lung functions. In this study, Busse et al (1999) reported that increasing doses of inhaled

corticosteroids lead to improved lung function and asthma control. Moreover, as shown in figure 2.21, a given dose of HFA-BDP requires 2.6 times the dose of CFC-BDP to achieve the same improvement in FEV_1. Similarly, several other clinical studies have also showed an improved asthma control with Qvar at half the daily dose of CFC-BDP (Davies et al., 1998; Gross et al., 1999; Magnussen, 2000).

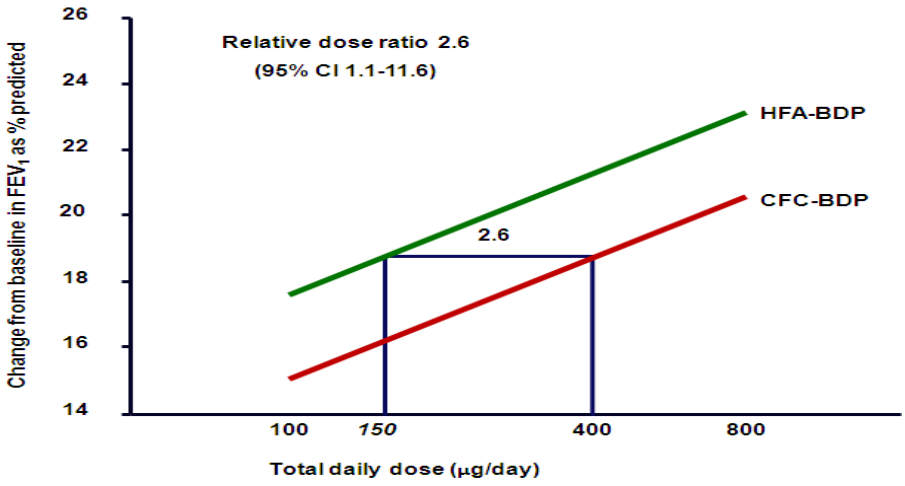


Figure 2.21: Dose-comparison calculation shows that it would take 2.6 times the dose of a large particle inhaled steroid (CFC-BDP) to achieve the same improvement in FEV_1 compared with an ultrafine particle inhaled steroid (HFA-BDP) reproduced from (Busse et al., 1999b)**.**

The increased distal lung deposition of Qvar might be expected to be associated with increased systemic effects, including adrenal suppression. However, reassuring data from several clinical trials have not documented any increased risk of systemic effects. Compared to other ICS, Qvar® inhalers have been found to be highly effective and well tolerated in both asthmatic adults (Van Schayck and Donnell, 2004a) and children (Szefler et al., 2002; Van Schayck and Donnell, 2004b). It produces equivalent asthma control to CFC-BDP at approximately half the daily dose with no clinically relevant adverse effects on adrenal function, bone metabolism or growth at recommended doses (Gentile and Skoner, 2010). The overall incidence of adverse effects that is related to

beclometasone dipropionate treatment was significantly lower with Qvar (11%) compared to CFC-BDP formulations (16%). In addition, the total incidence of local side effects such as dysphonia and cough was significantly lower in Qvar treated patients (8%) than in CFC-BDP treated ones (12%) (Thompson et al., 1998; Davies et al., 1999; Busse et al., 2000). Even without using spacers, its oropharyngeal deposition was efficiently reduced from 90% to 30% (Leach, 1998b). Furthermore, several clinical studies suggested that Qvar does not adversely affect the adrenal function at its maximum recommended dose and as shown in figure 2.22 may even produce less adrenal suppression than CFC-BDP at a comparable efficacious dose (Davies et al., 1998; Harrison et al., 1999a; Lipworth, 2000; Harrison, 2002).

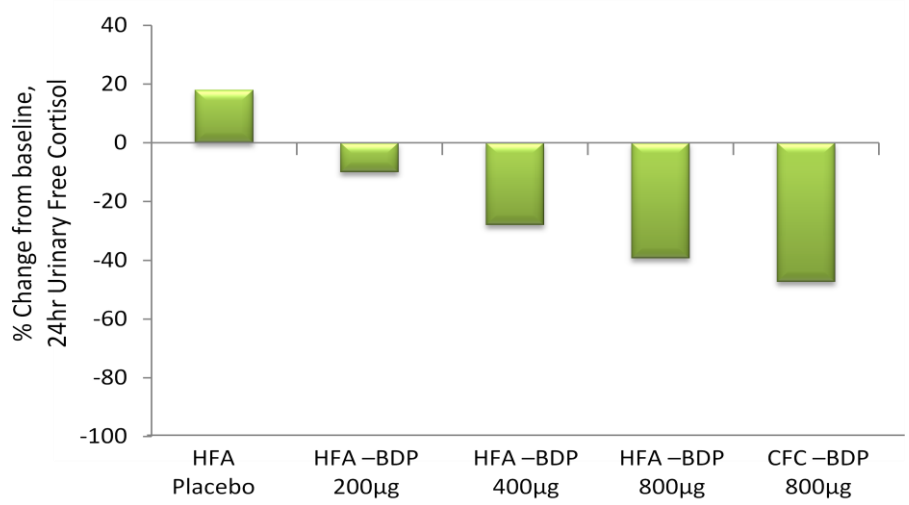


Figure 2.22: Mean Percent change from baseline in 24hr urinary free cortisol reproduced from (Harrison, 2002).

Even when the maximum daily doses are exceeded, the incidence of relevant systemic adverse effects is lower than expected (Lipworth, 2000). Some have suggested that this could be due to the shorter T_{max} for Qvar compared to CFC-BDP which may provide less stimulus for the HPA axis to change its output corticotrophin-releasing hormones and adrenocorticotropic hormone (Dekhuijzen and Honour, 2000). Furthermore, this extrafine spray plume does not produce serum or tissue accumulation when given at 12

hour intervals between doses (Lipworth, 2000). However, there is no firm evidence for the reduced systemic effects.

The overall therapeutic ratio of the HFA-BDP formulations is more favourable than that of the conventional CFC-BDP formulations due to equivalent efficacy with a lower dose and equivalent safety at the same dose (Thompson et al., 1998; Harrison et al., 1999a; Boulet et al., 2004). Although, smaller particles improve lung deposition, it also reduces systemic absorption from the upper respiratory tract and gastrointestinal tract and decrease adverse effects (Amirav et al., 2010). The enhanced delivery characteristics of the finer HFA-BDP aerosol even when inhaler technique is not ideal when compared to CFC-BDP suggest that it is possible to reduce the nominal BDP dose without compromising asthma control, which is considered a significant clinical advancement in asthma management.

2.5.1.2. The non extra-fine HFA formulation of beclometasone dipropionate (Clenil Modulite®; Chiesi, Italy)

Modulite® technology was originally applied to the development of BDP inhalers by matching CFC-BDP based inhalers in terms of aerosol characteristics and particles size, thus allowing a seamless transition of CFC-BDP MDI to HFA-BDP inhalers on a 1:1 nominal dose ratio basis (Bousquet and Cantini, 2002; Acerbi et al., 2007). The addition of glycerol as a non-volatile co-solvent together with the selection of an appropriate actuator orifice diameter, provides an aerosol with particle size characteristics, that closely resemble that of the conventional CFC-BDP (Ganderton et al., 2002).

Clenil Modulite® (Chiesi, Italy) is the first CFC-free metered dose inhaler directly interchangeable with CFC-containing inhalers**.** In the UK, it is currently the only available inhaled HFA-BDP MDI that can be used in place of the CFC-BDP without changing the prescribed dose of corticosteroid (Bousquet et al., 2009). The median

particle size in the aerosol generated by Clenil Modulite is 2.9µm and the distribution of particle sizes more closely matches that of CFC containing MDIs than Qvar (Ganderton et al., 2002). Several clinical studies have shown no differences in lung function, asthma control, tolerability, and systemic exposure between Clenil Modulite® and CFC inhalers in both healthy adults and asthmatic patients (Anderson et al., 2002; Bousquet and Cantini, 2002; Woodcock et al., 2002a). Consequently, the lung deposition from Clenil Modulite® is not as efficient as that with Qvar® which provides the same efficacy and safety profile as Clenil but at half the dose. However, this new non-extra fine HFA-BDP formulation allows a seamless transition to CFC-free BDP inhalers and minimizes difficulties for both patients and prescribers as the same dosage schedule is used. Clenil® Modulite is likely to minimise both NHS staff time and disruption for the patient. The differences between the newly developed CFC-free beclometasone inhalers; Clenil Modulite® and Qvar® inhalers are summarized in table 2.2.

Clenil®	**Qvar®**
- Requires no dose adjustment from CFC-BDP inhalers	- Requires 50 - 60% dose reduction from CFC-BDP inhalers
- Metered dose inhalers (MDIs) only	- Metered dose inhalers (MDIs), Autohalers, and Easi-Breathe inhalers
- 50 µg, 100 µg, 200 µg, and 250 µg strengths available.	- 50 µg and 100 µg strengths available
- Licensed in adults and children (any age)	- Licensed in adults, but not licensed in children <12 years.
- Similar lung and oropharynx deposition as CFC-BDP inhalers	- Increased lung deposition and reduced oropharynx deposition compared with CFC-BDP inhalers
- Optimal slow inhalation flow with good coordination is required	- Optimal slow inhalation with good coordination is less critical than when using traditional MDIs

Table 2.2: Differences between Clenil® and Qvar® inhalers.

Due to these differences, the Medicines and Healthcare Products Regulatory Agency (MHRA) have recommended that beclometasone MDIs must be prescribed by brand and not generically.

2.6. Methods of studying particle deposition

The assessment of drug deposition provides important information for evaluating the performance of inhalers and inhalation techniques. Studies have shown the important role of drug deposition in the lung on predicting clinical response and efficacy of inhaled drugs (Pauwels et al., 1997a; Snell and Ganderton, 1999; Newman, 2000). Several methods have been developed and validated for the assessment of pulmonary drug deposition.

2.6.1. Pharmacokinetic methods

Pharmacokinetic methods can be successfully used to predict lung deposition, bioavailability, and the systemic effects of an inhaled dose. They are indirect measurements that use plasma or urine concentrations to estimate the amount of drug which enters the systemic circulation via the pulmonary and the gastrointestinal routes (total systemic delivery), and thus provide valuable data which predict extra-pulmonary effects (Newnham et al., 1993).

As illustrated in Figure 2.23, after an inhalation, a portion of the dose is delivered to the lungs whilst the majority is swallowed (Chrystyn, 1997). The proportion of the dose that enters the lungs is cleared from the body, either by mucociliary clearance (Borgstrom et al., 1992) then swallowed or by absorption through the airway wall into the systemic circulation. It is this portion of the dose that has the potential to exert a therapeutic effect; this is termed the effective lung dose. Whether the dose is deposited in the lungs or swallowed, it will enter the systemic circulation. The total amounts delivered can therefore give rise to systemic side effects. Since inhaled medications are delivered

directly onto the therapeutic sites in the airways the dose is low; hence the potential for systemic side effects is markedly reduced (Chrystyn, 2001).

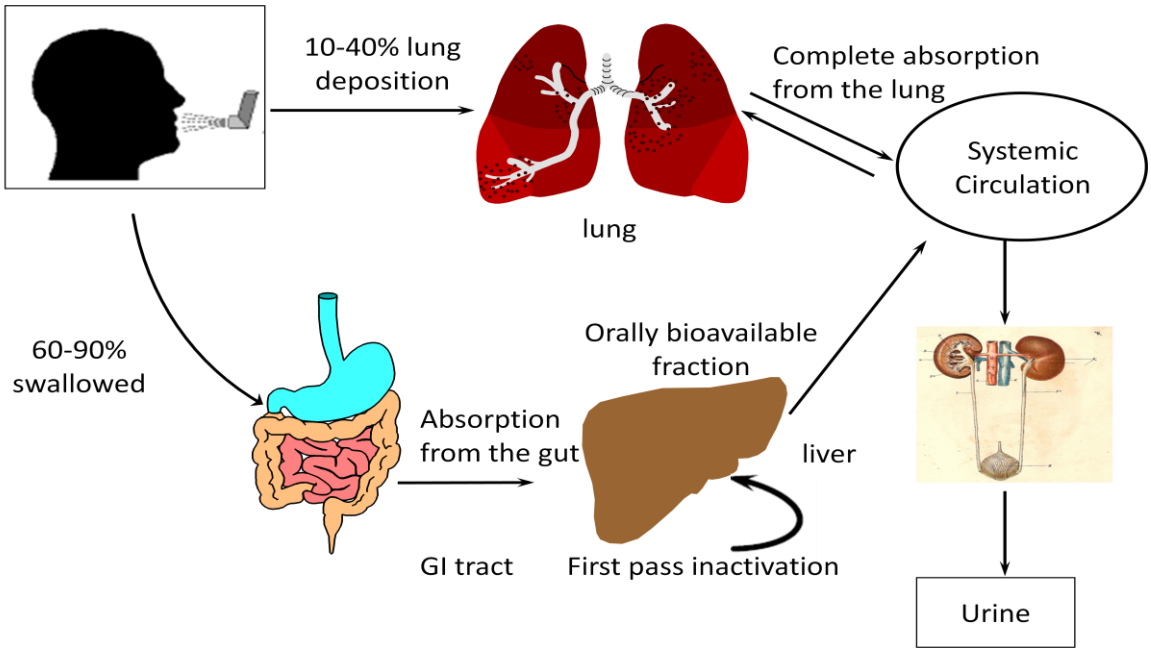


Figure 2.23: Schematic representation of the fate an inhaled drug (Chrystyn, 2001; Barnes, 2007).

Identification of lung deposition using pharmacokinetic methods requires absorption via the pulmonary and oral route to be separated except for drugs that are poorly absorbed, such as, sodium cromoglycate or drugs with a high first pass effect, such as, fluticasone propionate. Virtually, all the systemic delivery of fluticasone propionate is by the pulmonary route (Mollmann et al., 1998).

Borgstrom and Nelson (1990) first developed a charcoal block urinary excretion method to identify the relative lung deposition. They reported that the concurrent oral administration of activated charcoal blocked all absorption of the drug from the gastrointestinal tract. In this case the amount of drug eliminated in the urine gives an absolute value for the total lung dose. However, because this method uses oral charcoal it

would be unethical to extend it to patient studies due to the concomitant oral therapy patients receive (Chrystyn, 2001).

Drugs delivered to the lungs are very rapidly absorbed into the body whereas there is a lag time after oral administration before its delivery to the systemic circulation. The body starts eliminating drugs as soon as they are delivered to the body. Using this principle, plasma (Lipworth, 1996; Lipworth and Clark, 1997; Lipworth and Clark, 1998b) or urine samples (Hindle and Chrystyn, 1992; Hindle et al., 1993; Hindle and Chrystyn, 1994; Hindle et al., 1995) over the first 20 and 30 minutes, respectively, post inhalation have been used as useful indices of lung deposition. This is due to the negligible contribution of swallowed drug to systemic levels during these time periods (Hindle and Chrystyn, 1992).

Direct measurements of plasma salbutamol concentrations given by different inhaler devices over the first 20 minutes provided an effective and simple method to quantify and measure the relative bioavailability of salbutamol to the lung following inhalation (Lipworth, 1996). Indeed, the delayed gastrointestinal absorption compared to lung absorption and the very low oral bioavailability of salbutamol (<0.3) during the first 30 minutes post inhalation provided a sensitive index for lung deposition (Chrystyn et al., 1996; Clark and Lipworth, 1996a). This pharmacokinetic approach was successfully used to compare different inhaler devices (Lipworth and Clark, 1998b), different inhalation techniques (Engel et al., 1992), and to study the effects of multiple actuations and inhalations delay on lung deposition. Moreover, this pharmacokinetic method was used to investigate the effect of using an antistatic lining or washing the spacers to eliminate electrostatic charge, and evaluate the bioequivalence of different formulations (Clark et al., 1996; Clark and Lipworth, 1996b). This plasma pharmacokinetic method has also been used to compare the pharmacokinetic profile of Beclazone® (beclometasone

dipropionate) in its CFC and HFA based formulations and reported up to two fold greater drug absorption with the HFA-BDP than the CFC-BDP formulation at the same nominal dose (Lipworth and Jackson, 1999).

Hindle & Chrystyn (1992) were the first to report a urinary pharmacokinetic method to determine the relative bioavailability of salbutamol to the lung and to the body following an inhalation. As shown in figure 2.24, this study reported that the amount of salbutamol excreted in the urine over the first 30 minutes post inhalation was significantly greater than the amount eliminated following oral administration. They have validated how this index represents the amount of the inhaled dose deposited into the lungs. This measurement represents the effective lung dose because it measures the drug that is delivered to the body following passage through the airway wall. Hindle & Chrystyn (1992) also reported that the amount of salbutamol and its metabolites excreted in urine over the 24 hours period post-inhalation is an index of systemic delivery. This index is the relative bioavailability to the body following inhalation.

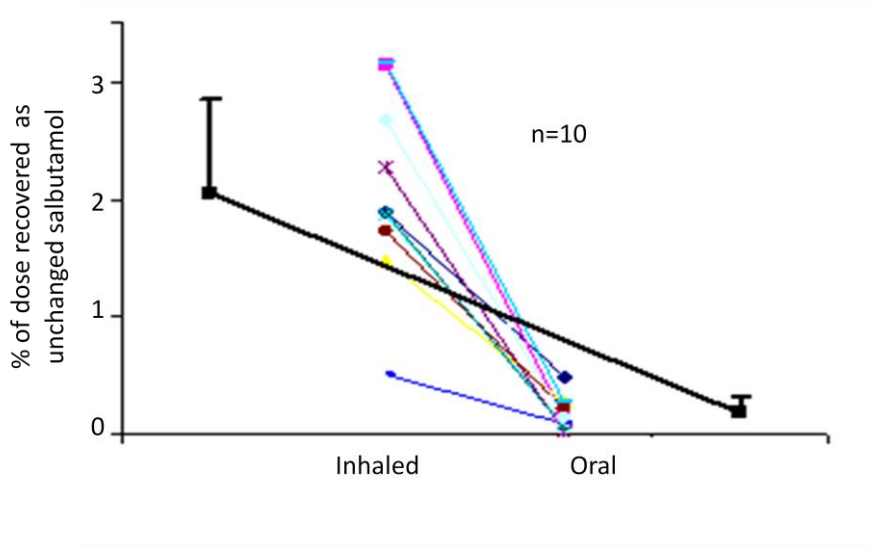


Figure 2.24: Mean and individual amounts of urinary salbutamol excreted 30 minutes post inhalation and oral dosing reproduced from (Hindle and Chrystyn, 1992).

The reproducibility of the Hindle and Chrystyn method has been reported (Tomlinson et al., 2003) and found to be more sensitive than a bronchoprovocation challenge test using methacholine in detecting a difference between inhalation techniques (Tomlinson et al., 2005). This index (relative lung bioavailability) of salbutamol to the lungs following inhalation has been useful to compare different inhalation devices, e.g. spacer devices (Chege and Chrystyn, 1994; Hindle and Chrystyn, 1994; Mazhar and Chrystyn, 2008), dry powder inhalers (Hindle et al., 1995; Hindle et al., 1997; Chege and Chrystyn, 2000), nebulisers (Silkstone et al., 2000; Silkstone et al., 2002; Mazhar et al., 2008), different inhalation techniques (Hindle et al., 1993), and different formulations (Chege and Chrystyn, 1995). The method is simple, non-invasive and has already been extended to determine the relative bioavailability of different drugs e.g, inhaled sodium cromoglycate (Aswania et al., 1997; Aswania et al., 1999; Aswania and Chrystyn, 2001; Aswania and Chrystyn, 2002), nedocromil (Aswania et al., 1998), gentamycin (Nasr and Chrystsyn, 1997; Al-Amoud et al., 2002; Al-Amoud et al., 2005) and formeterol (Nadarassan et al., 2007). However, the methodology has not been extended to inhaled corticosteroids.

The urinary pharmacokinetic method provides a simple and effective method of assessing the relative bioavailability of many drugs to the lung. An advantage of this method is that it uses the patient's own inhaler whereas other investigations of lung deposition following inhalation may require the use of a radiolabelled inhaled marker, which alters the formulation of the inhaled product. The disadvantage of this method is that it relates only to total lung deposition and does not differentiate between drug distributions into different regions of the lung; however, total lung deposition is probably more related to clinical response than regional lung deposition (Zainudin et al., 1990; Zanen et al., 1994; Zanen et al., 1996; Chrystyn, 1997; Chrystyn et al., 1998). The redistribution of drug deposited in the alveolar region is possible by the pulmonary circulation or via mucocilliary clearance (Chrystyn, 2001). Also, an even distribution of

drug throughout the lungs may not occur, especially in severe asthmatic subjects due to their altered airway calibre (Lipworth and Clark, 1997).

The plasma concentrations of drugs such as inhaled corticosteroids are very low, because of the small doses used and the very large volume of distribution of these drugs in the body. The analysis of these drugs in plasma requires highly sensitive analytical methods (Derendorf et al., 2001); however the concentrations of drugs in urine are much higher and offer a much easier solution to the required sensitivity of assays.

Several pharmacokinetic safety studies have provided useful information on drug absorption from different aerosol devices and answered several questions on the performance of these aerosols. Harrison et al (1999) compared the systemic delivery of the original CFC-BDP formulation to HFA-BDP formulations and found that the serum levels of beclometasone esters, as measured by AUC, was approximately 2.5 times greater following the HFA-BDP compared to the CFC-BDP. The smaller particle size of HFA-BDP resulted in a more rapid and greater efficiency of systemic delivery than did the larger particle size of the CFC formulation. Moreover, these pharmacokinetic safety studies were also successfully used in investigating the dose proportionality of BDP and the ability of one strength to substitute for the other strength by measuring 17-BMP maximum plasma concentrations (Harrison et al., 2002b) . The observation of the comparable efficacy of CFC-BDP to much lower doses of HFA-BDP suggested that HFA-BDP may have less safety concerns than CFC-BDP. Despite the fact that the measured serum profiles of beclometasone showed an increased extent of drug absorption of HFA-BDP compared to CFC-BDP, it was at least as favourable as CFC-BDP with regard to adrenal suppression (Harrison et al., 1999a).

2.6.2. Lung imaging techniques

The non-invasive imaging technique of gamma scintigraphy was developed to be used for the assessment of pulmonary drug delivery by labelling the formulation with a gamma ray emitting radionuclide, e.g. ^{99m}Tc. This process enables direct visualization and quantification of where the drug has been deposited by a gamma camera. In addition to providing accurate assessments of whole lung deposition, it provides data on regional deposition by dividing the lung into central, intermediate, and peripheral zones representing airways of different sizes. The peripheral zone/central zone deposition ratio enables differences in regional deposition between treatments regimens to be detected (Newman et al., 1989; Steed et al., 1997; Newman et al., 2003), but it has not been established whether efficacy depends upon whole or regional lung deposition (Chrystyn, 1997).

Originally, radiolabelled Teflon particles were used (Newman et al., 1981a); however, these techniques were soon replaced by methods to adhere the radionuclide (usually 99 m Technetium) to either the formulation or the drug molecule (Kohler et al., 1988).

Gamma scintigraphy is based on reformulating an existing inhaled product to incorporate a radiolabel. Prior to each study validation measurements are carried out *in-vitro* to show that the aerodynamic particle size characteristics are not altered in the radiolabeled product and similar to the original product (Farr, 1996; Snell and Ganderton, 1999; Newman et al., 2003).

There are two gamma scintigraphy methods, two-dimensional and three-dimensional imaging (Newman and Wilding, 1998a; Chrystyn, 2001). Two-dimensional gamma scintigraphy (planar imaging) studies drug deposition and the extent to which the drug is available at the site of action. If two inhalation products deliver the same amount of drug and have similar whole lung and regional deposition patterns then their clinical effect

within the lung should be the same. Thus, planar imaging provides a powerful tool to indicate if two delivery systems are either equivalent or not. Planar imaging studies have been used extensively to compare different inhalation devices, e.g. metered dose inhalers (Newman et al., 1995), spacer devices (Newman et al., 1981c; Newman et al., 1984; Vidgren et al., 1987; Newman et al., 1989; Newman and Newhouse, 1996), Dry powder inhalers (Vidgren et al., 1988; Borgstrom et al., 1994; Newman et al., 1994a) and nebulisers (Zainudin et al., 1990; Hardy et al., 1993; Newman et al., 1994b). A disadvantage of this method is that it is two-dimensional and thus some drug deposited in the smaller airways will be classified as in the central or the intermediate zones. Although, this is partially overcome by correcting for the distance from the imaging apparatus, the 2D problems do remain.

Three-dimensional imaging methods e.g., SPECT (single photon emission computed tomography) and PET (positron emission tomography) have been developed to overcome planar imaging problems. These more advanced techniques, SPECT and PET relate deposition pattern to anatomy better than planar imaging**,** as the gamma camera rotates through 360º allowing for a full three dimensional intrapulmonary deposition pattern. The three-dimensional imaging methods allow identification of pulmonary deposition with higher resolution and provide a better distinction between central and peripheral lung deposition (Usmani et al., 2005; Leach et al., 2006). This technique has been used by Newman, (2006) and revealed that ciclesonide deposition within the lungs was highest in the peripheral regions for HFA-MDI in asthmatic patients (Newman et al., 2006).

Positron emission tomography (PET) has an additional advantage in that it enables the drug itself to be labelled without modifying its chemical structure and can thereby overcome some of the limitations of gamma scintigraphy. It involves the direct chemical

incorporation of positron emitters such as ^{11}C, ^{18}F, ^{13}N, and ^{15}O into the drug molecule (Rhodes and Hughes, 1995; Newman et al., 2003). This technique has been applied to assess drug deposition of several drugs (Dolovich and Labiris, 2004; Dolovich, 2009).

Gamma scintigraphy produces data of the total lung dose that is absorbed through the lung and that is cleared by mucocilliary clearance. Since only the former is responsible for the therapeutic effect, then gamma scintigraphy will overestimate the effective dose reaching the lung (Chege and Chrystyn, 2000; Chrystyn, 2000). This is why gamma scintigraphy studies showed higher values than those of urinary excretion with charcoal block (Borgstrom et al., 1992; Newman et al., 1995). Similar results have been reported for sodium cromoglycate in that gamma scintigraphy indicates a total lung deposition of 8.8% (Newman et al., 1991a) while urinary excretion suggests less than 3% (Aswania et al., 1999). Gamma scintigraphy also involves modifications to the formulation of the inhaled product and consequently the product tested by this method is not the same as the one that the patient actually uses. Thus, accurate *in-vitro* studies are essential to ensure that the dose emission characteristics have not been changed (Newman et al., 1995). Some *in-vitro* studies have shown changes to the aerodynamic particle characteristics of the emitted dose when a radiolabel is incorporated into the inhaled product (Newman et al., 1982b). Despite the fact that, 3D scintigraphy gives more detailed information on deposition site, the larger doses of radiation dose invoke ethical consideration particularly if intended to be used in children (Newman et al., 1995; Chrystyn, 2001). In clinical practice, scintigraphic studies cannot accurately predict the performance of the inhaled drug in terms of efficacy and safety. It gives precise information about the distribution pattern of the aerosol without information of the subsequent fate of the drug.

2.6.3. Pharmacodynamic methods

The bioequivalence of inhaled products is complicated as the therapeutic effect is due to topical deposition of drug, whereas the safety is determined by systemic delivery via the oral and gastrointestinal route. Clinical endpoints such as spirometry and bronchoprovocation are regarded as the gold standard by the Regulatory Authorities to demonstrate the efficacy of inhaled products. However, systemic delivery using either pharmacokinetic or adrenal suppression methods is used to demonstrate the safety of inhaled products (Adams et al., 1994; Newman, 2000).

For inhaled medications, bioequivalence ensures that different doses of different drugs or different formulations of the same drug produce equivalent pharmacodynamic effect. Current guidelines recommend the use of a dose scale approach to demonstrate bioequivalence between different inhaled products. The bioequivalence of inhaled products is evaluated as the ratio of drug doses producing similar effects (relative potency) rather than comparing the magnitude of responses following the administration of different preparations. The most widely used dose scale approach is the Finney bioassay method that involves the determination of the relative potency of two inhaled products by calculating horizontal differences between their regression lines following inhaled administration (Adams et al., 1994; Lavorini et al., 2008a).

Measurement of lung functions by spirometry has been used to determine the degree of bronchodilatation produced and compare new inhalers. Busse et al (1999) reported that $FEV_{1,}$ which is a clinically relevant marker for asthma control, was sensitive enough to distinguish between increasing dose effects of BDP. This study showed that increasing doses of inhaled corticosteroids in patients with severe asthma led to an improved lung function and asthma control. In addition, they compared the effects of CFC-BDP and HFA-BDP on lung function by calculating the dose of each product required to obtain the

same improvement in FEV_1 (Finney Bioassay method). Their results showed that it would require 2.6 times the dose of CFC-BDP to obtain the same change in FEV_1. The improved lung deposition of HFA-BDP due to its smaller particle size compared to CFC-BDP formulations accounts for their clinically relevant difference in efficacy (Leach et al., 1998a).

However, this measurement alone is often insufficient to discriminate between the potency of drug products due to the maximum spirometric response from therapeutic inhaled doses (Chrystyn, 1994; Buck and Parry-Billings, 2001; Chrystyn, 2001). Some have argued that standard measures of improvement in baseline function may be too insensitive to detect real differences in potency. Measurement of FEV_1 failed to distinguish dose effects in previous studies with inhaled corticosteroids leading to the use of bronchoprovocation challenge testing to demonstrate dose effects (Barnes et al., 1998).

The fact that airway hyperresponsiveness (AHR) is a persistent key feature in asthma has encouraged the development of bronchoprovocation challenge testing as an objective diagnostic tool (Britton, 1998). The FDA guidelines have recommended bronchoprovocation challenge for assessing the equivalence of inhaled products by the determination of PD_{20} in asthmatic patients (Adams et al., 1994). The PD_{20} is the dose of bronchoconstrictor (usually histamine or methacoline) required to produce 20% reduction in FEV_1, following protection by the beta agonist. This method has been successfully used to compare the efficacy of different inhalation products (Wong et al., 1997; Eiser et al., 2001; Mallol et al., 2001; Creticos et al., 2002) and found to be a more sensitive measure than bronchodilatation alone (Buck and Parry-Billings, 2001).

However, there are several problems associated with direct measurements of the clinical response. As, although bronchodilators produce a rapid measurable response indicated by

rapid improvements in spirometric tests of lung function, their marketed doses are close to the top of the dose response curve. Consequently, two products that are deposited differently in the lungs may give a maximal response, causing the failure of spirometric tests to detect important differences in drug delivery between inhaled products (Newman et al., 1991b; Borgstrom et al., 1996; Snell and Ganderton, 1999; Mallol et al., 2001). On the other hand, inhaled corticosteroids show no rapid response, and the usual approach for comparing two inhalers is much more complex due to the need to conduct longer-term clinical trials of at least 4 weeks' duration (Barnes et al., 1998; Rhodes et al., 2001). Drug deposition in the lung should predict clinical response, but the flat nature of the dose response curve of most marketed doses of inhaled corticosteroids masks this relationship. Thus, for example, an 8-fold increase in deposition of beclometasone dipropionate from an HFA formulation (Leach, 1998b) is only associated with an observed 2-fold increase in efficacy (Davies et al., 1998; Busse et al., 1999b). Furthermore, the response to inhaled corticosteroids is highly variable, so that a large number of patients must be studied to achieve an appropriate statistical power (Zanen and Lammers, 1995; Newman and Wilding, 1998b).

The clinical response study failed to differentiate between different inhalation techniques (Giannini et al., 2000). In contrast differences have been observed for salbutamol urinary excretion (Silkstone et al., 2002; Tomlinson et al., 2005) and plasma drug concentrations (Lipworth and Clark, 1998b) and also when using gamma scintigraphy (Newman et al., 1984; Newman et al., 1991b). Newman et al. (1991) measured lung deposition in asthmatic subjects using both gamma scintigraphy and spirometry and reported that when subjects inhaled radiolabelled salbutamol from a MDI and a MDI attached to a large volume spacer the total lung deposition was 12.3 and 23.1% (of the dose), respectively, whilst, there was no difference in spirometry measurements (Newman et al., 1991b). The large inter-subject variability in the response to inhaled methacoline dosing

bronchoprovocation studies indicates that larger numbers need to be used in this type of bioequivalence study. Furthermore the bronchoprovocation agents may stimulate different receptors to those of the drug studied causing deterioration of lung function (Chrystyn, 2001; Barry and O'Callaghan, 2003).

In summary, the clinical response data should be combined with lung deposition data, in order to provide a much better assessment of inhaled drug delivery.

2.6.4. *In-vitro* methods

The particle size of inhalation aerosols is one of the key factors that governs the site and extent of drug deposition in the human respiratory tract and consequently affects its elicited clinical response. Therefore, *in-vitro* studies have been used extensively to reach a judgement about the relative efficiencies of different inhalation delivery systems (Bisgaard, 1996; Pauwels et al., 1997a; Weda et al., 2000). They are characterised by their relative ease of operation, high power to detect differences and the relatively low variability in the measurements compared to *in-vivo* experiments.

Several *in-vitro* studies have shown that the aerodynamic particle size distribution of inhalation aerosols correlates well with their *in-vivo* drug deposition in the lungs and can even predict clinically relevant differences in their systemic side effects (Zanen et al., 1996; Leach et al., 2002; Weda et al., 2004).

However, *in-vitro* studies can still be limited by the ability of the laboratory apparatus to mimic the complexity of the airway anatomy. In addition, it does not take into account patient handling factors and the difficulties that some patients have using their inhalers properly. Furthermore, *in-vitro* determinations use a fixed set of parameters that provides very consistent delivery at low variability and lacks inter- and intra-patient variability, which can greatly affect regional deposition in the lung. Thus, *in-vitro* measurements may not accurately predict the relative performance of drugs *in-vivo* and may even tend

to overestimate lung deposition (Holzner and Müller, 1995; Newman, 1998; Borgström et al., 2000; Newman et al., 2000b; Dunbar et al., 2002). Several gamma scintigraphic studies have shown that DPIs' whole lung deposition expressed as percent metered dose averaged 1.5 times that of MDIs, despite similar *in-vitro* performance. As shown in figure 2.25 the data shows that MDIs and DPIs have, on average, similar FPFs, but that DPIs actually deposit more drug in the lungs (Newman et al., 2003).

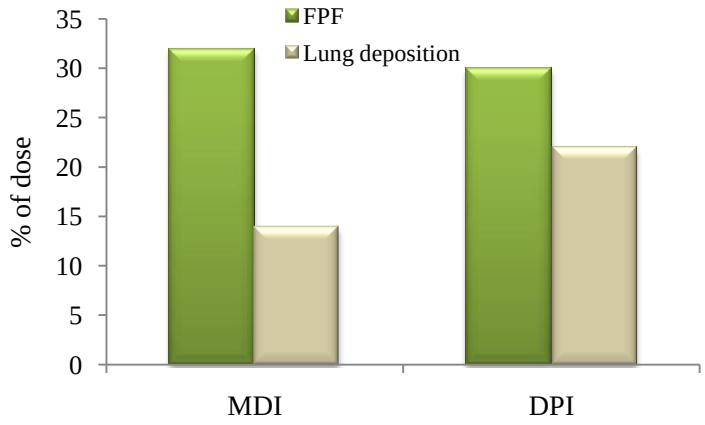


Figure 2.25: Comparison of *in-vivo* whole lung deposition data obtained by gamma scintigraphy and *in-vitro* fine particle fraction (FPF) data. Reproduced from (Newman et al., 2003).

Although good description of particle size distribution of inhaled aerosols may give predictive information on its intrapulmonary behaviour, *in-vivo* pulmonary deposition studies will always be necessary to bridge between *in-vitro* measurements and the clinical effect.

2.6.4.1. Characterisation of the emitted dose

The aerodynamic particle size distribution (APSD) of an aerosol cloud defines where the particles in the cloud are to be deposited following inhalation. Therefore, the APSD together with the delivered dose is widely known as a critical quality attribute in the *in-vitro* characterization of inhalation products. It is generally accepted that the

therapeutically effective particles should be in the range of 1-5μm. Above that range; particles tend to impact in mouth and throat and then swallowed. Below this range, particles will have the possibility to remain entrained in the airstream and then exhaled rather than deposition.

Inertial separation techniques have been widely considered the instruments of choice for measuring the APSD of inhaled products based on their mass and inertia for both regulators and pharmacopoeias. These systems are the golden standard for *in-vitro* inhaler testing, because they yield mass fractions of the drug dose in aerodynamic size classes that are relevant to particle deposition in the human respiratory tract (De Boer et al., 2002; Mitchell and Nagel, 2003).

The *in-vitro* methods vary from simple devices like the twin impinger to more complex apparatus with multiple collection stages including; the Multi-Stage Liquid Impinger (MSLI), the Andersen Cascade Impactor (ACI) and the more recently developed Next Generation Impactor (NGI) (Hickey, 2004; European Pharmacopoeia, 2005; British Pharmacopoeia, 2009). All these systems depend on the same principle, by which air is drawn through the system at a predetermined flow rate causing drug particles to be collected on a series of stages, each of which represents a certain size band. The stages are washed by a solvent to collect the drug and these solutions are analysed to obtain the mass of drug on each stage. Parameters such as the mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle fraction (FPF) and fine particle dose (FPD) are determined and used for comparisons of the *in-vitro* performance of different inhaler devices and drug combinations. The aerodynamic diameter of a given particle is defined as the diameter of a unit density sphere having the same settling velocity as the particle. The mass median aerodynamic diameter (MMAD) of an aerosol is the diameter that separates the mass of the particles equally by 50%. The GSD is a

measure of the polydispersity, or spread, of an aerosol. A monodisperse aerosol has a GSD of 1 and heterodisperse aerosol has a GSD greater than 1.2 (Newman, 1991). Of these parameters, the FPF and FPD have particular relevance, since they express respectively the percentage of the drug dose and the mass of drug contained in particles smaller than 5µm (respirable particles). The fine particle dose is the mass of particles less than 5µm, while the FPF can be represented as either the FPD divided by the nominal dose or by the emitted dose. This causes confusion because the nominal dose and the emitted dose are never the same.

In general, the higher the fine particle dose, the higher the proportion of the emitted dose that is likely to reach the lung (Newman et al., 2000b; European Pharmacopoeia, 2004). Particles with a diameter less than 5µm are often said to constitute the respirable range which provide an estimate of the fraction of the dose that has the potential to be deposited in the lung (Dolovich, 1993; Barry and O'Callaghan, 2003)

The reliability of Cascade Impactors data can be greatly influenced by several factors such as wall loss, particle bounce, and the nature of collection surfaces (Holzner and Müller, 1995; Dunbar and Mitchell, 2005; Kamiya et al., 2009). Upon contact with the collection plate, some particles may bounce due to impaction and be re-entrained into the airstream and carried to a lower stage. Several studies have found that coating of the collection surfaces of the Cascade Impactor with a media that provides a tacky surface (glycerol or silicone oil) is an appropriate precaution to minimize particle bounce and re-entrainment, especially when testing dry powder inhalers (DPIs) (Dunbar and Mitchell, 2005; United States Pharmacopeia, 2005; British Pharmacopoeia, 2009; Kamiya et al., 2009; Copley, 2010). It may also be required for some formulations delivered by pressurized metered dose inhalers (pMDIs), especially when measurements are being made with a low number of actuations from the inhaler (Nasr et al., 1997).

2.6.4.1.1. Andersen Cascade Impactor

The eight-stage Andersen Cascade Impactor (ACI) is widely used to characterize and control the aerodynamic particle size distribution emitted from therapeutic inhalation aerosols. The Andersen Cascade Impactor (ACI), illustrated in figure 2.26 is based on the assessment of the aerodynamic particle size of an emitted dose using a multi-stage approach yielding information about the mass fraction that has the potential to enter the deeper part of the lung. (European Pharmacopoeia, 2005; United States Pharmacopeia, 2005; British Pharmacopoeia, 2009).

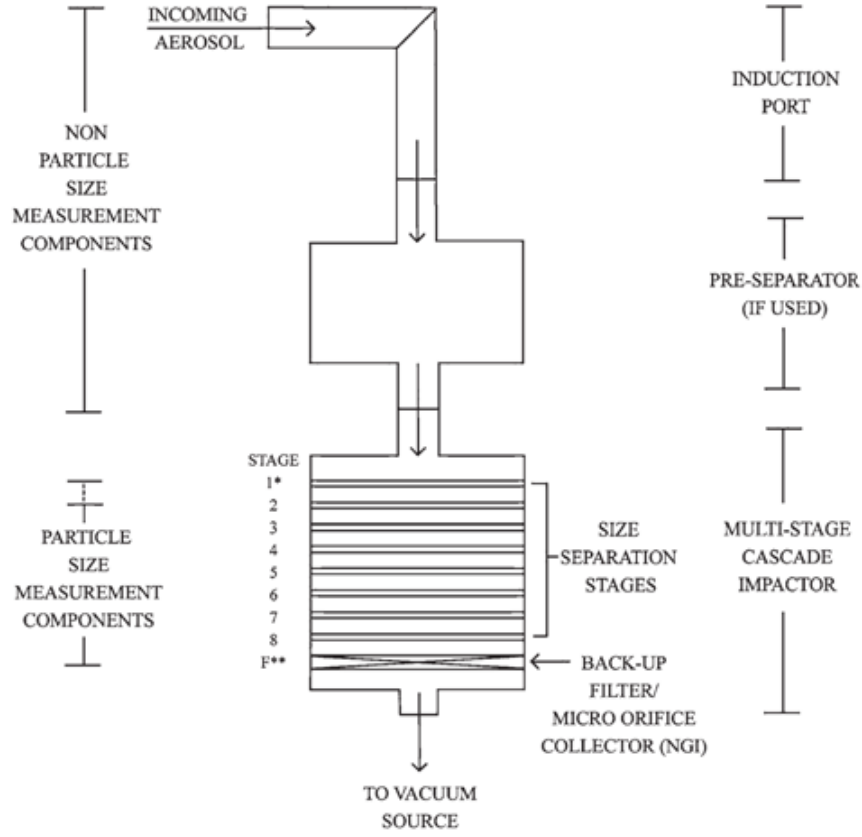


Figure 2.26: Schematic diagram of ACI components (Dunbar and Mitchell, 2005).

The ACI consists of a stack of eight plates with a series of precision drilled holes, and a final filter stage. They fractionate the incoming aerosol onto a series of stages arranged such that successively finer particles are removed as the aerosol passes through the instrument. Each stage of the impactor is associated with a cut-off diameter, a figure that defines the size of particles that are retained on the collection surface of that stage. All of

the particles above a certain size would be captured and those below it would pass through. At the end of the test, the amount of drug present on each collection stage is recovered using a suitable solvent and analysed. The ACI provides the required degree of resolution in the most important particle size range for inhalation products (0.5-5 µm). It operates at a flow rate of 28.3 L min^{-1} with cut-off diameters of 9, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4µm, respectively. It has also been modified to work at higher flow rate at 60 and 90 L min^{-1} whilst retaining the 28.3 L min^{-1} cut-off diameters. As shown in table 2.3 in the 60 L/min version, stages 0 and 7 are removed and replaced with two additional stages, -0 and -1. Similarly, in the 90L/min version, stages 0, 6, and 7 are removed and replaced with three additional stages, -0, -1, and -2. Changes are also made to the configuration of the collection plates (with and without centre holes).

Stage	Flow rate (L/min)		
	28.3	60	90
-2	----	----	9.0
-1	----	9.0	5.8
-0	----	5.8	4.7
0	9.0 - 10.0	----	----
1	5.8 - 9.0	4.7	3.3
2	4.7 - 5.8	3.3	2.1
3	3.3 - 4.7	2.1	1.1
4	2.1 - 3.3	1.1	0.7
5	1.1 - 2.1	0.7	0.4
6	0.7 - 1.1	0.4	----
7	0.4 - 0.7	---	----

Table 2.3: Stage cut-off diameter values (µm) for the various configurations of the Andersen cascade impactor at different flow rates (Copley, 2010).

Cascade Impactors have been extensively used for two distinct roles, the first is for quality control assessment of inhalers, and the second is for *in-vitro* bioequivalence studies of pulmonary drug products. The ACI is superior to both the twin impinger and the MSLI as it provides a more detailed particle size distribution of the aerosolised drug. As shown in figure 2.27, the particle size distribution obtained from the ACI simulates

the aerosol behaviour after leaving the inhaler and provides data that may be predictive of particle deposition in the respiratory tract (Weda et al., 2004; Dunbar and Mitchell, 2005; Mitchell et al., 2007a; British Pharmacopoeia, 2009).

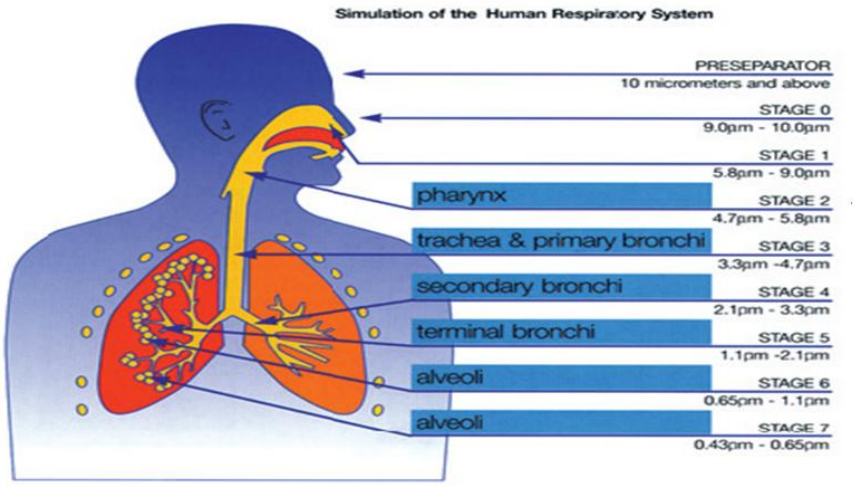


Figure 2.27: Presentation of regional lung deposition relative to ACI size range (Dunbar and Mitchell, 2005).

On MDI actuation, the drug dose delivered beyond the mouthpiece is typically separated into three fractions: the induction port deposition fraction (IPF), the coarse particle fraction (CPF), and the fine particle fraction (FPF). Induction port deposition approximates the drug that is deposited in the throat or mouth. The coarse particle fraction represents aerosol particles deposited in stages 0, 1, and 2 of the ACI. These large particles of 5-10 μm size deposit preferentially in the upper airways. The FPF represents aerosol particles deposited in stages 3, 4, and 5 of the ACI. These particles are 1-5 μm in size and have a high probability of penetrating into the deep lung (Guo et al., 2008). While, aerosol particle collected on stages 6 and 7 and in the final filter of the ACI correspond to particles less than 1μm in size (Asmus et al., 2003).

The ACI values provide a direct link with the mass of therapeutically active pharmaceutical agent and particle aerodynamic size by the precise determination of the

MMAD and the GSD, which have been shown to greatly affect lung deposition efficiency (Martonen and Katz, 1993; Newman, 1998; Thorsson and Geller, 2005). However, there are some limitations to the Andersen Cascade Impactor data, as it cannot perfectly simulate the respiratory tract, since it operates at a constant flow rate, while the real respiratory cycle has more variable flow rates. In addition, deposition in the impactor is by inertial impaction only, whereas in the respiratory tract particle deposition is also affected by sedimentation and diffusion, especially for small particles in deep lung regions. Besides, the method uses a vacuum pump generating a square wave airflow profile unlike the sinusoidal pattern of human inspiration(De Boer et al., 2002).

2.7. Inhaled corticosteroids

Inhaled corticosteroids (ICS) are the standard controller medications for asthma in both adults and children and additionally they are used in COPD treatment. Inhaled corticosteroids (ICS) have a positive effect on lung function, symptoms, exercise capacity, and may decrease disease exacerbations. At present there are many inhaled corticosteroids used to varying degrees in different countries for asthma treatment, e.g., beclometasone dipropionate (BDP), budesonide (BUD) , fluticasone propionate (FP), triamcinolone acetonide (TA), flunisolide, mometasone furoate and ciclesonide (CIC) (Derendorf et al., 2006; Barnes, 2007).

2.7.1. Mechanism of action

As illustrated in figure 2.28, the broad anti-inflammatory profile of corticosteroids and their ability to interfere with the multiple pathways involved in the inflammatory process accounts for their marked clinical effectiveness in asthma (Pelaia et al., 2003). Inhaled corticosteroids elicit their effects by diffusion across the cell membrane and subsequent binding to cytoplasmic glucocorticoid receptors in target cells; promoting their activation and translocation to the cell nucleus, where they bind to specific DNA sequences that

repress transcription of inflammatory genes and promote transcription of anti-inflammatory genes.

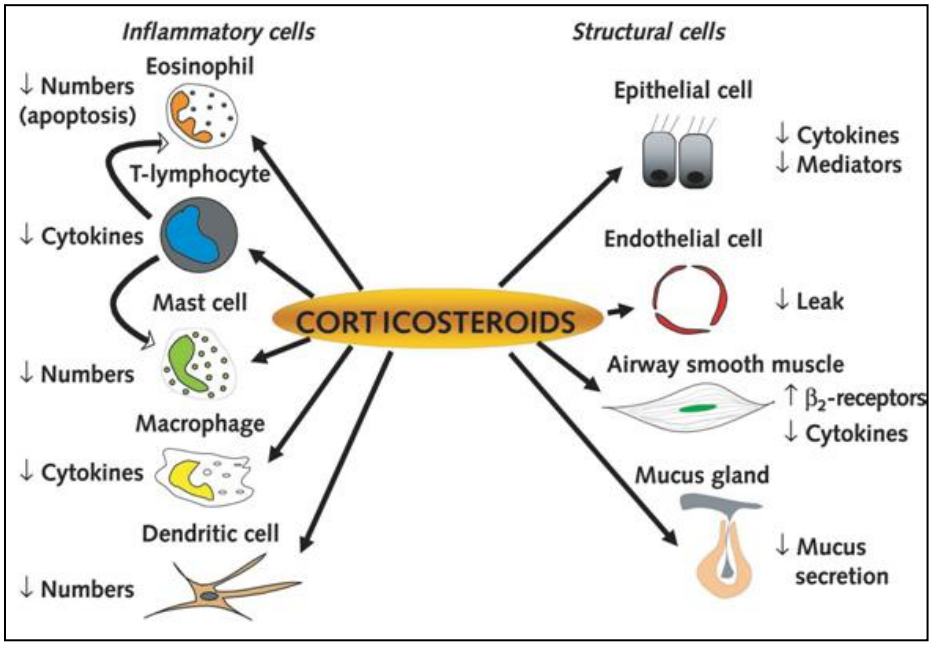


Figure 2.28: Cellular effects of corticosteroids (Barnes and Adcock, 2003).

In addition, ICS markedly reduce the survival of certain inflammatory cells, such as eosinophils, decrease mucosal mast cells number, and decrease the immediate response to allergen and exercise (Barnes and Adcock, 2003; Derendorf et al., 2006; Sobande and Kercsmar, 2008).

2.7.2. Adverse effects

The ideal goal of all inhaled corticosteroids is to provide a localized and long lasting therapeutic effect at the pulmonary target, minimize oral bioavailability, and minimize local and systemic side effects in combination with a convenient and easy to use inhaler (Cerasoli, 2006; Barnes, 2007). The therapeutic benefit from ICS is often achieved at relatively low doses (Masoli et al., 2004b), thus ICS have a very favourable benefit-to-risk ratio. However, despite their effectiveness and their improved safety profile relative to oral corticosteroids, there is still a concern about local (in the oropharyngeal cavity)

and systemic side effects (due to absorption of ICS into the circulation through the lungs and the GI tract), especially with high-dose and long-term use (Hanania et al., 1995; Kelly and Nelson, 2003). Although high pulmonary availability is required for efficacy, it may increase systemic absorption and the potential for unwanted side effects (Lipworth and Jackson, 2000; Barnes, 2007). However, these is not always the case, since higher lung deposition and more than double the systemic concentrations with 400µg/day HFA-BDP formulations, were associated with even less adrenal suppression with a comparable efficacious dose of 800µg/day CFC-BDP. The reason behind the reduced systemic effects is not known. In addition, equivalent doses of each product showed no difference in adrenal suppression despite the dose potency difference (Harrison et al., 1999a; Harrison, 2002). The key in reducing the risk of adverse events and achieving an optimal balance between safety and efficacy is to titrate the maintenance dose of ICS to the lowest possible dose that achieves asthma control.

2.7.2.1. Local side effects

The main local adverse effects of ICSs are oral candidiasis, dysphonia, and pharyngitis, as well as cough at the time of inhalation (Allen et al., 2003; Kelly and Nelson, 2003). Oral candidiasis is a dose related side effect that is observed in 5% of treated patients and can be prevented by rinsing the mouth with water or using a spacer or if required additional topical antifungal therapy . The decreased oral deposition of ciclesonide has led to decreased incidence of local candidiasis of 1% compared to 11% with fluticasone (Pedersen et al., 2006; Sobande and Kercsmar, 2008). The cough is due to a local irritation and is often resolved by changing the delivery device, pre-treatment with a bronchodilator, using a valved holding chamber, or slowing the rate of inhalation (Hanania et al., 1995; Sobande and Kercsmar, 2008). Dysphonia is also observed in patients receiving ICS, and it appears to be a direct effect of ICS administration, as it was absent when the propellant or excipients were administered without the ICS (Toogood et

al., 1980). It is caused by steroid induced myopathy of laryngeal muscles and it is reversible with cessation of the drug. However, this problem can be easily resolved by using a spacer device or mouth rinsing after inhalation (Hanania et al., 1995; Zainudin, 1997; Buhl, 2006).

2.7.2.2. Systemic side effects

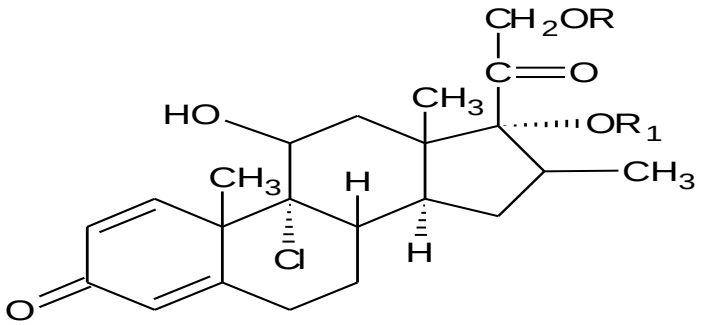
There are two routes by which inhaled corticosteroids can enter the systemic circulation. The majority of the inhaled fraction that is delivered into the lung easily enters the pulmonary circulation and is systemically available before inactivation in the liver takes place. The fraction deposited in the oropharynx is swallowed and its systemic availability depends on the gastrointestinal absorption and first-pass effect in the liver.

Systemic adverse effects are caused by long-term treatment with high doses of ICS. These systemic effects include, osteoporosis, skin thinning, skin bruising, cataracts, glaucoma, bone fractures, reduced bone mineral density, and suppression of hypothalamic-pituitary-adrenal axis function (HPA) (Kelly and Nelson, 2003; Cerasoli, 2006; Irwin and Richardson, 2006). However, despite some safety concerns, the over-riding evidence generally supports the conclusion that ICS are well tolerated and safe. At the recommended dosages, ICS produced no clinically significant adverse effects on bone density (Lung Health Study Research Group, 2000; Calverley et al., 2007; Anthracopoulos, 2008; Iles et al., 2008) or on suppression of the hypothalamic-pituitary-adrenal axis (The Childhood Asthma Management Program Research Group, 2000; Bisgaard et al., 2004; Martinovic, 2008; Skoner, 2008). In addition, inhaling steroids through spacers and mouth rinsing post inhalation may significantly reduce to some extent its local and systemic adverse effects (Selroos and Halme, 1991; Brown et al., 1993; Trescoli and Ward, 1998).

2.7.3. Beclometasone dipropionate

2.7.3.1. Chemical structure

Beclometasone dipropionate was the first inhaled corticosteroid used for the treatment of asthma in adults and children. It was first used in 1972 in a pressurized metered dose inhaler and later in a dry powder inhaler and an aqueous nasal spray.



	R	R_1
BDP	COC_2H_5	COC_2H_5
17-BMP	H	COC_2H_5
21-BMP	COC_2H_5	H
BOH	H	H

Figure 2.29: The structural formula of BDP, 17-BMP, 21-BMP, and BOH.

2.7.3.2. Pharmacokinetics and pharmacodynamics of inhaled beclometasone dipropionate

Pharmacokinetic (PK) and pharmacodynamic (PD) properties of ICS are directly related to safety and efficacy of the drug. The efficacy of an ICS is dependent on high glucocorticoid receptor binding, high pulmonary deposition and retention, enhanced lipophilicity, and lipid conjugation. Safety is optimised by low oral bioavailability, high protein binding and rapid systemic clearance (Derendorf et al., 2006; Quizon and Colin, 2010).

2.7.3.2.1. Prodrug

Beclometasone dipropionate is the parent compound and has low activity. As shown in figure 2.30, BDP is a prodrug that is metabolised by esterases in the human lung, liver and other parts of the body to three different metabolites, 17-beclometasone monopropionate (17-BMP), 21-beclometasone monopropionate (21-BMP) and beclometasone (BOH). 17-Beclometasone monopropionate (17-BMP) is the active metabolite whereas BOH and 21-BMP have a very low binding affinity to the glucocorticoid receptor (Foe et al., 2000; Derendorf et al., 2006; Rossi et al., 2007). This metabolism readily occurs in the lungs, hence, a high degree of pulmonary pre-systemic metabolism for BDP is essential for its topical activity in the lung (Wurthwein and Rohdewald, 1990).

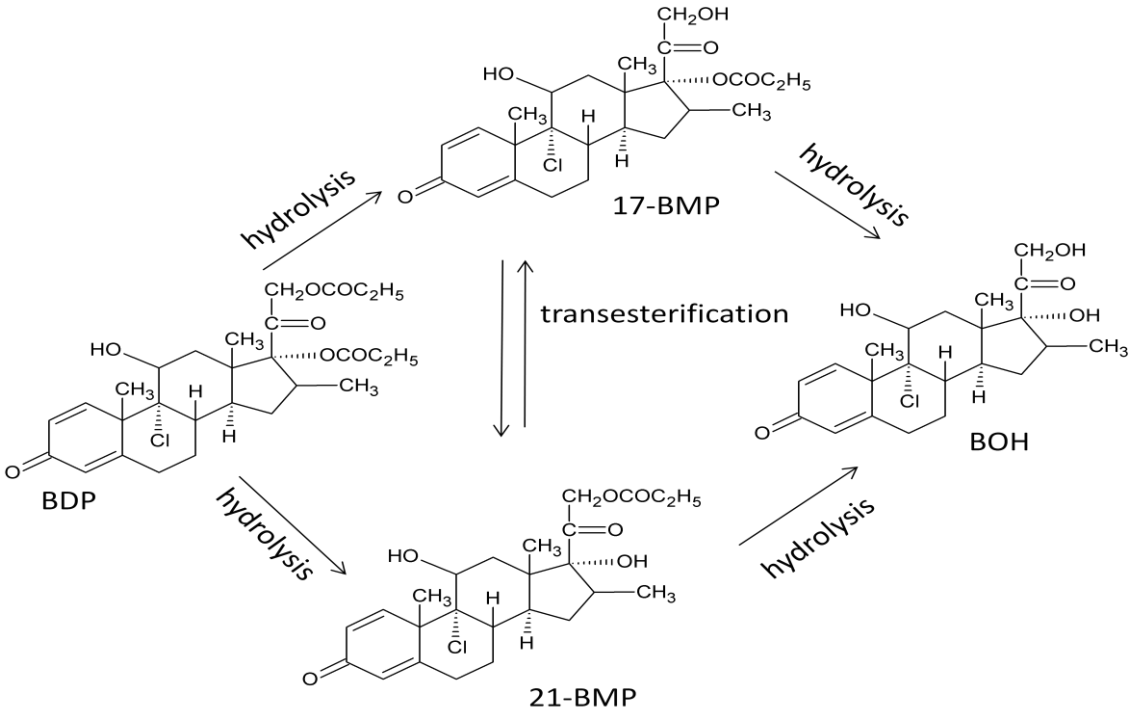


Figure 2.30: Major *in-vivo* degradation pathway for BDP and its main metabolites (Foe et al., 2000; Daley-Yates et al., 2001).

2.7.3.2.2. Receptor-binding affinity

The pharmacological effect of ICS is mediated through binding to glucocorticoid receptors that are widely distributed throughout the body. The receptor binding affinity has implications for the clinical safety profile, since both positive effects in the lung and side effects of the drug are mediated through the same receptors. It was found that the relative receptor binding affinity of 17-BMP to the glucocorticoid receptor is 30 and 18 times greater than the parent drug and BOH respectively, whereas 21-BMP is practically inactive. Therefore the anti-inflammatory activity of inhaled BDP is due mainly to the active metabolite 17-BMP, which is rapidly formed from the parent drug in lung tissues (Wurthwein and Rohdewald, 1990).

2.7.3.2.3. Bioavailability

Inhaled corticosteroids such as beclometasone dipropionate are intended to provide localized therapy in the lungs. There is a proportion of the ICS dose that is swallowed and systemically absorbed from the GI tract (oral bioavailability) and another proportion of the dose delivered to and absorbed by the lungs (pulmonary bioavailability). Consequently, the blood concentration of an ICS is a function of the sum of its pulmonary and orally absorbed fractions (Derendorf, 1997). The systemic bioavailability of an ICS has a very strong implication on the safety profile of the drug, since the orally absorbed fraction does not contribute to the beneficial pulmonary effects of the drug and only causes systemic side effects. It is advantageous, therefore, for the oral bioavailability of the ICS to be low. Inhaled BDP has a high systemic contribution from the swallowed fraction, due to its higher oral bioavailability and lower first pass inactivation compared to other ICS. The first pass inactivation values of BDP, fluticasone, and budesonide are 60-70%, 99, and 89% respectively (Lipworth, 1996; Trescoli and Ward, 1998).

The appearance of the new HFA-BDP formulations and the availability of adequate sensitive assays encouraged investigators to study the pharmacokinetics of inhaled BDP formulations.

Several pharmacokinetic studies used serum BOH concentrations to investigate the pharmacokinetic properties of BDP due to the initial lack of the appropriately sensitive analytical methods for 17-BMP (Harrison et al., 1997; Soria et al., 1998). However, due to the fact that beclometasone is only a small component of the material in the serum as compared with beclometasone esters, further pharmacokinetic studies have developed methods to measure the total amount of beclometasone in a hydrolyzed sample. The total BOH in a sample after hydrolysis should represent the sum of any BDP, 17-BMP, 21-BMP and BOH present (Seale and Harrison, 1998; Harrison et al., 1999a; Harrison et al., 1999b).

However, later on this approach was invalidated by a recent study by Daley-Yates et al (2001), who developed a sensitive assay to quantify 17-BMP and found that estimates of oral absorption and pulmonary bioavailability based on total BOH measurements were approximately half of those found for 17-BMP. Daley-Yates et al (2001), performed intravenous, intranasal, inhalation and oral studies to investigate 17-BMP pharmacokinetics. This study reported that following oral administration of BDP, either with or without activated charcoal, no unchanged BDP was detected in plasma while the total oral bioavailability of 17-BMP was 41% relative to an intravenous dose. However, the administration of traditional CFC-BDP by inhalation produced detectable concentrations of both BDP and BMP and the total inhaled bioavailability for 17-BMP was estimated as 62% relative to the intravenous dose. In addition, an oral charcoal procedure was used to differentiate the pulmonary absorbed and the orally absorbed 17-BMP in the total systemic available 17-BMP. The lung and gut were assumed to

contribute 36% and 26% to the systemic exposure, respectively (Daley-Yates et al., 2001).

Plasma levels of BDP post inhalation were very low due to its rapid hydrolysis to the active metabolite 17-BMP which is the main metabolite detected in plasma as indicated by several pharmacokinetic studies (Harrison et al., 2002a; Harrison et al., 2002b; Agertoft et al., 2003). However, it was surprising to find that the elimination of 17-BMP in plasma was not associated with a corresponding increase in BOH levels. These low levels of BOH in plasma may be explained either by that *in-vivo* 17-BMP is not eliminated primarily via metabolism to BOH or that BOH is very rapidly cleared from the plasma (Daley-Yates et al., 2001).

Systemic absorption of unchanged BDP occurs mainly through the lungs with negligible oral absorption of the swallowed dose. The rapid hydrolysis of swallowed BDP to 17-BMP will leave no intact BDP left after first pass metabolism and accounts for its negligible oral absorption. The charcoal block procedure did not affect the bioavailability of BDP, which confirmed the lack of oral absorption of unchanged BDP. Therefore, the detectable BDP after inhalation is due to the pulmonary-deposited BDP (Daley-Yates et al., 2001, Wang, 2003, Foe et al., 1998. On the other hand, the systemic absorption of 17-BMP arises mainly from lung deposition and to lesser extent from oral absorption of the swallowed dose. Using, the charcoal block procedure, 17-BMP plasma levels were only slightly reduced (less than 20%), confirming that the pulmonary absorption is the main source of systemic exposure to 17-BMP. There is an approximately linear increase in systemic exposure with increasing inhaled dose (Woodcock et al., 2002a).

The absence of detectable concentrations of BDP following oral administration is predictable due to its very high clearance, which leads to its extensive first pass metabolism. In contrast to BDP, the oral bioavailability of 17-BMP was high despite its

similar high clearance. These findings can be attributed to the predominance of systemic rather than first pass metabolism for 17-BMP elimination, While, the predominant elimination mechanism for BDP was through gut and hepatic metabolism (Daley-Yates et al., 2001).

Several pharmacokinetic studies showed that the extent of appearance of beclometasone esters from HFA-BDP in the serum as measured by the AUC was approximately 2-2.5 times greater following CFC-BDP. This can be explained due to the smaller particle size of HFA-BDP leading to its main lung deposition while most of the larger particle size CFC-BDP dose is swallowed and orally absorbed (Seale and Harrison, 1998; Harrison et al., 1999a; Harrison et al., 1999b; Harrison, 2002). This is in agreement with a previous pharmacokinetic study by Seale and Harrison (1998) who observed the poor orally bioavailability of beclometasone dipropionate as shown in figure 2.31. The same study also showed that T_{max} for the oral route was later than for inhaled HFA-BDP, thus suggesting a slower absorption with the oral route.

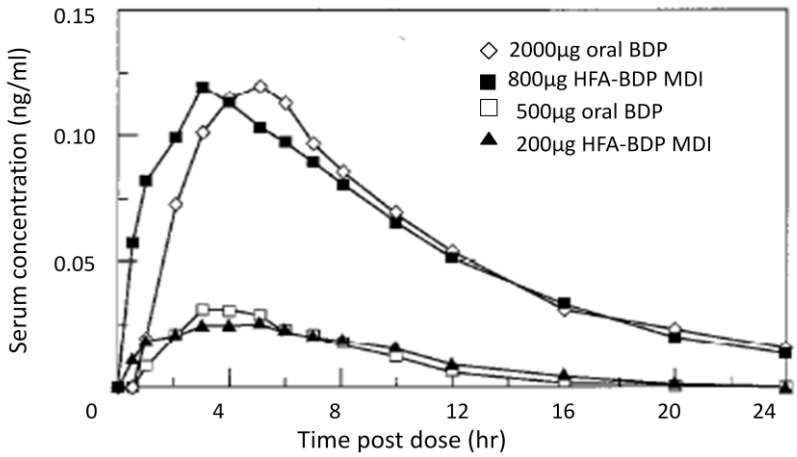


Figure 2.31: Inhaled HFA-BDP gives similar BOH concentration to 2.5 times the oral BDP dose (Seale and Harrison, 1998).

The more enhanced efficacy of the newly formulated HFA-BDP formulations compared to their CFC-BDP counterparts may be attributed to their different metabolic profiles.

Hydrofluoroalkane beclometasone dipropionate (HFA-BDP) showed better activation efficiency in the lung (Wang, 2003) and gave more of the active metabolite 17-BMP in the systemic circulation (after its main absorption from the lungs) in both adults (Harrison et al., 1999b; Lipworth and Jackson, 1999) and children (Agertoft et al., 2003). However, chlorofluorocarbon beclometasone dipropionate (CFC-BDP) gives mainly BOH in the systemic circulation after oral absorption and first pass metabolism in the liver (Seale and Harrison, 1998; Dekhuijzen and Honour, 2000; Wang, 2003).

The extensive (95%) pre-systemic conversion of BDP to the potent and more hydrophilic degradation product 17-BMP leads to extensive absorption of the drug from the lower respiratory tract into the systemic circulation. However, the high therapeutic index of inhaled BDP despite its high systemic absorption may result from a combination of high local potency in the lung, rapid metabolic inactivation and rapid clearance of BDP and its metabolites, especially 17- BMP, that reach the systemic circulation. Also, 17-BMP as the active metabolite, showed very high plasma protein binding and tissue binding in the liver, lung and kidney, suggesting a possible reason for the low systemic side effect of long term BDP treatment for asthma (Foe et al., 2000; Wang, 2003). Moreover, the high clearance values reported for both BDP and 17-BMP resulted in a very low accumulation ratio after multiple dosing with HFA-BDP (Seale and Harrison, 1998; Harrison et al., 1999a). Seale and Harrison (1998) examined the pharmacokinetics of total BOH measured following the first and 27^{th} steady state doses of three HFA-BDP dose levels administered twice daily for 14 days. As shown in figure 2.32, the small difference seen in total BOH serum concentrations confirmed the little accumulation on multiple dosing and the good proportionality of the pharmacokinetic parameters for the three HFA-BDP doses studied.

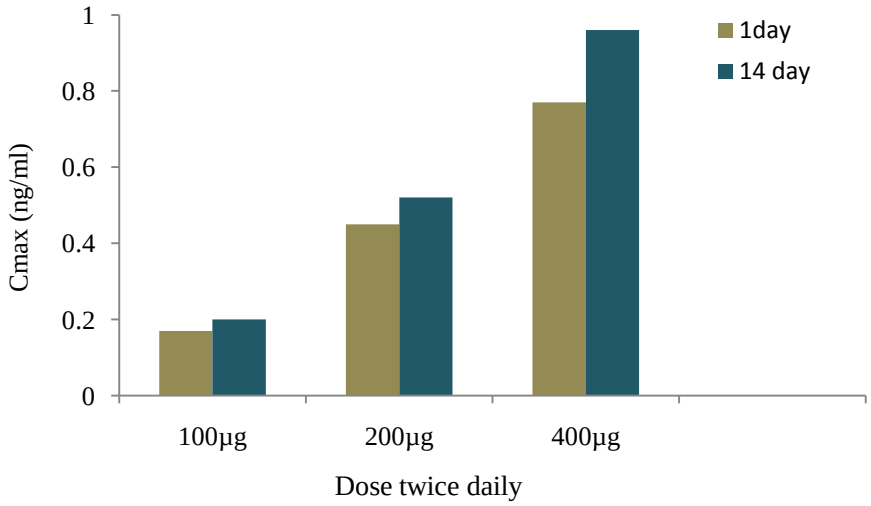


Figure 2.32: Comparison of total BOH C_{max} values on day 1 (single dose) and day 14 (steady state, reproduced from (Seale and Harrison, 1998).

This is in contrast to fluticasone propionate which has longer elimination half life; extensive tissue binding and prolonged receptor binding (Clark and Lipworth, 1997) that caused significant drug accumulation in plasma upon multiple dosing and hence an increased systemic safety risk (Thorsson et al., 1997). Irrespective of the route of administration (injection, oral or inhalation), BDP and its metabolites are mainly excreted in the faeces by biliary elimination while, approximately about 15 % of the dose is excreted as free and conjugated polar metabolites in the urine (Foye et al., 2008).

2.8. Summary

Inhaled corticosteroids are used extensively in the management of asthma and COPD. Beclometasone dipropionate is the first inhaled corticosteroid and it is widely prescribed. Beclometasone dipropionate MDIs have now been formulated with the ozone friendly HFA-propellants. This has resulted in two BDP MDIs with different dose recommendations, such that the MHRA has recommended that they should be prescribed by brand name and not generic. A urinary pharmacokinetic method has been applied to inhaled drugs to identify the relative lung and systemic bioavailability after an inhalation.

The feasibility of extending this urinary pharmacokinetic method for inhaled BDP needs to be investigated.

Similarly, the *in-vitro* aerodynamic particle size distribution of the dose emitted from the inhalation methods need to be determined to identify if these measurements can be related to *in-vivo* measurements of lung and systemic delivery. The application of this method could be demonstrated by comparing these two MDI formulations and investigate the effect of spacers.

Chapter 3: HPLC Materials and Methods

3.1 Introduction

Inhaled corticosteroids provide a favourable benefit/risk ratio for many therapeutic applications due to the low inhaled doses together with the large volume of distribution of these drugs. The resultant low plasma drug concentrations achieved, necessitate the use of highly sensitive analytical methods. This renders the evaluation of corticosteroid pharmacokinetics (PK) following inhaled administration a significant challenge. The concentrations of these drugs in urine are much higher and offer a much better assessment for lung and systemic bioavailability after an inhalation (Hindle and Chrystyn, 1992; Derendorf et al., 2001; Qu et al., 2007). Methods using liquid chromatography coupled to mass spectrometry (LC/MS) have been found to be useful techniques for solving the problem of corticosteroids analysis. This technique has been applied for the quantification of corticosteroids in biological fluids with high selectivity and sensitivity (Pujos et al., 2005). Previous attempts to measure the plasma systemic concentrations of BDP post administration relied on the conversion of BDP and 17-BMP to BOH, prior to measurement of total BOH (Harrison et al., 1997; Soria et al., 1998; Harrison et al., 1999b). However, this approach appeared to be not reliable and underestimated BDP oral and lung bioavailability. The use of total BOH data for pharmacokinetics data analysis is not accurate as some BDP would be counted more than once (as BDP and again after conversion to 17-BMP and BOH metabolites) and it is also unlikely that BDP, 17-BMP and BOH have the same clearance values (Daley-Yates et al., 2001). Several liquid chromatographic mass spectrometric methods (LC-MS) have been developed and validated for simultaneously quantifying BDP and its metabolites in rat and human plasma (Daley-Yates et al., 2001; Harrison et al., 2002a; Wang and Hochhaus, 2004) and in equine plasma and urine (Guan et al., 2003) for studying its detailed pharmacokinetics.

The aim of the work in this section was to develop a sensitive, robust, and reliable LC-MS assay for the determination of beclometasone dipropionate and its metabolites in methanolic samples for *in-vitro* testing of inhaled products and in human urine samples following oral and inhaled administrations to subjects. This method could, therefore be used to study the relative deposition of the drug in the lung. A solid phase extraction method was developed to separate and isolate BDP, 17-BMP and BOH from urine matrix interferences.

3.2 Analysis of beclometasone dipropionate in methanolic samples

3.2.1 Experimental

3.2.1.1 Chemicals

Beclometasone dipropionate (BDP): supplied by GlaxoSmithKline (GSK), UK.

Fluticasone propionate (FP): supplied by GlaxoSmithKline, (GSK), UK.

Methanol: HPLC grade; supplied by Fisher Scientific (UK).

3.2.1.2 Mobile phase

Acetonitrile: HPLC grade; supplied by Fisher Scientific (UK).

Water: HPLC grade; supplied by Fisher Scientific (UK).

3.2.1.3 Chromatographic conditions

Column: Sphereclone ODS (2) 5µm column, 2x250mm, Phenomenex, UK.

Mobile phase: Acetonitrile: water in the ratio of 60:40% v/v. The mobile phase was filtered through a 45mm membrane filter with a pore size of 0.45µm (Vaccubrand, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use.

Flow rate: 0.3ml/minute.

Pump: Merck Hitatchi L-6200 A (intelligent pump).

Injector: Rheodyne 7125 fitted with 150µl loop.

Temperature: Ambient temperature.

Mass spectrometer: Bruker Esquire HCT ion trap mass spectrometer.

Electrospray ionization source: positive ion source.

Desolvation temperature: 280 ºC.

Capillary and skimmer voltages: 4.0 kV and 40V respectively.

3.2.1.4 Preparation of standards

Each stock standard solution (1µg/ml) of beclometasone dipropionate (BDP) and the internal standard fluticasone propionate (FP) was prepared by dissolving the dry chemical powder in HPLC-grade methanol and stored at 4ºC. From the BDP stock solution, working standards were prepared by serial dilution to yield nominal beclometasone dipropionate concentrations of 30, 50, 70, 90, 100, 120, 140, and 160ng/ml (w/v). Working solutions were stored at 4ºC in well closed containers. From the FP stock solution, a working solution of 90ng/ml was prepared by dilution with methanol and stored at 4ºC. Stability studies through three thawing cycles, over 24 hours at room temperature and over 2 months at -20ºC showed no significant change in the analyte concentration.

3.2.2 Results

3.2.2.1 Calibration

An eight-point calibration curve was made using eight beclometasone dipropionate standards between 30ng/ml and 160ng/ml of BDP with 90ng/ml fluticasone propionate as the internal standard. Each standard was injected three times on three different days. The

peak area ratio of the extracted ion chromatogram of pseudo-molecular ions of beclometasone dipropionate (521.2m/z ratio) and the extracted ion chromatogram of pseudo-molecular ions of the internal standard fluticasone propionate (501m/z ratio) was plotted against the nominal concentration (x) of the calibration standards. A straight line was fitted to the data using linear regression. A representative plot, described by the equation y = 0.0184x-0.0689 (r^2=0.9986) was obtained as in figure 3.1. A representative chromatogram is shown in figure 3.2. The detector response was found to be linear over the concentrations range used with correlation coefficient of ≥ 0.9986.

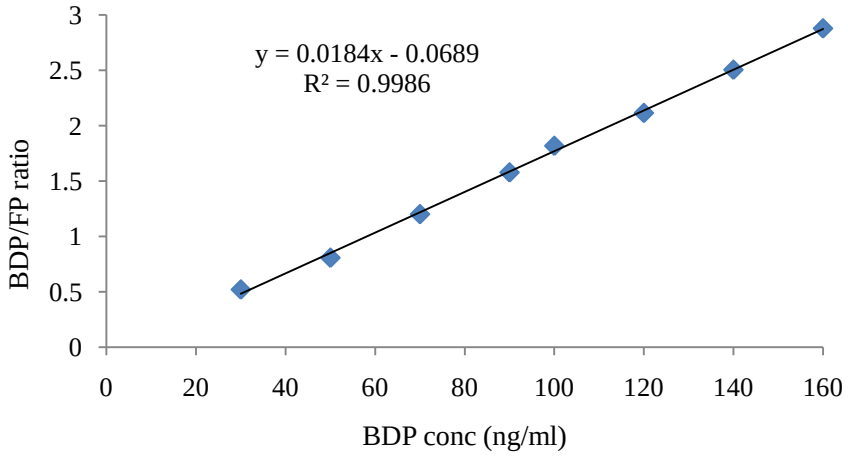


Figure 3.1: A representative calibration curve of the peak area ratio of beclometasone dipropionate and fluticasone propionate against the concentration of beclometasone dipropionate.

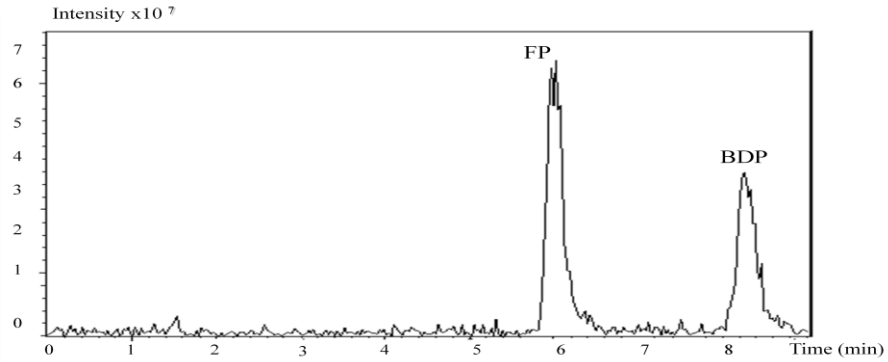


Figure 3.2: Extracted ion chromatogram obtained from the analysis of standard samples containing 120ng/ml beclometasone dipropionate (BDP), and 90ng/ml fluticasone propionate (FP).

3.2.2.2 Validation

The analytical method validation was carried out according to ICH method validation guidelines (ICH, 1994).

3.2.2.2.1 Precision

According to ICH and FDA guidelines, precision is the closeness of agreement (degree of scatter) between a series of individual measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (ICH, 1994; Food and Drug Administration (FDA), 2001). While the term precision relates to the concept of variation around a central value, imprecision is actually, what is measured. For a normal distribution, the measure of imprecision is the standard deviation (SD). The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV), except for the LOQ, where it should not exceed 20% (Chesher, 2008). The precision of the assay was determined by injecting three concentrations of BDP (low 35, medium 80 and high 150ng/ml) five times on the same day to determine the intra-day variation. The same experiment was repeated on five different days to determine the inter-day variation. The intra-day and inter-day variation, expressed as the coefficient of variation in peak area ratio, were calculated by dividing the standard deviation of the calculated concentrations by the mean concentration and multiplying by hundred. The intra-day assay variability, determined for the three standard concentrations of BDP on five occasions and the inter-day assay variability, determined at the same three concentrations and repeated for five different days are illustrated in table 3.1.

Table 3.1: Precision of the assay, (n=5).

Nominal BDP Concentration (ng/ml)	Intra-day %CV	Inter-day %CV
35	11.1	12.2
80	5.5	7.2
150	7.0	10.6

3.2.2.2.2 Accuracy

The accuracy of an analytical method describes the closeness of the test results obtained by the method to the true value (concentration of the analyte). Accuracy is determined by replicate analysis of a sample containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended (Food and Drug Administration (FDA), 2001).The accuracy of the assay was calculated as the percentage ratio of the measured concentration (obtained from the linear regression line over the concentration range investigated) to the nominal concentration. The results are shown in table 3.2.

Table 3.2: Accuracy of the assay, (n=5).

Nominal Concentration (ng/ml)	Mean ±SD ng/ml of measured Concentration	Mean ±SD % of measured Concentration (Accuracy)
Intra-assay variation		
35	33.4 ± 2.6	95.5 ± 7.3
80	73.2 ± 2.4	91.5 ± 3
150	143.1 ± 4.7	95.4 ± 3.1
Inter-assay variation		
35	35.7 ± 3.2	102.1 ± 9.2
80	78.2 ± 4.5	97.8 ± 5.6
150	144.4 ± 14.7	96.2 ± 9.8

3.2.2.2.3 Detection and quantification limits

According to ICH guidelines, the limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy under the standard operational conditions of the method. The ICH has listed two options available to determine both the LOD and the LOQ of an assay. One of these options are expressed as a concentration at a specified signal to noise ratio, usually 3:1 and 10:1 for the signal to noise ratio for LOD and LOQ respectively. The LOD and LOQ may also be calculated based on the standard deviation of the response (SD) and the slope (S) of five calibration curves using the linear regression method. The LOD and LOQ are calculated according to the following equations: The LOD = 3.3 (SD/S) and the LOQ = 10 (SD/S). The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines (ICH, 1994). The linear regression line method was used here to determine LOD and LOQ. The LOD and LOQ of beclometasone dipropionate with 10µl injection volumes were 6.3ng/ml and 19.0ng/ml, respectively.

3.2.3 Conclusion

According to ICH guidelines, the LC-MS assay developed in this section for the determination of beclometasone dipropionate in methanolic samples was found to be simple, sensitive and have acceptable limits for both accuracy and precision and has been successfully used to analyze samples from this study, and other subsequent studies.

3.3 Analysis of beclometasone dipropionate and its metabolites in human urine

3.3.1 Experimental

3.3.1.1 Chemicals

Beclometasone dipropionate (BDP): supplied by GlaxoSmithKline (GSK), UK.

Fluticasone propionate (FP): supplied by GlaxoSmithKline, (GSK), UK.

17-Beclometasone monopropionate (17-BMP): supplied by GlaxoSmithKline, (GSK), UK.

Beclometasone (BOH): supplied by GlaxoSmithKline, (GSK), UK.

Methanol: HPLC grade; supplied by Fisher Scientific (UK).

3.3.1.2 Mobile phase

Acetonitrile: HPLC grade; supplied by Fisher Scientific (UK).

Water: HPLC grade; supplied by Fisher Scientific (UK).

3.3.1.3 Solid phase extraction

Solid phase extraction cartridge: DSC-CN (cyanopropyl), 3ml/500mg, (Supelco, UK).

Extraction station: VAC-ELUT 10 manifold (Varian limited, UK).

Sample concentrator: Savant DNA 120, Speed Vac concentrator (Thermo Electron Corporation).

Methanol: HPLC grade; supplied by Fisher Scientific (UK).

Water: HPLC grade; supplied by Fisher Scientific (UK).

3.3.1.4 Chromatographic conditions

Column: Sphereclone ODS (2) 5µm column, 2x250mm, Phenomenex, UK.

Mobile phase: acetonitrile: water in the ratio of 60:40% v/v. The mobile phase was filtered through a 45mm membrane filter with a pore size of 0.45µm (Vaccubrand, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use.

Flow rate: 0.3ml/minute.

Pump: Merck Hitatchi L-6200 A (intelligent pump).

Injector: Rheodyne 7125 fitted with 150µl loop.

Temperature: ambient temperature.

Mass spectrometer: Bruker Esquire HCT ion trap mass spectrometer.

Electrospray ionization source: positive ion source.

Desolvation temperature: 280 ºC.

Capillary and skimmer voltages: 4.0 kV and 40V respectively.

3.3.2 Methodology

3.3.2.1 Preparation of standards

Primary stock solutions (1µg/ml) were prepared by dissolving beclometasone dipropionate, 17-beclomethasone monopropionate, beclometasone and fluticasone propionate (the internal standard) in HPLC-grade methanol. These were each stored at 4ºC. From the BDP, 17-BMP and BOH stock solutions, working standards were prepared by serial dilution using pooled 24 hour urine collected from six volunteers (3 females) to yield nominal concentrations of 30, 50, 70, 90, 100, 120, 140 and 160ng/ml. Working solutions were stored at -20ºC prior to analysis. Stability studies through three thawing cycles, over 24 hours at room temperature and over 2 months at -20ºC showed no significant change in the analyte concentrations.

3.3.2.2 Solid phase extraction method (SPE)

Analysis of drugs in biological fluids such as plasma or urine usually requires an initial pre-treatment step, in order to remove endogenous interfering compounds that may otherwise interfere with the assay and block the column. Techniques such as liquid-liquid and solid phase extraction are frequently used. Solid-phase extraction (SPE) is a separation process by which analytes of interest are isolated from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissue according to their physical and chemical properties. In SPE, a liquid sample is passed through a sorbent bed where analytes of interest are adsorbed while; other interfering compounds can be easily removed from the column by washing with suitable solvents. The desired analytes are then eluted from the column by using an appropriate elution solvent giving a highly pure sample.

The HPLC applications group of Supelco (2003) have developed a systematic extraction method for the recovery of steroidal compounds from urine. They compared the recovery of corticosteroids in urine from both conventional C18 and DSC-CN SPE cartridges. They reported that the subsequent eluate analysis of the C18 SPE urine extracts carried a yellow tint signifying insufficient removal of endogenous urine interferences and led to HPLC system failure due to high backpressure early in the run sequence. In contrast, the DSC-CN SPE provided cleaner chromatograms with good recoveries.

The solid phase extraction method using Discovery DSC-CN (monomerically bonded cyanopropyl chain) solid phase extraction cartridges developed by Supelco (2003) was applied in this study to extract BDP, 17-BMP and BOH from urine samples. The cartridges were prepared on a VAC Elut workstation (Varian limited, UK), allowing up to ten samples to be processed at the same time. The urine sample was first pre-treated by adding a 1ml urine sample to 1ml of the working concentration of the internal standard

and then diluted 1ml of HPLC grade water. Each DSC-CN cartridge was first conditioned with 3 ml methanol followed by equilibration with 3 ml HPLC grade water. This initial conditioning step is essential to wet the sorbent bed and ensure its interaction with the compounds of interest in the sample. Then 3ml of the pre-treated urine sample was then loaded to the cartridge and drawn through over 2-3 minutes. Interfering compounds were then removed by washing with 3ml 20% methanol. The column was then dried under a full vacuum for 5 minutes prior to elution with 1ml 100% methanol. After evaporating to dryness using a sample concentrator with a stream of nitrogen, the residue was reconstituted with 100µl of the mobile phase prior to injection and 10µl was injected into the LC-MS system.

3.3.3 Results

3.3.3.1 Calibration

An eight-point calibration curve was performed using eight urine samples containing standards between 30ng/ml and 160ng/ml of BDP, 17-BMP and BOH with 90ng/ml fluticasone propionate as the internal standard. Each standard was injected three times on three different days and averages were used to construct the calibration curve.

The peak area ratio of the extracted ion chromatograms of the pseudo-molecular ions of BDP (521.2m/z ratio), 17-BMP (465m/z ratio) and BOH (409m/z ratio) and the extracted ion chromatogram of the pseudo-molecular ion of the internal standard FP (501m/z ratio) (y) were plotted against the nominal concentration of the calibration standards (x). A straight line was fitted to the data using linear regression. The calibration curves obtained for BDP, 17-BMP and BOH when using fluticasone propionate (FP) as the internal standard are presented in figure 3.3. Figure 3.4 and figure 3.5 show the typical chromatograms for human blank urine and a human urine standard containing 100ng/ml BDP, 17-BMP and BOH and 90ng/ml I.S. The analysis time was 10 minutes and the

retention time for BDP, 17-BMP, BOH, and FP were 8.22, 3.98, 2.45, and 6.14 minutes respectively. Representative mass spectra are shown in figure 3.6. The detector response was found to be linear over the concentrations range used.

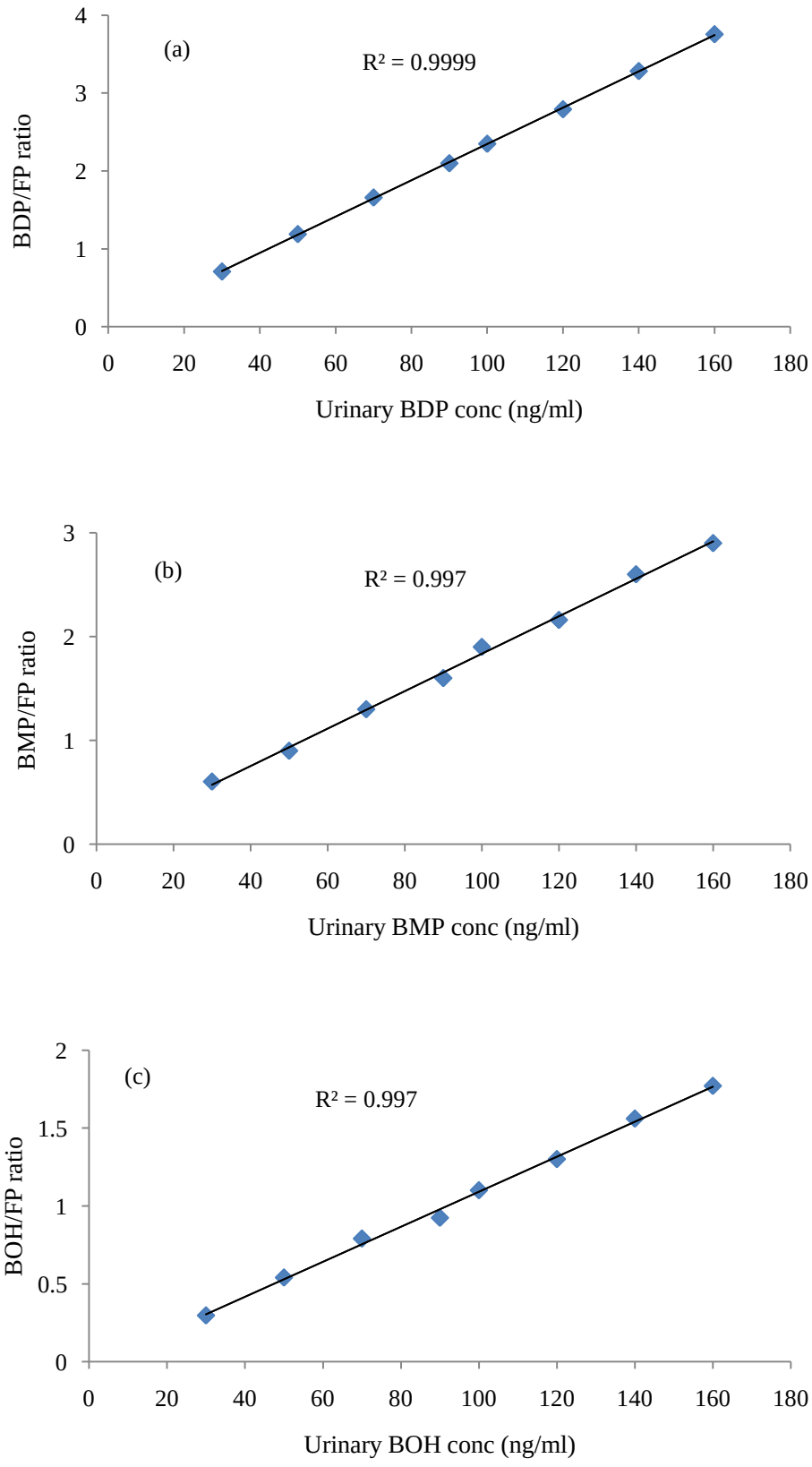


Figure 3.3: A representative calibration curve of the peak area ratio of the extracted ion chromatogram of (a) BDP/FP against the concentration of BDP (b) 17-BMP/FP against the concentration of 17-BMP (c) BOH/FP against the concentration of BOH.

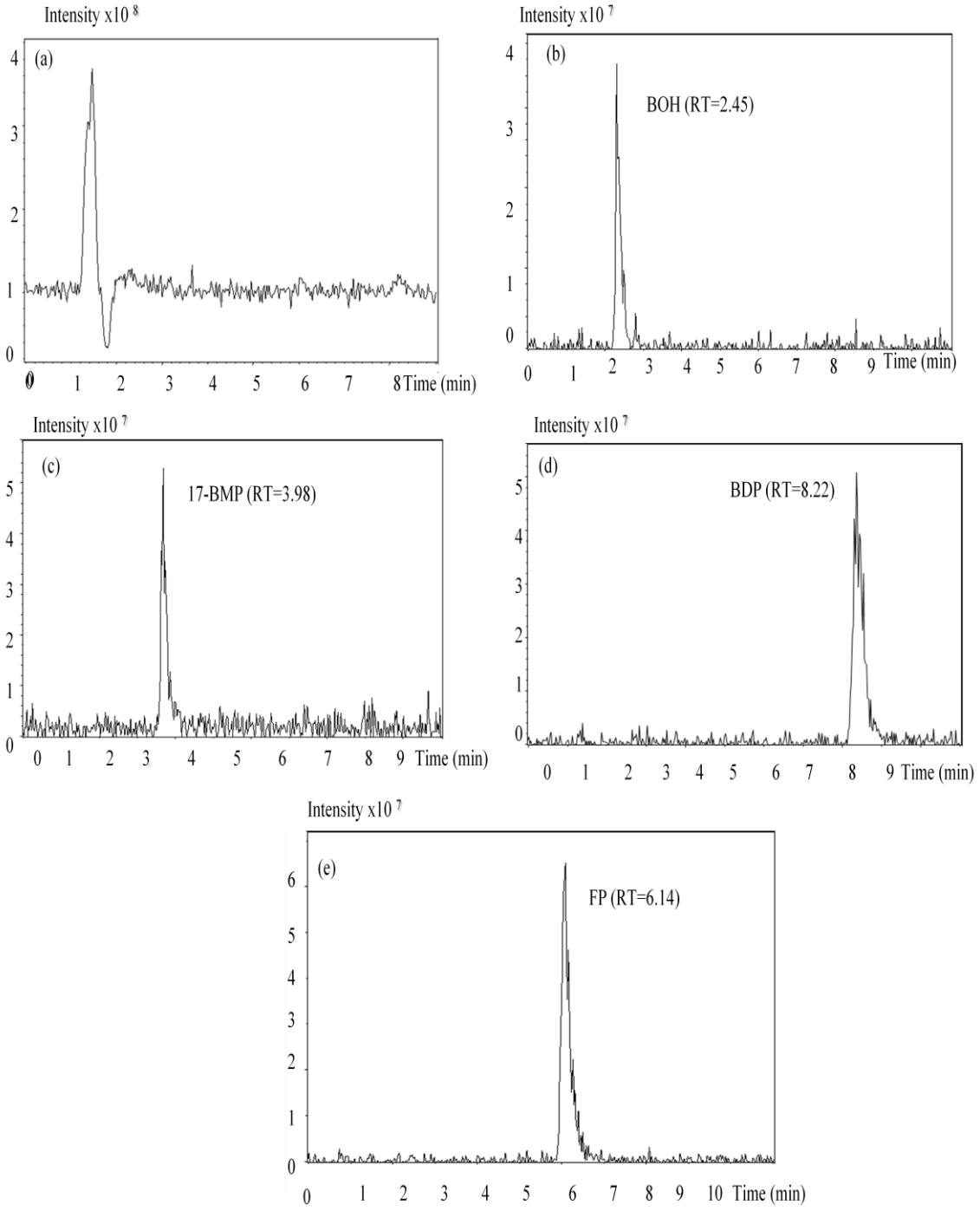


Figure 3.4: (a) Total ion chromatogram obtained from the analysis of an extracted blank urine sample, extracted ion chromatogram obtained from the analysis of (b) a standard urine sample containing 100ng/ml BOH (c) a standard urine sample containing 100ng/ml 17-BMP (d) a standard urine sample containing 100ng/ml BDP (e) a standard urine sample containing 90ng/ml FP.

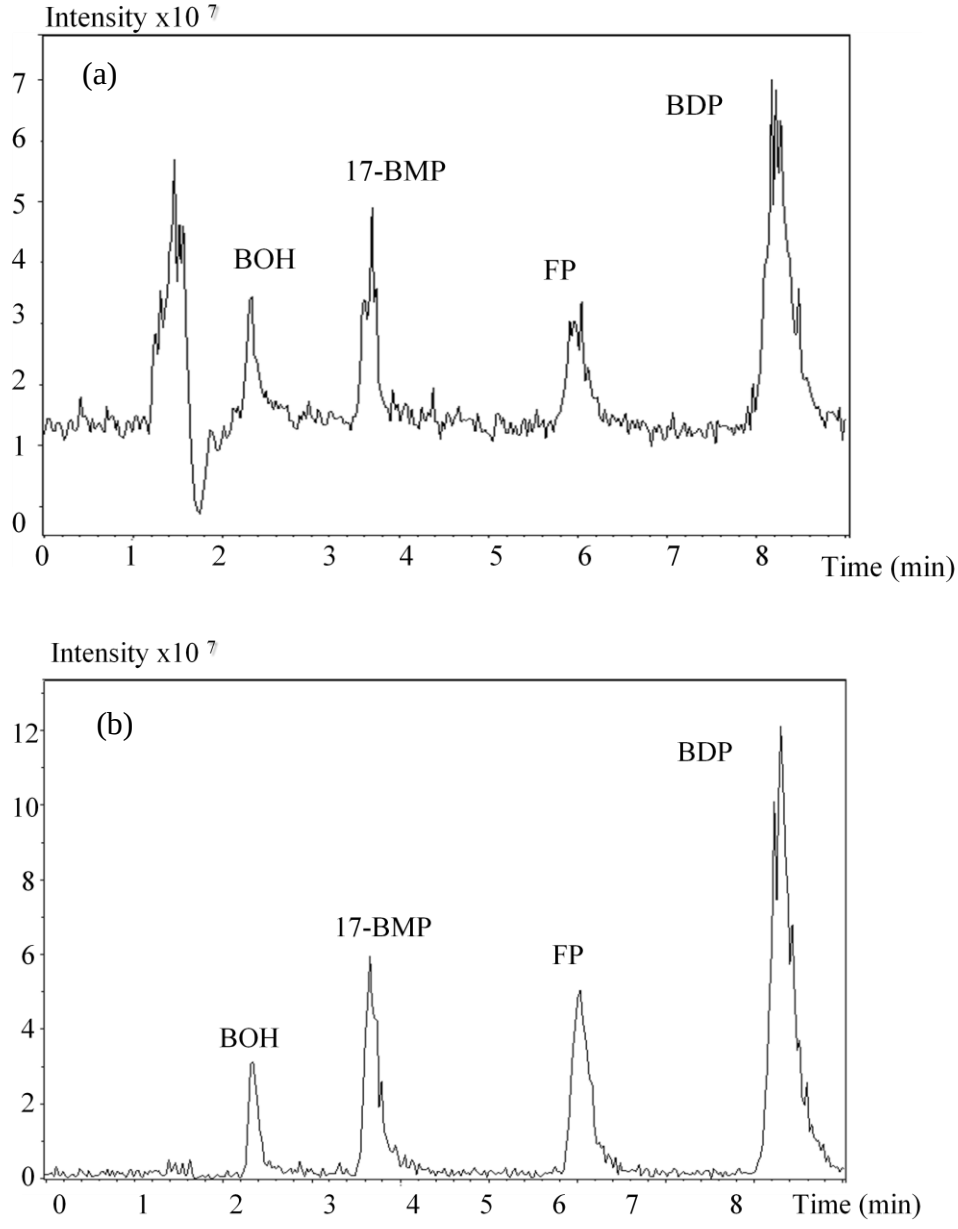


Figure 3.5: (a) Total ion chromatogram (b) extracted ion chromatogram of a volunteer urine sample 0-0.5hr post-inhalation of eight doses of beclometasone dipropionate from Clenil® Modulite MDI (250µg).

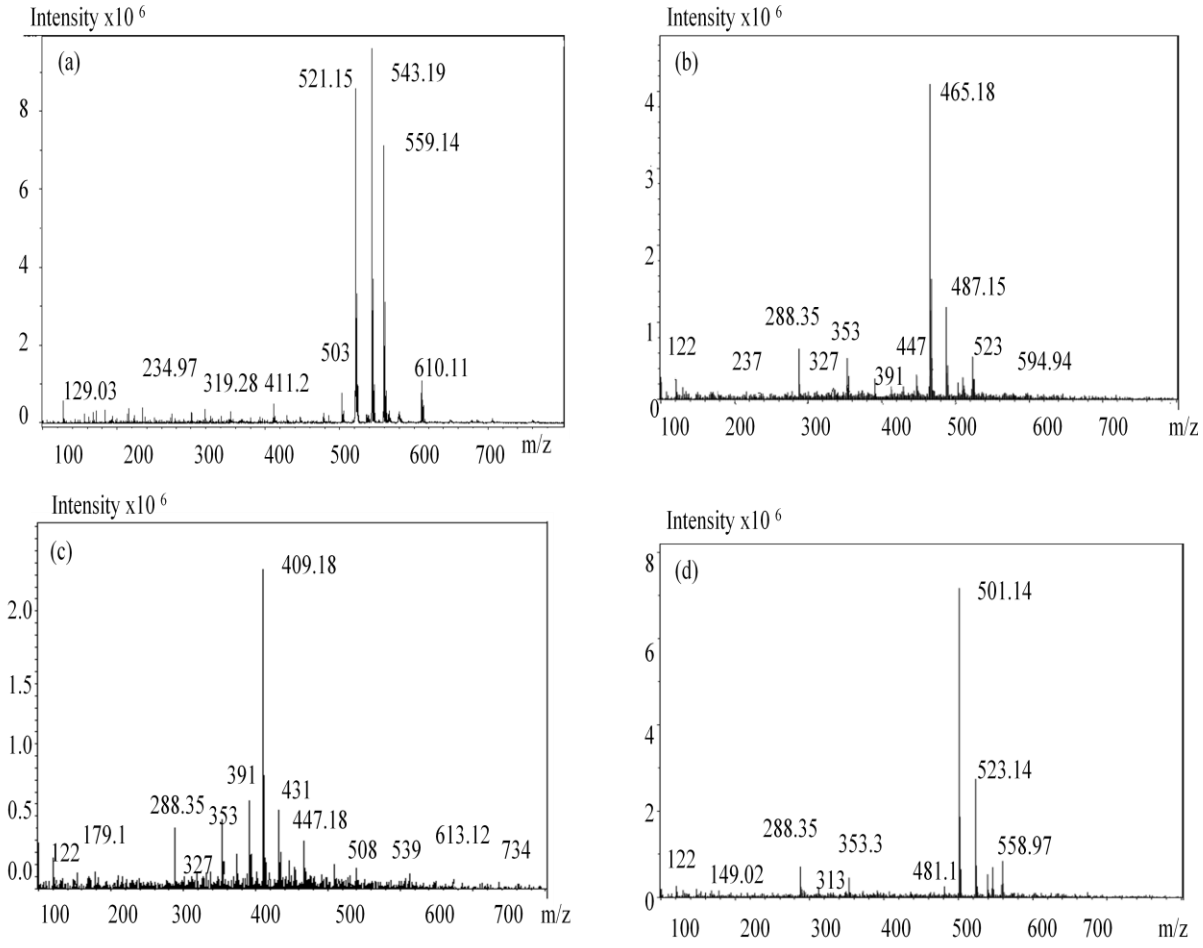


Figure 3.6: Positive ion mass spectrum of (a) beclometasone dipropionate (b) 17-beclometasone monopropionate (c) beclometasone, and (d) fluticasone propionate.

3.3.3.2 Validation

3.3.3.2.1 Recovery

Corticosteroids are characterised by a planar and relatively rigid configuration that contains a steroid nucleus with four fused rings, thus the aqueous nature of the sample matrix and the hydrophobic character of the analytes offers an excellent opportunity for reversed-phase retention. Most solid phase extraction methods use popular reversed phase chemistry such as DSC-18 cartridges due to their broad affinity for a wide range of compounds in aqueous solutions. However when dealing with contaminant rich samples

such as urine, their broad selectivity can lead to co-retention and elution of endogenous matrix interferences. However, the use of the less hydrophobic and more selective phase chemistry such as a cyanopropyl (CN) bonded silica bed as in this study can be more beneficial in discriminating between the analytes of interest and endogenous sample interferences (Supelco, 2003). The recovery of BDP, 17-BMP and BOH was determined by repeated solid phase extraction (n=3) of three quality control standards selected at high, mid and low points of the calibration range (35, 80 and 150ng/ml). The recovery was calculated by comparing the peak area of BDP, 17-BMP and BOH urine extracts to the peak area of BDP, 17-BMP and BOH obtained with the direct injection of methanolic standards assuming 100% recovery in order to provide an estimate of the extraction recovery. The results are illustrated in table 3.3. The same method was used to assess the recovery of the internal standard fluticasone propionate at the working concentration 90ng/ml, the mean (SD) % relative recovery for FP was found to be 94.3 (1.6) %.

Table 3.3: Recovery data of BDP, 17-BMP and BOH, (n=3).

Nominal Concentration (ng/mL)	% Relative Recovery (%RR)					
	BDP		17-BMP		BOH	
	% RR	%CV	% RR	%CV	% RR	%CV
35	95.3	2.6	89.6	4.5	94.1	2.0
80	92.2	1.6	92.3	3.2	90.5	1.9
150	94.4	1.1	90.8	4.3	93.6	2.9

3.3.3.2.2 Precision

The precision of the assay was determined by injecting three concentrations of BDP, 17-BMP and BOH (low 35, medium 80 and high 150ng/ml) five times on the same day to determine the intra-day variation. The same experiment was repeated on five different days to determine the inter-day variation. The intra-day and inter-day variation were assessed as the coefficient of variation in peak area ratio. The results are illustrated in table 3.4.

Table 3.4: Precision of the assay, (n=5).

Nominal concentration (ng/ml)	BDP		17-BMP		BOH	
	Intra-day %CV	Inter-day %CV	Intra-day %CV	Inter-day %CV	Intra-day %CV	Inter-day %CV
35	9.6	11.3	12.6	13.3	9.4	13.2
80	4.4	5.9	7.2	9.1	7.1	10.4
150	4.6	7.3	6.5	8.0	11.7	13.1

3.3.3.2.3 Accuracy

The accuracy of the assay was calculated as the percentage ratio of the measured concentration (obtained from the linear regression line over the concentration range investigated) to the nominal concentration. The Accuracy of the assay using FP as an internal standard is shown in tables 3.5.

Table 3.5: Accuracy of the assay using FP as an internal standard, (n=5).

Nominal Conc (ng/ml)	BDP		17-BMP		BOH	
	Mean ± SD measured Conc (ng/ml)	Mean ± SD % measured Conc	Mean ± SD measured Conc (ng/ml)	Mean ± SD % of measured Conc	Mean ± SD measured Conc (ng/ml)	Mean ± SD % of measured Conc
Intra-assay variation						
35	34.6 ± 3.5	98.7 ± 10.0	35.7 ± 2.6	102.1 ± 7.5	34.0 ± 1.6	97.3 ± 4.7
80	78.3 ± 2.4	97.8 ± 3.0	82.1 ± 6.6	102.6 ± 8.3	78.7 ± 2.0	98.3 ± 2.5
150	141.2 ± 8.7	94.1 ± 5.8	148 ± 5.7	98.8 ± 3.8	150.7 ± 15.0	100.5 ± 10.0
Inter-assay variation						
35	36.6 ± 3.0	104.6 ± 8.5	32.7 ± 4.4	93.4 ± 12.5	36.3 ± 3.2	103.6 ± 9.1
80	83.5 ± 4.5	104.4 ± 5.6	77.2 ± 3.6	96.4 ± 4.5	76.8 ± 4.5	96.0 ± 5.6
150	152.2 ± 13.3	101.5 ± 8.9	155.5 ± 9.4	103.7 ± 6.3	160 ± 8.0	106.7 ± 5.3

3.3.3.2.4 Detection and Quantification limits

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from the mean of the slope and SD of the intercept of five calibration curves when using fluticasone propionate as the internal standard. The LOD of BDP, 17-BMP, and BOH urine samples were 4.4, 3.6, and 6.6ng/ml, respectively. The LOQ of BDP, 17-BMP, and BOH urine samples were 13.3, 11.1, and 19.7ng/ml, respectively.

3.3.3.2.5 Stability

Freeze and thaw stability for BDP, 17-BMP and BOH in urine matrix, were determined after three freeze and thaw cycles by analyzing triplicate quality control samples at the concentrations of 35, 80, and 150ng/ml. The samples were frozen for 24 hours at -20ºC, and then left to thaw unassisted at room temperature; when completely thawed, the samples should be refrozen again for 24 hours under the same conditions, the process is again repeated, and the sample is analyzed on the third cycle. The short-term stability of the analytes was evaluated at the same concentrations after the samples were thawed and kept at room temperature for 24 hours. The long-term stability was evaluated after storing the above-mentioned concentrations at -20ºC for 2 months. Stability was expressed as the percentage ratio of measured concentration to the nominal concentration; the results are shown in table 3.6.

Table 3.6: Stability of BDP, 17-BMP and BOH under various conditions, (n=3).

	Nominal concentration (ng/ml)								
	BDP			**17-BMP**			**BOH**		
	35	**80**	**150**	**35**	**80**	**150**	**35**	**80**	**150**
Freeze-thaw (Three cycles)	95.6 (5.1)	94.6 (3.8)	93.6 (2.5)	90.6 (4.2)	89.6 (3.6)	91.3 (4.8)	93.6 (7.1)	90.7 (1.8)	94.3 (5.9)
Short term stability (24 hours)	91.8 (3.84)	89.6 (5.8)	92.6 (5.8)	93.3 (7.0)	90.6 (3.9)	88.9 (3.6)	90.9 (4.6)	92.9 (3.6)	94.6 (6.0)
Long term stability (2 months)	89.6 (1.9)	85.9 (7.6)	87.9 (5.2)	85.6 (3.9)	90.9 97.0)	88.9% (1.1)	90.3 (2.6)	91.6 (7.1)	86.9 (2.5)

3.3.4 Conclusion

A simple, sensitive and selective LC-(ESI+)-MS method was developed using a solid phase extraction procedure for simultaneously quantifying BDP and its two metabolites 17-BMP and BOH in human urine samples and suitable for routine clinical studies. Positive ESI (ESI+) was chosen for the better sensitivity. The solid phase extraction method using Discovery DSC-CN cartridges was successfully applied in this study to extract BDP, 17-BMP, and BOH from urine samples. No significant interferences were

observed at the retention times of BDP, 17-BMP and BOH and the internal standard FP in urine samples. Validation results have shown that the method has acceptable limits for both accuracy and precision (±15%) and has been successfully used to analyze samples from this study and subsequent studies. Fluticasone propionate can be used as the internal standard for all volunteers' urine samples. The calibration curves obtained with this LC-MS method were linear over the concentration range used. The SPE method was found to be reproducible and efficient as the recoveries were within the acceptable limits (± 15%). The stability results showed that the three analytes were stable under the conditions investigated in this study since the measured concentrations were all within 85-115% of nominal concentrations.

3.4 Preparative chromatography to produce the metabolites of beclometasone

Beclometasone dipropionate is a widely used inhaled corticosteroid for the inhalation therapy of asthma in both adults and children. Owing to the presence of the dipropionate ester functional group in its side chain, it is easily hydrolysed via esterases in the human lung, liver and other parts of the body to the more polar products 17-beclometasone monopropionate (17-BMP), 21-beclometasone monopropionate (21-BMP) and beclometasone (BOH). 17-beclometasone monopropionate is the active metabolite, whereas both 21-BMP and BOH have very low binding affinity to the glucocorticoid receptor. In this experiment, we reported the *in-vitro* hydrolysis of BDP using esterase enzyme as well as the isolation and characterisation of its degradation product.

3.4.1 Chemicals

Beclometasone dipropionate (BDP): supplied by GlaxoSmithKline (GSK), UK.

Esterase enzyme: from porcine liver, supplied by Sigma, UK.

Ethanol: HPLC grade; supplied by Fisher Scientific (UK).

Methanol: HPLC grade; supplied by Fisher Scientific (UK).

Acetonitrile: HPLC grade; supplied by Fisher Scientific (UK).

Water: HPLC grade; supplied by Fisher Scientific (UK).

3.4.2 Methodology

Incubation studies for metabolite preparation, separation and identification was first initiated by adding the solid esterase to a solution of BDP in ethanol / water (1:99 v/v) in order to yield a final concentration of 0.17mg/ml. This solution was incubated in a water bath shielded from light at 37ºC for 20hr. After incubation, the enzyme was removed using a 10.000 MWCO size-exclusion cartridge (Microcon, Millipore) by centrifugation and discarding the upper portion. Then the lower liquid portion was diluted with methanol to the desired concentration and the final product was purified by preparative HPLC. The gradient elution of metabolites were performed on a Dynamax C18 (21.4 mm X 250mm) column, the mobile phase used was acetonitrile: water (75: 25%) and the flow rate 10ml/min with UV detection at 240nm. The major peak was collected, freeze dried, and then reconstituted in 650µl deuterated solvent (d_4-methanol) for ^{1}H-NMR analysis. The NMR spectrum was recorded on a Bruker Avance NMR spectrometer operated at 500MHz.

3.4.3 Results

The ^{1}H-NMR spectrum of BDP and that of the hydrolysis reaction product is shown in figure 3.7 (a) and (b), respectively. As shown in Figure 3.7 (a) the signals corresponding to the protons in the 17- and 21-propionate groups of BDP appear at 2.43 ppm (CH_2, 5H) and 1.12 ppm (CH_3, 6H). The chemical shifts of these moieties are close together since their chemical environments are similar. On inspection of the signals from the enzyme hydrolysis product shown in figure 3.7 (b), it can be seen that one signal in each

environment has disappeared. Inspection of the integrals shows that two protons have been lost from the signal at 2.43 ppm and three from the signal at 1.12 ppm; this is consistent with cleavage of a propionate group. Evidence for the formation of 17-BMP (as opposed to the 21-BMP isomer) is provided by inspection of the signals for the protons at position 21 in the molecule. In the spectrum of BDP (top spectrum), these signals appear as doublets at 4.81 and 4.42 ppm (both 1H) indicating that the two protons of the CH_2 group are diastereotopic. This is likely due to hindered rotation of the propionate group in solution. Following the hydrolysis reaction (bottom spectrum), these signals collapse into a singlet at 4.04 ppm (2H) which indicates that the propionate group has been hydrolysed at the ester group forming the hydroxyl derivative; the CH_2 protons are now free to rotate and therefore become magnetically equivalent. The major shift in H_{21} and H_{21}´ confirmed that it is the 21-dipropionate group which has been cleaved.

The above results confirms previous findings that showed the rapid hydrolysis of BDP via the esterase enzyme to 17-BMP which was the major metabolite detected (Foe et al., 1998; Nave et al., 2007). The rapid hydrolysis of BDP to its active metabolite 17-BMP will favour a potent local anti-inflammatory action.

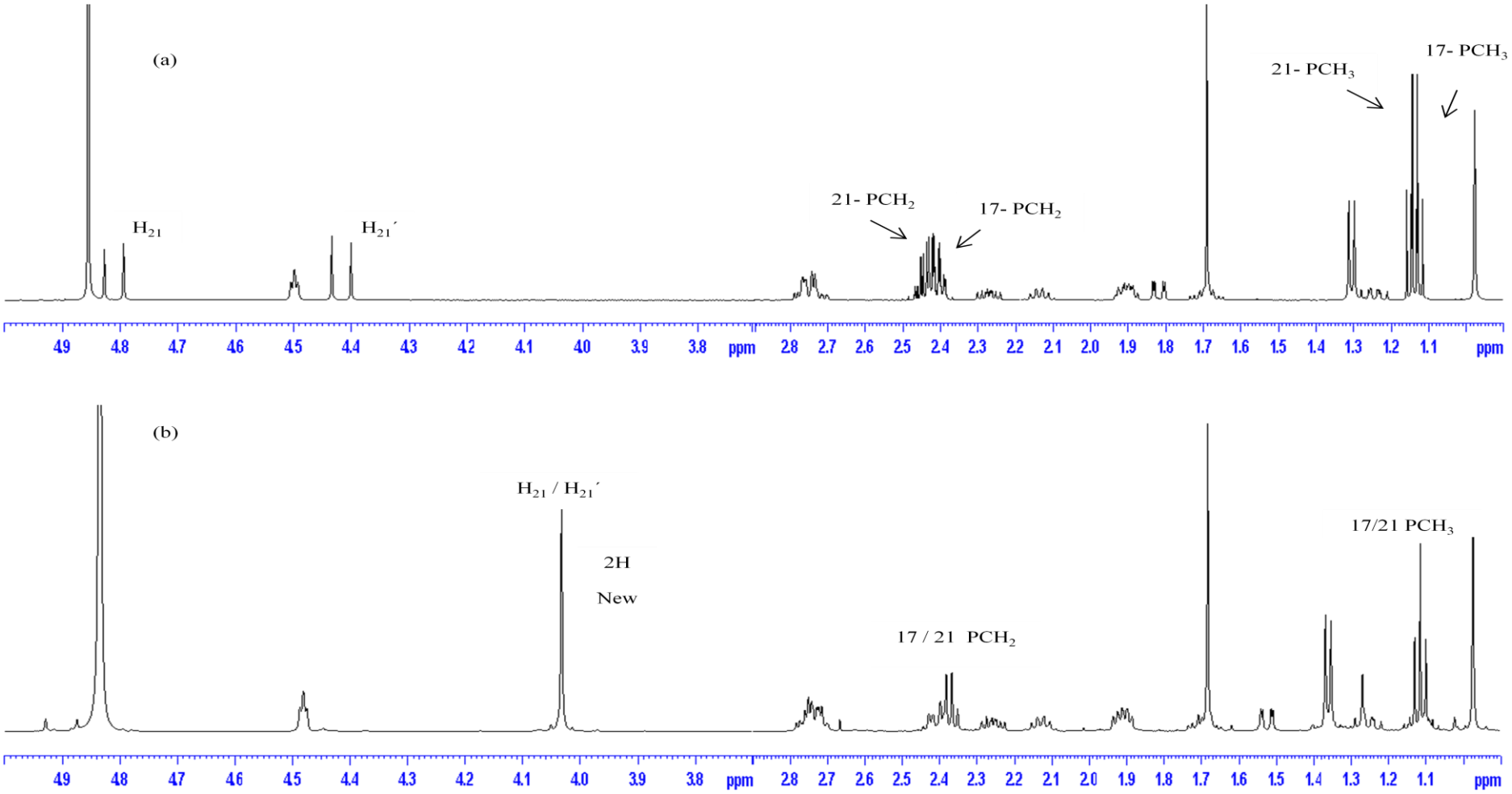


Figure 3.7: (a) ^{1}H-NMR spectrum of BDP (b) ^{1}H-NMR spectrum of the hydrolysis reaction product.

Chapter 4: Relative Bioavailability of Beclometasone to the Lung Following Inhalation using Urinary Excretion

4.1 Introduction

Aerosol inhalation is considered the optimal route of administering drugs for the treatment of obstructive airway diseases such as asthma and COPD (Everard, 2001). Reasons for this include both efficacy and safety. Inhaled drugs are delivered directly into the airways, producing higher local concentrations for better efficacy with significantly less systemic exposure and less risk of systemic side effects (Virchow et al., 2008; Broeders et al., 2009; Vincken et al., 2010). Following inhalation, a small portion of the inhaled dose is deposited in the airways while the majority is deposited in the mouth and subsequently swallowed (Newman et al., 1981c; Chrystyn, 2001; Barnes, 2007). The lung dose will be cleared either by mucociliary clearance or by absorption through the airway wall into the systemic circulation (Borgstrom et al., 1992). The latter is the fraction of the dose that will exert the clinical effect within the airway wall and it is termed the effective lung dose. The total amount of drug which enters the systemic circulation will be the sum of the amounts that entered via the pulmonary and gastrointestinal routes (Chrystyn, 2001).

The application of the traditional pharmacokinetic studies to determine lung deposition is difficult due to the low inhaled doses resulting in very low systemic concentration that is difficult to assay accurately (Rogers and Ganderton, 1995). In addition, these methods are unable to discriminate between the pulmonary and the orally absorbed fractions in the systemic concentrations (Newman et al., 1981c; Aswania et al., 1999). Pharmacokinetic methods using plasma or urine samples have been used to identify the relative lung deposition of the drug and total systemic delivery. Borgstrom and Nilsson, (1990) developed a charcoal block method to identify the relative lung deposition following an inhalation. They reported that the concurrent oral administration of activated charcoal blocked all absorption of the drug from the gastrointestinal tract. In this case the amount

of drug eliminated in the urine gives an absolute value of the total lung dose (Borgstrom and Nilsson, 1990). However, because this method uses oral charcoal it would be unethical to extend it to patient studies due to their concomitant oral therapy (Chrystyn, 2001).

Other pharmacokinetic methods have exploited the principle that drugs delivered to the lungs are very rapidly absorbed into the body whereas there is a lag time after oral administration before it is delivered to the systemic circulation. The body starts eliminating drugs as soon as they are delivered to the body. Using this principle, plasma concentrations (Lipworth, 1996; Lipworth and Clark, 1997; Lipworth and Clark, 1998b) and urinary excretion (Hindle and Chrystyn, 1992; Hindle et al., 1993; Hindle and Chrystyn, 1994; Hindle et al., 1995) of drugs over the first 20 and 30 minutes, respectively, post inhalation have been shown to be useful indices of lung deposition.

The urinary salbutamol pharmacokinetic method reported by Hindle and Chrystyn, (1992) demonstrated that the amount of salbutamol excreted in the urine over the first 30 minutes post oral administration was negligible and that significantly greater amounts ($p<0.001$) were excreted 30 minutes post inhalation. Thus, they reported that the 30 minutes urinary excretion post inhalation is representative of the amount of drug delivered to the lung because it measures the drug that is delivered to the body following passage through the airway wall. They called this index the relative bioavailability to the lungs following an inhalation. Hindle and Chrystyn, (1992) also reported that the amount of salbutamol and its metabolite excreted in urine over the 24 hour period post inhalation is an index of systemic delivery. They called this index the relative bioavailability to the body following inhalation. These two indices of salbutamol to the lung and to the body following inhalation have been shown to be useful to compare different inhalation devices, e.g. spacers (Chege and Chrystyn, 1994; Hindle and

Chrystyn, 1994; Mazhar and Chrystyn, 2008), dry powder inhalers (Hindle et al., 1995; Hindle et al., 1997; Chege and Chrystyn, 2000) nebulisers (Silkstone et al., 2000; Silkstone et al., 2002; Mazhar et al., 2008), different inhalation techniques (Hindle et al., 1993), and different formulations (Chege and Chrystyn, 1995). This simple and non-invasive method has also been extended to other drugs e.g, inhaled sodium cromoglycate (Aswania et al., 1997; Aswania et al., 1999; Aswania and Chrystyn, 2001; Aswania and Chrystyn, 2002), nedocromil (Aswania et al., 1998), gentamicin (Nasr and Chrystsyn, 1997; Al-Amoud et al., 2002; Al-Amoud et al., 2005), and formeterol (Nadarassan et al., 2007). However, the methodology has not been extended to inhaled corticosteroids.

Inhaled corticosteroids are the most effective drugs available to clinicians for the control of inflammation in asthma. Clinical studies have demonstrated their efficacy in reducing airway inflammation and hyper-responsiveness, as well as in preventing acute exacerbations, and improving lung functions in asthma. Beclometasone dipropionate (BDP) is a powerful topically active inhaled corticosteroid that is used in the treatment of asthma. It is actually a prodrug that is metabolised by esterases (in the human lung and elsewhere) to three different metabolites, 17-beclometasone monopropionate (17-BMP), 21-beclometasone monopropionate (21-BMP), and beclometasone (BOH). 17-BMP is the active metabolite whereas BOH and 21-BMP have a very low binding affinity to the glucocorticoid receptor (Wurthwein and Rohdewald, 1990; Derendorf et al., 2006).

A pressurized metered-dose inhaler (pMDI) is the most popular method for asthma inhalation therapy and it is well established as a safe and reliable delivery system. The phase out of chlorofluorocarbon (CFCs) propellants, stipulated in the Montreal protocol, due to its detrimental effect on the ozone layer (Montreal, 2000), led to the reformulation and design of pressurized metered dose inhalers with more environmentally safer propellants such as hydrofluoroalkane-134a (HFA). Clenil Modulite® and Qvar® are

newly developed CFC-free beclometasone inhalers. Clenil Modulite® (Chiesi, Italy) is the first CFC-free metered-dose inhaler directly interchangeable with CFC-containing inhalers and its particles distribution (MMAD 2.9μm) more closely matches that of CFC containing MDIs than Qvar (MMAD 1.2μm) (Ganderton et al., 2002). The smaller median particle size of the new Qvar® (HFA-BDP) has been shown to improve drug delivery compared with CFC-BDP in both adults and children, with a greater proportion of the drug deposited in the small airways and less deposited in the throat (Leach et al., 1998a; Leach et al., 2002; Janssens et al., 2003). Despite the improved lung deposition of HFA-BDP, it has a favourable safety profile (systemic and overall) compared with other inhaled corticosteroids (Thompson et al., 1998; Ayres et al., 1999). The fact that Qvar® produces equivalent asthma control to chlorofluorocarbon-based BDP inhalers, at approximately half the daily dose is largely attributed to its greater fine particle fraction (approximately 60%) compared to conventional CFC-BDP MDIs (approximately 30%) (Leach et al., 1998a).

The plasma concentrations of drugs such as inhaled corticosteroids are very low, because of the small therapeutic doses used and their very large volume of distribution in the body (Hindle and Chrystyn, 1992). Highly sensitive and reproducible analytical methods for the accurate assay of these low plasma concentrations is difficult (Derendorf et al., 2001). In contrast, the concentrations of drugs in urine are much higher. We have developed a sensitive and robust assay for BDP, 17-BMP, and BOH in urine and so we have extended the urinary salbutamol method of Hindle and Chrystyn (1992) to beclometasone dipropionate. Within the validation study of this pharmacokinetic method for beclometasone dipropionate, we have included a comparison between Clenil Modulite® and Qvar® Easi-Breathe inhalers.

4.2 Validation of relative bioavailability of beclometasone dipropionate to the lung following inhalation using urinary excretion

4.2.1 Method

The aim of this investigation was to identify and validate a urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of beclometasone following inhalation. The study was divided into two parts. In the first part, the dose of activated charcoal to completely block beclometasone dipropionate and its gastrointestinal absorption was determined. In the second part, the lung and systemic bioavailability of BDP given by a MDI was investigated. This was accomplished by identifying the amount of beclometasone dipropionate (BDP), 17-beclometasone monopropionate (17-BMP) and beclometasone (BOH) excreted in the urine after oral and MDI dosing with and without the co-administration of activated charcoal (concurrent oral charcoal will prevent absorption of any drug that is swallowed). Within this validation, this pharmacokinetic method has been applied to compare the urinary excretion of Qvar EB and Clenil MDI following inhalation.

4.2.1.1 Equipment and inhalation devices

- Inhaler devices:
  - Qvar® Easi-Breathe inhaler (EB) labelled as a nominal dose of 100µg beclometasone dipropionate per dose (Teva Pharmaceuticals, UK).
  - Clenil Modulite® metered dose inhaler (MDI) labelled as a nominal dose of 250µg beclometasone dipropionate per dose (Chiesi, UK).
- Activated Charcoal: Carbomix, Meadon, Laboratories Limited, UK.
- LC-(ESI+)-MS method conditions: sample preparation, analysis procedures, and chromatographic conditions were as reported in section 3.3.

4.2.1.2 Subjects and study design

Ethical approval for the study was obtained from the University of Huddersfield and healthy volunteers gave written consent to take part in the study. Subjects were non-smokers and were allowed no medication during the study period. Healthy volunteers were used to limit inter-individual variability of the airways as lung deposition is affected by airway calibre (Lipworth and Clark, 1997). It is necessary to include both males and females in the study in order to obtain meaningful pharmacokinetic data. For the first part of the study, the initial quantities of charcoal taken to completely block beclometasone dipropionate gastrointestinal absorption were determined. This was accomplished by identifying the urinary excretion of four healthy, non-smoking volunteers (two females) following the oral administration of a 20ml solution containing 20% ethanol and 2000μg beclometasone dipropionate with 5g activated charcoal (5g activated charcoal suspended in 50 ml water and given before and after the inhaled dose). The charcoal was given as a slurry in water, swirled around the mouth before swallowing. Urine samples were collected at 0.5, 1, 2, 3, 5, 8, 12, and 24 hours post study dose administration for analysis.

For the second part of the study, twelve healthy, non-smoking volunteers received the following study doses on separate study days, each separated by a minimum of 7 days:

- Oral administration of a 20ml (20%) ethanolic solution containing 2000μg beclometasone dipropionate [O].
- Oral administration of a 20ml (20%) ethanolic solution containing 2000μg beclometasone dipropionate with 5g activated charcoal (a suspension of 5g activated charcoal in 50 ml water given before and after the inhaled dose) [OC].
- Ten 100μg (1mg in total) inhalations of beclometasone dipropionate from a Qvar® Easi-Breathe (Teva Pharmaceuticals, UK) [IQ].

- Ten 100µg (1mg in total) inhalations of beclometasone dipropionate from a Qvar® Easi-Breathe with the concurrent oral administration of activated charcoal (5g in 50 ml water before and after the inhalation dose) [IQC].
- Eight 250µg (2mg in total) inhalations of beclometasone dipropionate from a Clenil® metered dose inhaler (Trinity; Chiesi, UK) [IC].

All subjects were trained on how to effectively use both the metered dose inhaler (MDI) and the Easi-breathe (EB) device according to the patient information leaflet. For the MDI, subjects were trained to remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth, and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and activate the MDI immediately after the start of this slow inhalation. This slow inhalation continued until their lungs were full of air (total lung capacity). After inhalation they held their breath for 10 seconds and the next dose was repeated 30 seconds later (Hindle et al., 1993). The same inhalation process was repeated for the EB device, the only difference was that subjects did not need to actuate the device during their inhalation, as the Easi-Breathe device would automatically deliver the dose upon inhalation. A check was made that the breath actuation process occurred (sound, taste and visual check of an external lever on the device that moves when a dose is released). Subjects voided their urine pre-dosing and then provided urine samples at 0.5, 1, 2, 3, 5, 8, 12, and 24 hours post study dose. The volume of urine excreted was recorded and aliquots of each sample were frozen at minus 20°C prior to analysis. The order of the study doses was randomized with a 7-day washout period between administrations.

4.2.2 Analysis

4.2.2.1 Urine sample analysis

The LC-(ESI+)-MS method with solid phase extraction that has been developed and validated for the assay of beclometasone dipropionate and its metabolites in urine samples (previously described in section 3.3) was used to identify the urinary amounts excreted.

4.2.2.2 Statistical analysis

Statistical comparisons of the urinary excretion data of beclometasone dipropionate and its metabolites following oral, MDI and MDI + C administration in urine samples produced at different time intervals were compared using one way analysis of variance (ANOVA) using SPSS V17.0 (SPSS Inc., Chicago, USA). The mean difference with 95% confidence interval was calculated. In addition, One-way analysis of variance with the application of Bonferroni correction was used to determine any difference between the urinary excretions from the inhalation methods. To identify equivalence of the urinary excretions between the inhalation methods, the 30 minutes and cumulative 24hr amounts, excreted for each inhalation method, were normalised for the nominal dose and then log transformed. From the mean square error of the analysis of variance, using patients and inhalation method as the main factors, the mean ratio (90% confidence interval) was calculated.

4.2.3 Results

Four healthy volunteers (two females), whose mean (SD) age, height and weight, were 29.5 (1.3) years, 165.3 (10.1) cm and 59.3 (5.7) kg, respectively participated in the first part of the study. No urinary excretion of BDP or its metabolites were detected up to 24hr following oral BDP (2mg in 20ml 20% ethanol solution) dosing with the concomitant administration of activated charcoal. The use of activated charcoal in this

dose was found to be sufficient to block the gastrointestinal absorption of beclometasone dipropionate and its metabolites in this study.

Twelve healthy volunteers (four females), whose mean (SD) age, weight and height, were 33.8 (11.6) years, 69.3 (11.4) Kg and 171.0 (8.6) cm, respectively completed the second part of the study. The demographic details of the subjects that participated in the first and second part of the study are shown in table 4.1 and 4.2, respectively.

Table 4.1: Demographic data of the volunteers that participated in the first part of the study, (n=4).

Subject	Sex	Age (years)	Height (cm)	Weight (kg)
1	Female	28	155	55
2	Male	30	178	60
3	Female	29	160	55
4	Male	31	168	67
Mean	**---**	**29.5 (1.3)**	**165.3 (10.1)**	**59.3 (5.7)**

Table 4.2: Demographic data of the volunteers that participated in the second part of the study, (n=12).

Subject	Sex	Age (years)	Height (cm)	Weight (kg)
1	Male	58	183	85
2	Female	50	165	60
3	Female	28	155	55
4	Male	30	178	60
5	Female	29	160	55
6	Male	31	168	67
7	Male	23	181	80
8	Male	32	170	69
9	Male	19	179	83
10	Male	23	174	69
11	Female	40	166	63
12	Male	42	173	85
Mean (SD)	**---**	**33.8 (11.6)**	**171.0 (8.6)**	**69.3 (11.4)**

No BDP, 17-BMP, or BOH was detected after oral dosing with activated charcoal. The individual and mean (SD) urinary excretion of beclometasone dipropionate and its metabolites post IC, IQ, IQC, and O study doses is presented in APPENDIX B.1-B.22 (refer to the enclosed DVD). All these urinary excretions of BDP and its metabolites are summarized in table 4.3 and APPENDIX B.23 (refer to the enclosed DVD) provide a summary of their excretion rate profiles.

Table 4.3: The mean (SD) cumulative urinary excretion of 17-beclometasone monopropionate, beclometasone and beclometasone dipropionate following the inhalation of 8 doses of Clenil Modulite® MDI (250 µg per actuation) [IC], 10 doses of Qvar® EB (100 µg per actuation) [IQ], 10 doses of Qvar® EB with simultaneous oral administration of 5g activated charcoal [IQC] and oral administration of an alcoholic solution of 2mgBDP [O], expressed in µg, (n=12).

Urine collection period (hours)	Amount of BOH (µg)				Amount of (17-BMP) (µg)				Amount of BDP (µg)			
	IC	IQ	IQC	O	IC	IQ	IQC	O	IC	IQ	IQC	O
0.5	5.1 (1.5)	6.0 (1.6)	5.1 (1.8)	0 (0)	3.1 (0.8)	3.9 (1.4)	3.2 (1.2)	0 (0)	3.9 (1.4)	4.2 (0.9)	3.7 (1.0)	0 (0)
1	12.0 (2.7)	13.5 (3.3)	11.3 (4.0)	2.5 (1.6)	7.7 (2.3)	8.9 (2.9)	6.7 (2.4)	0.8 (1.1)	8.8 (2.3)	9.1 (1.8)	8.9 (2.1)	0 (0)
2	28.5 (6.5)	31.2 (7.8)	23.5 (7.4)	6.7 (2.4)	17.5 (6.5)	18.7 (8.6)	12.6 (4.6)	3.7 (1.9)	13.3 (2.7)	13.1 (2.4)	13.4 (3.0)	0 (0)
3	47.4 (11.9)	52.1 (14.2)	34.9 (11.9)	11.4 (3.6)	21.3 (7.4)	24.6 (10.8)	15.0 (4.9)	6.3 (1.9)	16.6 (2.6)	16.1 (3.0)	16.7 (3.6)	0 (0)
5	61.1 (20.1)	66.8 (18.7)	42.1 (14.1)	17.7 (6.3)	24.5 (7.7)	27.4 (10.6)	17.1 (5.6)	9.1 (2.3)	20.3 (2.8)	19.2 (4.0)	20.1 (4.1)	0 (0)
8	69.8 (23.1)	76.6 (18.9)	48.5 (16.3)	23.5 (7.8)	26.5 (7.7)	30.2 (10.3)	19.0 (6.1)	12.4 (3.2)	24.1 (3.3)	20.9 (5.2)	21.8 (5.4)	0 (0)
12	74.8 (25.0)	81.4 (20.2)	53.5 (18.3)	28.1 (8.0)	28.5 (7.9)	32.5 (10.6)	20.9 (7.0)	14.6 (4.0)	24.1 (3.3)	20.9 (5.2)	22.2 (5.4)	0 (0)
24	78.5 (27.5)	86.2 (21.6)	57.8 (19.1)	33.6 (9.8)	30.2 (8.1)	34.4 (10.6)	22.7 (7.9)	16.1 (5.2)	24.1 (3.3)	20.9 (5.2)	22.2 (5.4)	0 (0)

Table 4.3 shows that no BDP, 17-BMP, or BOH was excreted in the first 30 minutes post oral administration. In contrast, significantly more amounts of BDP, 17-BMP, and BOH ($p<0.001$) were excreted post inhalation of both Qvar® and Clenil® study doses. In addition, no parent drug (BDP) was detected in any sample up to 24hrs post oral administration. The mean (SD) urinary excretion of BOH over the 0.5hr and 24hours period post dosing of inhaled Qvar®, inhaled Clenil®, inhaled Qvar® plus charcoal and oral administration is 6.0 (1.6), 5.1 (1.5), 5.1 (1.8), 0 (0) µg and 86.2 (21.6), 78.5 (27.5), 57.8 (19.1), 33.6 (9.8) µg, respectively. The 0.0-0.5hr and the 0-24hr urinary 17-BMP excretion following IQ, IC, IQC and O administration is 3.9 (1.4), 3.1 (0.8), 3.2 (1.2), 0 (0) µg and 34.4 (10.6), 30.2 (8.1), 22.7 (7.9), 16.1 (5.2) µg, respectively. The urinary BDP excreted were 4.2 (0.9), 3.9 (1.4), 3.7 (1), and 0 (0) µg during the first 0.5hr collection period and were 20.9 (5.2), 24.1 (3.33), 22.2 (5.4) and 0 (0) over the 24hr period post administration of IQ, IC, IQC and O study doses µg, respectively. Figures 4.1- 4.3 show the urinary excretion profiles for BOH, 17-BMP and BDP, while figures 4.4- 4.6 show their cumulative urinary excretion, respectively.

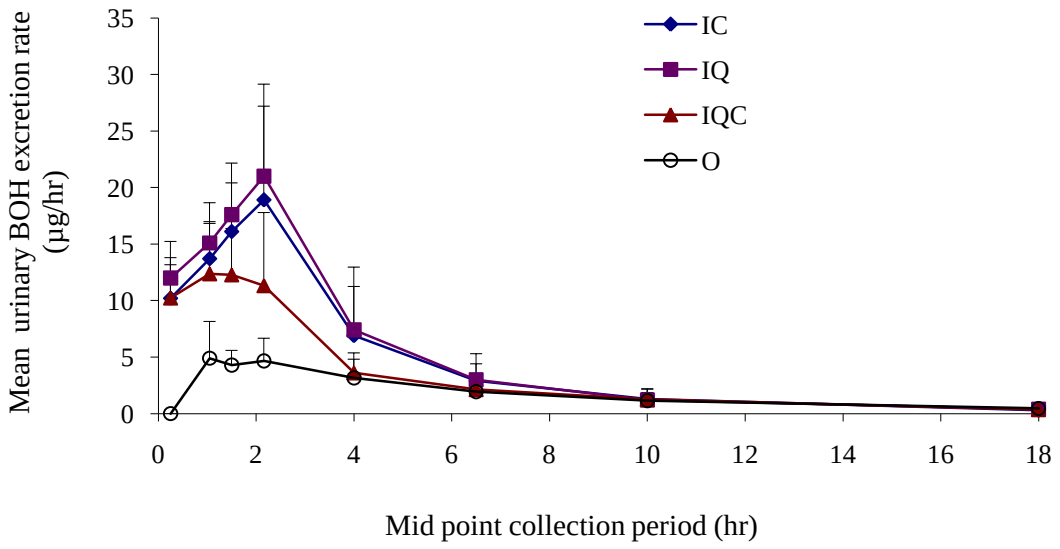


Figure 4.1: The mean (SD) urinary beclometasone (BOH) excretion rates post inhalation of IC, IQ, IQC and oral study doses, expressed in µg/hr, (n=12).

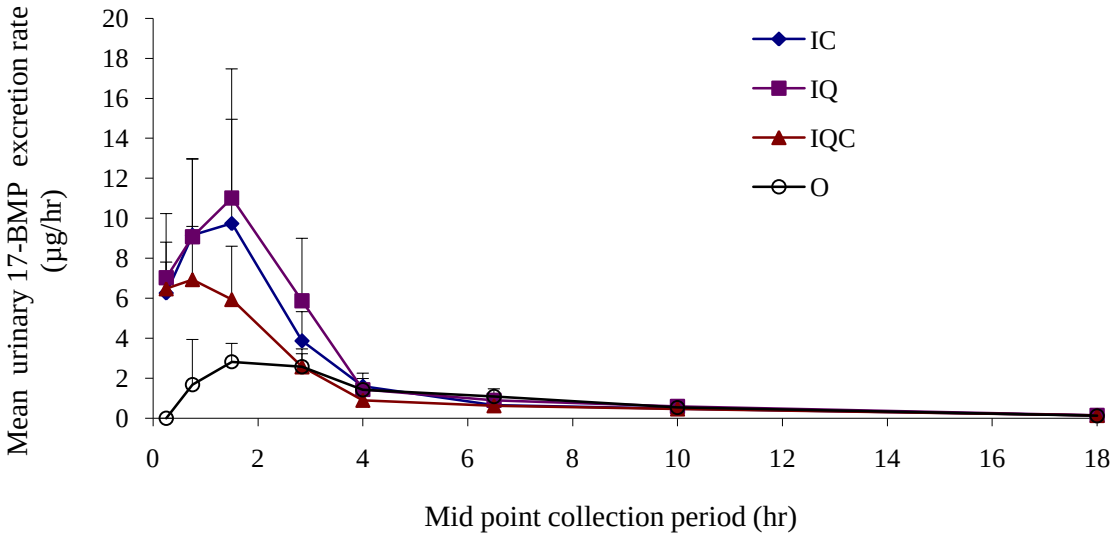


Figure 4.2: The mean (SD) urinary 17-beclometasone monopropionate (17-BMP) excretion rates post inhalation of IC, IQ, IQC and oral study doses, expressed in µg/hr, (n=12).

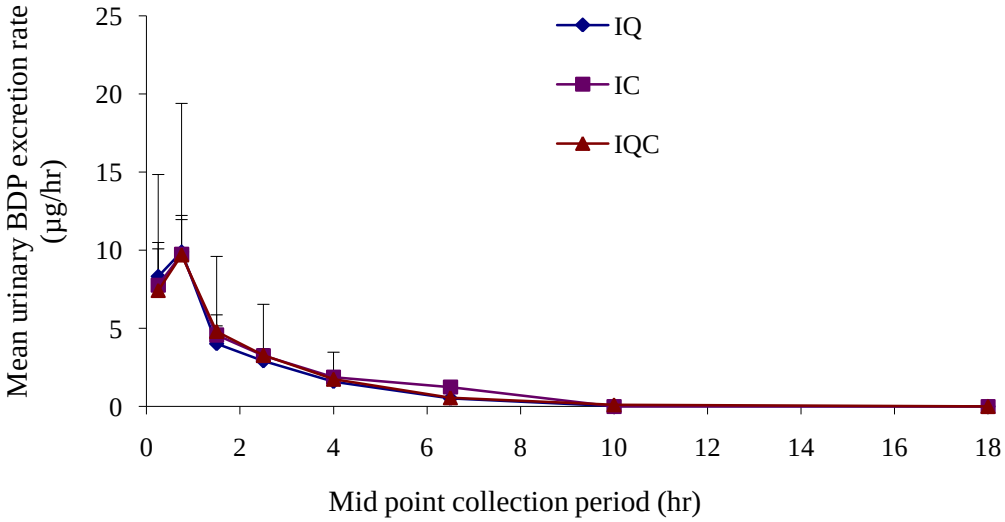


Figure 4.3: The mean (SD) urinary beclometasone dipropionate (BDP) excretion rates post inhalation of IC, IQ, IQC and oral study doses, expressed in µg/hr, (n=12).

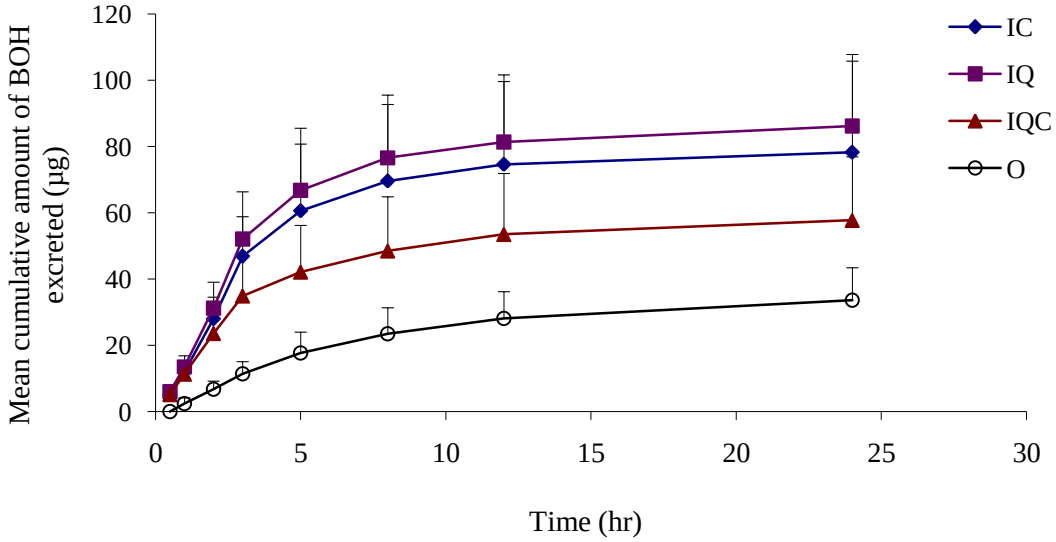


Figure 4.4: The mean (SD) cumulative urinary excretion of beclometasone (BOH) post study doses excreted in the urine post inhalation of IC, IQ, IQC and oral study doses, expressed in µg, (n=12).

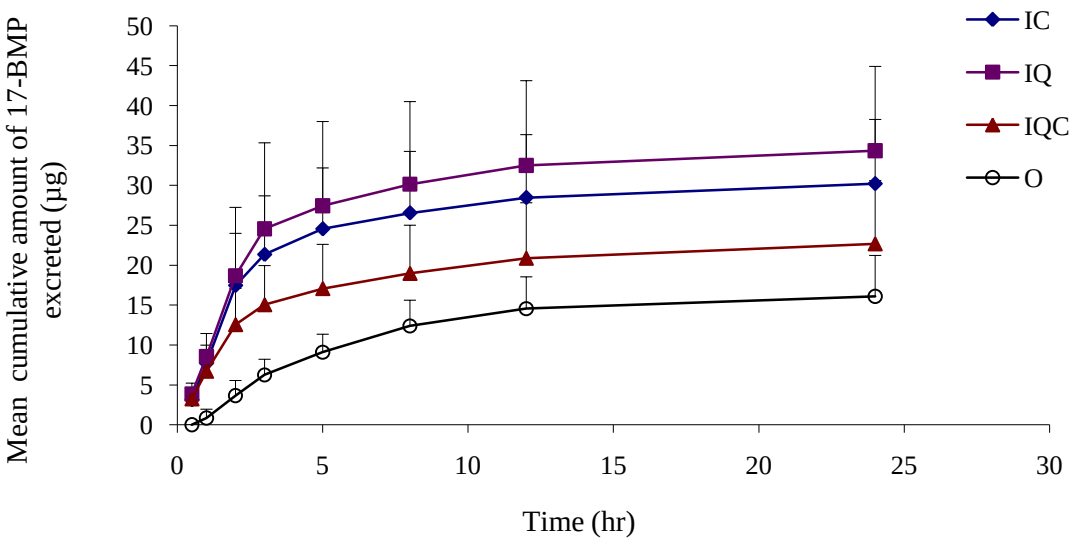


Figure 4.5: The mean (SD) cumulative urinary excretion of 17-beclometasone monopropionate (17-BMP) excreted in the urine post inhalation of IC, IQ, IQC and oral study doses, expressed in µg, (n=12).

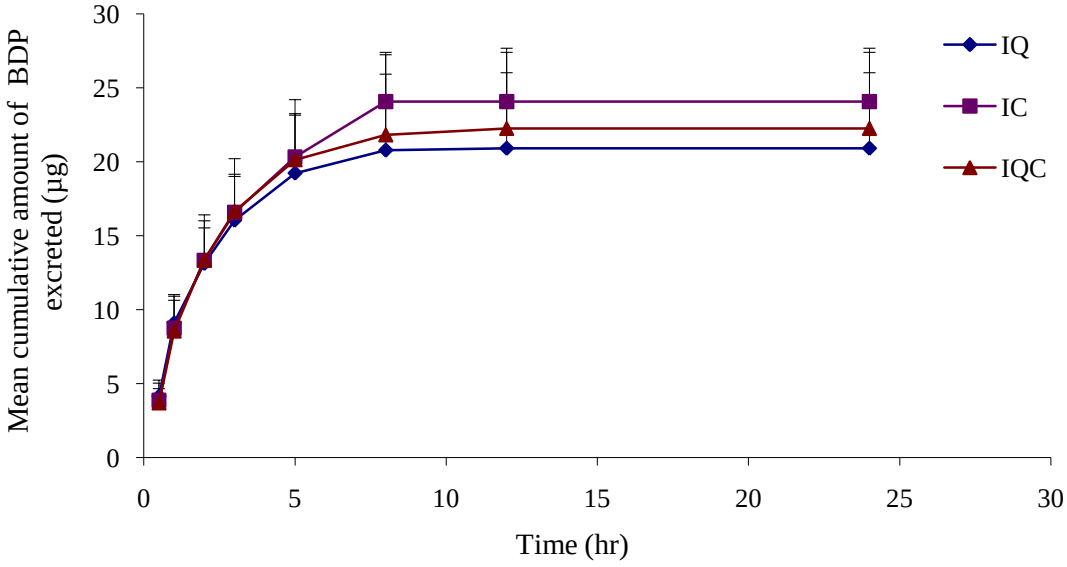


Figure 4.6: The mean (SD) cumulative urinary excretion of beclometasone dipropionate (BDP) excreted in the urine post inhalation of IC, IQ, IQC and oral study doses, expressed in µg, (n=12).

Table 4.4 describes the mean (SD) cumulative urinary excretion of BDP and its metabolites expressed as percentage of the nominal dose. The mean ratio of the cumulative urinary excretion excreted in the urine 0.5 and 24hrs, following the administration of [IC], [IQ], and [O] study doses is summarized in table 4.5. The individual 0.5hr urinary excretion of BDP and its metabolites recovered in urine post study doses expressed in µg and as % of nominal dose are presented in figures 4.7 and 4.8, respectively. These figures highlight that the 30 minutes urinary excretion post-oral administration shows that no BDP, 17-BMP, or BOH were detected in urine during that collection period. Figures 4.9 and 4.10 represent the individual 24hr urinary amounts of BDP and metabolites expressed in µg and as percentage of nominal dose, respectively.

Table 4.4: The mean (SD) of the cumulative urinary excretion of 17-beclometasone monopropionate, beclometasone and beclometasone dipropionate , expressed as percentage of nominal dose following the inhalation of 8 doses of Clenil Modulite® MDI (250 µg per actuation) [IC], 10 doses of Qvar® EB (100 µg per actuation) [IQ], 10 doses of Qvar® EB with simultaneous oral administration of 5g activated charcoal [IQC] and oral administration of an alcoholic solution of 2mg BDP [O], (n=12).

Urine collection period (hours)	Amount of BOH (%)				Amount of (17-BMP) (%)				Amount of BDP (%)			
	IC	IQ	IQC	O	IC	IQ	IQC	O	IC	IQ	IQC	O
0.5	0.3 (0.1)	0.6 (0.2)	0.5 (0.2)	0 (0)	0.2 (0.1)	0.4 (0.1)	0.3 (0.1)	0 (0)	0.2 (0.1)	0.4 (0.1)	0.4 (0.1)	0 (0)
1	0.6 (0.1)	1.4 (0.3)	1.1 (0.4)	0.1 (0.1)	0.4 (0.1)	0.9 (0.3)	0.7 (0.3)	0.1 (0.1)	0.4 (0.1)	0.9 (0.2)	0.9 (0.2)	0 (0)
2	1.4 (0.3)	3.1 (0.8)	2.4 (0.7)	0.3 (0.1)	0.9 (0.3)	1.9 (0.9)	1.3 (0.5)	0.2 (0.1)	0.7 (0.1)	1.3 (0.2)	1.3 (0.3)	0 (0)
3	2.4 (0.6)	5.2 (1.4)	3.5 (1.2)	0.6 (0.2)	1.1 (0.4)	2.5 (1.1)	1.5 (0.5)	0.3 (0.1)	0.8 (0.1)	1.6 (0.3)	1.7 (0.4)	0 (0)
5	3.1 (1.0)	6.7 (1.9)	4.2 (1.4)	0.9 (0.3)	1.2 (0.4)	2.8 (1.1)	1.7 (0.6)	0.5 (0.1)	1.0 (0.1)	1.9 (0.4)	2.0 (0.4)	0 (0)
8	3.5 (1.2)	7.7 (1.9)	4.9 (1.6)	1.2 (0.4)	1.3 (0.4)	3.0 (1.0)	1.9 (0.6)	0.6 (0.2)	1.2 (0.2)	2.1 (0.5)	2.2 (0.5)	0 (0)
12	3.7 (1.3)	8.1 (2.0)	5.4 (1.8)	1.4 (0.4)	1.4 (0.4)	3.3 (1.1)	2.1 (0.7)	0.7 (0.2)	1.2 (0.2)	2.1 (0.5)	2.2 (0.5)	0 (0)
24	3.9 (1.4)	8.6 (2.2)	5.8 (1.9)	1.7 (0.5)	1.5 (0.4)	3.4 (1.1)	2.3 (0.8)	0.8 (0.3)	1.2 (0.2)	2.1 (0.5)	2.2 (0.5)	0 (0)

Table 4.5: The mean ratio of the cumulative urinary excretion of 17-BMP, BOH and BDP, excreted in the urine 0.5 and 24hrs following the inhalation Clenil® MDI [IC], Qvar® EB [IQ], and the administration of the oral study doses [O], (n=12).

Time (hr)		**0.5**	**24**
O : IC Amount (µg)	**BOH**	---	1:2.3
	17-BMP	---	1:1.9
O: IQ Amount (µg)	**BOH**	---	1:2.6
	17-BMP	---	1:2.1
IC: IQ Amount (µg)	**BOH**	1:1.2	1:1.1
	17-BMP	1:1.2	1:1.1
	BDP	1:1.1	1:0.9
IC: IQ (%ND)	**BOH**	1:2.4	1:2.2
	17-BMP	1:2.0	1:2.0
	BDP	1:2.0	1:1.8

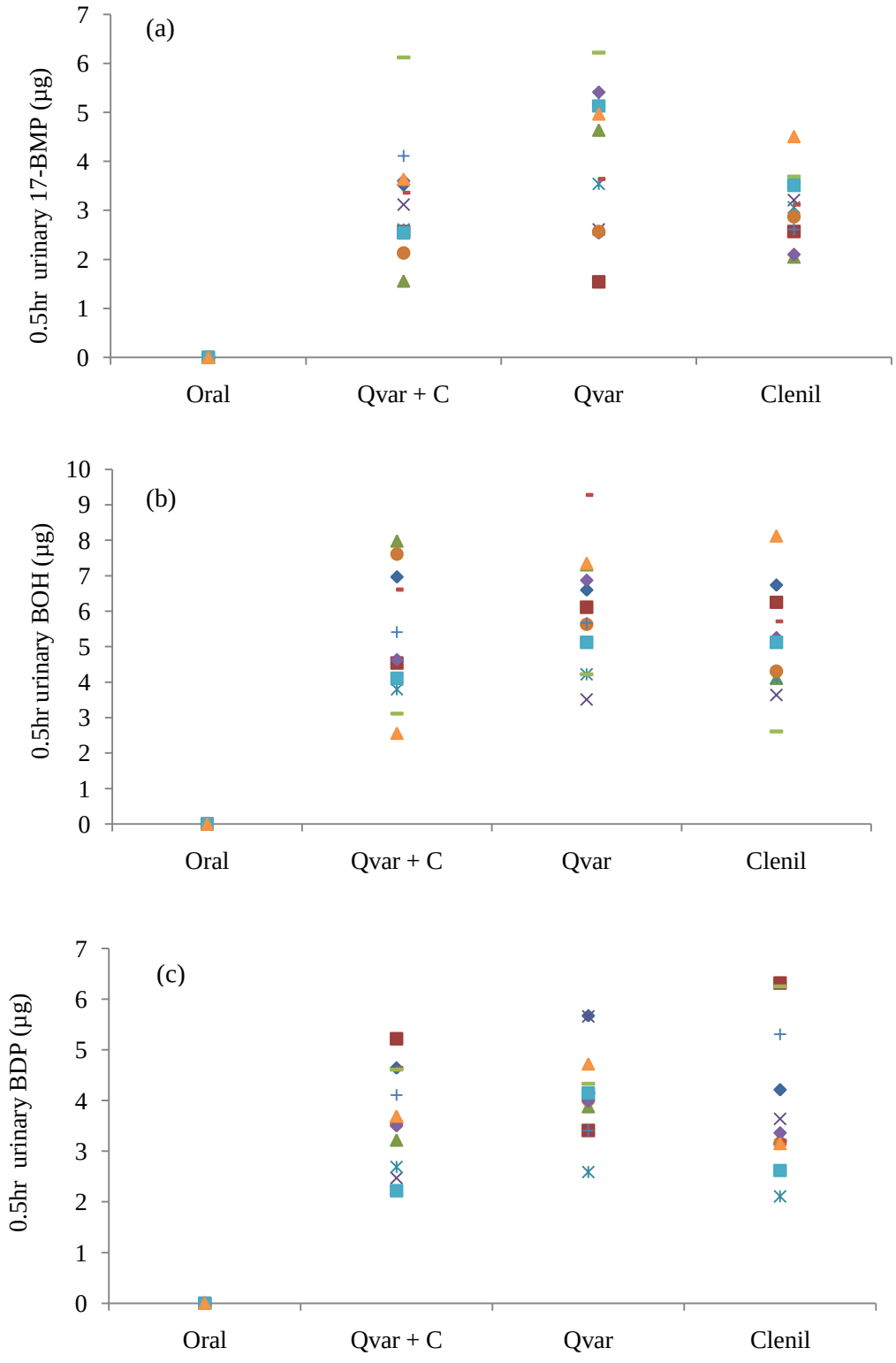


Figure 4.7: The 0.5hr urinary amounts of (a) 17-BMP (b) BOH (c) BDP recovered in urine post dosing via the oral solution, inhaled Clenil®, inhaled Qvar®, and inhaled Qvar® plus charcoal, expressed in µg, (n=12).

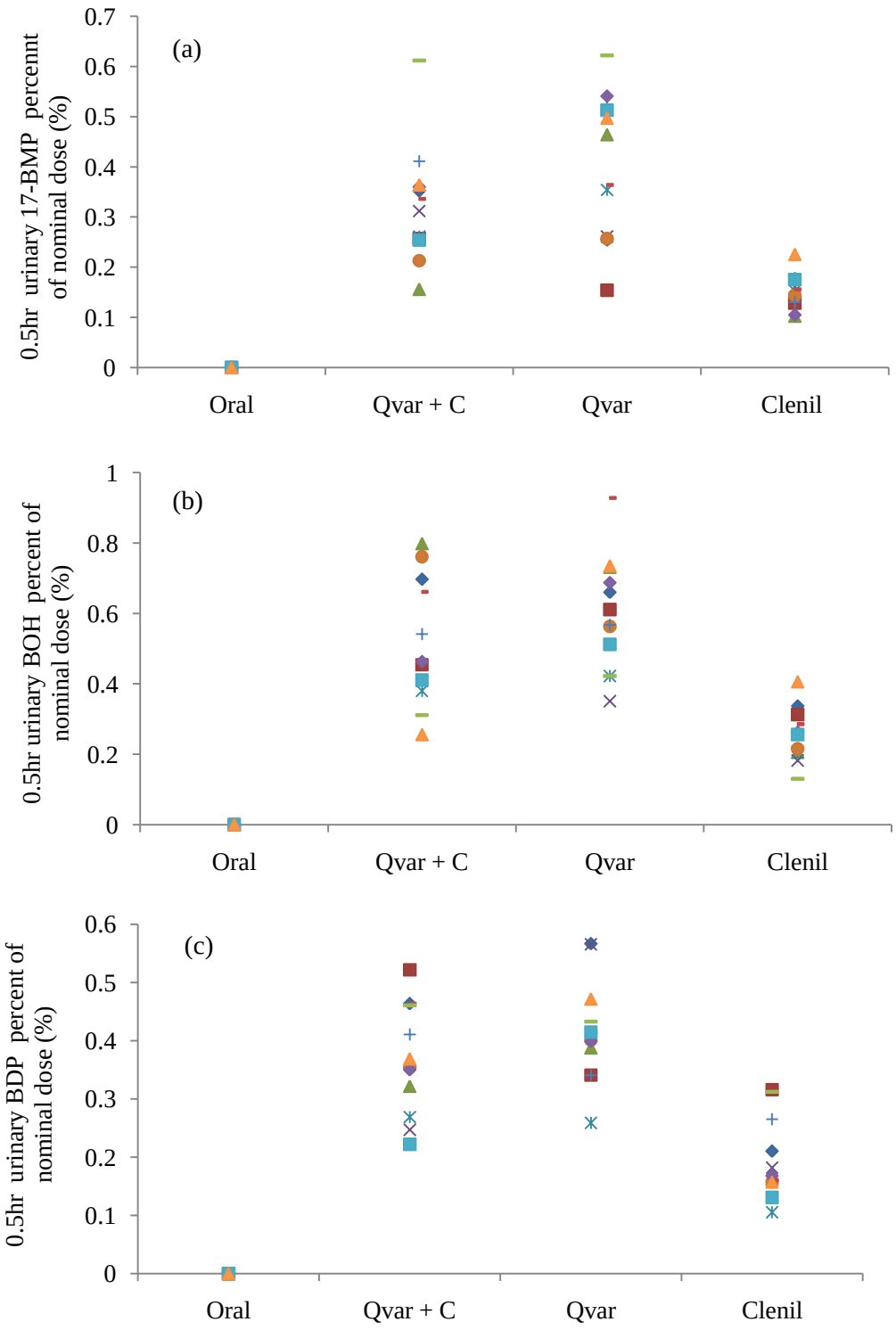


Figure 4.8: The 0.5hr urinary amounts of (a) 17-BMP (b) BOH (c) BDP recovered in urine post dosing via the oral solution, inhaled Clenil®, inhaled Qvar® , and inhaled Qvar® plus charcoal, expressed as % of nominal dose, (n=12).

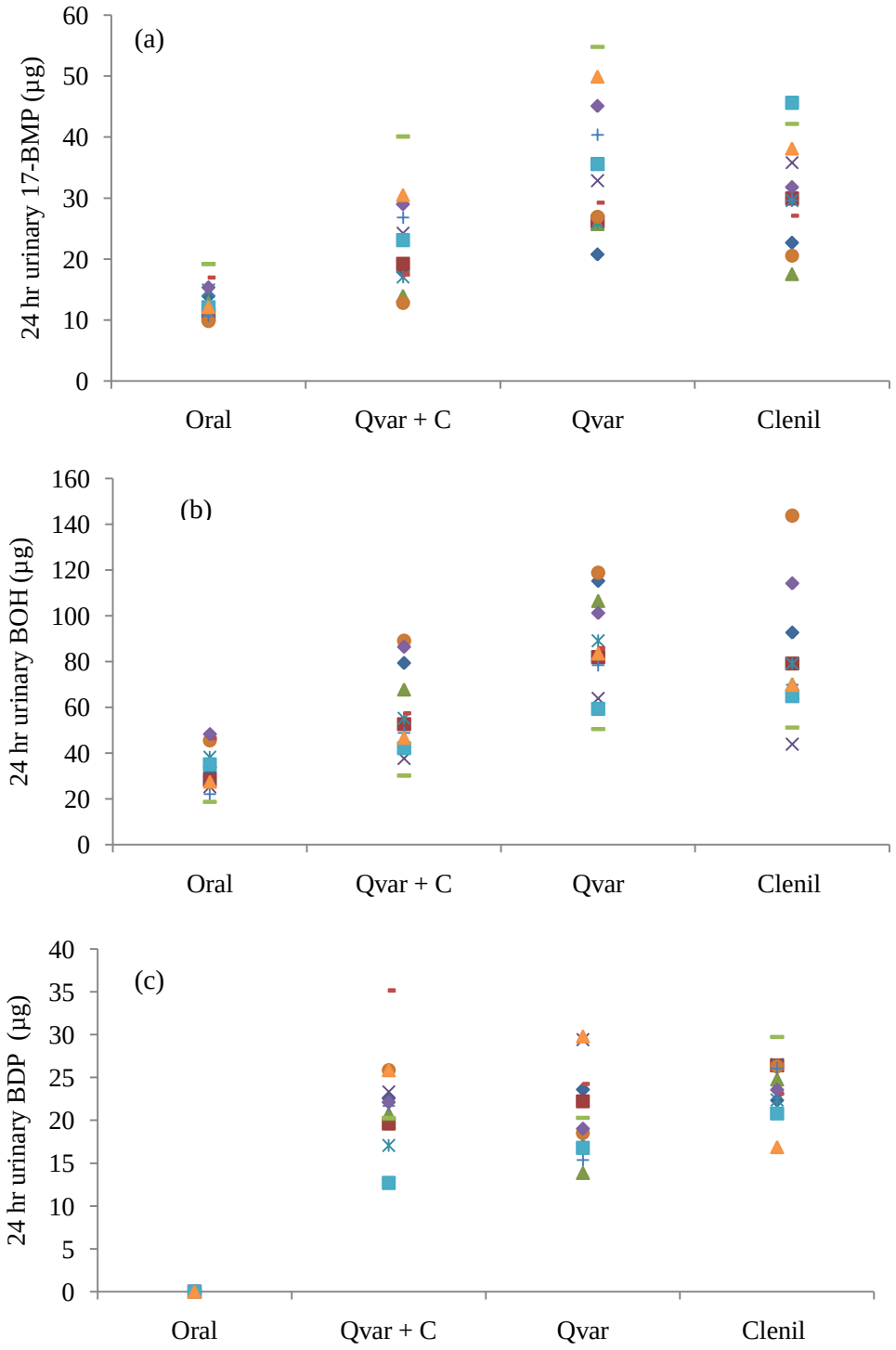


Figure 4.9: The 24hr urinary amounts of (a) 17-BMP (b) BOH (c) BDP recovered in urine post dosing via the oral solution, inhaled Clenil®, inhaled Qvar® , and inhaled Qvar® plus charcoal, expressed in µg, (n=12).

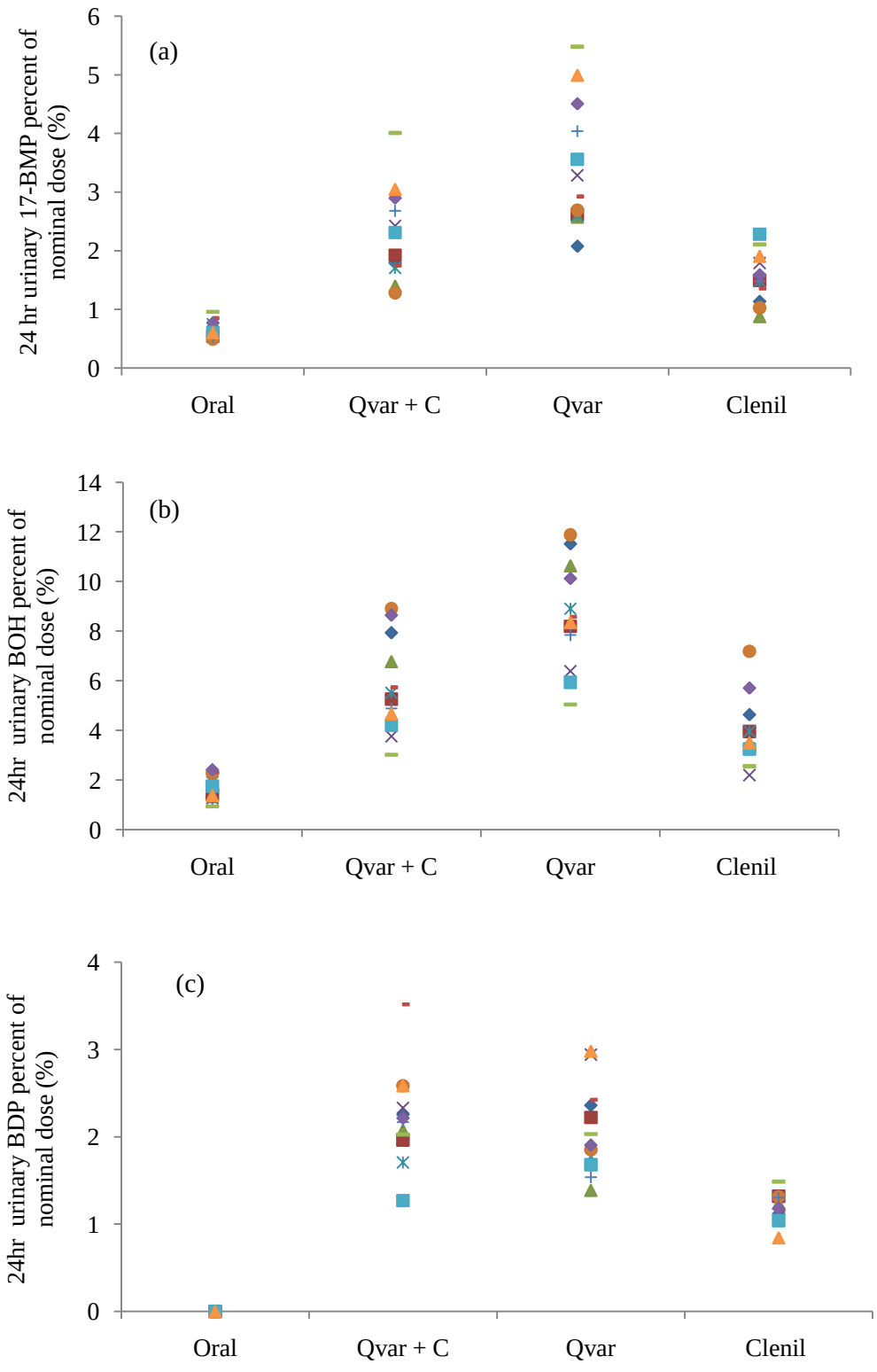


Figure 4.10: The 24hr urinary amounts of (a) 17-BMP (b) BOH (c) BDP recovered in urine post dosing via the oral solution, inhaled Clenil®, inhaled Qvar® , and inhaled Qvar® plus charcoal, expressed as % of nominal dose, (n=12).

A summary of the statistical analysis of the data obtained from different study doses is presented in tables 4.6 - 4.8. Figures 4.7 - 4.10 and tables 4.6 and 4.7 show that when comparing the cumulative amount of BOH and 17-BMP excreted in the urine following inhaled Qvar®, inhaled Clenil and inhaled Qvar® plus charcoal administration compared with oral administration, a significant difference ($p<0.001$) was found at all time intervals investigated. The mean difference (95% confidence interval) of 0.0-0.5hr urinary drug excretion following IQ, IC and IQC administration compared with oral administration were 3.9 (3.1,4.6) µg ,3.1 (2.4, 3.9) µg, 3.2 (2.5,4.0) µg and 5.5 (4.4, 6.6) µg ,4.6 (3.5, 5.7) µg, 4.6 (3.5,5.7) µg for 17-BMP and BOH respectively ($p<0.001$). The mean difference (95% confidence interval) of 0.0-24hrs cumulative urinary drug excretion following IQ, IC and IQC administration compared with oral administration were 18.2 (14.7, 21.7) µg , 14.7 (11.1, 18.2) µg, 6.6 (3.0, 10.1) µg and 52.5 (43.1, 61.9) µg , 44.9 (35.5, 54.2) µg, 24.2 (14.8, 33.5) µg for 17-BMP and BOH respectively ($p<0.001$). A summary of the mean ratio (90% confidence limits) between Qvar and Clenil with respect to the nominal dose and between each product and the oral dose is presented in table 4.9. These values are presented separately for BDP, 17-BMP, and BOH, as well as for all three metabolites combined. The latter, which represents an overall ratio, shows a mean ratio (90% confidence interval) for Qvar compared to Clenil of 231.4 (209.6 - 255.7)%, and 204.6 (189.6, 220.6) % for the 30 minute, and the 24hr urinary excretion, respectively. Figure 4.11 shows the mean ratio (90% confidence limits) between Qvar and Clenil with respect to the nominal dose.

Table 4.6: Statistical comparison of the mean difference (95% confidence interval) between the cumulative amounts of beclometasone excreted in the urine post different times of the following study doses; IQ vs O, IC vs O, IQC vs O, IQ vs IQC and IC vs IQ.

Time	IQ vs O (µg)	IC vs O (µg)	IQC vs O(µg)	IQ vs IQC(µg)	IC vs IQ(µg)
0.5	5.5 (4.4, 6.6)***	4.6 (3.5, 5.7)***	4.6 (3.5,5.7)***	0.9 (-0.2, 2.0)	-0.9 (-2, 0.2)
1	11.1 (8.9, 13.3)***	9.5 (7.3, 11.7)***	8.9 (6.6, 11.1)***	2.2 (0.0,4.5)*	-1.6 (-3.8, 0.7)
2	24.4 (19.8, 29.1)***	21.8 (17.1,26.4)***	16.9 (12.2, 21.5) ***	7.6 (2.9,12.2)**	-2.7 (-7.3, 2)
3	40.7 (33.2, 48.2)***	36.0 (28.5, 43.5)***	23.5 (16, 31)***	17.2 (9.7,24.7)***	-4.7 (-12.2,2.6)
5	49.1 (40.1, 58.2)***	43.4 (34.3, 52.4)***	24.4 (15.4, 33.5)***	24.7 (15.7, 33.8)***	-5.7 (-14.8, 3.3)
8	53.1 (44, 62.1)***	46.3 (37.2, 55.3)***	25.0 (16, 34.0)***	28.1 (19.0, 37.1)***	-6.8 (-15.8, 2.2)
12	53.3 (44.3, 62.3)***	46.7 (37.7, 55.7)***	25.4 (16.4, 34.4)***	27.9 (18.9, 36.9)***	-6.6 (-15.6, 2.4)
24	52.5 (43.1, 61.9)***	44.9 (35.5, 54.2)***	24.2 (14.8, 33.5)***	28.4 (19, 37.8)***	-7.7 (-17.1, 1.7)

Table 4.7: Statistical comparison of the mean difference (95% confidence interval) between the cumulative amounts of 17-beclometasone monopropionate excreted in the urine post different times of the following study doses; IQ vs O, IC vs O, IQC vs O, IQ vs IQC and IC vs IQ.

Time	IQ vs O (µg)	IC vs O (µg)	IQC vs O(µg)	IQ vs IQC(µg)	IC vs IQ(µg)
0.5	3.9 (3.1, 4.6)***	3.1 (2.4, 3.9)***	3.2 (2.5, 4)***	0.6 (-0.1, 1.4)	-0.7 (-1.5, 0.001)
1	7.7 (6.4, 9.1)***	6.9 (5.5, 8.2)***	5.9 (4.5, 7.2)***	1.9 (0.5, 3.2)**	-0.8 (-2.2, 0.5)
2	15.0 (11.9, 18.2)***	13.8 (10.6, 17)***	8.9 (5.7, 12)***	6.1 (3.0, 9.3)***	-1.2 (-4.4, 1.9)
3	18.3 (14.4, 22.3)***	15.1 (11.1, 19)***	8.8 (4.84, 12.75)***	9.5(5.6, 13.5)***	-3.2 (-7.2, 0.7)
5	18.4 (14.4, 22.3)***	15.4 (11.5, 19.4)***	8.0 (4.0, 11.9)***	10.4 (6.4, 14.3)***	-2.9 (-6.9, 1.0)
8	17.8 (14.1, 21.4)***	14.7 (11.1, 18.4)***	6.6 (3, 10.2)***	11.2 (7.6, 14.8)***	-3.04 (-6.7, 0.6)
12	18.0 (14.4, 21.5)***	14.5 (10.9, 18)***	6.3 (2.7, 9.8)**	11.7 (8.1, 15.2)***	-3.5 (-7, 0.1)
24	18.2 (14.7, 21.7)***	14.7 (11.12, 18.2)***	6.6 (3, 10.1)***	11.6 (8.1, 15.2)***	-3.5 (-7, 0.04)

For both tables* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference

Table 4.8: Statistical comparison of the mean difference (95% confidence interval) between the cumulative amounts of beclometasone dipropionate excreted in the urine post different times of IQ vs IQC and IC vs IQ study doses.

Time	IQ vs IQC(µg)	IC vs IQ(µg)
0.5	0.5 (-0.3, 1.2)	-0.3 (-1.1, 0.5)
1	0.6 (-1.2, 2.3)	-0.4 (-2.1, 1.4)
2	-0.2 (-2.5, 2.0)	0.2 (-2, 2.4)
3	-0.6 (-3.1, 1.9)	0.5 (-1.9, 3)
5	-0.9 (-3.9, 2.1)	1.1 (-1.9, 4.1)
8	-1.0 (-4.9, 2.8)	3.2 (-0.5, 7.1)
12	-1.3 (-5.1, 2.4)	3.2 (-0.6, 6.9)
24	-1.3 (-5.1, 2.4)	3.2 (-0.6, 6.9)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference

Table 4.9: Mean ratio (90% confidence interval) for Qvar compared to Clenil and between each product and the oral dose (when normalised for the nominal dose.

Urinary excretion	0.5hr urinary excretion	c24 hour urinary excretion		
	Qvar vs Clenil	Qvar vs Clenil	Qvar vs oral	Clenil vs oral
BDP	221.4(189.1,259.6)	170.7 (148.3,196.6)	---	---
17-BMP	236.6 (192.1, 291.2)	223.9 (202.2, 247.7)	430.6 (385.7, 480.2)	192.3 (172.3, 214.5)
BOH	236.8 (198.0, 283.5)	223.9 (206.7, 242.8)	517.6 (460.4, 581.8)	231.2 (205.6, 259.9)
All combined	231.4 (209.6, 255.7)	204.6 (189.6, 220.6)	451.3 (412.9, 492.8)	220.6 (202.0, 241.1)

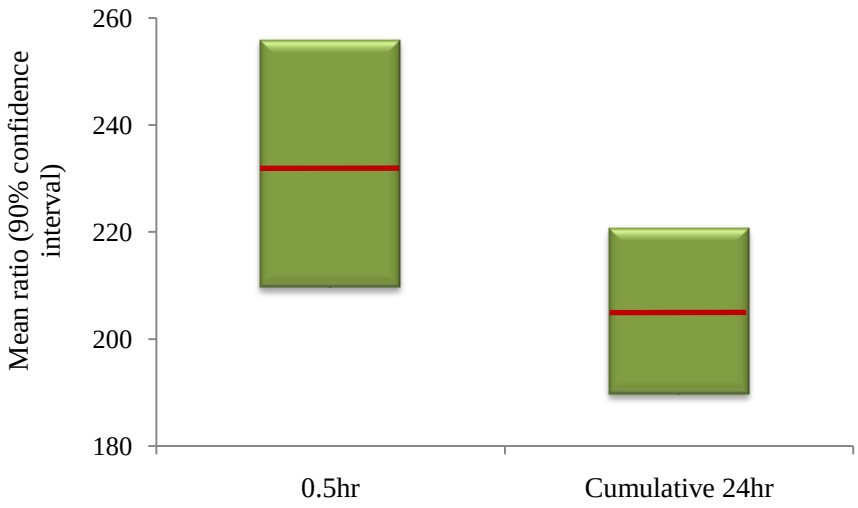


Figure 4.11: The overall mean ratio (90% confidence interval) for the 0.5hr and the 24hr urinary excretion between Qvar and Clenil with respect to the nominal dose.

As shown in tables 4.6 - 4.8 and figures 4.7 - 4.10, no significant difference (95% confidence interval) was found between the urinary amount of BOH, and 17-BMP excreted 0.5hr post dose following IQ and IQC administrations, while their 24hr urinary excretion results showed significance for the same treatment groups. The mean difference (95% confidence interval) of 0.0-0.5hr urinary drug excretion following inhaled Qvar® administration compared with inhaled Qvar® plus charcoal administration were 0.6 (-0.1, 1.4) µg , 0.9 (-0.2, 1.9) µg, and 0.5 (-0.3, 1.2) for 17-BMP, BOH, and BDP respectively. However, for BDP, no significant difference was found between the cumulative urinary BDP excretions post inhaled Qvar® and inhaled Qvar® plus charcoal administration at all time intervals investigated. As shown in tables 4.6 - 4.8, comparison of the amounts excreted for BDP and its metabolites between Qvar® and Clenil® at each sampling points showed no significant difference.

4.2.4 Discussion

Administration of beclometasone dipropionate by inhalation produced detectable concentrations of unchanged BDP, 17-BMP, and BOH in urine samples. However, the minor and inactive metabolite 21-BMP was not detected in this study, which is consistent with the failure of previous studies to detect it in most samples post-inhaled dosing (Falcoz et al., 1996; Daley-Yates et al., 2001; Harrison et al., 2002a). The absence of any BDP or metabolites detected in the urine post oral dose with charcoal administration is consistent with previous studies (Trescoli and Ward, 1998; Daley-Yates et al., 2001) and confirms the ability of charcoal to block the oral absorption of the portion of inhaled dose of beclometasone dipropionate that would be swallowed and subsequently absorbed following inhalation. Therefore, any BDP or metabolites excreted in the urine following inhalation with activated charcoal must have been absorbed via the lungs. As illustrated in table 4.3, following oral administration of BDP, none of the parent drug was detected in any of the urine samples at all time intervals investigated. In addition, none of the metabolites 17-BMP and BOH was excreted at 30 minutes post the oral dose.

The higher 30 minutes urinary excretions of 17-BMP, BDP, and BOH post inhalation compared to oral administration is due to its rapid and complete absorption from the lungs and the slow and negligible absorption from the gastrointestinal tract. This highlights the lag time for drug absorption from the gastrointestinal tract. This is in agreement with the salbutamol urinary excretion data post oral and inhaled administration initially reported by Hindle & Chrystyn (1992).

The non-significant difference found between the 30 minutes urinary excretion of 17-BMP, BDP and BOH in samples post inhalation from Qvar® EB and Qvar® EB with charcoal confirms the lag time for oral absorption and that charcoal blockage was not necessary. Moreover, the difference between the Qvar® and Qvar® plus charcoal is

similar to those expected post oral administration, which further confirms the prevention of oral absorption by the charcoal doses.

Significantly ($p<0.001$) more amounts of BDP, 17-BMP, and BOH were excreted in the urine following inhalation compared with oral administration at all the time intervals investigated. The ratios of 24hr urinary amounts of beclometasone recovered in urine following oral to inhaled Clenil® administrations and following oral to inhaled Qvar® administrations were 1:2.3, and 1:2.6, respectively. For 17-BMP, the 24hr urinary excretion of 17-BMP post oral to inhaled Clenil® and oral to inhaled Qvar® administration was at the ratio 1:1.9 and 1:2.1, respectively. These ratios of 24hr inhaled to oral for BOH and 17-BMP are consistent with AUC data previously reported (Daley-Yates et al., 2001).

The low oral to inhaled bioavailability of beclometasone dipropionate can be attributed to the efficient absorption of BDP from the lungs, but not form the gastrointestinal tract. This is consistent with a previous study that determined the relative bioavailability of oral versus inhaled beclometasone dipropionate from the HFA-BDP inhaler and reported that the fraction of an oral dose that reaches the systemic circulation was estimated as 40% relative to inhaled HFA BDP (Soria et al., 1998).

The significant differences ($p<0.001$) between the amounts of urinary 17-BMP and BOH in samples taken from 0.5-24hr collection periods post inhalation from the inhaled Qvar® and inhaled Qvar® with charcoal also highlights the contribution of the orally absorbed fraction. The urinary amounts of BOH and 17-BMP were only slightly reduced by the charcoal block, confirming that the pulmonary route was the predominant route for absorption of these metabolites.

The charcoal block did not affect the urine levels of BDP post inhalation, confirming that BDP, found in the systemic circulation arises from BDP absorbed unchanged from the

lung. The swallowed BDP is not available to the systemic circulation due to its extensive pre-systemic conversion. This is in agreement with previous studies that have also reported the absence of any detectable concentrations of BDP in the plasma following oral administration. This has been referred to the very high clearance of BDP, which would normally result in a high first pass metabolism. Although, 17-BMP also has high clearance values, it showed high oral bioavailability, suggesting that systemic rather than first pass metabolism predominated for 17-BMP elimination, while gut and hepatic metabolism predominated for BDP (Daley-Yates et al., 2001; Woodcock et al., 2002a).

The amounts of BOH, 17-BMP, and BDP excreted in the urine during the first 30 minutes post-inhalation can be used as an index of the relative bioavailability of beclometasone to the lungs following inhalation. This index could be used to compare the in-vivo lung deposition of different inhaled products/methods. The total amounts of beclometasone dipropionate and its metabolites excreted in the urine over the 24 hours post-inhalation represent the relative bioavailability of beclometasone to the body and can be used to compare the total systemic delivery following inhalation of different products or by different techniques.

Inspecting the results in table 4.5, highlights that the 30 minutes urinary excretion ratios post inhalation of eight doses of Clenil® (250µg) to ten doses of Qvar® (100µg), expressed as percentage of nominal dose were 1:2.4, 1:2 and 1:2 for BOH, 17-BMP and BDP, respectively. The 24hr urinary excretion ratio following the same IC and IQ study doses, expressed as percentage of nominal dose were 1:2.2, 1:2.0 and 1: 1.8 for BOH, 17-BMP and BDP, respectively. It is apparent from the small differences seen in the excretion ratios between BOH, 17-BMP, and BDP, that the 30 minutes excretions of any of them can be used to compare the bioequivalence of Qvar and Clenil. As shown in table 4.9 and figure 4.11, when combining all the data of the 0.5hr urinary excretion of

BDP and its metabolites, the overall mean ratio was 231.4% with 90% confidence interval of 209.6 - 255.7. While, the overall mean ratio was 204.6 with 90% confidence interval of 189.6 - 220.6 for the cumulative 24hr urinary excretion.

The above findings confirm that the urinary excretion of Qvar® was equivalent to Clenil® urinary excretion when administered at half the dose, these results are in agreement with previous findings that have shown that Qvar® as an extra fine aerosol with a particle size of 1.1µm has a relatively higher lung deposition. This is consistent with several previous *in-vitro*, gamma scintigraphy (Leach et al., 1998a), pharmacokinetic (Soria et al., 1998; Harrison et al., 1999b; Bousquet et al., 2009), and clinical (Davies et al., 1998; Busse et al., 1999b) studies that have confirmed that a given dose of Qvar HFA–BDP would result in approximately 2-2.5 fold greater potency compared with other CFC-containing beclometasone MDIs. Although Clenil® is a different formulation to the innovator CFC-product, it has been formulated as a seamless dose transition (Chaplin and Head, 2007).

4.3 Intra and inter- subject variability

4.3.1 Method

4.3.1.1 Equipment and inhalation devices

Inhaler devices: Qvar® Easi-Breathe inhaler (EB) labelled as a nominal dose of 100µg beclometasone dipropionate per dose (Teva Pharmaceuticals, UK).

LC-(ESI+)-MS method conditions: previously described in section 3.3 in this thesis.

4.3.1.2 Subjects and study design

Ethical approval for the study was obtained from the University of Huddersfield. Eight healthy, non-smoking subjects (4 females) gave written consent to participate in the study. All subjects were older than 18 years old with a FEV_1 > 90%. On separate study

days, each participant received eight 100μg (0.8mg in total) inhalations of beclometasone dipropionate from Qvar® EB (Teva Pharmaceuticals, UK) on five separate occasions to determine the reproducibility and the reliability of the 30 minutes urinary excretion method. The volunteers were first instructed to exhale to residual volume, then to put the Easi-Breathe inhaler into their mouth and seal their lips around the mouthpiece. They inhaled slowly and the Easi-Breathe device delivered the dose. This slow inhalation was continued to total lung capacity (until their lungs were full of air), with breath holding for about 10 seconds after each inhalation. For the next dose, this was repeated 30 seconds later (Hindle et al., 1993). Subjects voided their urine pre-dosing, provided urine samples 30 minutes after the start of the first dose, and cumulatively collected their urine for the 24 hours post study dose. The volume of urine excreted was recorded and aliquots of each sample were frozen at -20° C prior to analysis. There was a 7-day washout period between administrations. The amount of BDP and its metabolites excreted in the urine were measured using the previously validated LC-(ESI+)-MS method described in section 3.3.

4.3.2 Results

Eight healthy non-smoking subjects (4 females) with mean (SD) age, height, and weight of 27.4 (5.9) years, 167.8 (9.0) cm and 62.6 (7.8) kg respectively completed this reproducibility study. Their demographic data are shown in table 4.10.

Table 4.10: Demographic data of the volunteers that participated in the study.

Subject	Sex	Age (years)	Height (cm)	Weight (kg)
1	Female	29	155	55
2	Male	31	168	67
3	Male	23	175	65
4	Female	32	170	75
5	Male	19	180	66
6	Male	23	174	50
7	Female	25	160	60
8	Female	37	160	63
Mean (SD)	**---**	**27.4 (5.9)**	**167.8 (9.0)**	**62.6 (7.8)**

The individual 0.5hr and 24 hours urinary excretion data and coefficient of variation of beclometasone dipropionate and its metabolites from analysed urine samples are presented in APPENDIX B.24 - B.26 (refer to the enclosed DVD). A summary of the mean (SD) 0.5hr and 24hr intra-subject CV% and inter-subject CV% of BDP and its metabolites post eight inhalations from Qvar® EB (100µg) on five separate occasions are presented in table 4.11. The mean (SD) intra-subject coefficient of variation was 10.6 (4.2) %, 10.7 (5.2) %, and 9.5 (2.9) % for the 0.5hr urinary excretion and was 8.2 (2.6) %, 8.4 (1.5) %, and 8.9 (3.0) % for the 24hr urinary excretion for 17-BMP, BOH and BDP, respectively. The mean (SD) inter-subject coefficient of variation was 18.1 (3.2) %, 25.4 (3.1) %, and 33.4 (3.3) % for the 0.5hr urinary excretion and was 30.6 (3.8) %, 24.4 (1.4) %, and 27.7 (4.7) % for the 24hr urinary excretion for 17-BMP, BOH and BDP, respectively.

Table 4.11: The mean (SD) intra-subject and inter-subject CV% of 17-BMP, BDP, and BOH post inhalations.

	0.5hr			24hr		
	17-BMP	**BOH**	**BDP**	**17-BMP**	**BOH**	**BDP**
Intra-subject CV%	10.6 (4.2)	10.7 (5.2)	9.5 (2.9)	8.2 (2.6)	8.4 (1.5)	8.9 (3.0)
Inter-subject CV%	18.1 (3.2)	25.4 (3.1)	33.4 (3.3)	30.6 (3.8)	24.4 (1.4)	27.7 (4.7)

4.3.3 Discussion

The observed intra-subject variability in healthy volunteers in this study was generally low (<11%) and there was a higher inter-subject variability (ranged from 18.1-33.4%). The high inter-subject variability is largely attributed to the variability between subjects' lung deposition together with their renal excretion. This variability between subjects and within the same subject is consistent to that previously reported for salbutamol (Hindle and Chrystyn, 1992), gentamicin (Al-Amoud et al., 2005), formoterol (Nadarassan et al., 2007), sodium cromoglycate (Aswania and Chrystyn, 2002), and terbutaline (Abdelrahim et al., 2011). The urinary excretion pharmacokinetic method for the determination of the relative and total lung bioavailability of beclometasone dipropionate post-inhalation is reproducible, and can be effectively used to compare different inhalation products and techniques.

4.4 Conclusion

The comparison of the 30 minutes urinary excretion highlights the usefulness of this index as a measure of the relative bioavailability of beclometasone to the lung following inhalation. The amounts of BDP and its metabolites recovered in urine samples post study doses of inhaled Qvar® EB with an oral dose of activated charcoal represents the pulmonary absorbed fraction. Since, there was no difference found between the 0.5hr urinary excretion of inhaled Qvar® EB and inhaled Qvar® EB plus charcoal, then the use of activated charcoal is not necessary. The lack of BDP or metabolites in the urine samples at 30 minutes post oral dose together with their high significant amounts post inhalation highlights that the urinary salbutamol pharmacokinetic of Hindle and Chrystyn (1992) can be applied to beclometasone dipropionate post inhalation.

Chapter 5: *In-vitro* Dose Emission and Aerodynamic Particle Size Distribution, Relative Lung and Systemic Bioavailability of Beclometasone Inhaled From Clenil® MDI With and Without Spacer

5.1. Introduction

The assessment of pulmonary drug absorption and deposition is becoming increasingly important in drug development as this information can be effectively used to maximize pulmonary selectivity for locally acting drugs and to help determine the bioequivalence of generic inhalation products. There are several techniques available to describe lung deposition, including *in-vitro* approaches (the most well known being the Andersen Cascade Impactor). The use of *in-vitro* testing for inhalation methods has significantly improved the understanding of complex factors affecting aerosol delivery during inhalation. The information about size distribution of aerosol particles may be critical with regard to aerosol potential to deliver a dose to the lung. Cascade Impactors give information about the aerodynamic particle size distribution of the emitted dose. The Andersen Cascade Impactor (ACI) is the most commonly used impactor within the pharmaceutical industry for testing inhaled products (British Pharmacopoeia, 2009). *In-vitro* methods have been found to be simpler, less expensive to perform than *in-vivo* experiments with human subjects, have limited variability and allow a more detailed analysis. The standard Andersen Cascade Impactor (ACI) is designed to be operated at low flow rates, 28.3 L/min, however more recently, modifications are available that allow it to be operated at higher flow rates of 60L/min and 90 L/min. The understanding of the behaviour of different formulations under different airflow rates provides information on how patients can get the most out of their inhaler devices by using an optimum inhalation technique.

The potential of pharmacokinetic methods to successfully determine and predict lung deposition, bioavailability, and the systemic adverse effects of inhaled drug have been thoroughly investigated (Hindle and Chrystyn, 1992; Chege and Chrystyn, 1994; Hindle and Chrystyn, 1994; Hindle et al., 1997; Chege and Chrystyn, 2000; Aswania and

Chrystyn, 2001; Chrystyn, 2001; Aswania and Chrystyn, 2002). They are indirect measurements that uses plasma or urine concentrations to estimate the amount of drug which enters the systemic circulation via the pulmonary and the gastrointestinal tract routes (total systemic delivery), and thus provide valuable data which predict extra-pulmonary effects (Newnham et al., 1993).

Mazhar and Chrystyn (2008) used the Andersen Cascade Impactor and the urinary salbutamol pharmacokinetic method to compare the *in-vitro* and *in-vivo* drug delivery, respectively of a salbutamol MDI and when it was used with a Volumatic and an Aerochamber spacer. They found that there was no difference between the spacers. The fine particle dose for the spacers was similar to the MDI but the 30 minutes urinary excretion was greater due to inhalation from a static cloud, which occurs when using a spacer. The total dose emission was lower with the spacers, which was reflected by the lower 24hr urinary excretion.

The pressurized metered dose inhaler is still one of the most frequently prescribed inhaler devices despite the fact that most patients cannot use it correctly. The most common mistake made by patients using a MDI is failure to continuously inhale slowly after inhaler activation (Chrystyn and Price, 2009). In addition, the high velocity of the inhaled particles leads to most of the dose from metered dose inhalers to deposit on the throat causing both local and systemic effects even with good patient coordination between actuation and inhalation (Toogood et al., 1980; Aswania and Chrystyn, 2001). Consequently, the development of the spacer was a major addition to the use of MDIs. Spacers allow the aerosol jet emitted from the MDI orifice to slow down and thus decrease throat deposition and either improve or not affect pulmonary deposition (Terzano, 2001). However, most spacers are made from plastic materials that are prone to the accumulation of electrostatic charge on their surface, especially during patient

handling. This electrostatic charge (ESC) developed on spacer surfaces can attract the charged aerosol particles from a metered dose inhaler and thus they stay in the spacer and become not available for inhalation (Clark and Lipworth, 1996a). The presence of this electrostatic charge can dramatically affect drug output from spacers and requires cautious handling procedures to avoid it. Using a metal spacer (Bisgaard et al., 1995), washing it in detergent without subsequent rinsing (Pierart et al., 1999) or firing several puffs into the spacer, can avoid static charge accumulation (Berg et al., 1998; Kenyon et al., 1998).

There are now two brands of CFC-free beclometasone MDIs in the UK (Clenil Modulite® and Qvar®). These devices are not equipotent, and in order to limit prescribing errors and avoid confusion, the MHRA advises that CFC-free beclometasone MDIs should be prescribed by brand name. Qvar® contains beclometasone in solution and has been shown to deliver the drug as an extra-fine aerosol that results in a 2-2.5 fold greater potency compared with other CFC-containing beclometasone MDIs (Leach et al., 2002). Clenil Modulite® is equipotent to the CFC- innovator product (Becotide®), therefore, a straightforward substitution of doses can be performed (Chaplin and Head, 2007). The summary of product characteristics (SPC) for Clenil® MDI recommends using the Volumatic spacer.

The first study was designed to investigate the *in-vitro* dose emission characteristics for Clenil® MDI when used alone and when attached to different spacers. The second study was designed to use the urinary beclometasone dipropionate pharmacokinetic method to investigate the relative lung and systemic bioavailability of these spacers.

5.2. *In-vitro* dose emission and aerodynamic particle size distribution of the dose emitted from Clenil® inhaler

5.2.1. Method

The aim of this investigation is to use the Andersen Cascade Impactor (ACI) to determine the aerodynamic particle size distribution of the dose emitted from Clenil® MDI:

I. With different spacers at a flow rate of 28.3L/min.

II. Alone at different flow rates (28.3, 60, and 90 L/ min).

5.2.1.1. Equipment and inhalation devices

Equipment:

- MDI sampling apparatus: Copley Scientific Ltd, UK.
- Andersen MKII Cascade Impactor: Copley Scientific Ltd, UK.
- A/E fibre glass filter discs: 25mm; Pall Corporation, USA.
- GF 50 filter: Copley Scientific Ltd, UK.
- HCP5 pump: High Capacity Pump, Copley Scientific Ltd, UK.
- An Electronic Digital Flow Meter: DFM2000, Copley Scientific Ltd, UK.
- Parafilm M Laboratory film: Pechiney Plastic Packaging, USA.
- Silicone fluid spray: Releasil B silicone spray, Propower silicone lubricant, Premier Farnell, PLC, UK.
- Critical Flow Controller Model TPK2000: Copley Scientific Ltd, UK.

LC-(ESI+)-MS method conditions: sample preparation, analysis procedures, and chromatographic conditions were as reported in section 3.2.

Inhaler and spacer devices used as follows:

- Clenil® metered dose inhaler (MDI) labelled as a nominal dose of 250µg beclometasone dipropionate per dose (Chiesi, UK).

- The Aerochamber Plus spacer [APLUS], 145ml holding chamber, (Trudell Medical International Europe Ltd, UK).
- The Volumatic spacer device [VOL] 750ml holding chamber, (GlaxoSmithKline, UK).
- The Optimiser spacer [OPT], 50ml small plastic tube spacer having a cross section of 2.5 x 3.3cm (Teva Pharmaceuticals, UK).

5.2.1.2. Procedure

5.2.1.2.1. Total emitted dose

The nominal dose is the labelled dose and is the amount that is metered in the device during the inhalation process. The total emitted dose (TED) is the total amount of drug exiting the device and hence available to the user. The dose emitted from Clenil® MDI (labelled as a nominal dose of 250µg beclometasone dipropionate per puff, Chiesi, UK) was determined using the MDI Dose sampling unit (DSU) ; Copley Scientific Ltd, UK. Determinations were made for Clenil® metered dose inhaler alone and when it is attached to each of the following spacers:

- The Aerochamber Plus spacer that is washed in detergent solution, followed by either rinsing [APLUSR] or not rinsing [APLUSNR] with water, and then allowed to air dry.
- The Optimiser spacer that is washed in detergent solution, followed by either rinsing [OPTR] or not rinsing [OPTNR] with water, and then allowed to air dry.
- The Volumatic spacer device that is washed in detergent solution, followed by either rinsing [VOLR] or not rinsing [VOLNR] with water, and then allowed to air dry.

The MDIs were first primed by firing two doses to waste before use (Barry and O'Callaghan, 2003). The Clenil® MDI either alone or connected to each spacer was inserted tightly into the mouthpiece adaptor of the dose sampling unit (DSU) and aligned along the horizontal axis. A High Capacity Vacuum Pump (HCP5, Copley Scientific Ltd, UK) was connected to the apparatus outlet in order to achieve the desired airflow. The MDI sampling unit apparatus (Copley Scientific Ltd, UK) with a critical flow controller model TPK (Copley Scientific Ltd, UK) was used to produce sonic flow conditions according to Pharmacopoeia recommendations (European Pharmacopeia, 2001; British Pharmacopoeia, 2005; United States Pharmacopeia, 2005). The final filter was a 25 mm A/E fibreglass filter (Pall Corporation, USA). Parafilm M Laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus. Two separate doses from Clenil (250µg) were discharged into the DSU. The flow through each MDI / MDI + Spacer was 28.3 L min^{-1} with flow duration of 8.5 sec such that the inhalation volume was 4L. The flow was measured by an electronic digital flow meter (DFM2000, Copley Scientific Ltd, UK). Ten determinations were made for each dose emission (n=10). During each determination, one dose was discharged into the spacer followed by the *in-vitro* inhalation manoeuvre. The procedure was repeated until the set number of doses has been discharged. Following dose emission the dose sampling unit was dismantled and washed with 60:40% methanol: water and the filter was completely immersed in 60:40 methanol: water and sonicated for 5 minutes to remove any filter entrained drug. All solutions collected from the dose sampling unit post Clenil® MDI and Clenil® MDI + spacer actuation was made up to 250ml, and 50ml volume, respectively, while solutions collected from any spacer was made up to 100ml. The amount of drug in the dose sampling unit and the spacer was determined by using the previously developed and validated LC-(ESI+)-MS method previously described in section 3.2 in this thesis.

5.2.1.2.2. The aerodynamic particle size characterization

The aerodynamic particle size characterization is the size of particles or droplets that make the emitted aerosol cloud. It determines the percentage of the total emitted dose that reaches the lungs during an inhalation. The particle size analysis of aerosols from pressurized metered dose inhalers (pMDIs) was determined by using the Andersen Cascade Impactor (ACI) according to compendial procedures (European Pharmacopeia, 2001; United States Pharmacopeia, 2005). This technique provides a direct link with the mass of therapeutically active ingredient and the aerodynamic particle size of the emitted dose, which has been accepted as an indication of the likely site of particles deposition within the respiratory tract (Mitchell et al., 2003). The study was divided into two parts, in the first part the aerodynamic particle size distribution of Clenil® MDI either alone or plus spacers was measured with the Andersen Cascade Impactor (ACI) at a flow rate of 28.3L/min. In the second part, the aerodynamic particle size distribution of Clenil® MDI alone is determined at higher flow rates of 60L/min and 90L/min.

The Andersen Cascade Impactor (ACI) consists of eight stages and a final collection filter (25 mm A/E fibre glass filter, Pall Corporation, USA). All parts of the ACI were first washed in deionised water and acetone and allowed to dry. The collection plates were then coated with Silicone fluid spray (Releasil B silicone spray, Pro-power silicone lubricant, Premier Farnell, PLC, UK) and left to dry for one hour prior to analysis. Parafilm M Laboratory film (Pechiney Plastic Packaging, USA) was used to seal the apparatus. Two actuations from Clenil® MDI (250µg) were delivered into the impactor for each inhaler or inhaler/spacer combination. For the first part of the study, the ACI was assembled with the coated impaction plates according to the effective cut-off diameter of each stage at a flow rate of 28.3L/min for each Clenil® MDI or Clenil® MDI/spacer combination. A Critical Flow Controller model TPK2000 (Copley Scientific Ltd, UK) and an electronic digital flow meter (DFM2000, Copley Scientific Ltd, UK)

was used to adjust the flow rate at 28.3 L/min with flow duration of 8.5 seconds (equivalent to 4L inhalation volume).

For the second part of the study, the ACI was assembled and connected to Clenil® MDI alone to determine the aerodynamic particle size distribution at 60L/min and 90L/min. The modified ACI was used for operating at 60L/min and 90L/min. The Critical Flow Controller Model TK2000 (Copley Scientific, UK) was again used to ensure sonic flow and provide the required inhalation flow and volume. In the 60L/min version, stages, 0 and 7 are removed and replaced with two additional stages, -1 and -0. Similarly, in the 90L/min version, stages 0, 6, and 7 are replaced with three additional stages, -2,-1, and -0. The vacuum flow was provided by a HCP5 (High Capacity Vacuum Pump, Copley Scientific, UK). Five determination were made for each inhaler or inhaler/spacer combination (n=5). The apparatus was dismantled and washed with 60:40% methanol: water and the filter was completely immersed in 60:40 methanol: water and sonicated for 5 minutes to remove any filter entrained drug. All solutions collected from the induction port post Clenil® MDI and Clenil® MDI + spacer actuation was made up to 100ml and 25 ml volume, respectively, while solutions collected from any spacer and from different ACI stages post MDI and MDI+ spacer actuation was made up to volumes 100ml, and 25ml, respectively. The amount of beclometasone dipropionate deposited in the induction port (IP), spacer, and the various ACI stages were determined using the previously developed and validated LC-(ESI+)-MS method described in section 3.2. The amount deposited at the various stages was expressed in µg.

The mass mean aerodynamic diameter (MMAD), geometric standard deviation (GSD), total emitted dose (TED), percentage of fine particle fraction (%FPF) and fine particle dose (FPD) were calculated for each MDI and MDI + spacer using CITDAS software program (Copley Scientific Ltd, UK).

The total emitted dose (TED) is the dose that leaves the inhaler device and is available to the patient. The fine particle dose (FPD) is the cumulative amount of drug particles with size <5µm. The fine particle fraction (% FPF) is the FPD expressed as a percentage of the total amount deposited ex-mouth piece. The mass mean aerodynamic diameter (MMAD) was obtained from the logarithm of the effective cut-off diameter corresponding to 50% undersize. The geometric standard deviation (GSD) is the square root for the size corresponding to 84.1% less than the stated size divided by the square root of the size for 15.9% (GSD= $\sqrt{d84.1/d15.9}$), where d15.9 and d84.1 are the sizes corresponding to the mass-percentile values of 15.9% and 84.1% respectively, for the cumulative size distribution (United States Pharmacopeia, 2005).

5.2.2. Statistical analysis

Statistical analysis of the total emitted dose and aerodynamic particle size characterization of Clenil® MDI alone at different flow rates (28.3, 60, and 90 L/min) and Clenil® MDI alone or with different spacer combinations were carried out by one way analysis of variance (ANOVA) test using SPSS V17.0 (SPSS Inc., Chicago, USA).

5.2.3. Results

5.2.3.1. Total emitted dose

The individual total emitted dose of two 250 µg actuations of beclometasone dipropionate from Clenil® MDI alone or plus different spacers expressed in µg and as percentage of nominal dose is presented in APPENDIX B-27, and B-28 (refer to the enclosed DVD), respectively. A summary of the mean (SD) data is shown in table 5.1. The results are expressed graphically in figure 5.1.

Table 5.1: Mean (SD) Dose emission from two 250µg doses of BDP from Clenil® MDI determined at a flow 28.3 L min-1, expressed in µg and as percentage of nominal dose, (n=10).

		Dose (µg)	% of nominal dose
MDI	**TED**	390.8 (45.6)	78.2 (9.1)
VOLNR	**TED**	227.9 (21.1)	45.6 (4.2)
	Spacer	232.8 (34.9)	46.6 (7.0)
APLUSNR	**TED**	205.2 (48.5)	41.0 (9.7)
	Spacer	206.0 (47.4)	41.2 (9.5)
OPTNR	**TED**	158.7 (17.4)	31.7 (3.5)
	Spacer	220.9 (43.1)	44.2 (8.6)
VOLR	**TED**	163.0 (54.0)	32.6 (10.8)
	Spacer	252.3 (39.8)	50.5 (8.0)
APLUSR	**TED**	152.3 (31.5)	30.5 (6.3)
	Spacer	295.9 (38.3)	59.2 (7.7)
OPTR	**TED**	118.4 (24.1)	23.7 (4.8)
	Spacer	319.3 (47.0)	63.9 (9.4)

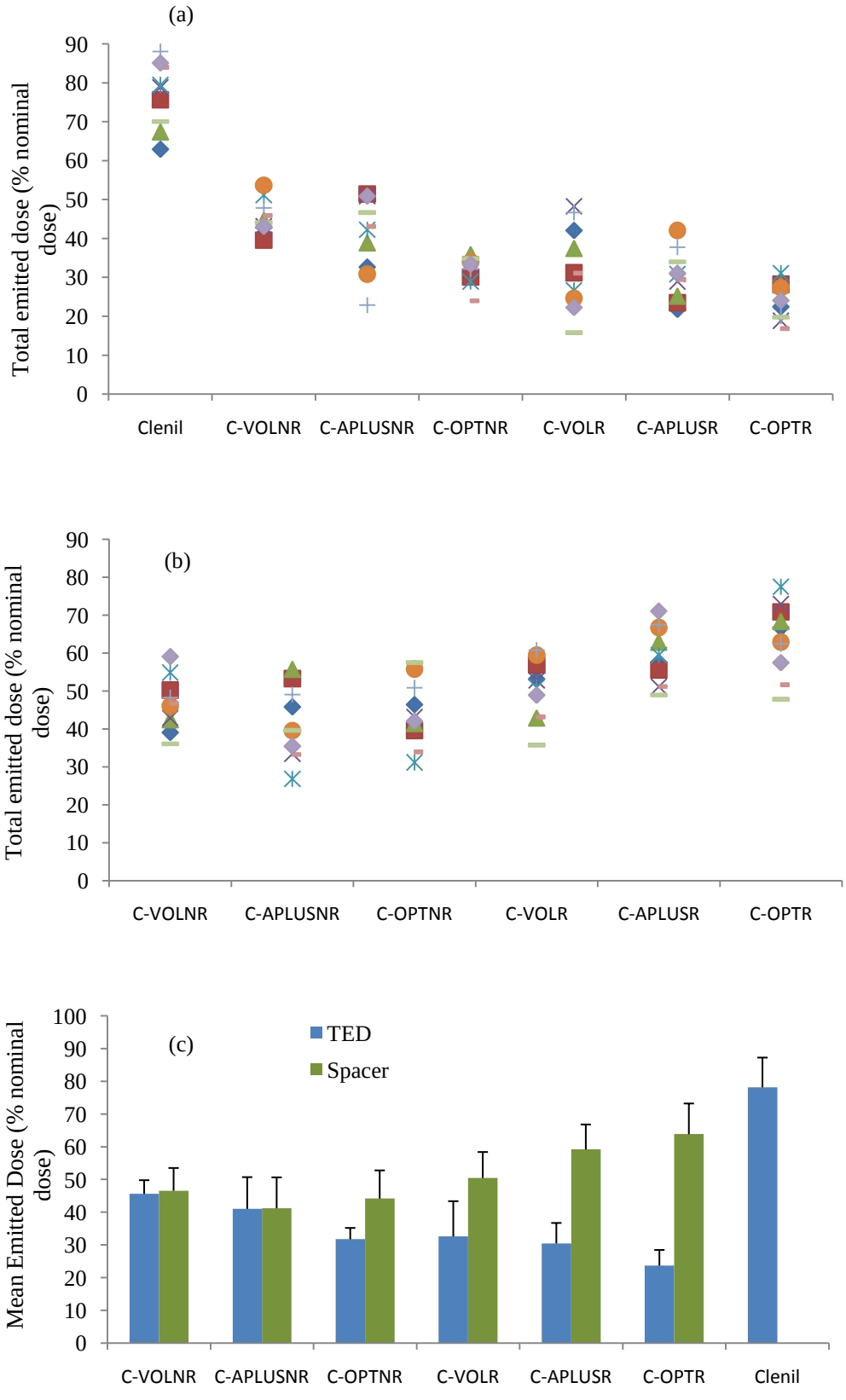


Figure 5.1: Beclometasone dipropionate amounts (a) total emitted dose (b) deposited in each spacer (c) mean (SD) total emitted dose and the amount deposited in each spacer expressed as a percent of the nominal dose obtained from Clenil® MDI at a flow rate 28.3 L min^{-1}, (n=10).

5.2.3.2. Aerodynamic particle size characterization

A summary of the aerodynamic particle size distribution data obtained from the Andersen Cascade Impactor (ACI) for Clenil® MDI (250µg) either alone or plus different spacers at 28.3 L/min are illustrated in table 5.2 and figure 5.2. In addition, the effect of flow rate on particle size distribution of Clenil® MDI was investigated. The results obtained from the Andersen Cascade Impactor for Clenil® MDI at 28.3, 60, and 90L/min are summarized in table 5.3 and figures 5.3 - 5.5.

Table 5.2: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following two actuations of Clenil® MDI (250µg) either alone or plus different spacers at 28.3 L/min. Values quoted in µg unless specified, (n=5).

	Stage Cut-off	MDI	VOLNR	APLUSNR	OPTNR	VOLR	APLUSR	OPTR
Amount left in spacer		-------	224.3 (35.0)	233.9 (25.7)	240.9 (26.6)	271.8 (20.9)	301.6 (49.3)	305.5 (33.9)
Induction Port (IP)		251.3 (22.0)	27.5 (5.7)	31.6 (10.7)	28.7 (7.1)	19.2 (3.4)	26.2 (5.4)	24.3 (6.7)
0	10	7.3 (1.3)	11.2 (2.9)	13.2 (2.9)	11.5 (0.6)	9.0 (1.5)	11.5 (0.6)	5.9 (1.7)
1	9	15.3 (4.1)	12.6 (3.0)	16.1 (3.2)	15.2 (3.1)	12.9 (3.5)	16.0 (2.6)	10.1 (3.6)
2	5.8	15.5 (2.3)	20.4 (5.0)	17.6 (5)	20.1 (7.6)	15.3 (2.4)	15.7 (4.1)	14.2 (3.7)
3	4.7	19.6 (2.8)	24.1 (4.9)	19.3 (5.8)	18.0 (6.4)	21.8 (2.3)	18.4 (6.3)	14.2 (2.6)
4	3.3	18.8 (5.4)	34.2 (4.8)	28.6 (7.9)	19.7 (4.0)	28.3 (5.4)	25.5 (4.7)	18.4 (4.7)
5	2.1	24.8 (9.4)	41.6 (9.7)	36.9 (3.6)	20.6 (2.1)	25.8 (1.9)	17.2 (8.5)	10.6 (0.5)
6	1.1	13.8 (2.7)	23.9 (5.2)	28.8 (8.1)	12.9 (3.3)	14.5 (2.7)	9.8 (2.7)	6.4 (1.6)
7	0.7	11.8 (1.7)	14.2 (4.9)	13.1 (4.6)	8.7 (4.0)	7.0 (2.1)	7.1 (2.4)	3.9 (2.0)
Filter	0.4	3.8 (1.4)	9.4 (1.2)	6.9 (2.9)	7.9 (2.7)	8.4 (1.7)	7.5 (3.3)	4.4 (2.6)
Total emitted dose (TED) (µg)		**381.8 (6.3)**	**218.9 (23)**	**212.1 (21.0)**	**163.4 (15.2)**	**162.2 (13)**	**155.3 (15.4)**	**112.5 (8.0)**
Total emitted dose (% of nominal dose)		**76.4 (1.7)**	**43.8 (4.6)**	**42.4 (4.2)**	**32.7 (3.0)**	**32.44 (2.6)**	**31.1 (3.1)**	**22.5 (1.6)**
FPD (µg)		**97.6 (20.8)**	**153.9 (19.4)**	**138.8 (22.2)**	**93.3 (17.6)**	**110.6 (7.4)**	**90.6 (18.8)**	**62.7 (8.2)**
% FPF of nominal dose		**19.5 (4.2)**	**30.8 (3.9)**	**27.8 (4.4)**	**18.8 (3.5)**	**22.1 (1.5)**	**18.1 (3.8)**	**12.5 (1.6)**
%FPF of TED		**25.6 (5.4)**	**70.3(5.6)**	**65.2 (4.9)**	**57.6 (5.3)**	**68.3 (2.1)**	**58.1 (7.2)**	**55.6 (3.6)**
MMAD (µm)		**2.8 (0.4)**	**2.3 (0.1)**	**2.2 (0.1)**	**3.1 (0.2)**	**2.7 (0.2)**	**3.1 (0.3)**	**3.3 (0.3)**
GSD (no units)		**2.2 (0.2)**	**2.3 (0.2)**	**2.7 (0.3)**	**2.3 (0.1)**	**2.2 (0.1)**	**2.2 (0.1)**	**1.8 (0.1)**

Table 5.3: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following two actuations of Clenil® MDI (250µg) alone at 28.3, 60, and 90 L/min flow rates. Values quoted in µg unless specified, (n=5).

	28.3L/min	**60L/min**	**90L/min**
Induction Port (IP)	251.3 (22.0)	200.8 (19.4)	187.3 (14.7)
-2	---	---	17.2 (4.8)
-1	---	13.1 (3.1)	17.1 (3.6)
-0	---	19.1 (3.3)	14.7 (3.5)
0	7.3 (1.3)	---	---
1	15.3 (4.1)	17.4 (2.4)	12.2 (1.7)
2	15.5 (2.3)	20.1 (4.1)	20.2 (5.7)
3	19.6 (2.8)	23.0 (2.4)	42.1 (3.2)
4	18.8 (5.4)	38.6 (2.1)	19.6 (1.1)
5	24.8 (9.4)	27.9 (1.5)	10.2 (1.0)
6	13.8 (2.7)	14.5 (6.0)	---
7	11.8 (1.7)	8.6 (0.7)	---
Filter	3.8 (1.4)	---	8.0 (2.0)
Total emitted dose (TED) (µg)	**381.8 (6.3)**	**383.2 (22.7)**	**348.6 (19.0)**
Total emitted dose (% of nominal dose)	**76.4 (1.7)**	**76.6 (4.5)**	**69.7 (3.8)**
FPD (µg)	**97.6 (20.8)**	**138 (7.1)**	**116.5 (5.7)**
% FPF of nominal dose	**19.5 (4.2)**	**27.6 (1.4)**	**23.4 (1.1)**
%FPF of TED	**25.6 (5.4)**	**36.1 (3.3)**	**33.6 (0.5)**
MMAD (µm)	**2.8 (0.4)**	**2.2 (0.1)**	**2.2 (0.2)**
GSD (no units)	**2.2 (0.2)**	**2.9 (0.2)**	**3.3 (0.2)**

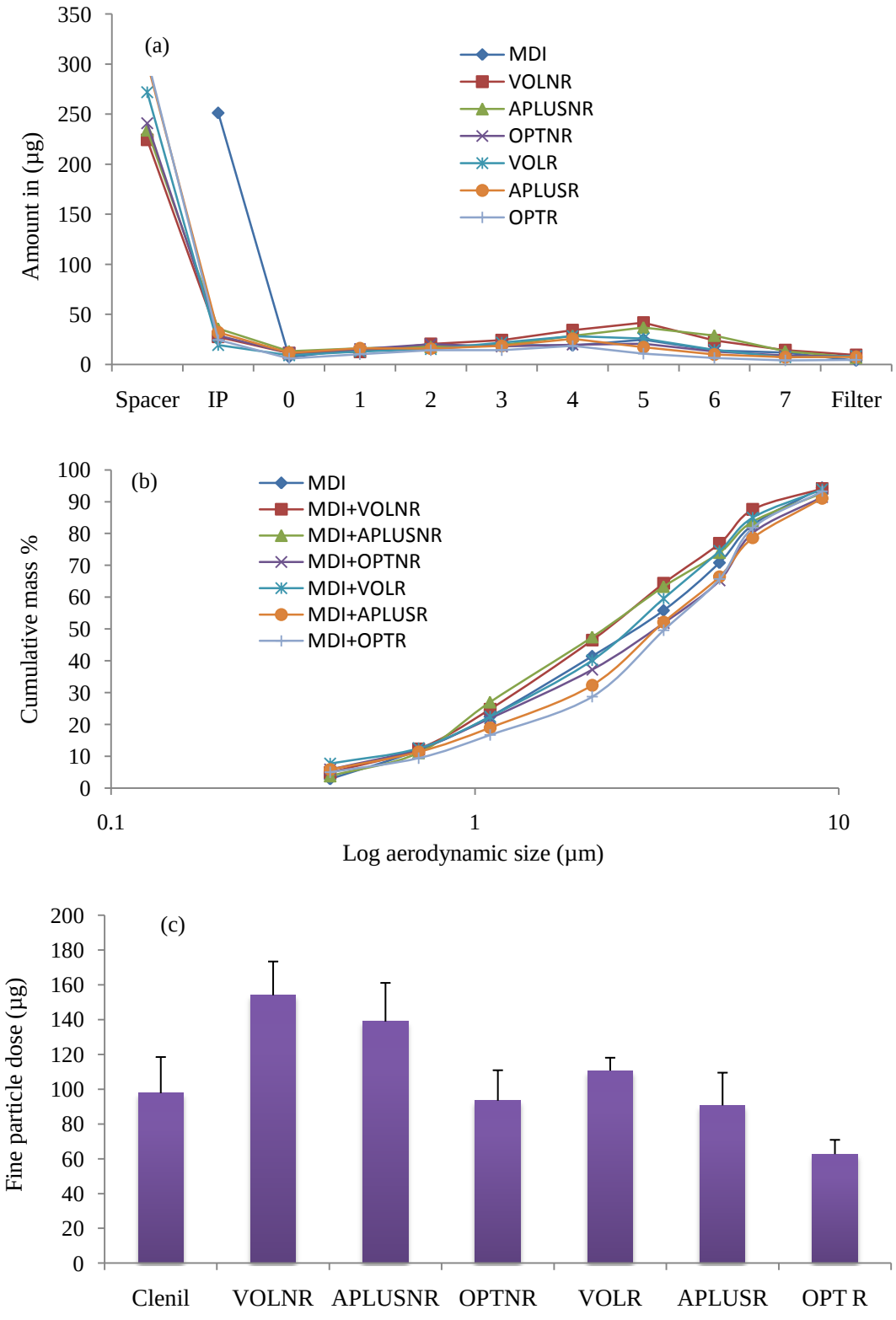


Figure 5.2: Mean beclometasone dipropionate emitted from Clenil MDI alone or with different spacers at a flow rate of 28.3 L min^{-1} (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose, (n=5).

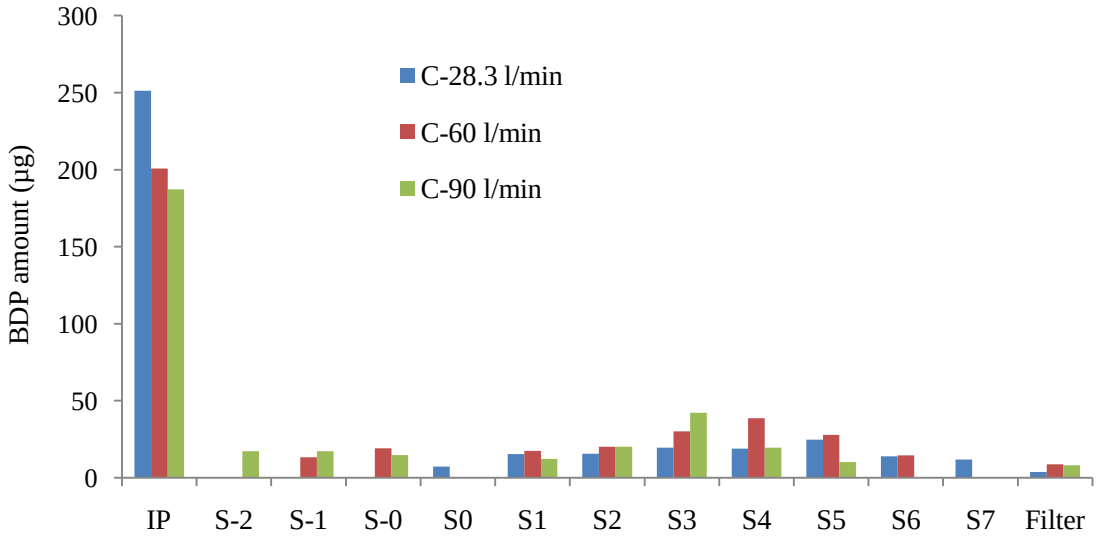


Figure 5.3: Mean amount of beclometasone dipropionate deposited in each stage of the ACI from Clenil® MDI alone at 28.3, 60, and 90 L min^{-1} flow rates, expressed in µg, (n=5).

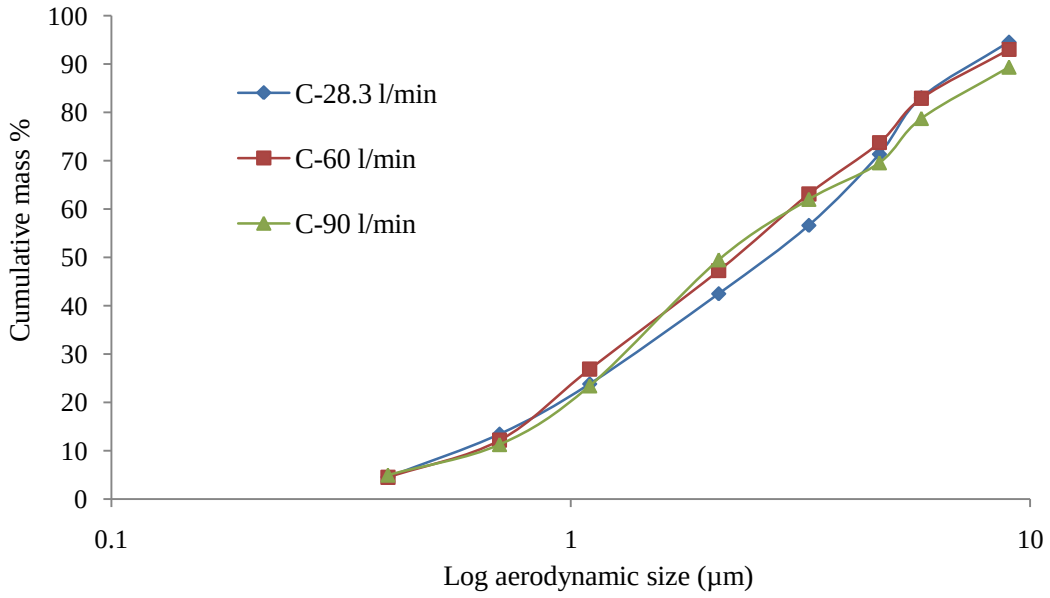


Figure 5.4: The mean aerodynamic distribution of the emitted dose of beclometasone dipropionate emitted from Clenil® MDI alone at 28.3, 60, and 90 L min^{-1} flow rates, (n=5).

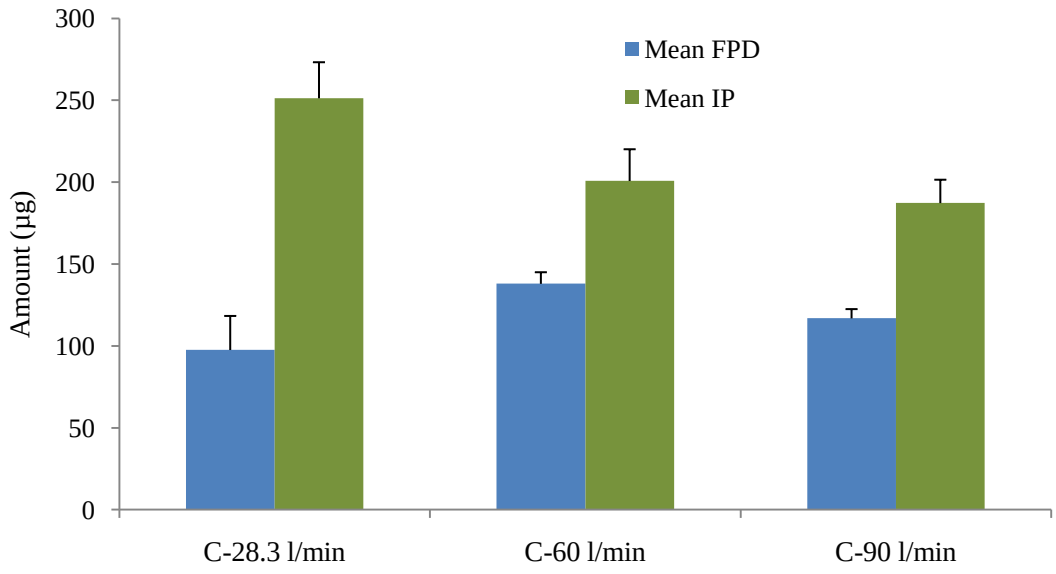


Figure 5.5: Mean (SD) fine particle dose and induction port deposition of beclometasone dipropionate emitted from Clenil MDI alone at 28.3, 60, and 90 L min-1 flow rates, expressed in µg, (n=5).

5.2.3.3. Statistical analysis

A summary of the statistical analysis when using different spacers with Clenil® MDI at 28.3L/min is presented in table 5.4. The statistical analysis data of Clenil® MDI operated at different flow rates is summarized in table 5.5. The FPD and % FPF of the emitted dose of the MDI operated at 28.3L/min was significantly lower than that at 60 L/min ($p<0.05$). In addition, the amount of drug deposited in the induction port was significantly lower ($p<0.05$) for the 90L/min when compared to that obtained at 28.3L/min.

Table 5.4: Mean difference (95% confidence interval) for different spacers used with Clenil® MDI.

Comparator		FPD (µg)	FPF%	MMAD	TED (µg)	Spacer deposition(µg)
VOLNR	C-MDI	56.3 (26.5,86.2)**	44.7 (35.8, 53.6)***	-0.5 (-1,-0.04)*	-162.9 (-192.4, -133.4)***	---
	APLUSNR	-15.07 (-44.9,14.8)	-5.1 (-14,3.9)	-0.03 (-0.5,0.4)	-6.9 (-36.4, 22.6)	9.7 (-55.3, 74.6)
	OPTNR	60.6 (30.7, 90.4)***	12.7 (3.7, 21.6)**	-0.8 (-1.3, -0.3)**	55.5 (26, 85)**	-16.7 (-81.6, 48.3)
	VOLR	43.3 (13.5, 73.1)**	2.0 (-6.9,10.9)	-0.4 (-0.9,0.1)	56.7 (27.2, 86.2)**	-47.6 (-112.5, 17.4)
	APLUSR	63.3 (33.5,93.2)***	12.2 (3.2, 21.1)*	-0.8 (-1.3,-0.4)**	63.7 (34.2, 93.2)***	-77.3 (-142.2,-12.4)*
	OPTR	91.3 (61.4, 121.1)***	14.7 (5.8, 23.6)**	-1.0 (-1.5, -0.6)***	106.5 (77, 136)***	-81.2 (-146.2, -16.2)*
APLUSNR	C-MDI	41.3 (11.4, 71.1)*	39.6 (30.7, 48.6)***	-0.5 ((-1,-0.08)*	-169.7 (-199.2, -140.2)***	---
	OPTNR	45.5 (15.7,75.3)**	7.6 (-1.3,16.5)	-0.8 (-1.3,-0.4)**	48.6 (19.1,78.1)**	-7.0 (-72,58)
	VOLR	28.2 (-1.6,58.1)	-3.1 (-12,5.9)	-0.4 (-0.9,0.02)	49.9 (20.4,79.4)**	-37.9 (-102.9,27.1)
	APLUSR	48.3 (18.4, 78.1)**	7.1 (-1.8,16)	-0.9 (-1.3, -0.4)**	56.8 (27.3, 86.1)**	-67.7 (-132.6,-2.7)*
	OPTR	76.2 (46.3,106)***	9.6 (0.7,18.6)*	-1.1 (-1.5,-0.6)***	99.6 (70.1, 129.1)***	-71.5 (-136.5, -6.6)*
OPTNR	C-MDI	-4.2 (-34.1,25.6)	32.0 (23.1, 41)***	0.3 (-0.2, 0.8)	-218.4 (-247.9, -188.9)***	---
	VOLR	-17.3 (-47.1,12.6)	-10.7 (-19.6,-1.7)*	0.4 (-0.06,0.9)	1.2 (-28.3, 30.7)	-30.9 (-95.9,34.1)
	APLUSR	2.8 (-27.1,32.6)	-0.5 (-9.4,8.4)	-0.03 (-0.5,0.4)	8.2 (-21.3,37.7)	-60.7 (-125.6, 4.3)
	OPTR	30.7 (0.8, 60.5)*	2.0 (-6.9,11)	-0.2 (-0.7,0.2)	51.0 (21.5, 80.5)**	-64.5 (-129.5, 0.5)
VOLR	C-MDI	13.0 (-16.8,42.9)	42.7 (33.8, 51.6)***	-0.1 (-0.6, 0.4)	-219.6 (-249.1, -190.1)***	---
	APLUSR	20.0 (-9.8,49.9)	10.2 (1.2, 19.1)*	-0.4 (-0.9,0.02)	6.9 (-22.6,36.4)	-29.8 (-94.7, 35.2)
	OPTR	48.0 (18.1,77.8)**	12.7 (3.8, 21.6)**	-0.6 (-1.1, -0.2)*	49.7 (20.2, 79.2)**	-33.6 (-98.6, 31.3)
APLUS R	C-MDI	-7.0 (-36.8,22.8)	32.5 (23.6, 41.5)***	0.3 (-0.1, 0.8)	-226.5 ((-256, -197)***	---
	OPTR	27.9 (-1.9, 57.8)	2.5 (-6.4, 11.5)	-0.2 (-0.7, 0.3)	42.8 (13.3, 72.3)**	-3.9 (-68.8, 61.1)
OPTR	C-MDI	-34.9 (-69.8, -5.1)*	30.0 (17.5, 42.5)***	0.5 (0.08,1)*	-269.3 (-298.8, -239.8)***	---

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 5.5: Mean difference (95% confidence interval) for Clenil® MDI operated at different flow rates.

Comparator		FPD (µg)	FPF%	MMAD	TED (µg)	Induction port deposition (µg)
28.3L/min	60 L/min	-40.4 (-71.5, -9.3)*	-10.6 (-20.6, -0.5)*	0.6 (-0.07,1.27)	-1.4 (-37.6, 34.9)	50.6 (-1.4, 102.5)
	90L/min	-19.3 (-50.5, 11.7)	-8.0 (-18.1, 2.1)	0.6 (-0.07,1.27)	33.2 (-3.0, 69.5)	64.0 (12.1, 116)*
60 L/min	90L/min	21.04 (-10.1, 52.1)	2.6 (-7.5, 12.6)	$-2.4x10^{-16}$ (-0.7, 0.7)	34.6 (-1.7, 70.9)	13.5 (-38.5, 65.4)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

5.2.4. Discussion

This study clearly demonstrates that the total amount of drug as well as the FPD and the FPF obtained from the same dose of Clenil® MDI was greatly affected by the different spacers used. The results show that the total emitted dose from the MDI alone is significantly ($p<0.001$) greater than that from all MDI/spacers combinations used. This is consistent with the markedly greater amounts of drug deposited in the induction port when using the MDI compared to other inhalation methods using the spacer. This amount deposited in the induction port is considerably important as it represents the oropharyngeal cavity of the patient. This is very beneficial in the case of inhaled steroids as the spacer walls becomes the major site of drug deposition and not the oropharynx. The reduction in the oropharyngeal drug deposition by spacers limits the occurrence of local side effect (e.g., oral candidiasis and dysphonia) (Salzman and Pyszczynski, 1988; Fergusson et al., 1991; Hanania et al., 1995; Hardy et al., 1996; Zainudin, 1997; Buhl, 2006) and systemic side effects (Brown et al., 1990; Selroos and Halme, 1991; Meeran et al., 1995; O'Callaghan and Barry, 1999) following inhaled corticosteroids therapy. Salzman and Psyszczynski (1988) compared the administration of BDP to systemic steroid dependent patients using the MDI alone or the MDI attached to the Aerochamber spacer. The addition of the Aerochamber spacer in this study was very advantageous as besides eliminating the oropharyngeal thrush and reducing candida colonisation from 66% to 33%, it also improved the FEV_1 gradually leading to cessation of the systemic corticosteroid therapy to many patients over 6 months.

This different behaviour of the MDI when used with or without a spacer is due to the space that the spacer provides. This distance reduces the primary droplet size by providing extra time for the complete evaporation of the propellant and slows down the fast moving aerosol. Thereby, it increases the sedimentation of these large particles on the spacer walls. In contrast, when using the MDI alone, the aerosol particles travel at

high speed, which enhances their deposition in the induction port and therefore, decreases the FPF. This is in agreement with a previous beclometasone dipropionate study that reported a significant reduction in the amount of non-respirable BDP available for inhalation when using a spacer. In this study using the large Volumatic spacer device increased the amount of drug delivered to the lung while decreasing the total steroid dose available to the patient (O'Callaghan et al., 1994).

It is evident from the above results that differences in handling and washing the spacers greatly affected the aerodynamic particle size distribution of inhaled aerosols. This may be explained by the different electrostatic properties of both not rinsed (NR) and rinsed (R) spacers. Several studies have demonstrated that most commercially available MDIs are highly charged especially the new HFA-formulations, which were found to even have greater electrostatic charge than their CFC predecessors (Peart et al., 2003; Kwok et al., 2006; Mitchell et al., 2007b). In addition to the charge of the aerosol from the MDI, the electrically insulated material of plastic spacers is also prone to develop electrostatic charge (ESC) due to frictional contact during handling. Thus, when the highly charged aerosol particles comes into contact with the plastic spacer device inherent electrostatic charge, mutual repulsion between the charged particles causes them to move to the periphery of the aerosol cloud and contact the spacer walls. Consequently, this leads to aerosol drug retention within these devices, resulting in a significant reduction of the drug aerosol available for inhalation. However, several methods reported in the literature have been found to significantly avoid this electrostatic charge accumulation on spacer surfaces, thus allowing its optimum drug delivery. *In-vitro* studies have shown that coating plastic spacer with an antistatic lining increased the fine particle dose of sodium cromoglycate from a Fisonair spacer (O'Callaghan et al., 1993), and the fine particle dose of budesonide from the Nebuhaler spacer (Barry and O'Callaghan, 1995). Alternatively,

washing plastic spacers in detergent and leaving it to drip dry was also described as an effective method for reducing the electrostatic charge in spacer devices.

As shown from the results, washing the spacer in detergent and leaving it to drip dry without subsequent water rinsing was found to give significantly more emitted dose and FPD from the spacer than when the same spacer is rinsed with water following detergent use. In addition, the lower MMAD of the not rinsed spacers compared to the rinsed ones implies that there are differences in the aerosol particles behaviour and distribution with these two different handling methods. This was also confirmed by the observed decrease in the amount of drug deposited in the not rinsed spacers compared to the rinsed ones. The mean (SD) amounts of BDP deposited in the spacer were 224.3 (35) vs 271.8 (20.9), 233.9 (25.7) vs 301.6 (49.3), and 240.6 (26.6) vs 305.5 (33.9) for the VOLNR vs VOLR, the APLUSNR vs APLUSR, and for the OPTNR vs OPTR. This difference can be explained by the different levels of electrostatic charge accumulated on the surface of these NR and R plastic spacers. Soaking the spacer in detergent solution without subsequent rinsing was more successful in eliminating the electrostatic charge from the spacer surface leading to less attraction of the charged aerosol particles on the spacer walls, thus increasing drug output. In contrast, the detergent coated followed by water rinsing washing protocol did not provide adequate protection against electrostatic charge, which may further leads to inconsistent drug delivery. These findings are consistent with many studies that found this washing procedure more effective in reducing the spacers charge and increasing lung deposition. Coating with detergents without subsequent rinsing has been shown to increase the fine particle fraction of salbutamol by approx. 55% to 70% compared to unwashed highly charged spacers (Wildhaber et al., 1996b). Pierart et al (1999) observed that water rinsed spacers had a substantial electrostatic charge and lower salbutamol fine particle delivery. The mean (SD) percentages of the

label claim emitted mass/actuation, were 53.1(3.1) % vs. 36.2 (3.5) % for the detergent treated when not rinsed and the detergent treated rinsed spacers, respectively.

In addition, the results from this study show that the amount of drug in the potentially respirable aerodynamic particle size range varies considerably for a particular MDI, depending on the spacer used. The results clearly show a more significant FPD from the not rinsed Volumatic (VOLNR) at $p<0.01$ and the not rinsed Aerochamber Plus (APLUSNR) at $p<0.05$ combination with C-MDI than when using the MDI alone. However, the FPD from the MDI alone was non-significant when compared with the rest of the spacers used except with the rinsed Optimiser (OPTR) spacer that significantly decreased the FPD obtained from Clenil® inhaler ($p<0.05$). On the other hand, the % FPF of the emitted dose from the MDI alone was significantly lower ($p<0.001$) than that obtained when using any MDI/spacer combination.

Terzano and Mannino (1999) have shown that using the Volumatic spacer with beclometasone dipropionate significantly reduced the MMAD and increased the percentage of fine particles than when using the MDI alone. This is also in accordance with another *in-vitro* study by Feddah et al (2001) who reported a significant increase in the fine particle mass (FPM) of BDP from different commercially available MDI products with the Volumatic spacer and suggested that the respirable dose appears to be a function of the shape and volume of the spacer device. Other studies investigated the effect of using spacer devices with an HFA solution formulation of BDP and reported a marked increase in the FPF when using the Aerochamber plus and the Ace spacer devices than when using the MDI alone (Williams et al., 2001; Smyth et al., 2004).

The results showed that when comparing the three detergent-washed not water rinsed spacers, more FPD and lower MMAD was delivered at the following order via the Volumatic (VOLNR), the Aerochamber Plus (APLUSNR), and then, the Optimiser

(OPTNR). The not rinsed Volumatic (VOLNR) spacer used with the MDI showed the least amount of drug deposition inside the spacer with more BDP delivered to the impactor and consequently a higher FPF than all other methods. Both the not rinsed Aerochamber Plus (APLUSNR) and the not rinsed Volumatic (VOLNR) significantly increased the FPD when compared to the MDI alone while other spacers did not.

This different BDP aerosol behaviour form these spacers may be due to their different shapes, sizes, and washing procedures. Despite of the spacers' simple concept and structure, these variations between spacers were found to affect the amount of drug available for inhalation by altering its dose emission characteristics. When a dose is discharged into a spacer, impaction of particles on its walls is expected to increase with decreasing the spacer size due to the greater plume velocity in smaller spacers. Furthermore, the larger volume spacer such as the Volumatic would result in a more efficient evaporation of the aerosol dose (Mazhar and Chrystyn, 2008). Thus, in this study the smaller size of the Optimiser spacer may not have been sufficient to allow complete evaporation of the aerosol propellant before reaching the impactor. This was further confirmed by the smaller emitted dose from the smaller volume spacers compared to the larger volume ones. In this study the mean (SD) of the total emitted dose of the not rinsed Volumatic (750ml), the not rinsed Aerochamber Plus (150ml), and the not rinsed Optimiser (50ml) were 218.9 (23.0), 212.0 (21.0), and 163.4 (15.2), respectively.

Mazhar and Chrystyn (2008) compared the *in-vitro* aerodynamic particle size distribution and the *in-vivo* drug delivery obtained from Ventolin Evohaler (GlaxoSmithKline, UK) when attached to the Volumatic (VOL) and Aerochamber Plus valved holding chamber (APLUSVHC). This study reported a higher fine particle dose, smaller MMAD, and small increase in lung bioavailability when using the larger Volumatic spacer when compared to the smaller APLUSVHC spacer.

Barry and O'Callaghan (1996) compared the output of sodium cromoglycate, salbutamol, and budesonide from different spacer devices and reported considerable differences in their drug delivery. In this study, the dose of sodium cromoglycate in small particles recovered from the large volume Fisonair and the small volume spacer were 118% and 33%, respectively than that recovered from the MDI alone. However, this large difference in the spacer behaviour with sodium cromoglycate did not occur with budesonide as the amount of budesonide recovered from the larger and the small volume spacer were 92 and 78%, respectively which indicates that the effects of spacers can also change with the type of drug used. In addition, a year later O'Callaghan (1997) has reported that the output of sodium cromoglycate particles with an aerodynamic diameter of less than 5 μm increases with spacer length and diameter. Several previous studies have reported that more small particle beclometasone, fluticasone (125μg) and salmeterol was recovered from the Volumatic spacers than from the Aerochamber ($p<0.001$) (Barry and O'Callaghan, 1999). Similarly, a recent *in-vitro* study reported an increased respirable dose of salbutamol exiting the large volume spacers (>500ml) compared to smaller ones (<250ml) (Hall et al., 2011). Thus, as previously published (Agertoft and Pedersen, 1994; Ahrens et al., 1995; Barry and O'Callaghan, 2000) and further confirmed in this study, the size of the spacer may affect the drug amount available for inhalation.

It was previously reported that small volume spacers (<100ml) can actually reduce the amount of respirable drug available to the patient, compared to the use of the MDI alone, and they offer no protection against hand-breath coordination. In contrast, large volume spacers have been shown to offer good protection against poor hand breath coordination as well as reducing oropharyngeal deposition without reducing the respirable dose available to the patient (Kim et al., 1987; Wilkes et al., 2001).

The influence of higher inspiratory flow rates (60 and 90L/min) on the aerosol particle size distribution of Clenil® MDI was also evaluated and compared to that at 28.3L/min. As shown from the results illustrated in tables 5.3 and 5.5, increasing the flow rate from 28.3 L/min to 60 L/min showed a lower MMAD and a significant increase ($p<0.05$) in both the FPD and the % FPF. Also increasing the flow rate from 28.3L/min to 90 L/min were associated with lower MMAD and higher FPD for the 90L/min flow rate compared to the 28.3L/min flow rate, however, the results were non-significant ($p>0.05$).

These results are consistent with a previous *in-vitro* study that reported a significant increase in the fine particle mass (FPM) of salbutamol when the flow rate was increased from 30 to 55L/min (Smith et al., 1998). Similarly a later study reported that the FPM of Becotide® inhaler (100µg BDP), Flixotide® inhaler (250µg FP), and Pulmicort® aerosol (200µg BUD) were significantly increased when the flow rate increased from 30L to 60L/min and not much affected when the flow rate increased from 60L to 90L/min. This same study also demonstrated the effect of flow rate on the MMAD and showed significantly lower MMAD when increasing the flow rate from 30 to 60L/min for BDP and FP inhalers and when increasing the flow rate from 60 to 90L/min for the Budesonide inhaler (Feddah et al., 2000). These findings were further confirmed by a more recent study that demonstrated that the effect of different flow rates on the aerosol particles performance. This study reported that increasing the flow rate form 30L/min to 60L/min led to a significant increase in the FPF from 35.4±0.5% to 41.5±1.3% and from 35.9±0.5% to 44.7±0.98% for FP in Flixotide®, and Seretide® inhalers, respectively (Hoe et al., 2009). However, these *in-vitro* studies are in contrast with previous *in-vivo* (Newman et al., 1982b; Tomlinson et al., 2005) and *in-vitro* lung deposition studies that showed that higher flows reduce (Terzano and Mannino, 1999) or even not affect lung deposition (Ross and Schultz, 1996).

Theoretically, if the flow is increased the impaction would be greater. However, the results show the opposite. This could be due to the design and the material of the induction port. However, the decrease in amounts deposited in the induction port needs some examination. The results show that particle bounce may be occurring. This should increase the amounts deposited on the first few stages. It is expected that the fine particle dose should remain the same. Whether particle bounce is occurring is difficult to explain because the plates were coated with silicone. At this stage no conclusion can be drawn except that others have replicated the same phenomenon (Smith et al., 1998) and thus warrants further investigation.

5.3. Relative lung and systemic bioavailability of beclometasone dipropionate inhaled from Clenil® metered dose inhaler with different spacers using urinary drug excretion post inhalation

5.3.1. Method

The aim of this investigation is to apply the urinary pharmacokinetic method of beclometasone dipropionate after an inhalation to highlight the advantage of spacers to improve lung deposition, reduce systemic delivery, and compare different spacers when attached to a Clenil® metered dose inhaler. In addition, to determine the effect of different spacer handling procedures on drug delivery by comparing drug output from either water rinsed or not rinsed detergent coated spacers.

5.3.1.1. Equipment and inhalation device

Inhaler and spacer devices used as follows:

- Clenil® metered dose inhaler (MDI) labelled as nominal dose of 250µg beclometasone dipropionate per dose (Chiesi, UK).
- The Aerochamber Plus spacer [APLUS], 145ml holding chamber, (Trudell Medical International Europe Ltd, UK).

- The Volumatic spacer device [VOL], 750ml holding chamber, (GlaxoSmithKline, UK).
- The Optimiser spacer [OPT], 50ml small plastic tube spacer having a cross section of 2.5 x3.3cm (Teva Pharmaceuticals, UK).

LC-(ESI+)-MS method conditions: sample preparation, analysis procedures, and chromatographic conditions were as reported in section 3.3 in this thesis.

5.3.1.2. Subjects and study design

Ethical approval for the study was obtained from the University of Huddersfield. Twelve healthy (six females), non-smoking volunteers older than 18 years with an average FEV_1> 90% of predicted, gave their written consent to take part in the study. Clenil Modulite® MDI was examined with different spacers, APLUS, VOL, and OPT. Each spacer-MDI combination was assessed following adequate washing of the spacer with detergent followed by either thoroughly rinsing (R) or not rinsing with water (NR). All spacers were allowed to air dry before each study. The order of administration was randomised and there was a 7-day break between each study inhalation.

On separate study days, following a light breakfast each subject inhaled the following doses.

Eight 250µg (2 mg in total) inhalations of beclometasone dipropionate from a Clenil Modulite® metered dose inhaler (Chiesi, UK) used with

- No spacer [Clenil® MDI].
- The Aerochamber Plus spacer that is pre-washed in detergent solution, followed by either rinsing [C-APLUSR] or not rinsing [C-APLUSNR] with water, and then allowed to air dry.

- The Optimiser spacer that is pre-washed in detergent solution, followed by either rinsing [C-OPTR] or not rinsing [C-OPTNR] with water, and then allowed to air dry.
- The Volumatic spacer device that is pre-washed in detergent solution, followed by either rinsing [C-VOLR] or not rinsing [C-VOLNR] with water, and then allowed to air dry.

All subjects were trained on how to use the inhaler devices according to the patient information leaflet. When using the MDI, subjects were trained to remove the cap, exhale slowly as far as comfortable, put the MDI into their mouth, and seal their lips round the mouthpiece. They were then instructed to start a slow inhalation through their mouth and actuate the MDI immediately after the start of this slow inhalation. This slow inhalation continued until their lungs were full of air (total lung capacity) usually over 3-5 seconds. After inhalation they held their breath for 10 seconds and the next dose was inhaled 30 seconds later (Hindle et al., 1993). All subjects were also trained to standardize their inhalation technique when using spacers according to the instructions produced by the manufacturer. When using spacers, subjects exhaled to residual volume as much as possible, the dose was discharged into the spacer and within one second subjects inhaled slowly and deeply for about 3 to 5 seconds. This was followed by a breath hold for at least 10 seconds. The doses were repeated as required after waiting for about 30 seconds between doses. Subjects emptied their bladder prior to each study dose and then urine samples were collected at 30 minutes, and cumulatively for 24 hours post dosing of each study dose. The volume of urine excreted was recorded and aliquots of each sample were frozen at -20°C prior to analysis.

5.3.2. Analysis

5.3.2.1. Sample analysis

The LC-(ESI+)-MS method with solid phase extraction that has been developed and validated for the assay of beclometasone dipropionate and its metabolites from urine samples was used to identify amounts excreted in the urine samples as explained in section 3.3. In addition, the amount of drug left in each spacer device was determined by the LC-(ESI+)-MS method described in section 3.2.

5.3.2.2. Statistical analysis

Statistical analysis of the 30 minutes and the 24hr urinary excretion of beclometasone dipropionate and its metabolites following administration of Clenil® MDI either alone or with water rinsed or not rinsed detergent coated spacers and the amount left in the spacers were accomplished using a one way analysis of variance (ANOVA) test using SPSS V17.0 (SPSS Inc., Chicago, USA). The mean difference with 95% confidence interval was calculated for each inhalation method. In addition, One-way analysis of variance with the application of Bonferroni correction was used to determine any difference between the urinary excretions of Clenil when used alone and when it is attached to each spacer. To identify equivalence of the urinary excretions between the inhalation methods, the 30 minutes and cumulative 24hr amounts, excreted for each inhalation method, were normalised for the nominal dose and then log transformed. From the mean square error of the analysis of variance, using patients and inhalation method as the main factors, the mean ratio (90% confidence interval) was calculated.

5.3.3. Results

Twelve (six females) healthy non smoking subjects completed the study. Their mean (SD) age, weight and height was 31.2 (8.9) years, 66.3 (8.1) kg and 166.7 (7.6) cm, respectively. The demographic details of the participants are described in table 5.6.

Table 5.6: Demographic data of the volunteers that participated in the study, (n=12).

Subject	Sex	Age (years)	Height (cm)	Weight (kg)
1	Female	27	160	65
2	Male	30	165	75
3	Male	28	166	55
4	Male	33	178	60
5	Female	29	155	60
6	Male	23	168	67
7	Male	32	174	71
8	Female	19	160	71
9	Male	23	168	71
10	Female	51	161	63
11	Female	37	165	56
12	Male	42	180	82
Mean (SD)	**-**	**31.2 (8.9)**	**166.7 (7.6)**	**66.3 (8.1)**

The individual urinary amounts of beclometasone dipropionate and its metabolites 17-BMP and BOH excreted at 0.5hr, and 24hr post dose, for each of the twelve volunteers post eight inhalation from Clenil® MDI alone or with different spacers are shown in APPENDIX B.29-B.31 (refer to the enclosed DVD) and figures 5.6 - 5.7. The amount of beclometasone dipropionate left in each spacer device following inhalation of Clenil® MDI (250µg) study doses is shown in APPENDIX B.32 (refer to the enclosed DVD). A summary of the mean (SD) amounts of urinary BDP and its metabolites excreted from the twelve subjects 0.5hr, 24 hours and the amount retained in each spacer device post inhalation from Clenil® MDI either alone or with different spacers is represented in table 5.7 and figures 5.8 - 5.9.

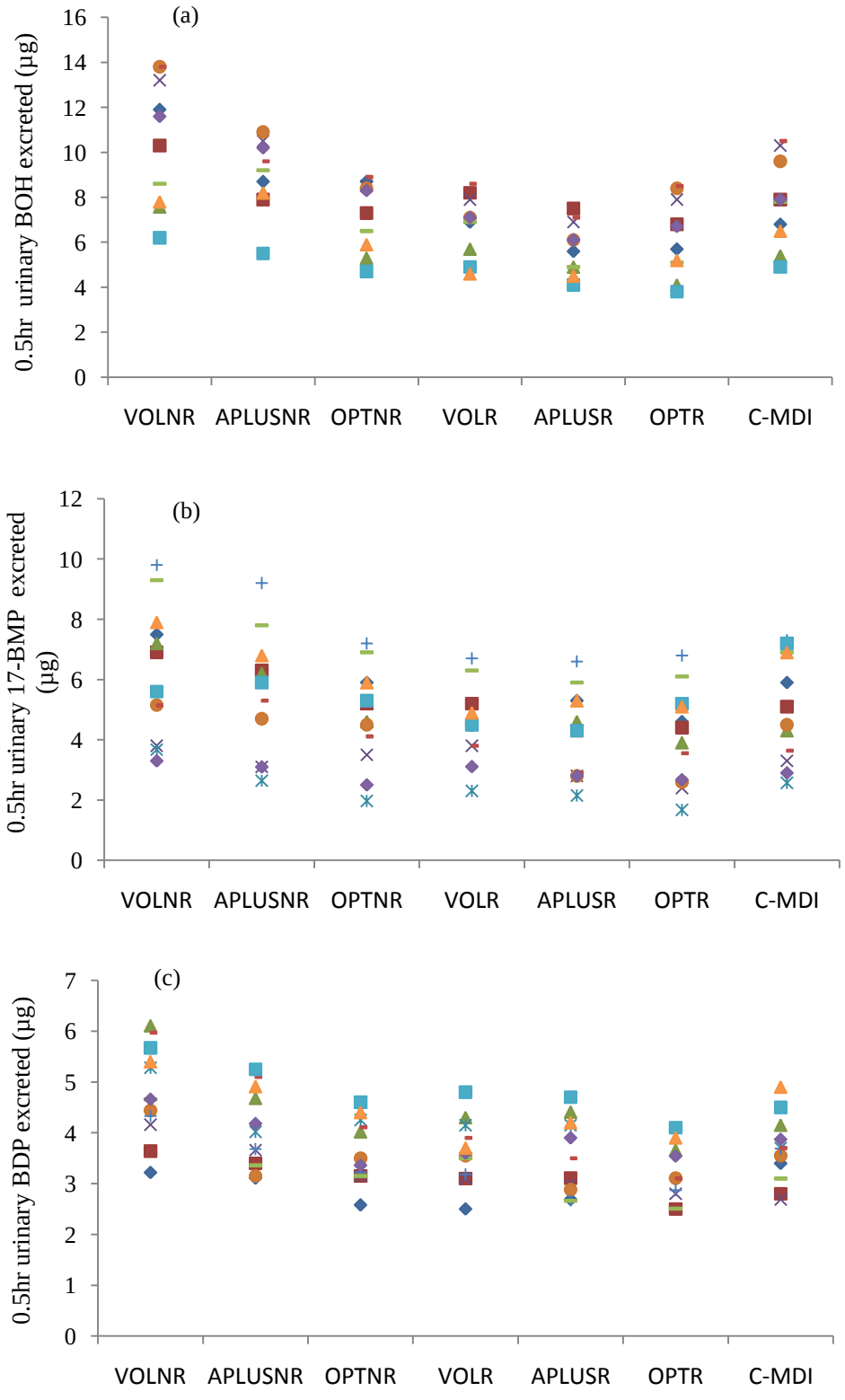


Figure 5.6: The 0.5hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted in urine post inhalation of Clenil MDI study doses with and without spacer, (n=12).

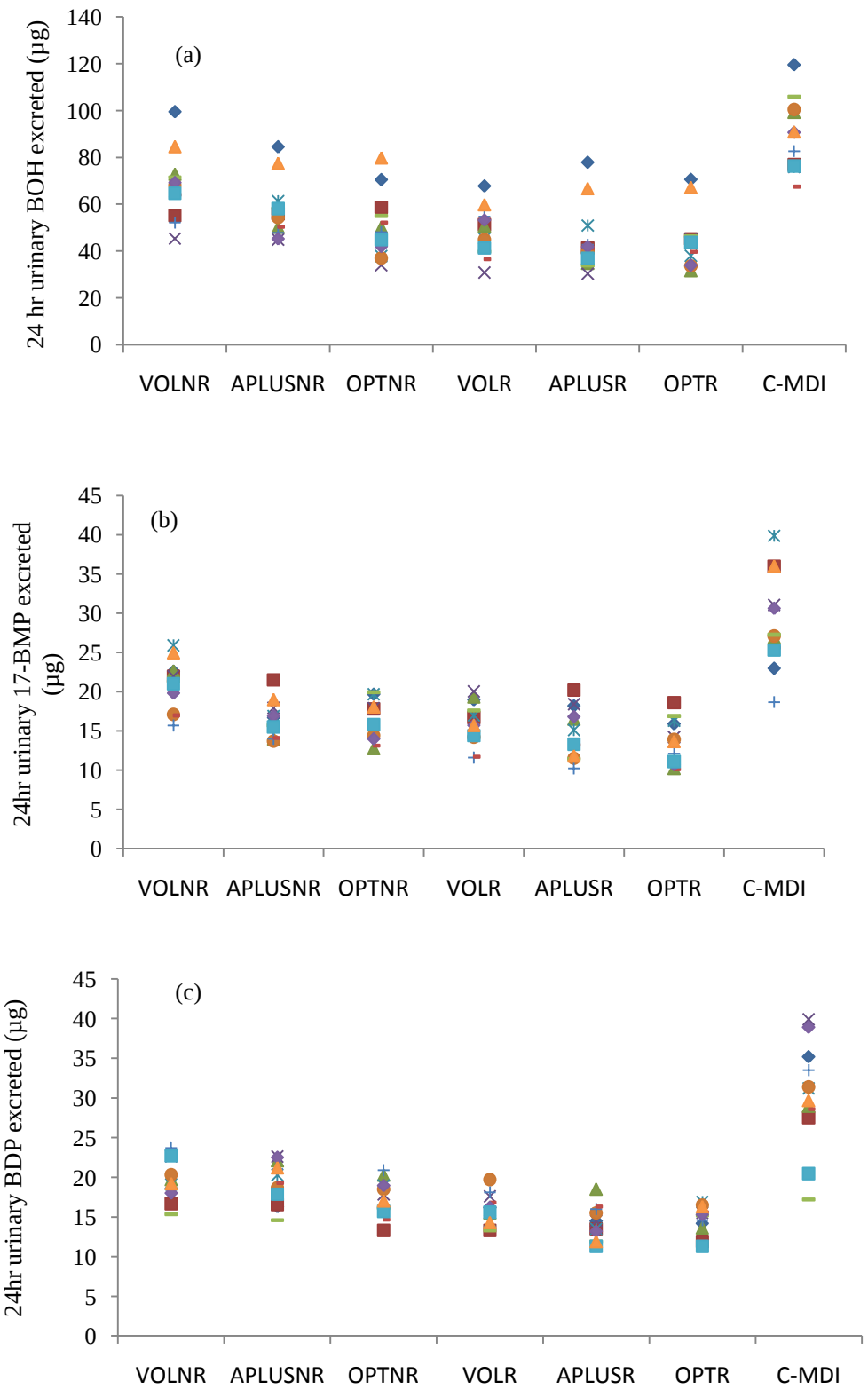


Figure 5.7: The 24hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted in urine post inhalation of Clenil MDI study doses with and without spacers, (n=12).

Table 5.7: Mean (SD) amounts of beclometasone dipropionate and its metabolites excreted 0.5hr, and 24hr post inhalation of Clenil® MDI study doses with and without spacers, expressed in µg, (n=12).

Device	Amount left in spacer (µg)	17-BMP (µg)		BOH (µg)		BDP (µg)	
		0.5hr	24hr	0.5hr	24hr	0.5hr	24hr
MDI	---	5.0 (1.8)	28.9 (6.0)	7.4 (1.9)	88.5 (15.4)	3.7 (0.6)	30.2 (6.6)
VOLNR	670.8 (74.4)	6.3 (2.2)	21.0 (3.1)	10.0 (2.8)	67.7 (14.4)	4.8 (0.9)	19.8 (2.6)
APLUSNR	758.0 (136.5)	5.6 (2.0)	16.3 (2.4)	8.6 (1.6)	57.4 (12.3)	4.0 (0.8)	19.4 (2.7)
OPTNR	705.4 (84.4)	4.8 (1.6)	16.4 (2.7)	7.1 (1.4)	50.8 (13.7)	3.6 (0.6)	17.4 (2.3)
VOLR	732.9 (74.9)	4.6 (1.2)	16.1 (2.8)	6.7 (1.1)	48.0 (10.4)	3.6 (0.6)	15.9 (1.9)
APLUSR	784.8 (46.9)	4.2 (1.4)	14.7 (3.3)	5.7 (1.1)	44.8 (14.0)	3.5 (0.8)	14.2 (2.1)
OPTR	807.0 (120.5)	4.1 (1.6)	13.6 (2.9)	6.2 (1.6)	44.9 (12.3)	3.3 (0.6)	14.7 (1.8)

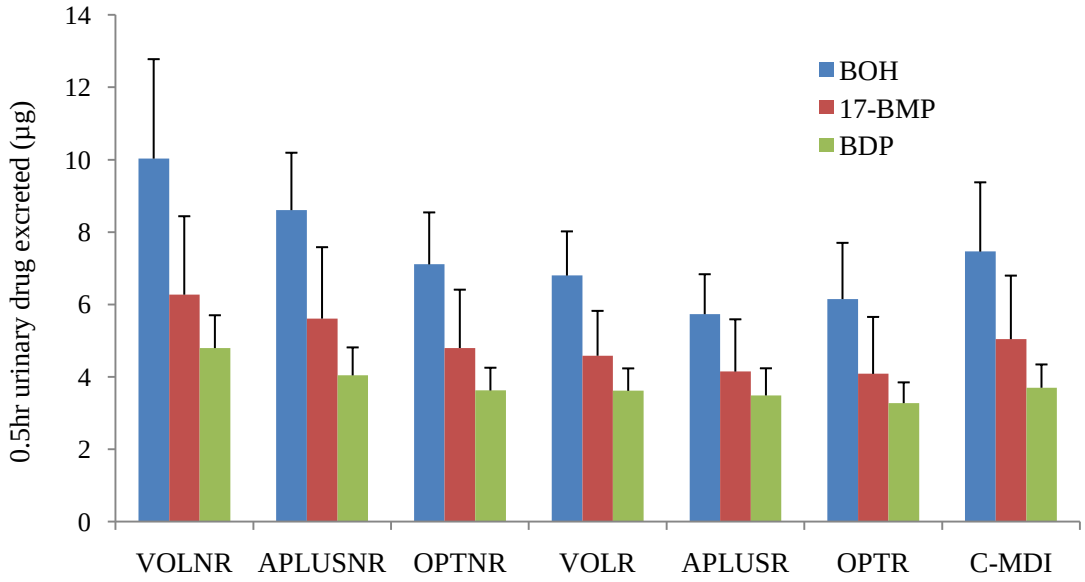


Figure 5.8: The 0.5hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Clenil MDI study doses with and without spacers, (n=12).

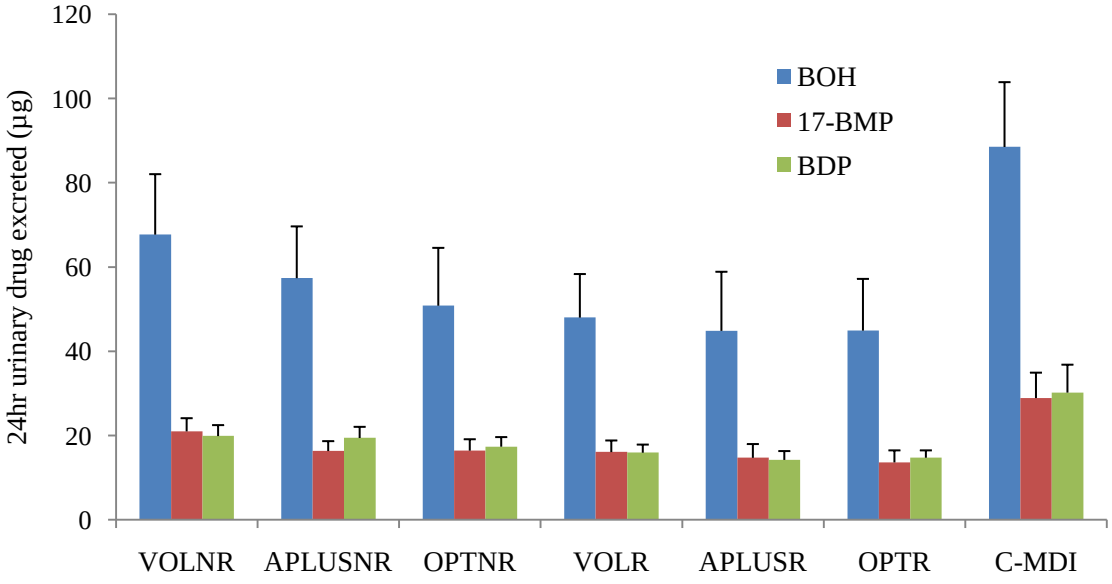


Figure 5.9: The 24hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Clenil MDI study doses with and without spacers, (n=12).

A summary of the statistical comparison between urinary amounts of BDP and its metabolites excreted 30 minutes and 24hrs post inhalation from Clenil® MDI for the twelve subjects is presented in tables 5.8 and 5.9, respectively. A summary of the statistical comparison between the amounts of beclometasone dipropionate retained in each spacer post inhalation of Clenil® MDI (250µg) study dose via different detergent prewashed spacers that is followed by either rinsing or not rinsing with water is presented in table 5.10. A summary of the mean ratio (90% confidence limits) between Clenil when used alone compared to when it is attached to each spacer with respect to the nominal dose is presented in table 5.11. These values are presented separately for BDP, 17 BMP, and BOH, as well as for all three metabolites combined.

Table 5.8: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate and its metabolites excreted post 30 minutes using Clenil® MDI and Clenil® MDI + spacers.

Inhaler	Comparator	MDI	VOLNR	APLUSNR	OPTNR	VOLR	APLUS R
17-BMP	VOLNR	-1.3 (-1.7,-0.8)***	---	---	---	---	---
	APLUSNR	-0.6 (-1,-0.1)*	0.7 (0.2,1.1)**	---	---	---	---
	OPTNR	0.2 (-0.2,0.7)	1.5 (1,1.9)***	0.8 (0.4,1.3)***	---	---	---
	VOLR	0.5 (0, 0.9)*	1.7 (1.2,2.1)***	1.0 (0.6,1.5)***	0.2 (-0.2,0.7)	---	---
	APLUSR	0.9 (0.4,1.4)***	2.1 (1.7,2.6)***	1.5 (1, 1.9)***	0.7 (0.2,1.1)**	0.4 (-0.02, 0.9)	---
	OPTR	1.0 (0.5,1.4)***	2.2 (1.7,2.6)***	1.5 (1.1,2)***	0.7 (0.3,1.2)**	0.5 (0.1,1)*	0.1 (-0.4, 0.5)
BOH	VOLNR	-2.6 (-3.3,-1.8)***	---	---	---	---	---
	APLUSNR	-1.1 (-1.9, -0.4)**	1.4 (0.7,2.2)***	---	---	---	---
	OPTNR	0.4 (-0.4,1.1)	2.9 (2.2,3.7)***	1.5 (0.8,2.2)***	---	---	---
	VOLR	0.7 (-0.1,1.4)	3.2 (2.5,4)***	1.8 (1.1,2.5)***	0.3 (-0.4,1)	---	---
	APLUSR	1.7 (1,2.5)***	4.3 (3.6,5)***	2.9 (2.1,3.6)***	1.4 (0.6,2.1)***	1.1 (0.3,1.8)***	---
	OPTR	1.3 (0.6,2)***	3.9 (3.2,4.6)***	2.5 (1.7,3.2)***	1.0 (0.2,1.7)*	0.7 (-0.1,1.4)	-0.4 (-1.1,0.3)
BDP	VOLNR	-1.1 (-1.4,-0.8)***	---	---	---	---	---
	APLUSNR	-0.4 (-0.7,-0.1)*	0.8 (0.5,1.1)***	---	---	---	---
	OPTNR	0.1 (-0.2,0.3)	1.2 (0.9,1.5)***	0.4 (0.1,0.7)**	---	---	---
	VOLR	0.1(-0.2,0.3)	1.2 (0.9,1.5)***	0.42 (0.12,0.7)**	0.009 (-0.3,0.3)	---	---
	APLUSR	0.2 (-0.1,0.5)	1.3 (1,1.6)***	0.6 (0.3,0.9)***	0.1 (-0.16,0.44)	0.1 (-0.7,0.4)	---
	OPTR	0.4 (0.1,0.7)*	1.5 (1.2,1.9)***	0.8 (0.5,1.1)***	0.4 (0.05,0.7)*	0.3 (0.04,0.6)*	0.2 (-0.1,0.5)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 5.9: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate and its metabolites excreted post 24hr using Clenil® MDI and Clenil® MDI + spacers.

Inhaler	Comparator	MDI	VOLNR	APLUSNR	OPTNR	VOLR	APLUS R
17-BMP	VOLNR	7.9 (5.8, 10.1)***	---	---	---	---	---
	APLUSNR	12.6 (10.5, 14.7)***	4.7 (2.0, 7.3)***	---	---	---	---
	OPTNR	12.5 (10.4, 14.6)***	4.6 (1.9, 7.2)**	-0.1 (-2.8, 2.6)	---	---	---
	VOLR	12.9 (10.7, 15.0)***	4.9 (2.2, 7.6)***	0.2 (-2.4, 2.9)	0.3 (-2.3,3.0)	---	---
	APLUSR	14.2 (12.1, 16.4)***	6.3 (3.6, 9.0)***	1.6 (-1.1, 4.3)	1.7 (-1.0, 4.4)	1.4 (-1.3, 4.0)	---
	OPTR	15.3 (13.2, 17.4)***	7.4 (4.7, 10.0)***	2.7 (0.01, 5.4)	2.8 (0.1, 5.5)*	2.4 (-0.2, 5.1)	1.1 (-1.6, 3.8)
BOH	VOLNR	20.8 (14.4, 27.1)***	---	---	---	---	---
	APLUSNR	31.1 (24.8, 37.4)***	10.3 (3.5, 17.2)**	---	---	---	---
	OPTNR	37.7 (31.3, 44.0)***	16.9 (10.0, 23.7)***	6.5 (-0.3, 13.4)	---	---	---
	VOLR	40.5 (34.2, 46.8)***	19.7 (12.9, 26.5)***	9.4 (2.5, 16.2)**	2.8 (-4.0, 9.7)	---	---
	APLUSR	43.7 (37.3, 50.0)***	22.9 (16.0, 29.7)***	12.5 (5.7, 19.3)***	6.0 (-0.9, 12.8)	3.2 (-3.7, 10.0)	---
	OPTR	43.6 (37.3, 49.9)***	22.8 (16.0, 29.6)***	12.4 (5.6, 19.3)***	5.9 (-0.9, 12.7)	3.1 (-3.7, 9.9)	-0.1 (-6.9, 6.8)
BDP	VOLNR	10.3 (8.1, 12.5)***	---	----	---	---	---
	APLUSNR	10.8 (8.6, 13.0)***	0.5 (-1.8, 2.7)	---	---	---	---
	OPTNR	12.8 (10.6, 15.1)***	2.5 (0.3, 4.7)*	2.1 (-0.2, 4.3)	---	---	---
	VOLR	14.3 (12.1, 16.5)***	4.0 (1.7, 6.2)***	3.5 (1.3, 5.7)**	1.4 (-0.8, 3.6)	---	---
	APLUSR	16.0 (13.8, 18.2)***	5.7 (3.5, 7.9)***	5.2 (3.0, 7.4)***	3.1 (0.9, 5.4)**	1.7 (-0.5, 3.9)	---
	OPTR	15.5 (13.3, 17.7)***	5.1 (2.9, 7.3)***	4.7 (2.5, 6.9)***	2.6 (0.4, 4.8)*	1.2 (-1.0, 3.4)	-0.5 (-2.7, 1.7)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 5.10: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate retained in each spacer post inhalations of from Clenil® MDI.

Inhaler	Comparator	VOLNR	APLUSNR	OPTNR	VOLR	APLUS R
Clenil® MDI	**APLUSNR**	-87.1 (-151.1, -23.2)**	---	---	---	---
	OPTNR	-34.6 (-98.5, 29.3)	52.5 (-11.4, 116.4)	---	---	---
	VOLR	-62.1 (-126, 1.9)	25.1 (-38.8, 89.0)	-27.4 (-91.4, 36.5)	---	---
	APLUSR	-136.4 (-200.4, -72.5)***	-49.3 (-113.2, 14.6)	-101.8 (-165.7, -37.9)**	-74.4 (-138.3, -10.5)*	---
	OPTR	-114 (-177.9, -50.0)***	-26.8 (-90.8, 37.1)	-79.4 (-143.3, -15.4)*	-51.9 (-115. 8,12)	22.5 (-415,86.4)

Table 5.11: Mean ratio (90% confidence interval) for Clenil MDI compared to Clenil MDI/spacer (when normalised for the nominal dose).

Urinary excretion	0.5hr			
	BDP	**17-BMP**	**BOH**	**All combined**
APLUSNR	91.0 (85.1, 97.3)	90.2 (83.3, 97.8)	85.5 (79.1, 92.5)	88.9 (84.9, 93.1)
APLUSR	106.0 (99.1, 113.4)	121.4 (112.0, 131.5)	128.4 (118.8, 139.0)	118.2 (112.9, 124.0)
VOLNR	76.8 (71.8, 82.1)	80.3 (73.6, 87.5)	74.8 (68.9, 81.0)	77.3 (73.9, 80.7)
VOLR	101.5 (94.9, 108.5)	107.5 (98.5, 117.2)	108.2 (99.8, 117.2)	105.7 (101.1, 110.4)
OPTNR	101.2 (95.3, 107.6)	105.3 (97.6, 113.7)	96.3 (90.3, 102.6)	102.2 (93.7, 111.4)
OPTR	112.1 (105.5, 119.1)	125.5 (116.3, 135.4)	121.3 (113.9, 129.3)	120.3 (110.4, 131.3)
24hr				
APLUSNR	153.0 (139.4, 168.0)	175.4 (159.2, 193.3)	155.1 (139.8, 172.1)	160.8 (152.0, 170.2)
APLUSR	209.2 (190.6, 229.8)	197.2 (179.0, 217.3)	202.2 (182.2, 224.3)	202.8 (191.7, 214.5)
VOLNR	149.3 (136.1, 164.0)	136.6 (125.9, 148.1)	131.7 (120.7, 143.5)	139.0 (132.3, 145.9)
VOLR	186.3 (169.6, 204.4)	179.0 (165.0, 194.0)	185.9 (170.6, 202.8)	183.7 (174.9, 192.9)
OPTNR	171.1 (154.8, 188.9)	174.9 (159.4, 192.1)	177.4 (157.8, 199.2)	170.7 (157.1, 185.7)
OPTR	201.2 (182.2, 222.3)	212.3 (193.3, 233.3)	200.4 (178.2, 225.2)	212.8 (195.8, 231.1)

5.3.4. Discussion

The effectiveness of inhaled therapy for topical diseases such as asthma depends on the ability of the inhalation device to deliver the correct dose of active drug substance to the lung, which is the site of action with minimal deposition to other unwanted regions that have no role in therapy and only contribute to side effects. Thus, the use of spacer devices is highly recommended with inhaled steroids therapy as they always reduce oropharyngeal deposition, may correct for poor hand-breath coordination and may increase lung deposition compared with MDI alone, and thus improve lung-targeting (Newman and Newhouse, 1996; Newman, 2004).

Following Clenil MDI inhalation, the use of the Volumatic and the Aerochamber Plus spacers without rinsing (VOLNR and APLUSNR) resulted in significantly higher amounts of urinary beclometasone dipropionate and its metabolites after 30 minutes post dosing compared to the MDI alone which indicates more efficient delivery of drug to the lungs using these spacers. However, the results were not significant when using the Optimiser spacer without rinsing (OPTNR). The above findings are consistent with several studies that confirmed that the use of spacers may be associated with a significant increase in the relative lung bioavailability compared to the MDI alone (Newman et al., 1984; Hindle and Chrystyn, 1994; Aswania and Chrystyn, 2001; Silkstone et al., 2002; Mazhar and Chrystyn, 2008).

As previously shown from the results, the use of any of the spacer devices whether large or small resulted in significantly ($p<0.001$) lower urinary excretion of BDP and its metabolite 24 hours post dosing from Clenil MDI for all the individuals. This decrease in systemic delivery of drug is due to deposition of part of the dose on the walls of the spacer devices themselves instead of deposition in the mouth (Newman and Newhouse, 1996). This is due to the spacer ability to trap large particles and allow smaller particles

to pass through to the patient, hence only a small fraction of the inhaled dose is deposited in the oropharynx. This is consistent with previous studies using the urinary excretion method (Chege and Chrystyn, 1994; Hindle and Chrystyn, 1994).

Silkstone et al (2002) used a urinary pharmacokinetic method to compare the lung and systemic delivery of salbutamol following inhalation from a MDI, a MDI attached to a spacer (MDI + SP), and a nebuliser (NEB). This study reported that doses inhaled from a metered dose inhaler attached to a spacer delivered more to the lungs and less to the systemic circulation than either the same doses from a MDI used alone or five times the dose given via a jet nebuliser (Silkstone et al., 2002).

The decrease in the quantity of beclometasone dipropionate that is deposited in the oropharynx after inhalation is highly important for inhaled corticosteroids as it diminishes the risk of topical adverse effects like thrush and dysphonia, as well as minimizing oral beclometasone absorption that could results in unwanted systemic side effects (Derendorf, 1997). Other several studies have investigated the effect of using spacer devices with beclometasone dipropionate MDIs on the suppression of free cortisol levels, which is considered to be a sensitive marker of adrenal activity and hence systemic delivery and safety. These studies reported a reduction in systemic effect from the high dose inhaled corticosteroids with spacers without detrimental effect on control of asthma symptoms (Prahl and Jensen, 1987; Brown et al., 1990; Farrer et al., 1990). The results from this study and from previous findings clearly demonstrated the improved therapeutic index of ICS when used with spacer devices and accounts for the recent guidelines recommending using spacers when delivering high doses of beclometasone (BTS/SIGN, 2008).

In all cases, more urinary amounts of BDP and its metabolites were excreted at 30 minutes and 24hrs post dosing from detergent prewashed spacers without subsequent

rinsing (NR spacers) compared to the same type of spacer prewashed in detergent solution then rinsed (R spacers). The higher lung deposition with these not rinsed spacers is most likely explained by the anti-static effect of the detergent coating. These results are in agreement with previous *in-vivo* studies that found a small increase in the output of salbutamol from spacers only after soaking it in soapy water without subsequent rinsing and found it to be as effective as an antistatic lining in reducing the effect of electrostatic charge on drug delivery (Clark and Lipworth, 1996a; Wildhaber et al., 2000a). Similarly, Pierart et al (1999) reported an increase in mean lung deposition of radio labelled salbutamol in healthy subjects from 11.5% through a static spacer to 45.6% through a detergent-coated spacer and further indicated that the antistatic property of detergent can lasts for at least four weeks. The influence of an electrostatic charge in the Babyhaler and Aerochamber spacers with HFA salbutamol MDI was investigated and found to reduce drug delivery to the lung by more than two fold (Anhoj et al., 1999). The use of detergent coating signifies a reduction of electrostatic charge on spacer surfaces by lowering their surface potentials (Kwok et al., 2006) which increases the half life of medication within the spacer (Barry and O'Callaghan, 1999). Thus, the electrostatic charge present on the walls of the spacer can have a profound effect on the behaviour of the aerosol cloud within the holding chamber and decrease drug output from it. Conversely, reduction or elimination of electrostatic charges on spacer surfaces improves drug delivery. It was previously reported that pre-washing spacers with detergent solution and then air drying without subsequent water rinsing is a highly effective method that improved lung deposition (Kenyon et al., 1998; Pierart et al., 1999), and enhanced the clinical effect (Wildhaber et al., 2000b). This accounts for recommending detergent washing of spacers on a regular basis in at least one guideline (BTS/SIGN, 2008). These results support the Aerochamber Plus patient information leaflet (PIL) recommending its detergent washing

without subsequent rinsing and contradicts the patient information leaflet (PIL) recommendation to rinse the Volumatic after washing.

The 30 minutes urinary excretion results also indicate that the use of the not rinsed Volumatic (VOLNR) spacer resulted in significantly higher amounts of urinary excretion of BDP and its metabolites compared to all other inhalation methods. This is in agreement with other studies that stated that large volume holding chambers such as the Volumatic appear to augment lung deposition to a greater degree than tube spacers or small holding chambers such as the Aerochamber (Newman and Newhouse, 1996).

Another pharmacokinetic study that used plasma salbutamol as indicative of lung deposition showed considerable variations in lung deposition between different large and small volume spacers from an HFA inhaler system. This study reported that the relative lung deposition was greater when the MDI attached to a Volumatic spacer compared with the Aerochamber, and the latter was similar to the MDI used alone (Lipworth and Clark, 1998a). Aswania et al (2001) compared Cromogen® MDI either alone or attached to the Volumatic spacer. The mean (SD) urinary excretion of sodium cromoglycate was 34.1 (20.2) and 211.7 (123.5) µg following MDI and MDI + Volumatic spacer, respectively. This shows that the MDI attached to a large volume spacer delivers more sodium cromoglycate to the lungs than the MDI alone (Aswania and Chrystyn, 2001). Other radiolabelled and clinical studies in both adults and children have shown that large volume spacers were more effective than the MDI alone especially during an asthma attack, whereas small volume spacers were only as effective as the optimally used MDI (Cushley et al., 1983; Newman et al., 1984; Levison et al., 1985; Keeley, 1992).

The better performance of large volume spacers compared to smaller ones may be due to the increased drug residence time in the bigger spacer of the large volume spacer with better chance for drug delivery. In addition, small volume spacers have an increased

likelihood of particle impaction on device walls compared to large volume spacers. Inhalation from large volume spacers with few second delays between actuation and inhalation have the advantage of not significantly affecting drug deposition, while for small volume spacers, this delay would result in even greater loss of drug in the device (Newman et al., 1988; Pedersen, 1996).

The difference in the 30 minutes urinary excretion for the not rinsed Volumatic compared to the MDI alone is not as large as that for other drugs. This agrees with previous evidence that suggests that HFA-MDIs lung deposition is not greatly affected by the addition of a spacer (Dubus et al., 2001; Woodcock et al., 2002a). This is may be due to the multimodal aerosol particle size distribution of HFA-MDIs; this is a phenomenon in which primary droplets can break up into smaller secondary droplets. Thus, the aerosol emitted from HFA MDIs has only a small portion of its volume occupied by droplets and appears to be pre-atomized prior to reaching the atomization nozzle. As a result, it will be expected to undergo limited particle size reduction following passage through the atomization nozzle. Furthermore, the presence of ethanol in these formulations, which is a vapour pressure suppressant, will further reduce the initial velocity required for the post nozzle break up of HFA-MDIs droplets (Smyth and Hickey, 2003; Smyth et al., 2004). Another explanation is that the results could be due to the greater electrostatic charge of the CFC-free beclometasone MDI (Kwok et al., 2006; Mitchell et al., 2007b)

The *in-vitro* and *in-vivo* studies of BDP inhaled from Clenil® MDI discussed in this chapter suggest that all spacer devices employed substantially reduced the amount of drug deposited in the oropharynx. This was clearly indicated by the lower 24hr urinary excretions of BDP and metabolites and the lower amount of drug deposited in the induction port of the impactor with spacers use following the *in-vivo* and *in-vitro* studies respectively. Indeed, the *in-vitro* higher emitted dose for the MDI alone compared with

that of the spacers did translate into more *in-vivo* drug delivery to the systemic circulation. This is in agreement with several previous *in-vitro* (O'Callaghan et al., 1994; Feddah et al., 2001) and *in-vivo* (Vidgren et al., 1987; Hindle and Chrystyn, 1994; Aswania and Chrystyn, 2001) studies which demonstrated similar effects when using spacers with MDIs.

The combination of the higher fine particle dose together with the higher total emitted dose of the not rinsed Volumatic in the *in-vitro* study accounts for its significantly higher 30 minutes and 24hr urinary excretion amounts post inhalation compared to all other inhalation methods. Also, comparison of the MDI vs the not rinsed Optimiser (OPTNR) post inhalation showed significantly more 24hr urinary excretion ($p<0.001$) and non significant 30 minutes urinary excretion which was consistent with the *in-vitro* significant increase in TED ($p<0.001$), and the non-significant difference in the FPD. Yet, their *in-vitro* results showed non-significant difference in their MMAD and significant difference for their % FPF at $p<0.001$. In addition, the *in-vivo* significant difference ($p<0.001$) of BDP and metabolites between the not rinsed and the rinsed Volumatic spacer is consistent with the significant *in-vitro* FPD difference ($p<0.01$) for the same inhalation group; however, their % FPF and MMAD difference showed non-significant difference.

When comparing the MDI alone or plus the not rinsed spacers, both the *in-vitro* FPD and the *in-vivo* 30 minutes urinary excretion showed the same following order: the Volumatic > Aerochamber Plus > MDI > Optimiser. However, the *in-vitro* TED and the *in-vivo* 24hr urinary excretion following the same inhalation methods decreased in the following order; MDI > Volumatic > Aerochamber Plus > Optimiser.

To sum up, the *in-vitro* and *in-vivo* results in this study showed that the FPD together with the TED are more important *in-vitro* parameters that represent the 30 minutes and

24hr urinary drug excretion, respectively post dosing. Seale and Harrison (1998) confirmed that the increase in fine particle mass is directly correlated to an increased lung absorption and hence an increased airway availability. This study further suggested that calculation of the fine particle mass of an administered dose rather the absolute dose given to the patient correlates well to the *in-vivo* drug delivery of both HFA-BDP and CFC-BDP. In addition, another study reported that determination of the *in-vitro* FPD of salbutamol from the ACI was found to be the most suitable impactor fraction that represents good *in-vitro-in-vivo* correlations (Weda et al., 2004). The observations from this study provide further evidence of good *in-vitro- in-vivo* correlations and in accordance with previous suggestions (Seale and Harrison, 1998; Silkstone et al., 2002; Barry and O'Callaghan, 2003; Mazhar and Chrystyn, 2008).

5.4. Conclusion

The significant reduction in the *in-vitro* amount deposited in the induction port, which represents the oropharyngeal cavity of the patient, and the significant reduction of the *in-vivo* 24 hr urinary excretion results when using any of the spacers clearly emphasized the importance of using spacers with inhaled corticosteroids therapy. This supports the British Thoracic Society recommendation for the management of asthma to use spacers regularly especially with high doses of inhaled corticosteroids.

Each brand of spacer device has different drug delivery characteristics and the efficiency of spacers depends upon its size and the control of electrostatic charge effects, which causes drug delivery to vary considerably according to how the spacer is handled. Large volume spacer devices improved drug delivery to the lungs from Clenil® MDI when compared to smaller volume ones, which was consistent with previous findings. These results were further supported by the in-vitro measurements of inhaled Clenil fine particle dose. In addition, simple variations in spacers handling techniques altered the

level of the electrostatic charge on the spacer walls. Both *in-vitro* and *in-vivo* studies confirmed the superiority of the both small and large detergent coated spacers without subsequent rinsing in improving lung deposition compared to the water rinsed ones. This emphasizes the potential of the electrostatic charge as a key determinant limiting aerosol drug delivery from MDI/spacer combination. However, it is still unknown whether these differences in handling will have a clinically significant effect. The use of the large volume spacer that is properly prewashed in soapy solution without subsequent rinsing to minimize the effects of static charge was the most efficient inhaler device used.

The results of this chapter demonstrate that the urinary pharmacokinetic method that has been previously developed and validated for beclometasone dipropionate in Chapter 4 is a potential tool to compare different inhalation methods.

Chapter 6: *In-vitro* Dose Emission and Aerodynamic Particle Size Distribution, Relative Lung and Systemic Bioavailability of Beclometasone Inhaled From Qvar® MDI and Qvar® EB With and Without Spacer

6.1. Introduction

Beclometasone dipropionate is a well established inhaled corticosteroid in the prophylactic management of mild, moderate and severe asthma in adults and children. The combination between beclometasone dipropionate (BDP) and HFA-134a propellant results in an aerosol with much smaller particles than those produced by CFC-BDP inhalers. An example of an HFA-BDP inhaler is Qvar® developed by Teva Pharmaceuticals, UK that has reported a MMAD of 1.1 μm (Leach et al., 1998a; Smyth and Hickey, 2003). Qvar® is available as a metered dose inhaler (MDI) and as a breath-actuated inhaler device. The extra-fine properties of Qvar® formulation accounts for its improved lung deposition, better penetration into the peripheral airways, improved asthma control and health related quality of life (Juniper et al., 2002). Previously, such peripheral airways could only be reached using systemically administered therapy (Leach et al., 2002; Skoner, 2008). Several clinical studies have shown that Qvar is effective at half the dose of CFC-BDP formulations (Davies et al., 1998; Leach et al., 1998a; Busse et al., 2000; Agertoft et al., 2003). This improved efficacy of Qvar® at a lower dose leads to equivalent asthma control and even fewer side effects (Lipworth and Jackson, 2000). Furthermore, the large proportion of extra-fine particles in this HFA-BDP formulation results in lung doses to become less dependent on breathing pattern compared with CFC-BDP (Janssens et al., 2003; Leach et al., 2005). In addition, the use of devices such as the Easi-Breathe (Qvar® EB) aids coordination by actuating at a pre-determined point during inspiration and thus it does not require synchronisation of actuation and inhalation. These devices are highly valuable for individuals with poor inhalation techniques, however, they do not protect against oropharyngeal deposition (Newman et al., 1991c).

The therapeutic ratio is the ratio between the clinical effect and the systemic effect of an inhalation. The systemic effect of an inhaled corticosteroid depends on the systemic absorption of both the amount of drug deposited in the airways and the amount of drug

that reaches the gastrointestinal tract, whereas the clinical effect only depends on the amount of drug deposited in the airways (Pedersen, 1996). Therefore, minimizing the amount of drug that reaches the gastrointestinal tract that has no therapeutic value and only contributes to systemic side effects is highly advantageous with inhaled corticosteroid therapy (Wildhaber et al., 2000a; Roller et al., 2007) and this is the reason behind the fact that spacers are highly recommended with inhaled corticosteroid therapy. Spacers reduce both the velocity and the size of the aerosol particles as they provide extra time for complete evaporation of the propellant and therefore eliminate the need for patient co-ordination between actuation of the MDI and inhalation of the aerosol (Newman, 2004). Moreover, spacers have a size selective function and retain the non-breathable large particles by impaction on the spacer walls thus reducing the "cold-Freon effect" and drug deposition in the oropharynx, with fewer local side effects from steroid aerosols such as coughing, hoarseness, throat discomfort, and oral candidiasis (Newman et al., 1981a; Terzano and Mannino, 1999). However it is still debatable whether or not spacers improve drug delivery to the airways, as spacers may offer no additional benefit to patients with good inhaler technique (Donnell, 2001).

The fact that most spacers are constructed with lightweight plastic materials for portability and durability makes it highly prone to electrostatic charge accumulation that adversely affects drug output and lung deposition (Barry and O'Callaghan, 1995; Dewsbury et al., 1996). Several methods have been described to reduce static charge accumulation on plastic spacer surfaces (O'Callaghan et al., 1993; Barry and O'Callaghan, 1995; O'Callaghan, 1997; Kenyon et al., 1998). However the most simple and popular method is coating spacers with dilute surfactant solutions by simply immersing the spacer in a detergent solution followed by drip-drying without rinsing with water (Wildhaber et al., 1996a; Pierart et al., 1999).

6.2. *In-vitro* dose emission and aerodynamic particle size distribution of the dose emitted from Qvar® EB and Qvar® metered dose inhaler

6.2.1. Method

6.2.1.1. Equipment and inhalation devices

- Equipment:

As, described in section 5.2.1.1 of this thesis.

- Inhaler and spacer devices used as follows:

- Qvar® metered dose inhaler (MDI), and Qvar® EB labelled as nominal dose of 100µg beclometasone dipropionate per shot (Teva Pharmaceuticals, UK).
- The Aerochamber Plus spacer [APLUS], 145ml holding chamber, (Trudell Medical International Europe Ltd, UK).
- The Volumatic spacer device [VOL] 750ml holding chamber, (GlaxoSmithKline)
- The Optimiser spacer [OPT], 50ml small plastic tube spacer having a cross section of 2.5 x 3.3cm (Teva Pharmaceuticals, UK).

- LC-(ESI+)-MS method conditions: sample preparation, analysis procedures, and chromatographic conditions were as reported in section 3.2.

6.2.1.2. Procedure

6.2.1.2.1. Total emitted dose

In the present work, *in-vitro* measurements of the total emitted dose was performed with extrafine hydrofluoroalkane (HFA)-BDP inhalers available as Qvar® MDI and Qvar® EB either alone or when connected to different spacers using the metered dose inhaler dose sampling unit (DSU). Each type of spacer was tested after thoroughly washing in detergent solution then followed by either rinsing (R) or not rinsing with water (NR), and then air-dried.

Measurements were carried out for Qvar® EB either alone or when attached to each of the following spacers the Volumatic, the Aerochamber Plus, and the Optimiser (with and without rinsing). In addition, the total emitted dose determinations were carried out for Qvar® MDI either alone or when attached to the Aerochamber Plus (with and without rinsing). Each inhaler was first primed by firing two doses to waste before use. Each inhaler/inhaler-spacer was connected to the DSU and operated as previously described in section 5.2.1.2.1 and four separate doses from Qvar EB (100µg) or Qvar MDI (100µg) were discharged into the ACI. On each occasion, one dose was introduced into the spacer followed by the *in-vitro* inhalation manoeuvre. The procedure was repeated until the set number of doses has been discharged. The amount of drug in the dose sampling unit and spacer was determined by using the previously developed and validated LC-(ESI+)-MS method previously described in section 3.2 in this thesis.

6.2.1.2.2. The aerodynamic particle size characterization

The aerodynamic particle size distributions of Qvar® EB and Qvar® MDI used either alone or when attached to spacers at a flow rate of 28.3L/min and the aerodynamic particle size distribution of the same inhalers used alone without spacers at higher flow rates (60, and 90 L/min) were determined. These distributions were measured with the Andersen Cascade Impactor (ACI). Four separate actuations from Qvar EB (100µg), and Qvar MDI (100µg) were delivered into the Andersen Cascade Impactor for each inhaler or inhaler/spacer combination. The procedure details were as previously described in section 5.2.1.2.2. Five determination were made for each inhaler or inhaler/spacer combination (n=5). The amount of beclometasone dipropionate expressed in µg deposited in the induction port (IP) and the various ACI stages were determined using the previously developed and validated LC-(ESI+)-MS method described in section 3.2 in this thesis.

6.2.2. Statistical analysis

The data was statistically analyzed using one way analysis of variance (ANOVA) to compare the total emitted dose and aerodynamic particle size characterization of different MDIs and MDI/spacer combinations at a flow rate of 28.3 L/min and the aerodynamic particle size characterization of different inhalers used alone at higher flow rates using SPSS V17.0 (SPSS Inc., Chicago, USA).

6.2.3. Results

6.2.3.1. Total emitted dose

The individual emitted doses of four 100µg actuations of beclometasone dipropionate from Qvar® EB alone or when attached to different spacers expressed in µg and as percentage of nominal dose are presented in APPENDIX B.33 and B.34 (refer to the enclosed DVD), respectively. While the individual emitted doses of Qvar® MDI with or without spacers expressed in µg and as percentage of nominal dose are shown in APPENDIX B.35 (refer to the enclosed DVD). A summary of the mean (SD) emitted doses for Qvar EB and Qvar MDI are illustrated in table 6.1. The results for Qvar® EB and Qvar® MDI are expressed graphically in figures 6.1, and 6.2, respectively.

Table 6.1: Mean (SD) dose emission from four 100µg doses of beclometasone dipropionate from a Qvar® EB and Qvar MDI determined at a flow 28.3 L min¯1, expressed in µg and as percent of nominal dose, (n=10).

		Qvar-EB		Qvar MDI	
		Dose (µg)	**% of nominal dose**	**Dose (µg)**	**% of nominal dose**
MDI	**TED**	354.6 (29.9)	88.7 (7.5)	329.2 (37.3)	82.3 (9.3)
APLUSNR	**TED**	221.0 (34.2)	55.2 (8.6)	203.1 (34.2)	50.8 (8.6)
	Spacer	101.1 (28.2)	25.3 (7.1)	113.4 (16.7)	28.3 (4.2)
APLUSR	**TED**	170.9 (46.4)	42.7 (11.6)	164.4 (43.5)	41.1 (10.8)
	Spacer	189.8 (30.0)	47.5 (7.5)	163.3 (48.0)	40.8 (12.0)
VOLNR	**TED**	197.3 (30.3)	49.3 (7.6)	---	---
	Spacer	106.0 (23.4)	26.5 (5.9)	---	---
VOLR	**TED**	152.9 (52.9)	38.2 (13.2)	---	---
	Spacer	166.6 (39.8)	41.6 (10.0)	---	---
OPTNR	**TED**	212.8 (31.6)	53.2 (7.9)	---	---
	Spacer	146.3 (25.7)	36.6 (6.4)	---	---
OPTR	**TED**	165.7 (48.2)	41.4 (12.1)	---	---
	Spacer	217.4 (29.4)	54.4 (7.4)	---	---

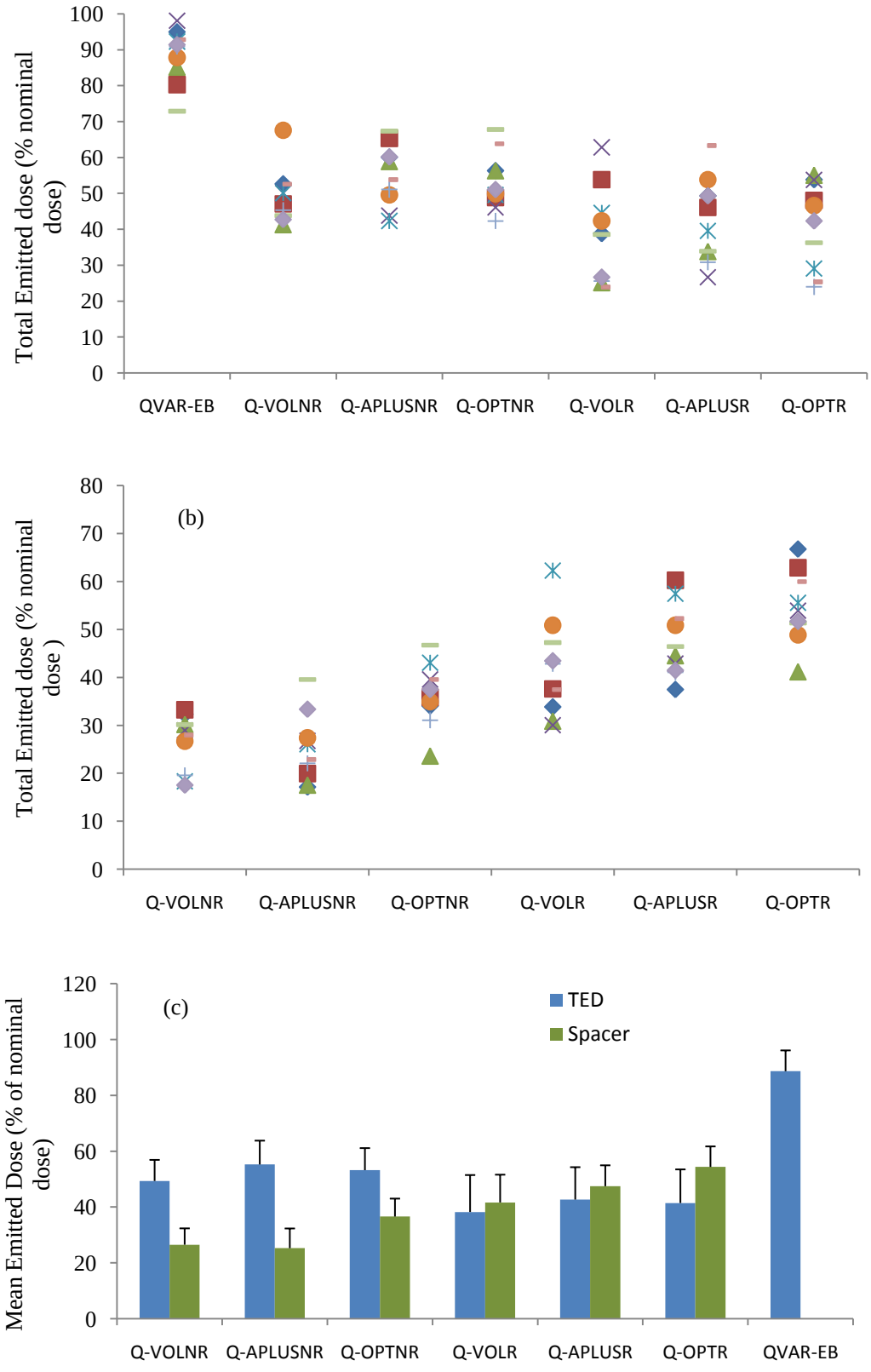


Figure 6.1: Beclometasone dipropionate (a) total emitted dose (b) deposited in each spacer (c) mean (SD) total emitted dose and the amount deposited in each spacer expressed as a percent of the nominal dose from Qvar EB at a flow rate 28.3 L min^{-1}, (n=10).

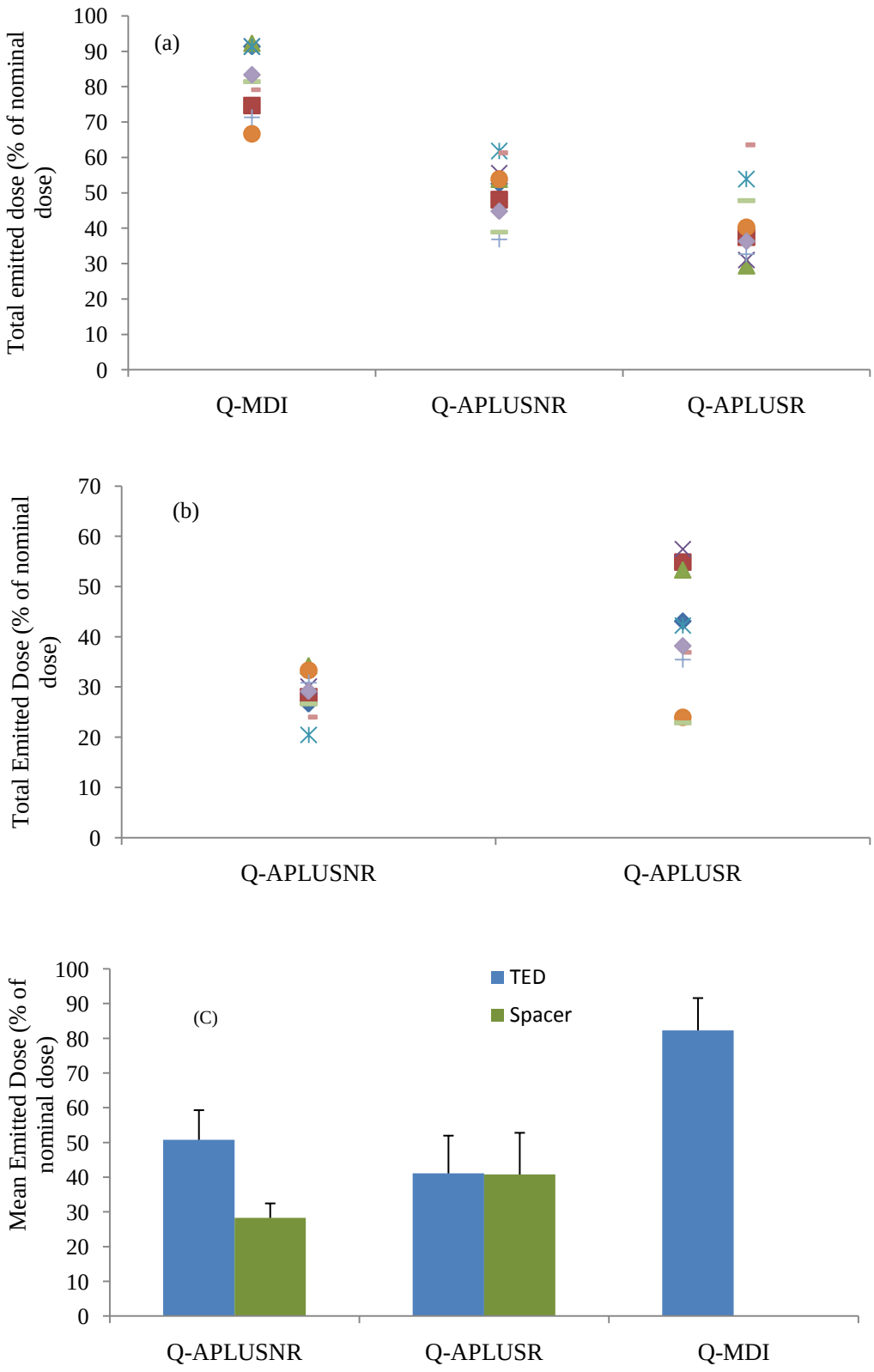


Figure 6.2: Beclometasone dipropionate (a) total emitted dose (b) deposited in each spacer (c) mean (SD) total emitted dose and the amount deposited in each spacer expressed as a percent of the nominal dose from Qvar MDI at a flow rate 28.3 L min^{-1}. (n=10).

6.2.3.2. Aerodynamic particle size characterization

A summary of the aerodynamic particle size distribution data obtained from the Andersen cascade impactor for Qvar® EB and Qvar® MDI either alone or plus different spacers at 28.3 L/min flow rate are shown in tables 6.2, 6.3, and figures 6.3 and 6.4 respectively.

In addition, the effect of higher flow rates conditions on the aerosol particle size distribution of both Qvar EB and Qvar MDI were investigated. Tables 6.4, and 6.5 and figures 6.5, and 6.6 represent the aerodynamic particle size distribution data obtained at different flow rates (28.3, 60, and 90L/min) from the Andersen Cascade Impactor for Qvar® EB and Qvar® MDI, respectively.

Table 6.2: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following four actuations of Qvar EB® (100µg) either alone or plus different spacers at 28.3 L/min. Values quoted in µg unless specified, (n=5).

	Stage Cut-off	Qvar-EB	VOLNR	APLUSNR	OPTNR	VOLR	APLUSR	OPTR
Amount left in spacer		----	169.2 (18.7)	117.7 (16.5)	126.4 (8.1)	228.1 (20.1)	225.8 (36.9)	191.2 (22.9)
Induction Port (IP)		121.8 (15.5)	5.0 (2.6)	9.3 (1.9)	7.5 (3.6)	3.4 (2.0)	4.7 (1.5)	3.6 (1.0)
0	10	9.2 (1.8)	8.5 (4.1)	7.5 (2.2)	7.7 (2.4)	9.9 (3.3)	2.9 (2.0)	3.2 (0.9)
1	9	12.9 (1.5)	9.6 (1.6)	10.3 (2.2)	7.8 (1.4)	10.9 (3.0)	4.6 (1.8)	4.0 (0.5)
2	5.8	15.8 (4.9)	10.5 (1.8)	11.5 (3.6)	7.5 (2.4)	7.6 (1.7)	8.5 (3.4)	9.6 (4.5)
3	4.7	20.5 (5.4)	13.8 (2.6)	21.8 (7.2)	12.9 (1.9)	9.7 (2.3)	10.3 (2.4)	9.7 (3.2)
4	3.3	33.0 (5.5)	15.7 (1.6)	22.5 (4.5)	14.8 (3.2)	16.1 (5.7)	15.0 (4.6)	12.4 (1.3)
5	2.1	34.8 (8.1)	41.1 (26.4)	30.4 (4.1)	37.4 (8.0)	24.0 (7.2)	17.5 (5.3)	23.4 (14.7)
6	1.1	50.8 (13.6)	34.3 (4.9)	45.1 (15.4)	46.9 (17.1)	24.7 (10.3)	26.9 (11.6)	37.7 (12.1)
7	0.7	37.0 (11.7)	36.3 (1.0)	37.9 (10.7)	40.2 (6.6)	19.3 (2.3)	21.1 (10.9)	25.6 (11.2)
Filter	0.4	35.1 (8.9)	16.7 (2.9)	34.6 (9.2)	24.8 (7.9)	12.5 (0.8)	22.1 (5.5)	9.6 (1.4)
Total emitted dose (TED) (µg)		**372.6 (27.1)**	**191.6 (20.8)**	**230.7 (30.1)**	**207.5 (9.6)**	**138.1 (20.3)**	**133.6 (18.4)**	**138.8 (16.5)**
Total emitted dose (% of nominal dose)		**93.16 (6.8)**	**47.9 (5.2)**	**57.7 (7.5)**	**51.9 (2.4)**	**34.5 (5.1)**	**33.4 (4.6)**	**34.7 (4.1)**
FPD (µg)		**218.0 (29.1)**	**161.3 (22.2)**	**196.2 (27.0)**	**179.6 (15.1)**	**108.2 (19)**	**115.9 (22.9)**	**121.9 (20.9)**
% FPF of nominal dose		**54.5 (7.3)**	**40.3 (5.6)**	**49.0 (6.8)**	**44.9 (3.8)**	**27.0 (4.7)**	**28.9 (5.7)**	**30.5 (5.2)**
%FPF of the TED		**58.4 (5.2)**	**84.0 (2.4)**	**84.9 (1.4)**	**86.5 (4.1)**	**78.2 (2.5)**	**86.3 (5.5)**	**87.5 (5.0)**
MMAD (µm)		**1.2 (0.1)**	**1.2 (0.1)**	**1.1 (0.3)**	**1.0 (0.2)**	**1.5 (0.2)**	**1.2 (0.5)**	**1.1 (0.2)**
GSD (no units)		**3.8 (0.3)**	**3.8 (1.0)**	**3.8 (1.1)**	**3.4 (0.01)**	**4.2 (1.1)**	**3.3 (0.7)**	**2.6 (0.01)**

Table 6.3: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following four actuations of Qvar® MDI (100µg) either alone or plus different spacers at 28.3L/min flow rate. Values quoted in µg unless specified, (n=5).

	Stage Cut-off	Q-MDI	APLUSNR	APLUSR
Amount left in spacer		----	183.4 (30)	239.6 (66.2)
Induction Port (IP)		130.9 (26.3)	10.1 (3.2)	6.8 (1.9)
0	10	6.3 (1.4)	8.3 (1.6)	3.6 (0.9)
1	9	9.03 (3.8)	16.0 (3.8)	5.8 (2.2)
2	5.8	12.7 (3.1)	13.1 (4.9)	10.5 (2.8)
3	4.7	17.8 (3.9)	22.4 (3)	13.2 (3.7)
4	3.3	32.1 (8.2)	24.2 (3.7)	15.6 (4.3)
5	2.1	37.7 (6.4)	31.4 (10.9)	18.3 (5.6)
6	1.1	43.4 (3.1)	36.0 (4.4)	28.7 (9.4)
7	0.7	34.9 (4.9)	36.7 (6.0)	27.2 (2.9)
Filter	0.4	36.9 (1)	37.1 (8.4)	20.4 (9.5)
Total emitted dose (TED) (µg)		**362.2 (34.7)**	**235.3 (29.0)**	**150.1 (28.9)**
Total emitted dose (% of nominal dose)		**90.6 (8.7)**	**58.8 (7.2)**	**37.5 (7.2)**
FPD (µg)		**208.9 (16.3)**	**191.6 (23.8)**	**127.2 (27.2)**
% FPF of nominal dose		**52.2 (4.1)**	**47.9 (6.0)**	**31.8 (6.8)**
%FPF of TED		**57.6 (6.2)**	**81.4 (0.4)**	**84.5 (3.5)**
MMAD (µm)		**1.2 (0.2)**	**1.1 (0.1)**	**1.1 (0.1)**
GSD (no units)		**3.5 (0.4)**	**4.2 (0.3)**	**4.1 (0.4)**

Table 6.4: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following four actuations of Qvar® EB (100µg) alone at 28.3, 60, and 90 L/min flow rates. Values quoted in µg unless specified, (n=5).

	28.3L/min	**60L/min**	**90L/min**
Induction Port (IP)	121.8 (15.5)	100.2 (18.6)	81.6 (18.1)
-2	---	---	4.1 (0.5)
-1	---	7.3 (1.3)	6.8 (1.3)
-0	---	9.1 (1.7)	16.0 (3.6)
0	9.2 (1.8)	---	---
1	12.9 (1.5)	10.4 (1.4)	24.3 (3.8)
2	15.8 (4.9)	29.5 (5.1)	38.3 (6.1)
3	20.5 (5.4)	31.9 (5.5)	45.2 (5.5)
4	33.0 (5.4)	37.0 (8.6)	74.3 (11.5)
5	34.8 (8.1)	58.3 (3.9)	27.5 (4.9)
6	50.8 (13.6)	36.1 (7.2)	---
7	37.0 (11.7)	---	---
Filter	35.1 (8.9)	29.1 (8)	19.8 (5.7)
Total emitted dose (TED) (µg)	**372.6 (27.1)**	**349.1 (41.9)**	**338.0 (22.2)**
Total emitted dose (% of nominal dose)	**93.2 (6.8)**	**87.3 (10.5)**	**84.5 (5.5)**
FPD (µg)	**218.0 (29.1)**	**225.9 (26.6)**	**235.7 (12.2)**
% FPF of nominal dose	**54.5 (7.3)**	**64.8 (8.1)**	**58.9 (3.1)**
%FPF of TED	**58.4 (5.2)**	**59.6 (1.9)**	**69.8 (3.8)**
MMAD (µm)	**1.2 (0.17)**	**1.2 (0.2)**	**1.2 (0)**
GSD (no units)	**3.8 (0.3)**	**3.4 (0.3)**	**3.1 (0.25)**

Table 6.5: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) following four actuations of Qvar® MDI (100µg) alone at 28.3, 60, and 90 L/min flow rates. Values quoted in µg unless specified, (n=5).

	28.3L/min	**60L/min**	**90L/min**
Induction Port (IP)	130.9 (26.3)	116.8 (27.8)	99.6 (4.3)
-2	---	---	10.9 (3.2)
-1	---	8.9 (2.5)	10.7 (3.5)
-0	---	10.1 (3.8)	17.2 (2.1)
0	6.3 (1.4)	---	---
1	9.0 (3.7)	15.1 (4.8)	19.0 (5.6)
2	12.7 (3.1)	20.5 (4.7)	41.3 (7.7)
3	17.8 (3.9)	36.7 (6.7)	52.6 (7.4)
4	32.1 (8.2)	49.0 (8.3)	65.9 (4.5)
5	37.7 (6.4)	58.8 (11.1)	36.3 (11)
6	43.4 (3.1)	44.3 (4.3)	---
7	34.9 (4.9)	---	---
Filter	36.9 (1.0)	27.2 (5.8)	26.5 (7.1)
Total emitted dose (TED) (µg)	**362.2 (34.7)**	**387.4 (16.6)**	**380.0 (10.4)**
Total emitted dose (% of nominal dose)	**90.6 (8.7)**	**96.9 (4.2)**	**95.0 (2.6)**
FPD (µg)	**208.9 (16.3)**	**241.8 (32.7)**	**247.4 (9.5)**
% FPF of nominal dose	**52.2 (4.1)**	**60.4 (8.2)**	**61.8 (2.4)**
%FPF of TED	**57.6 (6.2)**	**62.3 (6.1)**	**70.7 (7.7)**
MMAD (µm)	**1.2 (0.2)**	**1.2 (0.1)**	**1.3 (0.2)**
GSD (no units)	**3.5 (0.4)**	**3.4 (0.3)**	**3.4 (0.9)**

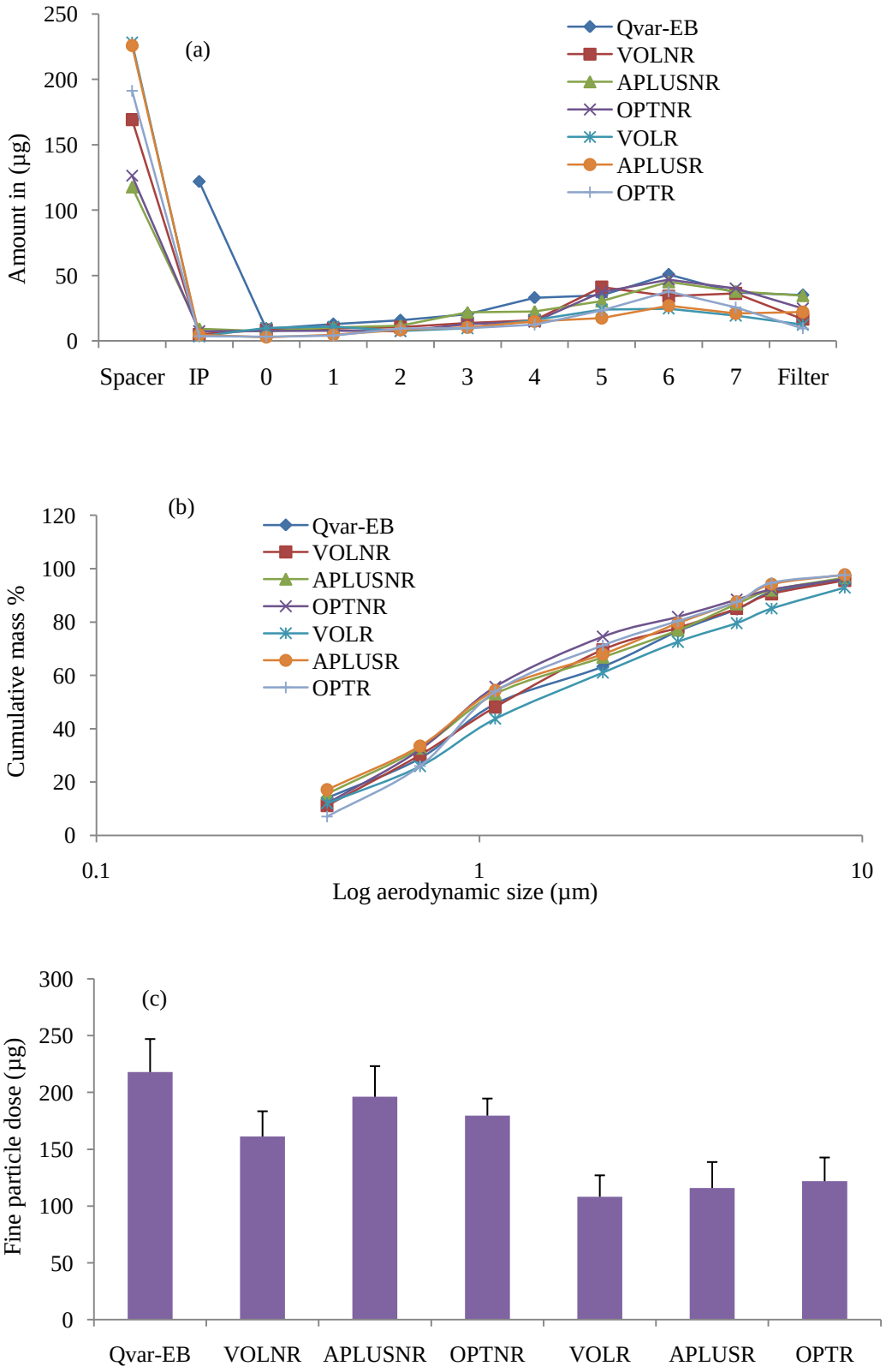


Figure 6.3: Mean beclometasone dipropionate emitted from Qvar® EB alone or with different spacers at a flow rate 28.3 L min^{-1} (a) deposited in each stage of the ACI (µg) (b) the aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose, (n=5).

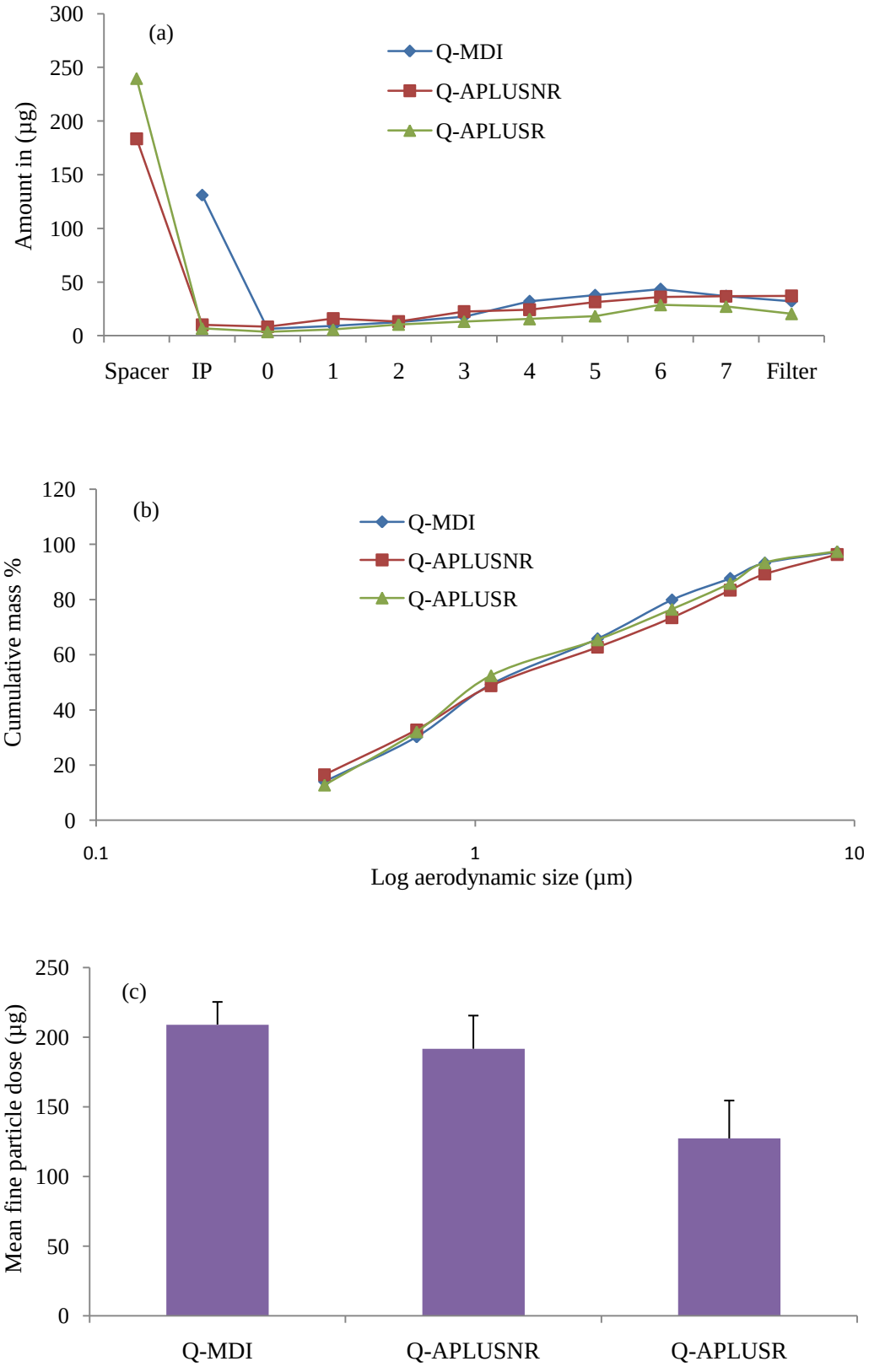


Figure 6.4: Mean beclometasone dipropionate emitted from Qvar® MDI alone or with spacer at a flow rate 28.3 L min^{-1} (a) deposited in each stage of the ACI (µg) (b) the aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose, (n=5).

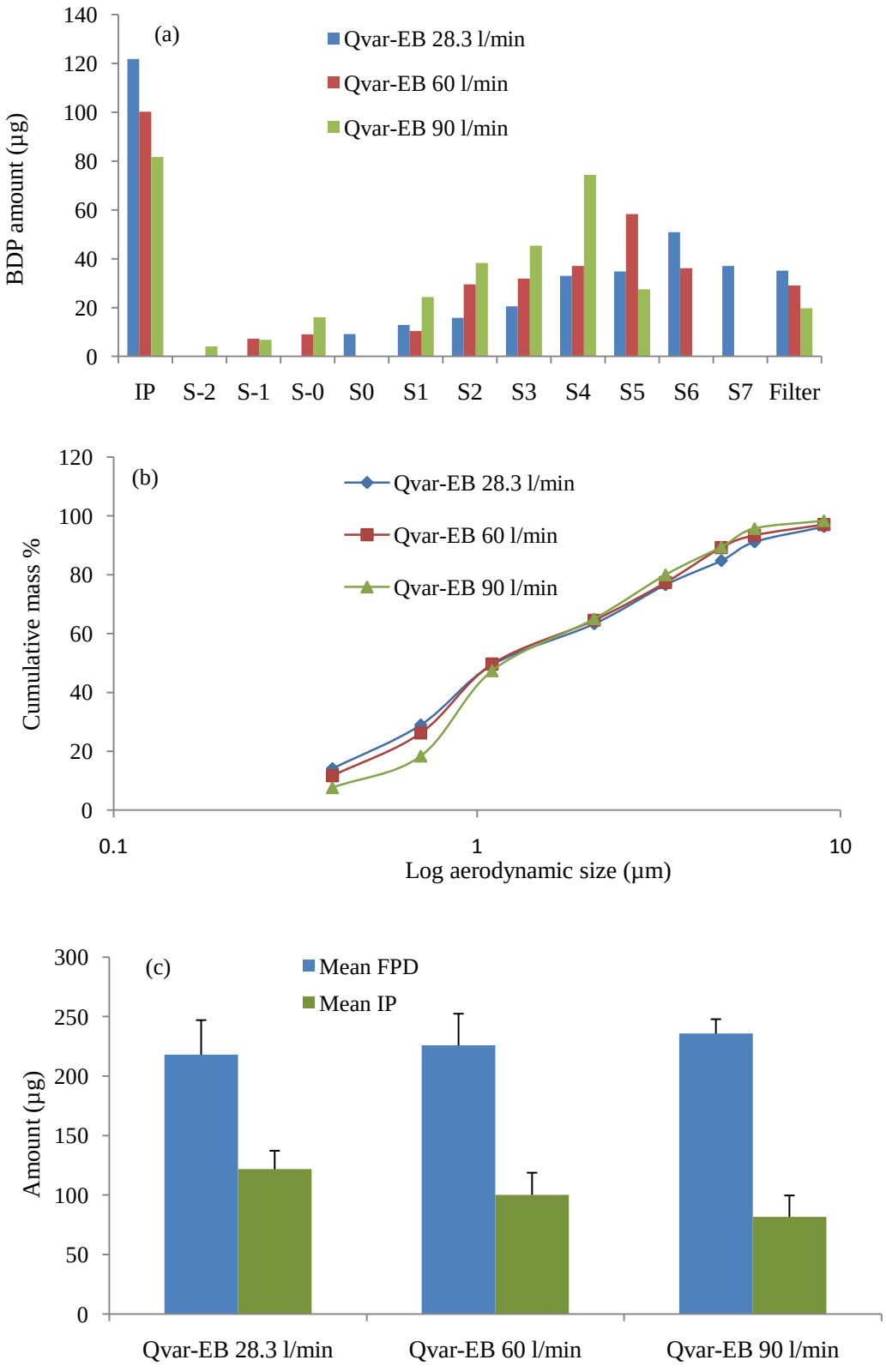


Figure 6.5: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) the aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose and induction port deposition emitted from Qvar® EB alone at 28.3, 60, and 90 L min^{-1} flow rates, expressed in µg, (n=5).

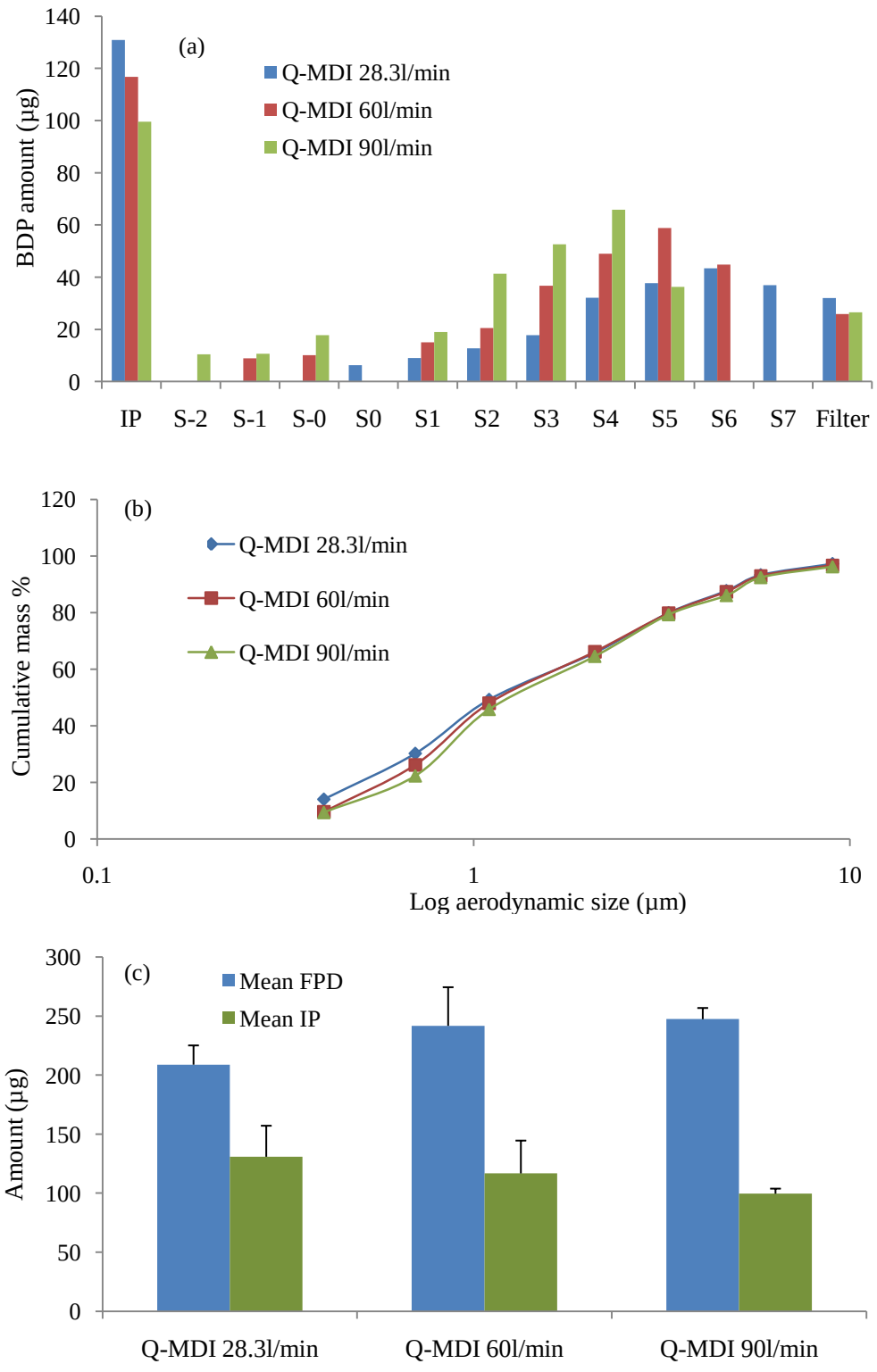


Figure 6.6: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) the aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose and induction port deposition emitted from Qvar® MDI alone at 28.3, 60, and 90 L min^{-1} flow rates, expressed in µg, (n=5).

6.2.3.3. Statistical analysis

Table 6.6 and table 6.7 represent a summary of the statistical analysis of data obtained from Qvar® EB and Qvar® MDI, respectively operated at 28.3L/min using the Andersen Cascade Impactor. The total emitted dose significantly decreased for both Qvar® EB and Qvar® MDI, when using any of the spacers studied than when using each inhaler alone. Table 6.8 summarizes the statistical analysis data for Qvar® EB and Qvar® MDI, operated at different flow rates.

Table 6.6: Mean difference (95% confidence interval) for different spacers used with Qvar® EB.

Comparator		FPD (µg)	FPF%	MMAD	TED (µg)	Spacer deposition(µg)
VOLNR	Qvar-EB	-56.7 (-94.6, -18.8)**	25.6 (18.3, 32.9)***	-0.03 (-0.5, 0.5)	-181 (-214.5, -147.5)***	---
	APLUSNR	-34.9 (-72.8, -18.8)	-0.9 (-8.2, 6.4)	0.07 (-0.4, 0.6)	-39 (-72.6, -5.5)*	51.5 (15.1, 87.9)*
	OPTNR	-18.3 (-56.2, 19.6)	-2.5 (-9.8, 4.8)	0.1 (-0.4, 0.6)	-15.9 (-49.4, 17.7)	42.8 (6.4, 79.2)*
	VOLR	53.1 (15.2, 91)*	5.8 (-4.5, 13.1)	-0.3 (0.8, 0.2)	53.5 (20, 87.1)**	-58.9 (-95.3, -22.5)**
	APLUSR	45.3 (7.4, 83.2)*	-2.3 (-9.6,5)	-0.07 (-0.6, 0.4)	58 (24.5, 91.6)**	-56.6 (-93, -20.2)**
	OPTR	36.1 (-2.8, 75)	-4.4 (-11.9, 3.1)	0.09 (-0.4, 0.6)	49.4 (15, 83.8)**	-17.4 (-54.9, 20.1)
APLUSNR	Qvar-EB	-21.8 (-59.7, 16.1)	26.5 (19.2, 33.8)***	-0.1 (-0.6, 0.4)	-142 (-175.5, -108.5)***	---
	OPTNR	16.6 (-21.3, 54.5)	-1.6 (-8.9, 5.7)	0.07 (-0.4,0.6)	23.2 (-10.4, 56.7)	-8.6 (-45, 27.8)
	VOLR	88 (50.1, 125.9)***	6.7 (-0.6, 14)	-0.04 (-0.9,0.1)	92.6 (59.1, 126.1)***	-110.3 (-146.7, -73.9)***
	APLUSR	80.2 (42.3, 118.1)***	-1.4 (-8.7, 5.9)	-0.1 (-0.6, 0.4)	97.1 (63.6, 130.6)***	-108.1 (-144.5,-71.7)***
	OPTR	71 (32.1, 109.9)**	-3.5 (-10.9, 4)	0.02 (-0.5, 0.52)	88.5 (54.1, 122.8)***	-68.9 (-106.4, -31.4)**
OPTNR	Qvar-EB	-38.4 (-76.3, -0.5)*	28.1 (20.8, 35.4)***	-0.2 (-0.7, 0.3)	-165.1 (-198.7, -131.6)***	---
	VOLR	71.4 (33.5, 109.3)**	8.3 (1, 15.6)*	-0.4 (-0.9, 0.05)	69.4 ((35.9, 102.9)***	-101.7 (-138.1, -65.3)***
	APLUSR	63.7 (25.8, 101.6)**	0.2 (-7.1, 7.5)	-0.2 (-0.7, 0.3)	73.9 (40.4, 107.4)***	-99.5 (-135.9, -63.1)***
	OPTR	54.4 (15.5, 93.3)*	-1.9 (-9.4, 5.6)	-0.04 (-0.5, 0.45)	65.3 (30.9, 99.7)**	-60.2 (-97.8, -22.7)**
VOLR	Qvar-EB	-109.8 (-147.7, -71.9)***	19.8 (12.5, 27.1)***	0.3 (-0.2, 0.8)	-234.5 (-268.1, -201)***	---
	APLUSR	-7.8 (-45.7, 30.1)	-8.1 (-15.4, -0.8)*	0.2 (-0.3, 0.7)	4.5 (-29, 38)	2.2 (-34.2, 38.6)
	OPTR	-17 (-55.9, 21.9)	-10.2 (-17.7, -2.7)*	0.4 (-0.1, 0.9)	-4.1 (-38.5, 30.3)	41.5 (3.9, 79)
APLUS R	Qvar-EB	-102.1 (-140, -64.2)***	27.9 (20.6, 35.2)***	0.03 (-0.5, 0.5)	-239 (-272.6, -205.5)***	---
	OPTR	-9.2 (-48.2, 29.7)	-2.1 (-9.6, 5.4)	0.2 (-0.3, 0.7)	-8.6 (-43, 25.8)	39.2 (1.7, 76.7)*
OPTR	Qvar-EB	-92.8 (-131.7, -53.9)***	30 (22.5, 37.5)***	-0.1 (-0.6, 0.4)	-230.4 (-264.8, -196)***	---

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 6.7: Mean difference (95% confidence interval) for Qvar® MDI plus APLUS spacer rinsed and not rinsed.

Comparator		Spacer deposition (µg)	FPD (µg)	FPF%	MMAD	TED (µg)
APLUSNR	Q-MDI	---	-17.3 (-70.2, 35.5)	23.8 (14.7, 32.9)**	-0.03 (-0.3, 0.2)	-127 (-174.2, -79.6)**
	APLUSR	-56.2 (-292.8, 180.4)	64.4 (11.6, 117.3)*	-3.1 (-12.2, 6)	0.07 (-0.2, 0.3)	85.2 (37.9, 132.5)**
APLUSR	Q-MDI	---	-81.8 (-134.6, -28.9)*	26.9 (17.8, 36)**	-0.1 (0.5, -0.3)	-212.2 (-259.5, -164.8)**

Table 6.8: Mean difference (95% confidence interval) for Qvar® EB and Qvar® MDI operated at different flow rates.

	Comparator		FPD (µg)	FPF%	MMAD	TED (µg)	Induction port deposition (µg)
Qvar-EB	28.3L/min	60 L/min	-7.9 (-67.7, 51.9)	-1.2 (-7.8, 5.5)	0.03 (-0.4, 0.4)	23.6 (-58.7, 105.9)	21.6 (-5.7,48.8)
		90L/min	-17.7 (-77.5, 42.1)	-11.4 (-18.1, -4.8)**	$1.1x10^{-15}$ (0.4, 0.41)	34.6 (-47.7, 116.9)	40.2 (12.9, 67.5)*
	60 L/min	90L/min	9.8 (-69.6, 50)	-10.2 (-16.9, -3.6)	-0.03 (-0.4, 0.4)	11.1 (-71.2, 93.4)	18.6 (-8.6, 45.9)
Qvar MDI	28.3L/min	60 L/min	-32.8 (-71.8,6.2)	-4.6 (-23.2,13.9)	-0.03 (-0.4, 0.3)	-25.2 (-55.8, 5.5)	14.1 (-40, 68.2)
		90L/min	-38.4 (-77.4,0.6)	-13.1 (-31.6, 5.5)	-0.1 (-0.5, 0.3)	-17.8 (-48.5, 12.8)	21 (-22.8, 85.3)
	60 L/min	90L/min	-5.6 (-44.6, 33.4)	-8.4 (-27, 10.1)	-0.07 (-0.4, 0.3)	7.4 (-23.3, 38)	17.1 (-37, 71.2)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

6.2.4. Discussion

The significant decrease in drug deposition in the induction port of the ACI when using spacers was evident for both Qvar® inhaler devices studied. Again, this was due to significant drug deposition on the spacer walls instead of the induction port of the ACI. Concomitantly, the deposition in the spacer led to a significant decrease in the total emitted dose delivered from it. This was consistent with several previous *in-vitro* (Rahmatalla et al., 2002) and *in-vivo* studies (Leach, 1998b; Leach et al., 1998a; Leach, 1999).

The fine particle dose is defined as the dose of the aerosolized drug particles with an aerodynamic diameter <5μm that is capable of penetrating the lung during inhalation (respirable). The fine particle fraction (% FPF) is the percentage ratio of FPD to the total recovered dose (the dose that leaves the inhaler device and is available to the patient) (Newman et al., 2000b; Zeng et al., 2002). The addition of the spacer to Qvar® EB inhaler was associated with either not affecting the FPD as in the case of the not rinsed Aerochamber Plus (APLUSNR) or with a small decrease in the FPD as with the rest of spacers used. However, the use of the not rinsed Volumatic (VOLNR) spacer caused even more lowering to the FPD compared to the not rinsed Optimiser (OPTNR) spacer. The above results revealed that the addition of a small volume spacer such as the Aerochamber Plus or the Optimiser had less effect on decreasing the FPD from Qvar® EB inhaler than the larger volume Volumatic spacer. The previous results clearly suggest that it is not necessary to use large volume spacers with such extra-fine aerosols. The small volume spacer devices maintained the extra-fine properties of these formulations. As shown from the above results, the non-significant influence of the not rinsed Aerochamber Plus (APLUSNR) spacer when attached to Qvar® inhalers on its aerodynamic particle size distribution together with its ability to significantly decrease the quantity of BDP trapped in the *in-vitro* oropharynx make it advisable to be used by

patients. The use of spacers with inhaled corticosteroids is preferred as they diminish the risk of topical side effects like thrush and dysphonia, as well as minimising unwanted systemic side effects that could results from oral beclometasone absorption (Derendorf, 1997).

These results are in accordance with previous studies that observed no significant changes in the *in-vivo* and *in-vitro* lung deposition with an Aerochamber spacer attached to the extrafine Qvar® formulation (Leach et al., 1998b; Rahmatalla et al., 2002). Similarly, another *in-vitro* study investigated the effect of spacers on the respirable dose delivery from Qvar MDI post adding the Aerochamber Plus valved holding chamber or the Optichamber valved holding chamber. Surprisingly, the results from this study did not demonstrate an equivalent *in-vitro* performance from these two spacers despite their similar sizes, which may be due to differences in their valve design and material. The mean ± SD respirable dose (1-5μm) of BDP from the Aerochamber Plus valved holding chamber (27.2±10μg/actuation) was not significantly different from the respirable dose produced by the MDI alone (29±7.0μg/actuation). In contrast, the Optichamber dramatically decreased the respirable dose to less than half that produced by either the MDI alone or with the Aerochamber Plus valved holding chamber (Asmus et al., 2003).

In addition, the effect of the spacers' electrostatic charge on the aerosol behaviour from Qvar® EB and Qvar® MDI was also investigated. Similar to the Clenil® MDI, the washing of the spacer with soap solution followed by water rinsing apparently significantly decreased the drug output from spacers. The FPD emitted from any Qvar® inhaler/spacer combination significantly decreased when using the water rinsed spacers than that obtained when not rinsing the same detergent coated spacer. Although some manufacturers have mentioned rinsing detergent washed spacers with water, it appears

that this rinsing actually removes the detergent from the spacer and thus the antistatic effect of the detergent coating is lost.

This is in agreement with a previous study by Kwok et al (2006) who investigated the effect of detergent coated Aerochamber Plus spacers on drug output from Qvar (100μg) inhaler. In this study, coating the APLUS with detergent removed the surface charge leading to lower electrostatic retention of drugs and higher drug output from the spacer. Similarly, Dewsbury et al (1996) reported that the presence of high electrostatic charge on spacer surfaces gave the lowest respirable fraction whilst neutralisation of that charge gave the highest respirable fraction.

The mass mean aerodynamic diameter obtained from the Andersen Cascade Impactor for Qvar EB and Qvar MDI was 1.2, which is consistent with previous studies (Leach, 1998b; Stein, 1999).

The effect of higher flow rates on Qvar® EB aerosol performance was investigated. The results showed that the amount of drug deposited in the induction port of the ACI decreased significantly ($P<0.05$) when increasing the flow rate from 28.3 to 90L/min, while this induction port deposition difference was non-significant when increasing the flow rate from 28.3 to 60L/min and from 60 to 90L/min. For the Qvar® MDI, a similar reduction in the induction port deposition was indicated when increasing the flow rate; however, the results were not significant. In addition, the results from this study show that increasing the flow rates from 28.3L/min to 60, and 90L/min have little effect on the FPD delivered from both inhalers. The mean FPD (SD) obtained from Qvar® EB were 218 (29.1), 225.9 (26.6), and 235.7 (12.2) at a flow rate of 28.3, 60 and 90L/min, respectively. The mean FPD (SD) for Qvar® MDI were 208.9 (16.3), 241.8 (32.7), and 247.4 (9.5) at a flow rate of 28.3, 60 and 90L/min, respectively. These results are consistent with previous studies that have shown a significant decrease in the induction

port deposition of HFA-BDP with increasing the flow rate from 28.3 L/min to 90 L/min and suggested that BDP in this extra-fine formulation have greater accessibility to the lung at higher flow rates (Rahmatalla et al., 2002). Other studies also reported a limited effect of higher inspiratory flow rates of 60 and 90L/min on respirable dose from MDIs when compared to that achieved at 30L/min (Smith et al., 1998; Feddah et al., 2000).

6.3. Relative lung and systemic bioavailability of beclometasone dipropionate inhaled from Qvar® EB and Qvar® MDI with different spacers using urinary drug excretion post inhalation

6.3.1. Method

The aim of this investigation is to apply the urinary pharmacokinetic method of beclometasone dipropionate after an inhalation to compare the effect of different spacers on drug output from Qvar® EB and Qvar® MDI inhalers. Although, Qvar EB is not recommended to be used with spacers, its mouthpiece fits into the Volumatic, the Aerochamber Plus, and the Optimiser spacer. Qvar MDI can only be used with the Aerochamber Plus spacer.

6.3.1.1. Equipment and inhalation device

Inhaler and spacer devices used as follows:

- Qvar® Easi-Breathe inhaler (EB) labelled as nominal dose of 100µg beclometasone dipropionate per dose (Teva Pharmaceuticals, UK).
- Qvar® metered dose inhaler (MDI) labelled as nominal dose of 100µg beclometasone dipropionate per dose (Teva Pharmaceuticals, UK).
- The Aerochamber Plus spacer [APLUS], 145ml holding chamber, (Trudell Medical International Europe Ltd, UK).
- The Volumatic spacer device [VOL] 750ml holding chamber, (GlaxoSmithKline).

- The Optimiser spacer [OPT], small plastic tube spacer having a cross section of 2.5x 3.3cm (Teva Pharmaceuticals, UK.
- LC-(ESI+)-MS method conditions: sample preparation, analysis procedures and chromatographic conditions were as reported in section 3.3.

6.3.1.2. Subjects and study design

Twelve healthy non-smoking volunteers between 18-45 years consented to take part in the study and approval was obtained from the University of Huddersfield Ethics Committee. Healthy subjects received Qvar® EB or Qvar® MDI either alone or attached to different spacers. The order of these doses was randomized and each study dose was separated by 7 days. Each subject inhaled eight doses of the following study doses.

Eight 100µg (800µg in total) inhalations of beclometasone dipropionate from a Qvar® MDI (Teva Pharmaceuticals, UK) with:

- No spacer [Qvar® MDI].
- The Aerochamber Plus spacer that is washed in detergent solution, followed by either rinsing [Q-APLUSR] or not rinsing [Q-APLUSNR] with water, and then allowed to air dry.

Eight 100µg (800µg in total) inhalations of beclometasone dipropionate from a Qvar® Easi breathe inhaler (Teva Pharmaceuticals, UK) with:

- No spacer [Qvar EB®].
- The Aerochamber Plus spacer that is washed in detergent solution, followed by either rinsing [QEB-APLUSR] or not rinsing [QEB-APLUSNR] with water, and then allowed to air dry.

- The Optimiser spacer that is washed in detergent solution, followed by either rinsing [QEB-OPTR] or not rinsing [QEB-OPTNR] with water, and then allowed to air dry.
- The Volumatic spacer device that is washed in detergent solution, followed by either rinsing [QEB-VOLR] or not rinsing [QEB-VOLNR] with water, and then allowed to air dry.

On each occasion, there were eight separate actuations with each actuation followed by an inhalation. Each volunteer was trained on how to use the inhaler devices according to the patient information leaflet. The lungs were emptied as far as comfortable, the MDI was placed between the lips, actuated and at the same time the subjects breathed in through the mouth taking 5-10 seconds to fully inhale, then removed the inhaler, held their breath for 10 seconds, and slowly exhaled. The same inhalation steps were repeated for the Easi-Breathe device, however subjects were asked to preclude MDI actuation during inhalation, where the dose was delivered automatically as they breathed. The inhalation manoeuvre was a deep breath to ensure optimal drug delivery. A check was made that the breathe actuation process occurred (sound, taste and visual check of an external lever on the device that moves when a dose is released). All subjects were trained on how to use each spacer according to the instructions produced by the manufacturer. When using spacers subjects exhaled to residual volume as much as possible, the dose was discharged into the spacer and within one second subjects inhaled slowly and deeply for about 3 to 5 seconds. This was followed by a breath hold for at least 10 seconds. This inhalation manoeuvre was repeated every 30 seconds for each inhaled dose.

Urine samples were collected at 30 minutes, and then cumulatively pooled up to 24hrs after inhalation. The volume of urine excreted was recorded and aliquots of each sample

were frozen at -20°C prior to analysis. The amount of drug left in each spacer device was also determined.

6.3.2. Analysis

6.3.2.1. Sample analysis

The amount of BDP and its metabolites excreted in urine and the amount of BDP retained in each spacer device were measured using the previously developed and validated LC-(ESI+)-MS method described in section 3.3 and 3.2, respectively.

6.3.2.2. Statistical analysis

Statistical analysis of the 30 minutes and the 24 hours urinary excretion of beclometasone dipropionate and its metabolites following administration of Qvar-EB and Q-MDI either alone or with water rinsed or not rinsed detergent coated spacers and the amount left in each spacer were accomplished using a one way analysis of variance (ANOVA) test using SPSS V17.0 (SPSS Inc., Chicago, USA). In addition, One-way analysis of variance with the application of Bonferroni correction was used to compare the urinary excretions when using each inhaler alone and when it is attached to each spacer. The 30 minutes and the cumulative 24hr amounts excreted for each inhalation method were normalised for the nominal dose and then log transformed. From the mean square error of the analysis of variance, using patients and inhalation method as the main factors, the mean ratio (90% confidence interval) was calculated.

6.3.3. Results

Twelve (six females) healthy non smoking subjects completed the study. Their mean (SD) age, weight and height was 31.2 (8.9) years, 66.3 (8.1) kg and 166.7 (7.6) cm, respectively. Their demographic details were previously described in table 5.1.

The individual urinary excretion data of BOH, BDP, 17-BMP, and the amount of BDP retained in each spacer post inhalation of Qvar® EB and Qvar® MDI study doses either alone or with different spacers are presented in APPENDIX B.36 - B.42 (refer to the enclosed DVD). These urinary excretion data of BOH, BDP, and 17-BMP from Qvar® EB and Qvar® MDI are expressed graphically in figures 6.7 - 6.10. A summary of the mean (SD) amounts of parent drug and metabolites obtained from the twelve subjects post inhalation from Qvar® EB, Qvar® MDI either alone or with different spacers and the amount retained in each spacer are represented in table 6.9 and figures 6.11 - 6.14. Statistical analysis of the data is shown in tables 6.10 - 6.13.

A summary of the mean ratio (90% confidence limits) between Qvar EB when used alone and when it attached to each spacer with respect to the nominal dose is presented in table 6.14. The mean ratio (90% confidence limits) between the Aerochamber Plus (with and without rinsing) and Qvar MDI is summarized in table 6.15. These values are presented separately for BDP, 17 BMP, and BOH, as well as for all three metabolites combined**.**

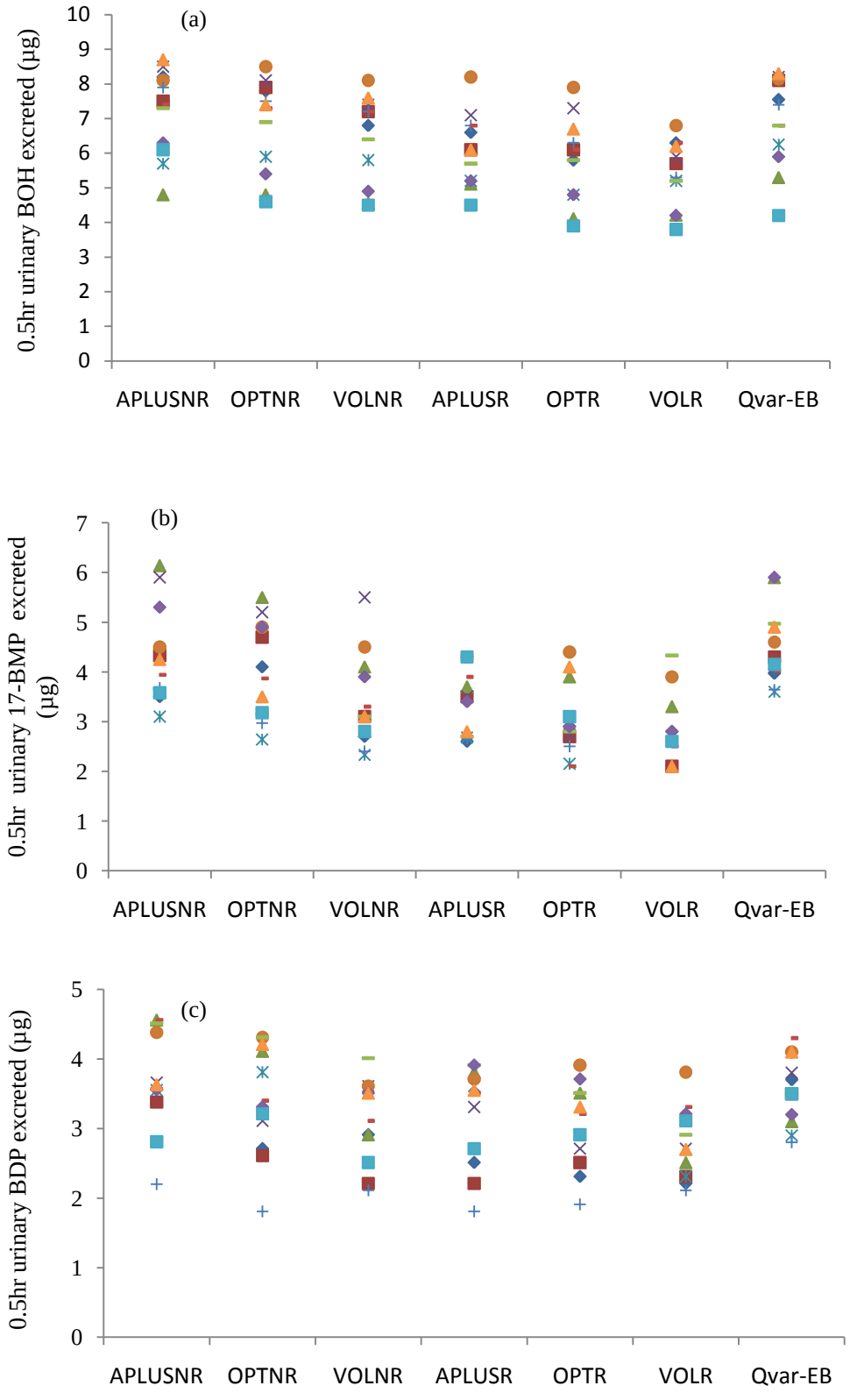


Figure 6.7: The 0.5hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted post inhalation from Qvar® EB (100µg) alone or via different spacers, expressed in µg, (n=12).

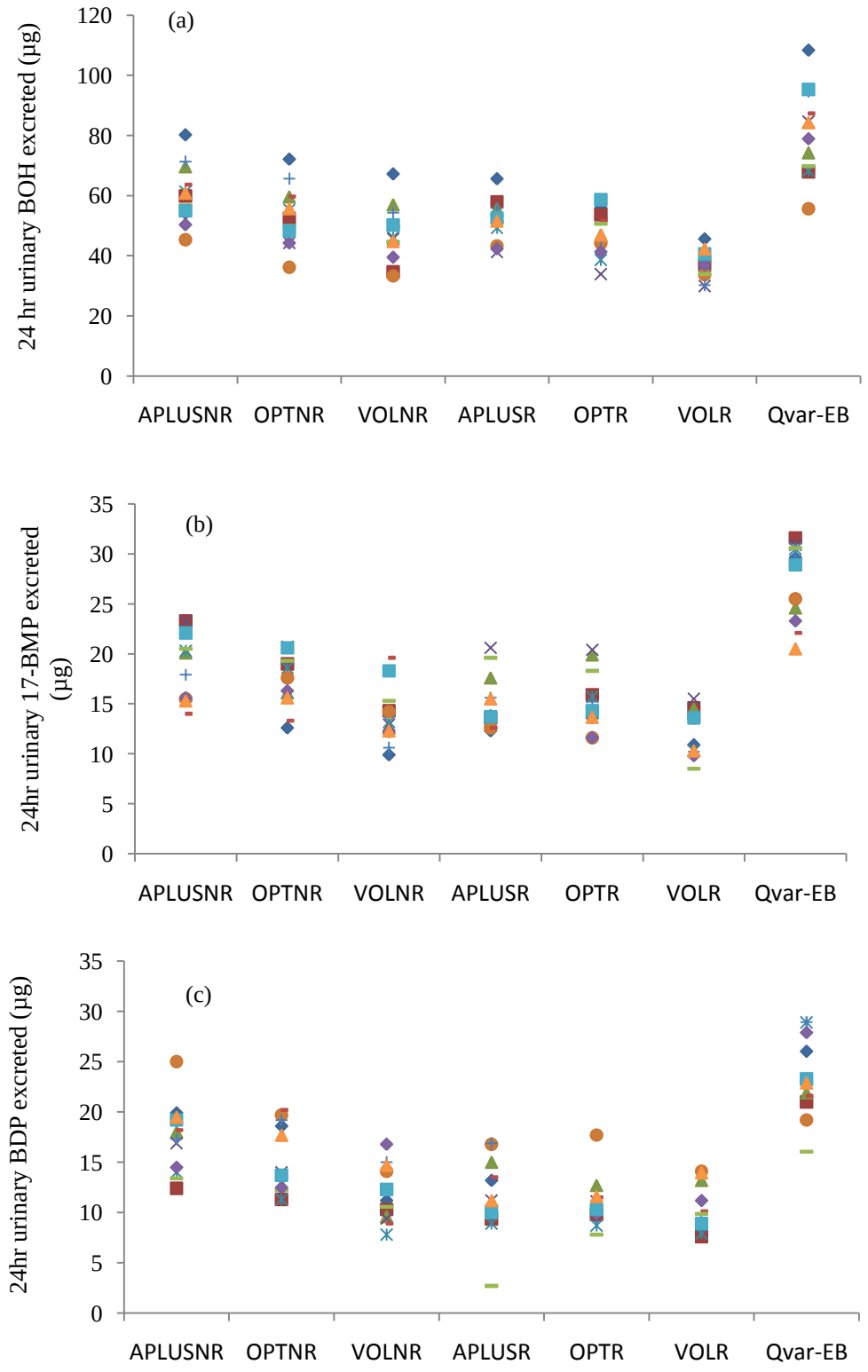


Figure 6.8: The 24hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted post inhalation from Qvar® EB (100µg) alone or via different spacers, expressed in µg, (n=12).

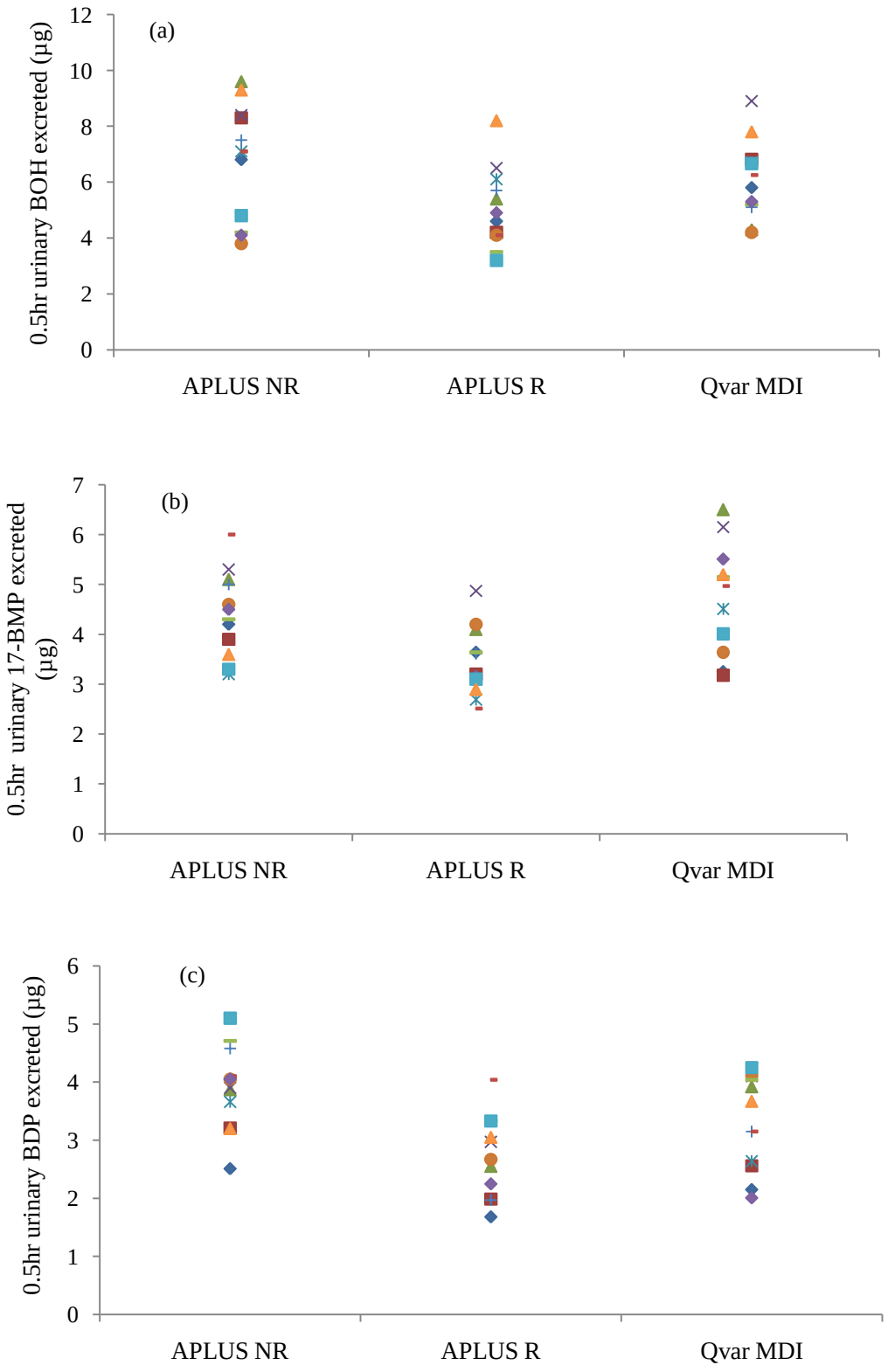


Figure 6.9: The 0.5hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted post inhalation from Qvar® MDI (100µg) alone or via different spacers, expressed in µg, (n=12).

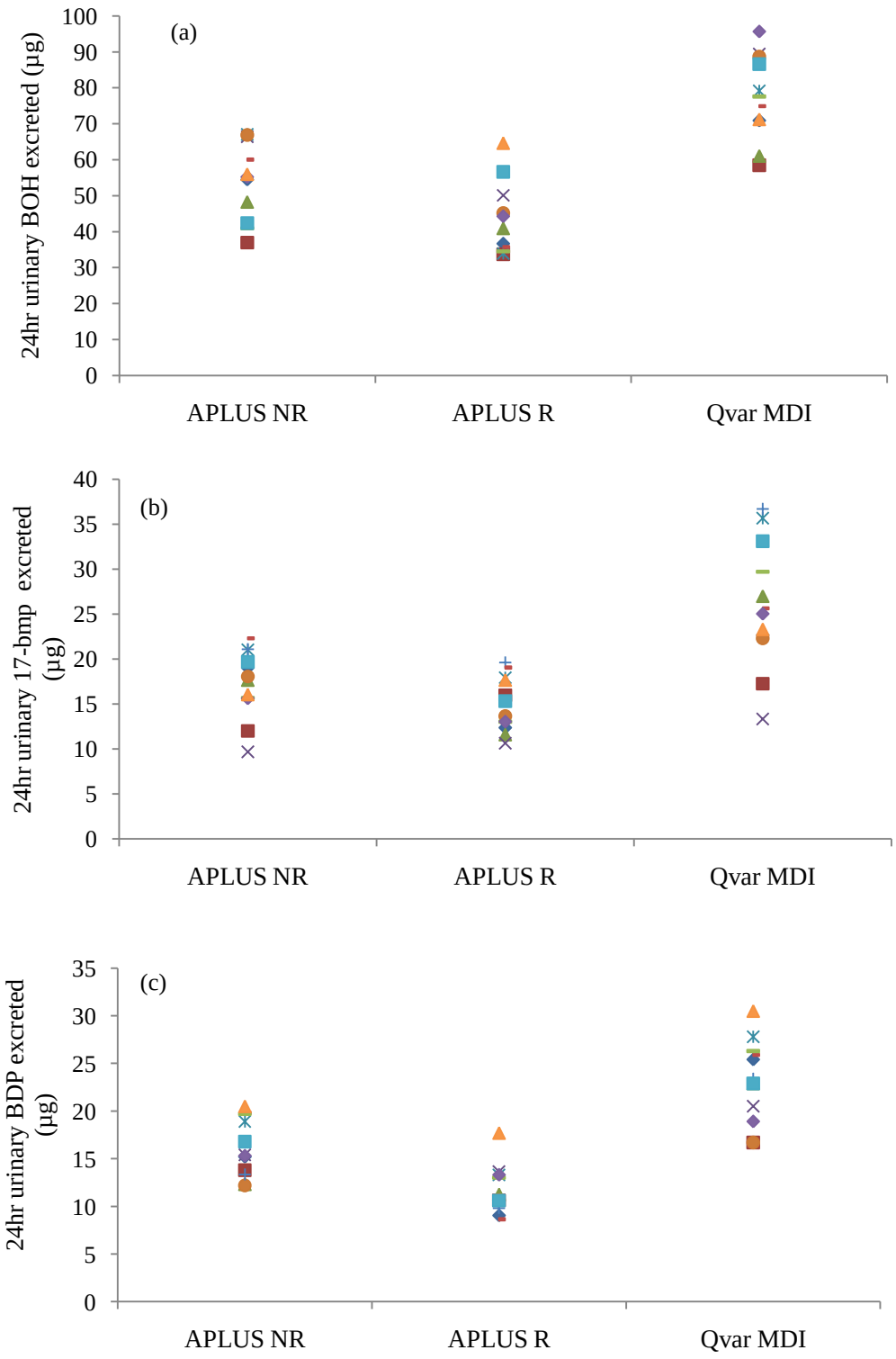


Figure 6.10: The 24hr individual amounts of (a) beclometasone (b) 17-beclometasone monopropionate (c) beclometasone dipropionate excreted post inhalation from Qvar® MDI (100µg) alone or via different spacers, expressed in µg, (n=12).

Table 6.9: Mean (SD) amount of beclometasone dipropionate and its metabolites excreted 0.5hr, and 24hr post inhalation of different study doses with and without spacers, expressed in µg, n=12.

Inhaler	Device	Amount left in spacer (µg)	17-BMP (µg)		BOH (µg)		BDP (µg)	
			0.5hr	24hr	0.5hr	24hr	0.5hr	24hr
Qvar® EB	EB	---	4.5 (0.8)	27.3 (3.9)	6.9 (1.4)	80.8 (14.6)	3.5 (0.5)	23.4 (3.9)
	VOLNR	355.5 (52.6)	3.4 (0.9)	13.7 (2.9)	6.5 (1.2)	47.2 (9.5)	3.1 (0.6)	11.7 (2.8)
	APLUSNR	336.9 (89.1)	4.3 (1.0)	18.6 (3.4)	7.2 (1.2)	60.5 (9.8)	3.7 (0.7)	17.4 (3.5)
	OPTNR	455.5 (76.8)	4.1 (1.0)	17.1 (2.6)	6.8 (1.3)	53.6 (10.0)	3.4 (0.8)	15.3 (3.5)
	VOLR	428.4 (52.5)	2.9 (0.7)	12.1(2.3)	5.5 (1.0)	37.2 (4.8)	2.8 (0.5)	10.3 (2.3)
	APLUSR	403.4 (97.8)	3.3 (0.6)	15.1 (2.8)	6.1 (1.0)	51.6 (7.0)	3.2 (0.7)	11.6 (3.9)
	OPTR	513.6 (101.8)	3.0 (0.7)	15.4 (2.9)	5.7 (1.2)	47.3 (7.3)	3.0 (0.6)	11.0 (2.5)
Qvar® MDI	MDI	---	4.7 (1.1)	25.8 (7.0)	6.1 (1.4)	77.7 (11.3)	3.3 (0.8)	23.1 (4.3)
	APLUSNR	370.1(67.5)	4.4 (0.8)	17.3 (3.8)	6.8 (2.1)	53.3 (10.7)	3.9 (0.7)	16.1 (3.0)
	APLUSR	431.4 (76.3)	3.5 (0.7)	15.0 (3.0)	5.0 (1.4)	43.0 (9.8)	2.8 (0.7)	11.8 (2.5)

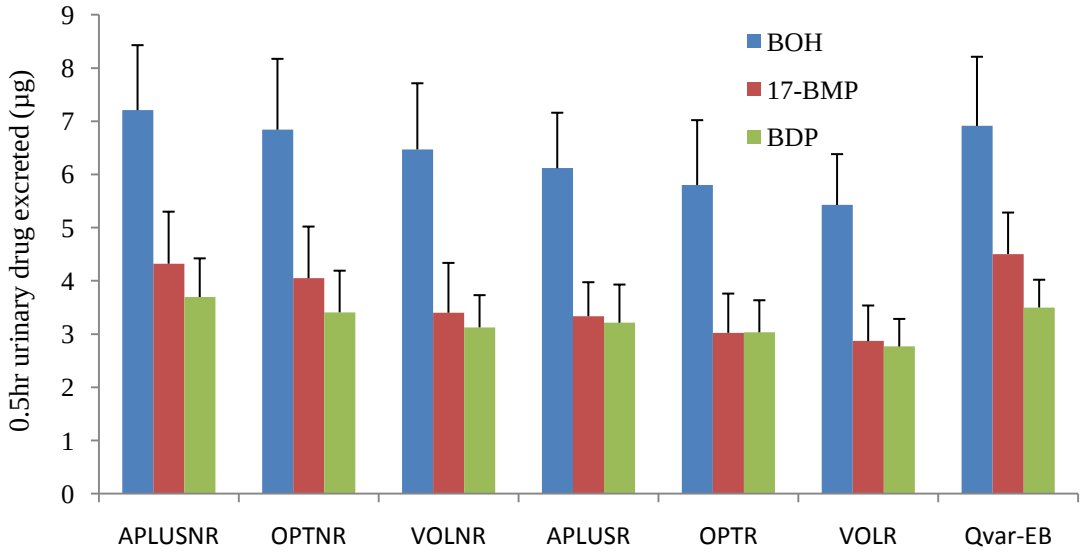


Figure 6.11: The 0.5hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Qvar EB study doses with and without spacers, (n=12).

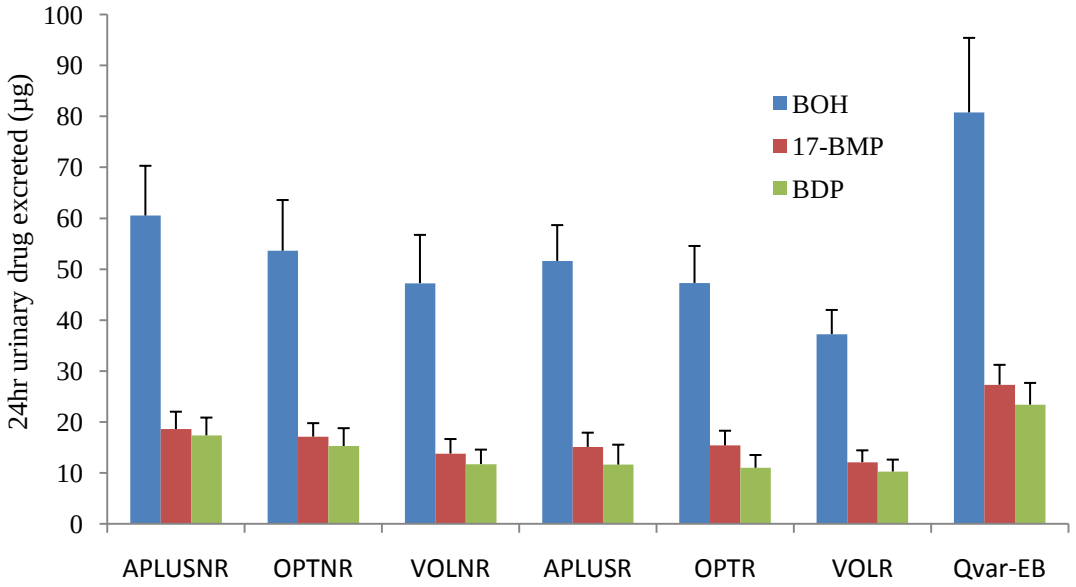


Figure 6.12: The 24hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Qvar EB study doses with and without spacers, (n=12).

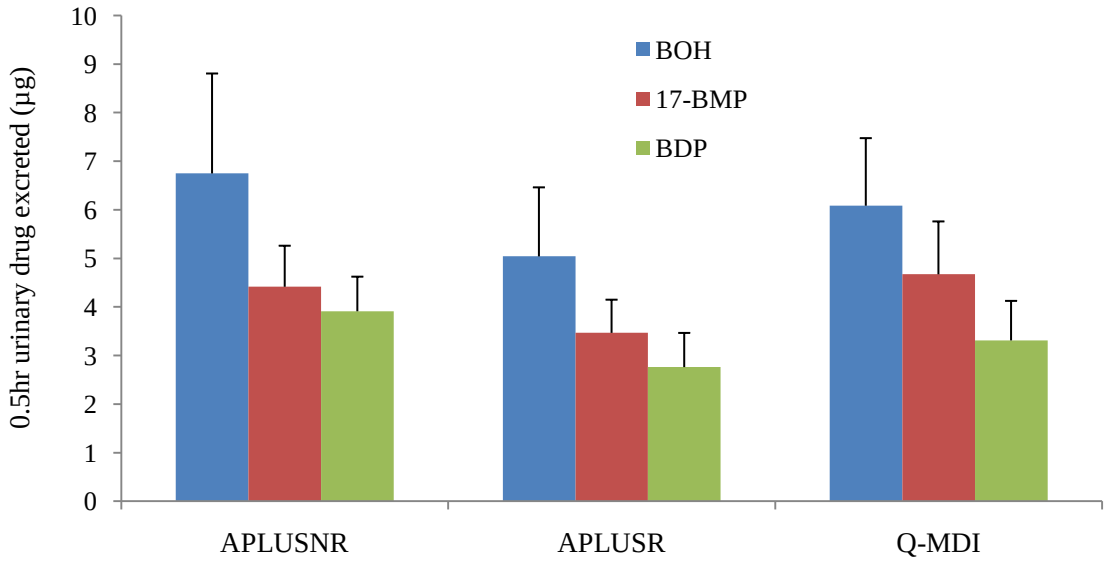


Figure 6.13: The 0.5hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Qvar EB study doses with and without spacers, (n=12).

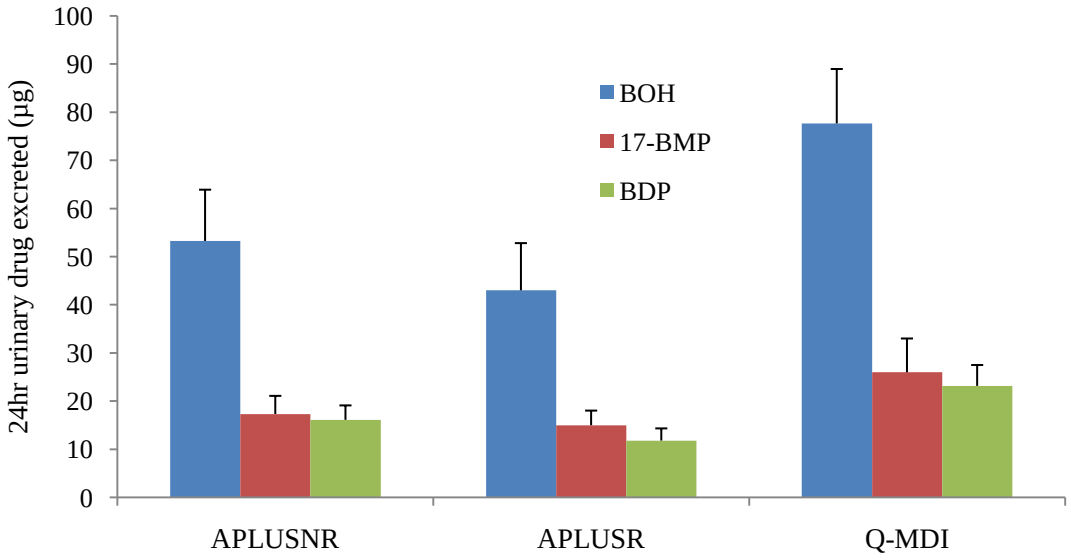


Figure 6.14: The 24hr mean (SD) amounts of beclometasone dipropionate and its metabolites excreted post inhalation of Qvar EB study doses with and without spacers, (n=12).

Table 6.10: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate and its metabolites excreted post 30 minutes using Qvar® EB and Qvar® EB+ spacers.

Inhaler	Comparator	Qvar-EB	APLUSNR	OPTNR	VOLNR	APLUSR	OPTR
17-BMP	APLUSNR	0.2 (-0.3, 0.7)	---	---	---	---	---
	OPTNR	0.5 (-0.1, 1.0)	0.3 (-0.2, 0.8)	---	---	---	---
	VOLNR	1.1 (0.6, 1.6)*	0.9 (0.4, 1.4)**	0.6 (0.2, 1.1)*	---	---	---
	APLUSR	1.2 (0.7, 1.7)*	1.0 (0.5, 1.5)**	0.7 (0.2, 1.2)*	0.1 (-0.4, 0.6)	---	---
	OPTR	1.5 (1.0, 2.0)**	1.3 (0.8, 1.8)***	1.0 (0.5, 1.5)*	0.4 (-0.1, 0.9)	0.3 (-0.2, 0.8)	---
	VOLR	1.6 (1.1, 2.1)***	1.5 (1.0, 2.0)***	1.2 (0.7, 1.7)***	0.5 (0.03, 1.0)*	0.5 (-0.1, 1.0)**	0.2 (-0.3, 0.7)
BOH	APLUSNR	-0.3 (-0.6, 0.04)	---	---	---	---	---
	OPTNR	0.1 (-0.3,0.4)	0.4 (0.03,0.7)*	---	---	---	---
	VOLNR	0.4 (0.1, 0.8)*	0.7 (0.4,1.1)***	0.4 (0.03,0.7)*	---	---	---
	APLUSR	0.8 (0.5,1.1)***	1.1 (0.8,1.4)***	0.7 (0.4,1.1)***	0.4 (0.02,0.7)*	---	---
	OPTR	1.1 (0.8,1.4)***	1.4 (1.1,1.7)***	1 (0.7,1.4)***	0.7 (0.3,1)***	0.3 (-0.02,0.7)	---
	VOLR	1.5 (1.1,1.8)***	1.8 (1.4,2.1)***	1.4 (1.1,1.8)***	1 (0.7,1.4)***	0.7 (0.4,1)***	0.4 (0.04,0.7)*
BDP	APLUSNR	-0.2 (-0.5, 0.2)	---	---	---	---	---
	OPTNR	0.1 (-0.2, 0.5)	0.3 (-0.1, 0.6)	---	---	---	---
	VOLNR	0.4 (0.1, 0.7)**	0.6 (0.3, 0.9)**	0.3 (-0.1, 0.6)	---	---	---
	APLUSR	0.3 (0.1, 0.6)*	0.5 (0.2, 0.8)**	0.2 (-0.1, 0.5)	-0.1 (-0.4, 0.2)	---	---
	OPTR	0.5 (0.2, 0.8)***	0.7 (0.3, 1.0)***	0.4 (0.1, 0.7)*	0.1 (-0.2, 0.4)	0.2 (-0.2, 0.5)	---
	VOLR	0.7 (0.5, 1.1)***	0.9 (0.6, 1.2)***	0.6 (0.3, 1.0)***	0.4 (0.02,0.7)*	0.4 (0.1, 0.8)**	0.3 (-0.1, 0.6)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 6.11: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate and its metabolites excreted post 24hr using Qvar® EB and Qvar® EB+ spacers.

Inhaler	Comparator	Qvar-EB	APLUSNR	OPTNR	VOLNR	APLUSR	OPTR
17-BMP	APLUSNR	8.7 (6.7, 10.7)***	---	---	---	---	---
	OPTNR	10.2 (8.2, 12.2)***	1.5 (-0.5, 3.5)	---	---	---	---
	VOLNR	13.5 (11.5, 15.5)***	4.8 (2.8, 6.8)***	3.3 (1.3, 5.3)**	---	---	---
	APLUSR	12.2 (10.2, 14.2)***	3.5 (1.5, 5.5)***	2.0 (0.02, 4.0)*	-1.3 (-3.3, 0.7)	---	---
	OPTR	11.9 (9.9, 13.9)***	3.2 (1.3, 5.2)**	1.7 (-0.3, 3.7)	-1.6 (-3.6, 0.4)	-0.3 (-2.3, 1.7)	---
	VOLR	15.2 (13.2, 17.2)***	6.5 (4.5, 8.5)***	5.0 (3.0, 7.0)***	1.7 (-0.3, 3.7)	3.0 (1.0, 5.0)**	3.3 (1.3, 5.3)**
BOH	APLUSNR	20.3 (15.1, 25.4)***	---	---	---		---
	OPTNR	27.2 (22.0, 32.3)***	6.9 (1.8, 12.0)**	---	---	---	---
	VOLNR	33.5 (28.4, 38.6)***	13.3 (8.2, 18.4)***	6.4 (1.4, 11.5)**	---	---	---
	APLUSR	29.2 (24.0, 34.3)***	8.9 (3.8, 14.0)***	2.0 (-3.1, 7.1)	-4.4 (-9.5, 0.7)	---	---
	OPTR	33.5 (28.4, 38.6)***	13.2 (8.1, 18.4)***	6.3 (1.2, 11.4)	-0.1 (-5.2, 5.1)	4.3 (-0.8, 9.5)	---
	VOLR	43.5 (38.4, 48.7)***	23.3 (18.2, 28.4)***	16.4 (11.3 (21.5)***	10.0 (4.9, 15.1)***	14.4 (9.3, 19.5)***	10.1 (4.9, 15.2)***
BDP	APLUSNR	6.1 (3.9, 8.2)***	---	---	---	---	---
	OPTNR	8.1 (6.0, 10.3)***	2.1 (-0.1, 4.3)	---	---	---	---
	VOLNR	11.7 (9.6, 14.0)***	5.6 (3.5, 7.8)***	3.5 (1.4, 5.7)**	---	---	---
	APLUSR	11.8 (9.6, 14.0)***	5.7 (3.6, 7.9)***	3.6 (1.5, 5.8)**	0.1 (-2.1, 2.3)	---	---
	OPTR	12.4 (10.3, 14.6)***	6.4 (4.2, 8.5)***	4.3 (2.1, 6.4)***	0.7 (-1.4, 2.9)	0.5 (-1.5, 2.8)	---
	VOLR	13.1 (11.0, 15.3)***	7.1 (4.9, 9.3)***	5.0 (2.8, 7.2)***	1.5 (-0.7, 3.6)	1.4 (-0.8, 3.5)	0.7 (-1.4, 2.9)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 6.12: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate and its metabolites excreted post 30 minutes and 24hr using Qvar® MDI and Qvar® MDI + spacers.

Inhaler	Time	Comparator	Q-MDI	APLUSNR
17-BMP	0-0.5hr	APLUSNR	0.3 (-0.4,0.9)	---
		APLUSR	1.2 (0.6,1.8)***	0.9 (0.3,1.6)**
	0-24hr	APLUSNR	8.7 (5.8,11.5)***	---
		APLUSR	11 (8.1,13.9)***	2.3 (-0.5,5.2)
BOH	0-0.5hr	APLUSNR	-0.7 (-1.7,0.3)	---
		APLUSR	1.0 (0.,2.0)*	1.7 (0.7,2.7)**
	0-24hr	APLUSNR	24.4 (16.9,31.9)***	---
		APLUSR	34.7(27.2, 42.2)***	10.3 (2.8,17.8)*
BDP	0-0.5hr	APLUSNR	-0.6 (-1.0,-0.2)*	---
		APLUSR	0.5 (0.1,1)*	1.1 (0.7,1.6)***
	0-24hr	APLUSNR	7.1 (5,9.1)***	---
		APLUSR	11.4 (9.3,13.4)***	4.3 (2.3, 6.3)***

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 6.13: Mean difference (95% confidence interval) for the amount of beclometasone dipropionate retained in each spacer post inhalations of beclometasone dipropionate Qvar EB and Qvar® MDI.

Inhaler	Comparator	VOLNR	APLUSNR	OPTNR	VOLR	APLUS R
Qvar® EB	APLUSNR	18.6 (-44.5,81.7)	---	---	---	---
	OPTNR	-100 (-163.1,-36.9)**	-118.6 (-181.7,-55.5)***	---	---	---
	VOLR	-72.9 (-136,-9.8)*	-91.5 (-154.6,-28.4)**	27.1 (-36,90.2)	---	---
	APLUSR	-47.9 (-111.0,15.1)	-66.5 (-129.6,-3.5)*	52.1 (-11,115.1)	24.9 (-38.1,88.0)	---
	OPTR	-158.1 (-221.2,-95.0)***	-176.7 (-239.8,-113.6)***	-58.1 (-121,5.0)	-85.2 (-148.3,-22.0)**	-110.2 (-173.0,-47.0)***
Qvar® MDI	APLUSR	---	-61.3 (-111, -11.6)*	---	---	---

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 6.14: Mean ratio (90% confidence interval) for Qvar EB compared to Qvar EB/spacer (when normalised for the nominal dose).

Urinary excretion	0.5hr			
	BDP	**17-BMP**	**BOH**	**All combined**
APLUSNR	96.9 (87.7, 107.0)	105.1 (95.1, 116.2)	95.5 (89.9, 101.4)	99.1 (94.2, 104.3)
APLUSR	112.3 (101.6, 124.0)	135.5 (122.6, 149.8)	112.4 (105.9, 119.2)	119.6 (113.7, 125.9)
VOLNR	114.4 (104.4, 125.5)	134.9 (119.4, 152.5)	106.8 (102.6, 111.2)	118.2 (111.9, 124.9)
VOLR	128.9 (117.6, 141.2)	158.6 (140.2, 179.1)	127.0 (122.0, 132.2)	137.4 (130.0, 145.2)
OPTNR	105.9 (96.2, 116.6)	112.7 (101.2, 125.7)	101.1 (97.5, 104.8)	106.5 (101.0, 112.2)
OPTR	117.9 (107.1, 130.0)	150.8 (135.4, 168.2)	119.5 (115.3, 123.9)	128.7 (122.0, 135.5)
24hr				
APLUSNR	135.5 (113.0, 162.6)	147.7 (135.7, 160.8)	133.0 (123.9, 142.8)	138.5 (128.7, 149.2)
APLUSR	215.5 (179.7, 258.6)	181.8 (167.0, 198.0)	155.4 (144.8, 166.9)	182.6 (169.6, 196.6)
VOLNR	202.2 (176.3, 232.1)	200.0 (177.0, 225.9)	171.4 (157.6, 186.6)	187.0 (178.6, 203.6)
VOLR	230.0 (200.6, 264.1)	227.7 (201.6, 257.3)	215.3 (197.8, 234.4)	224.3 (210.0, 239.4)
OPTNR	155.0 (136.3, 176.1)	160.0 (146.5, 174.7)	150.8 (137.6, 165.4)	155.3 (145.9, 165.0)
OPTR	214.4 (188.7, 243.8)	178.6 (163.2, 195.0)	170.0 (155.1, 186.6)	186.8 (176.0, 198.6)

Table 6.15 : Mean ratio (90% confidence interval) for Qvar MDI compared to Qvar MDI/APLUS spacer (when normalised for the nominal dose).

Urinary excretion	0.5hr			
	BDP	**17-BMP**	**BOH**	**All combined**
APLUSNR	83.4 (74.2, 93.9)	104.9 (92.7, 118.8)	92.4 (80.3, 106.4)	93.1 (86.7, 100.0)
APLUSR	120.0 (106.6, 134.9)	133.6 (118.2, 151.3)	122.0 (106.0, 140.5)	125.1 (116.4, 134.4)
24hr				
APLUSNR	143.6 (129.8, 159.0)	148.3 (133.4, 164.7)	147.3 (131.7, 164.7)	146.4 (137.9, 155.4)
APLUSR	196.8 (177.7, 217.7)	170.2 (153.1, 189.1)	182.8 (163.3, 204.4)	182.9 (172.2, 194.3)

Significantly, more amounts of beclometasone dipropionate and its metabolites were excreted in the urine 30 minutes following inhalation by Qvar EB inhaler alone compared to that when attached to the Volumatic spacer with or without rinsing or when attached to the rinsed Aerochamber Plus and the rinsed Optimiser. However, as shown in table 6.10, the results were found to be non-significant when comparing Qvar EB alone vs attaching it with either the Aerochamber Plus or the Optimiser spacer without rinsing. Table 6.11 shows that the 24 hours urinary excretion post inhalation of Qvar EB and all differently treated spacers (VOLNR, APLUSNR, OPTNR, VOLR, APLUSR, and OPTR) showed a significant difference ($p < 0.001$).

More significant amounts of BDP and metabolites were excreted at 30 minutes and 24hrs post inhalation from the Volumatic NR vs R. However, the results were non-significant with the 24hr urinary BDP and 17-BMP amounts. Similarly, more 30 minutes and 24hr urinary excretion from QEB-APLUSNR vs QEB-APLUSR and QEB-OPTNR vs OEB-OPTR was obtained. The results were statistically significant, except when comparing the 24hr urinary excretion of BMP and BOH for the not rinsed Optimiser vs the rinsed.

For Qvar® MDI, the 30 minutes urinary amounts of BDP and metabolites excreted were similar post inhalation from Qvar® MDI alone or when attached to the Aerochamber Plus spacer without rinsing. While using the rinsed Aerochamber Plus (APLUSR) spacer led to a significant decrease in the 30 minutes urinary excretion of BDP ($p<0.05$), 17-BMP ($p<0.001$), and BOH ($p<0.05$), compared to that when using the MDI alone. The use of Aerochamber Plus spacer with and without rinsing significantly ($p<0.001$) decreased the 24hr urinary excretion of BDP and metabolites than that when using the MDI alone. The 30 minutes urinary amounts of BDP and metabolites excreted post inhalation from Q-APLUSNR was significantly higher than that obtained from Q-APLUSR spacers. The results were significant at $p<0.01$for BOH and 17-BMP and at $p<0.001$for BDP. The

24hr urinary amounts excreted post inhalation from Q-APLUSNR vs Q-APLUSR was significant at $p<0.001$ for BDP and at $p<0.05$ for BOH and non-significant for 17-BMP.

6.3.4. Discussion

The mouthpiece of the Qvar EB fitted tightly into the Aerochamber Plus, the Volumatic, as well as the Optimiser Spacer.

Non-significant urinary amounts of BDP and its metabolites were excreted at 30 minutes post inhalation from Qvar® EB alone compared to inhalation via the Qvar EB attached to the not rinsed Aerochamber Plus and the not rinsed Optimiser. However, significant more amounts of drug and metabolites were excreted from Qvar EB alone compared to when it was attached to any of the rest of spacers used. Similarly, for the Qvar® MDI, comparable amounts of BDP and metabolites were excreted when using the MDI alone or when attached to the not rinsed Aerochamber Plus spacer. In contrast, the Q-APLUSR spacer combination significantly reduced drug delivery. These results imply that patients with asthma would receive the same dose of beclometasone dipropionate from Qvar® EB alone or via the not rinsed Aerochamber Plus or the not rinsed Optimiser and from Qvar® MDI alone or via the not rinsed Aerochamber Plus if using an optimal inhaler technique. In addition, the 30 minutes and the 24hr urinary excretion post inhalation via the not rinsed Aerochamber Plus were higher than all other spacers used. The results were significant at all times except when comparing the 30 minutes and the 24hr urinary amounts of BMP and BDP delivered from the not rinsed Aerochamber Plus vs the not rinsed Optimiser where it failed to reach significance.

The use of any of the spacer devices with Qvar® MDI or Qvar® EB resulted in significantly ($p<0.001$) lower amounts of BDP and metabolites 24 hours post dosing. This was due to deposition of part of the dose on the walls of the add-on device instead of the patient's throat.

This is in agreement with a recent *in-vivo* gamma scintigraphy study that showed that the small particle HFA-BDP lung deposition averaged 52% and was not affected by the use of the Aerochamber. This study also reported a reduction in the oropharyngeal deposition of HFA-BDP from approximately 28% to 4% with the Aerochamber (Leach and Colice, 2010). Another study showed that the use of the Aerochamber Plus spacer with the highly extra fine formulation of Ciclesonide MDI did not affect its pharmacokinetics, suggesting a similar lung deposition when using the Ciclesonide MDI with or without a spacer (Drollmann et al., 2006).

Similarly, other studies reported that using small tube spacers (50ml) with HFA formulations were found to markedly reduce oropharyngeal deposition either without affecting (Hardy et al., 1996) or with increasing lung deposition (Richards et al., 2001). Previous findings by Hardy et al (1996) reported that the use of the Optimiser spacer with the Easi-Breathe inhaler removed most of the non-respirable drug, without compromising the fine particle dose delivered from the Easi-Breathe inhaler and significantly reduced oropharyngeal deposition in healthy subjects by 80%. Thus, the combination of a breath-operated inhaler with a small volume spacer offers the advantages of improved co-ordination and reduction in oropharyngeal deposition, without the inconvenience of a large volume spacer (Hardy et al., 1996).

The inhalation of Qvar EB via the not rinsed Aerochamber Plus (APLUSNR) and the not rinsed Optimiser spacer (OPTNR) produced more drug and metabolites excreted than that inhaled via the not rinsed Volumatic. This may be attributed to that small volume spacers such as the Optimiser and Aerochamber Plus may be more suitable in maintaining the extra-fine particle fraction better than large volume spacers such as the Volumatic spacer.

Also, the comparison between different treatment methods for the same spacer showed superior lung deposition of the detergent coated spacers without subsequent water rinsing to those followed by rinsing for both Qvar® EB and Qvar® MDI. These results follow the same trend as those with Clenil®.

Plastic spacers are highly prone to the build up of static charge through contact and friction, so when contacting the highly charged aerosol cloud confined inside the spacer, mutual repulsion between the charged particles cause them to move to the periphery of the aerosol cloud and contact the spacer walls. This drug retention within the spacer device results in significant reduction in the drug aerosol available for inhalation. Highly charged spacers have been shown to significantly reduce both *in-vitro* (O'Callaghan et al., 1993; Barry and O'Callaghan, 1995) and *in-vivo* (Kenyon et al., 1998; Anhoj et al., 1999) drug output. These differences in dose delivered are due to less electrostatic attraction of charged aerosol particles to walls of the non-electrostatic spacer (detergent prewashed and air-dried). Another study showed that removal of the electrostatic charge from Nebuhaler, Volumatic, and the smaller Aerochamber by soaking in a household detergent increased drug output from CFC- and HFA-MDIs through all spacers by 17-82% (Chuffart et al., 2001).

As previously published and further confirmed in this study, the fine details of adequate handling of spacers can have a significant effect on maximizing drug delivery from various spacers and inhaler devices, thus improve therapeutic responses, and reduce treatment costs.

The findings of this study of reduced FPD after rinsing are in line with the 30 minutes urinary excretion data. According to the results from the previous *in-vitro* and *in-vivo* studies, the FPD and the TED *in-vitro* parameters are more important in predicting the *in-vivo* 30 minutes and the 24hr urinary drug excretion, respectively. This is in agreement

with a previous study that reported that there is no discernible relationship between the MMAD and the in vivo indices of salbutamol lung deposition and suggested that the FPD is the more appropriate indicator for *in-vivo* lung deposition (Richardson et al., 2007). In addition, Harrison et al (1997) showed good correlation between the *in-vitro* fine particle mass and the *in-vivo* drug delivery of three different strengths of an HFA-BDP formulation. Similarly, it was previously reported that plotting the dose response curve in terms of the emitted dose (for systemic response) and fine particle dose for (pulmonary effects) better represent the doses causing these specific responses when comparing different ICSs (Martin et al., 2002; Parameswaran et al., 2003).

6.4. Conclusion

The use of spacers with Qvar® inhalers always significantly reduced the oropharyngeal deposition, however, they did not increase the amount of drug excreted 30 minutes post dosing. The presence of the electrostatic charge on the surface of water rinsed spacers following detergent washing would have contributed to significant loss of drug output from the spacer compared to those not water rinsed post detergent treatment.

Overall, the previous *in-vivo* and *in-vitro* results showed good correlation with each other. According to the results from these studies, the FPD and the TED *in-vitro* parameters are more important in predicting the *in-vivo* 30 minutes and the 24hr urinary drug excretion, respectively.

Chapter 7: Comparison of *In-Vitro* Aerodynamic Particle Size Distribution and *In-Vivo* Relative Lung and Systemic Bioavailability of Beclometasone Inhaled from the Extra-Fine Qvar® and the Non-Extrafine Clenil® Modulite Inhalers

7.1. Introduction

Inhalation aerosols such as metered dose inhalers are used largely for the treatment of lung diseases. Chlorofluorocarbons (CFCs) were the most widely used propellants in MDIs as it is cheap, safe and efficient, but recently its use has been universally restricted due to its deleterious effects on the ozone layer. The International Montreal Protocol agreement on phasing out the ozone depleting chlorofluorocarbons propellants (CFCs) has led to the search of a suitable alternative propellant for MDIs. Beclometasone dipropionate has been recently reformulated with the safer and more environmentally friendly HFA propellants. Despite the complexities and challenges faced in this reformulation process, considerable success has been achieved. The new Modulite® platform technology, allows for the manipulation of inhaled HFA based solution formulations to tailor the desired particle size for optimum lung deposition. These development in aerosol designs and technologies have facilitated the transition to CFC-free products at unchanged doses, or to replace existing drugs at reduced nominal daily dose in case of extra-fine formulations.

Beclometasone dipropionate has been recently reformulated into two CFC-free beclometasone inhalers that are available in the UK and licensed for asthma treatment. The extrafine Qvar® formulation (1.1µm) delivers most of the inhaled dose to both central and peripheral airways resulting in a uniform treatment of inflammation and bronchoconstriction throughout the lower respiratory tract. This feature is of particular interest for inhaled corticosteroids treatment as the asthmatic inflammation affects both large and small airways considerably. It was not until HFA solution technology was introduced that it was possible to produce a MDI with lung deposition values greater than 50%. This improved lung deposition require halving the dosage down when switching patients from a CFC- inhaler to Qvar® (Davies et al., 1998).

The non-extrafine formulation Clenil® Modulite (2.9µm MMAD, Chiesi, UK), allows for an easy transition from CFC-BDP to HFA-BDP products as it is designed to deliver an aerosol with particle size and properties that more closely resembles those of a CFC-MDI. Many studies have confirmed that Clenil® provided similar asthma control, efficacy (Anderson et al., 2002; Rocca-Serra et al., 2002; Woodcock et al., 2002a) and comparable adverse effects (Acerbi et al., 2007) when compared to CFC-BDP inhalers. In the UK, Clenil Modulite is the only available HFA-BDP that can be used in place of CFC-BDP without changing the prescribed dose of corticosteroid. Therefore, it has solved the technical difficulties in switching patients from CFC to HFA-BDP inhalers (Ganderton et al., 2002; Bousquet et al., 2009).

7.2. *In-vitro* comparison of aerodynamic particle size distribution of beclometasone dipropionate post inhalation from different inhalers

The aim of this section is to compare the aerodynamic particle size distribution of beclometasone dipropionate emitted from Qvar® EB (100µg), Qvar® MDI (100µg), and Clenil® MDI (250µg) with and without spacers by using the previously reported results in chapter 5.2 and 6.2 of this thesis.

7.2.1. Statistical analysis

The data was statistically analyzed using one way analysis of variance (ANOVA) to compare the aerodynamic particle size characterization of different inhalers and inhalers/spacer combinations at a flow rate of 28.3 L/min and the aerodynamic particle size characterization of different inhalers used alone at higher flow rates using SPSS V17.0 (SPSS Inc., Chicago, USA).

7.2.2. Results

7.2.2.1. Aerodynamic particle size characterization

A summary of the *in-vitro* data obtained from the ACI for Clenil® (250µg), Qvar® EB (100µg), and Qvar® MDI (100µg) at a flow rate of 28.3L/min with different spacers and at different flow rates of 28.3, 60, and 90L/min without spacers are summarized in tables 7.1, and 7.2, respectively. Figures 7.1, 7.2, and 7.3 represent the data obtained from the three inhalers with the Optimiser, the Volumatic, and the Aerochamber Plus spacer respectively at a flow rate of 28.3L/min. Figures 7.4, 7.5, and 7.6 represent the data obtained from the three inhalers alone when operated at flow rates of 28.3, 60, and 90L/min, respectively. A summary of the statistical analysis of the *in-vitro* data obtained from each of the three inhalers plus different spacers at a flow rate of 28.3L/min and obtained from each inhaler alone at different flow rates are shown in table 7.3, and 7.4, respectively.

Table 7.1: A summary of the mean (SD) data obtained from the Andersen Cascade Impactor (ACI) for Clenil ® (250µg), Qvar® EB (100µg), and Qvar MDI (100µg) used alone at 28.3, 60, and 90L/min flow rates. Values quoted in µg unless specified, (n=5).

Inhalation method	Spacer used	Spacer deposition	TED* (µg)	TED (% ND)*	FPD (µg)	%FPF of (ND)*	%FPF of (TED)*	MMAD (µm)	GSD (No units)
Clenil MDI	----	------	381.8 (6.3)	76.4 (1.7)	97.6 (20.8)	19.5 (4.2)	25.6 (5.4)	2.8 (0.4)	2.2 (0.2)
	VOLNR	224.3 (35.0)	218.9 (23.1)	43.8 (4.6)	153.9 (19.4)	30.8 (3.9)	70.3 (5.6)	2.3 (0.1)	2.3 (0.2)
	APLUSNR	233.9 (25.7)	212.1 (21)	42.4 (4.2)	138.8 (22.2)	27.8 (4.4)	65.2 (4.9)	2.3 (0.1)	2.7 (0.3)
	OPTNR	240.9 (26.6)	163.4 (15.2)	32.7 (3.0)	93.3 (17.6)	18.7 (3.5)	57.6 (5.3)	3.1 (0.2)	2.3 (0.1)
	VOLR	271.8 (20.9)	162.2 (13)	32.4 (2.6)	110.6 (7.4)	22.1 (1.5)	68.3 (2.1)	2.7 (0.2)	2.2 (0.1)
	APLUSR	301.6 (49.3)	155.3 (15.4)	31.1 (3.1)	90.6 (18.8)	18.1 (3.8)	58.1 (7.2)	3.1 (0.3)	2.2 (0.1)
	OPTR	305.5 (33.9)	112.5 (8.0)	22.5 (1.6)	62.7 (8.2)	12.5 (1.6)	55.6 (3.6)	3.3 (0.3)	1.8 (0.1)
Qvar EB	-----	-----	372.6 (27.1)	93.2 (6.8)	218.0 (29.1)	54.5 (7.3)	58.4 (5.2)	1.2 (0.2)	3.8 (0.3)
	VOLNR	169.2 (18.7)	191.6 (20.8)	47.9 (5.2)	161.3 (22.2)	40.3 (5.6)	84 (2.4)	1.2 (0.1)	4.0 (1.0)
	APLUSNR	117.7 (16.5)	230.7 (30.1)	57.7 (7.5)	196.2 (27.0)	49 (6.8)	84.9 (1.4)	1.1 (0.3)	3.8 (1.1)
	OPTNR	126.4 (8.1)	207.5 (9.6)	51.9 (2.4)	179.6 (15.1)	44.9 (3.8)	86.5 (4.1)	1.0 (0.2)	3.4 (0.01)
	VOLR	228.1 (20.2)	138.1 (20.3)	34.5 (5.1)	108.2 (19)	27 (4.7)	78.2 (2.5)	1.5 (0.23)	4.17 (1.1)
	APLUSR	225.8 (36.9)	133.6 (18.4)	33.4 (4.6)	115.9 (22.9)	28.9 (5.7)	86.3 (5.5)	1.2 (0.49)	3.3 (0.7)
	OPTR	191.2 (22.9)	138.8 (16.5)	34.7 (4.1)	121.9 (20.9)	30.5 (5.2)	87.5 (5)	1.1 (0.15)	2.6 (0.01)
Qvar MDI	-----	-----	362.2 (34.7)	90.5 (8.7)	208.9 (16.3)	52.2 (4.1)	57.6 (6.2)	1.2 (0.15)	3.5 (0.4)
	APLUSNR	183.4 (30.0)	235.3 (29.0)	58.8 (7.2)	191.6 (23.8)	47.9 (6)	81.4 (0.4)	1.1 (0.06)	4.2 (0.3)
	APLUSR	239.6 (66.2)	150.1 (28.9)	37.5 (7.2)	127.2 (27.2)	31.79 (6.8)	84.5 (3.5)	1.1 (0.12)	4.1 (0.4)

* TED: Total emitted dose, ND: Nominal dose.

Table 7.2: A summary of the data obtained from the ACI for Clenil® (250µg), Qvar® EB (100µg), and Qvar MDI (100µg), (n=5)

	Inhaler	28.3L/min	60L/min	90L/min
Total emitted dose (TED) (µg)	Clenil	381.8 (6.3)	383.2 (22.7)	348.6 (19.0)
	Qvar EB	372.6 (27.1)	349.1 (41.9)	338.0 (22.2)
	Qvar MDI	362.2 (34.7)	387.4 (16.6)	380.0 (10.4)
TED (% of nominal dose)	Clenil	76.4 (1.7)	76.6 (4.5)	69.7 (3.8)
	Qvar EB	93.2 (6.8)	87.3 (10.5)	84.5 (5.5)
	Qvar MDI	90.6 (8.7)	96.9 (4.2)	95.0 (2.6)
FPD (µg)	Clenil	97.6 (20.8)	138.0 (7.1)	116.5 (5.7)
	Qvar EB	218 (29.1)	225.9 (26.6)	235.7 (12.2)
	Qvar MDI	208.9 (16.3)	241.8 (32.7)	247.4 (9.5)
% FPF of nominal dose	Clenil	19.5 (4.2)	27.6 (1.4)	23.4 (1.1)
	Qvar EB	54.5 (7.3)	64.8 (8.1)	58.9 (3.1)
	Qvar MDI	52.2 (4.1)	60.4 (8.2)	61.8 (2.4)
%FPF of TED	Clenil	25.6 (5.4)	36.1 (3.3)	33.6 (0.5)
	Qvar EB	58.4 (5.2)	59.6 (1.9)	69.8 (3.8)
	Qvar MDI	57.6 (6.2)	62.3 (6.1)	70.7 (7.7)
MMAD (µm)	Clenil	2.8 (0.4)	2.2 (0.1)	2.2 (0.2)
	Qvar EB	1.2 (0.2)	1.2 (0.2)	1.2 (0)
	Qvar MDI	1.2 (0.2)	1.2 (0.1)	1.3 (0.2)
GSD (no units)	Clenil	2.2 (0.2)	2.9 (0.15)	3.3 (0.2)
	Qvar EB	3.8 (0.3)	3.4 (0.3)	3.1 (0.3)
	Qvar MDI	3.5 (0.4)	3.4 (0.3)	3.4 (0.9)
Induction port deposition (µg)	Clenil	251.3 (22.0)	200.8 (19.4)	187.3 (14.7)
	Qvar EB	121.8 (15.5)	100.2 (18.6)	81.6 (18.1)
	Qvar MDI	130.9 (26.3)	116.8 (27.8)	99.6 (4.3)

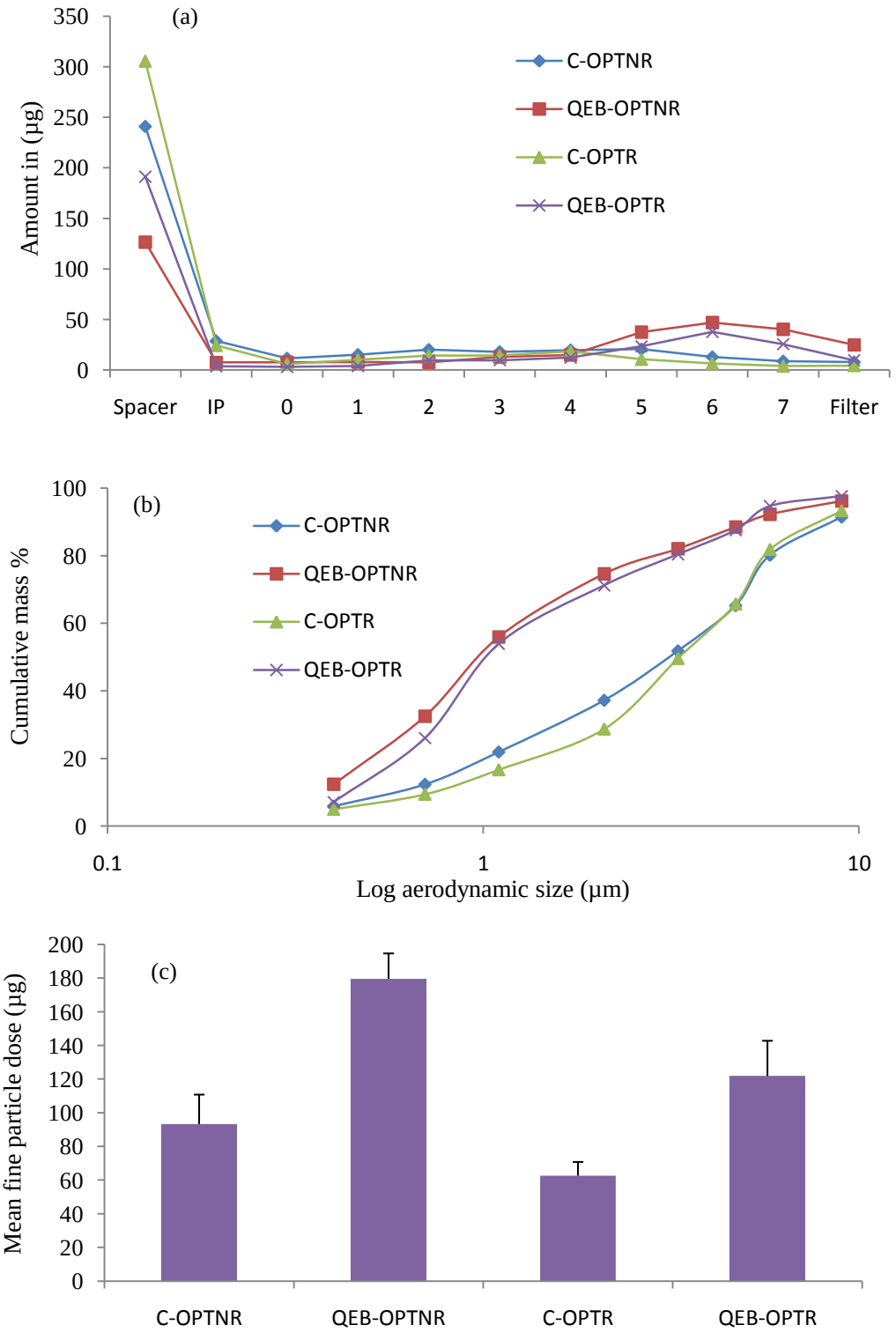


Figure 7.1: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose emitted from different inhalers with the Optimiser spacer at a flow rate 28.3 L min^{-1}, (n=5).

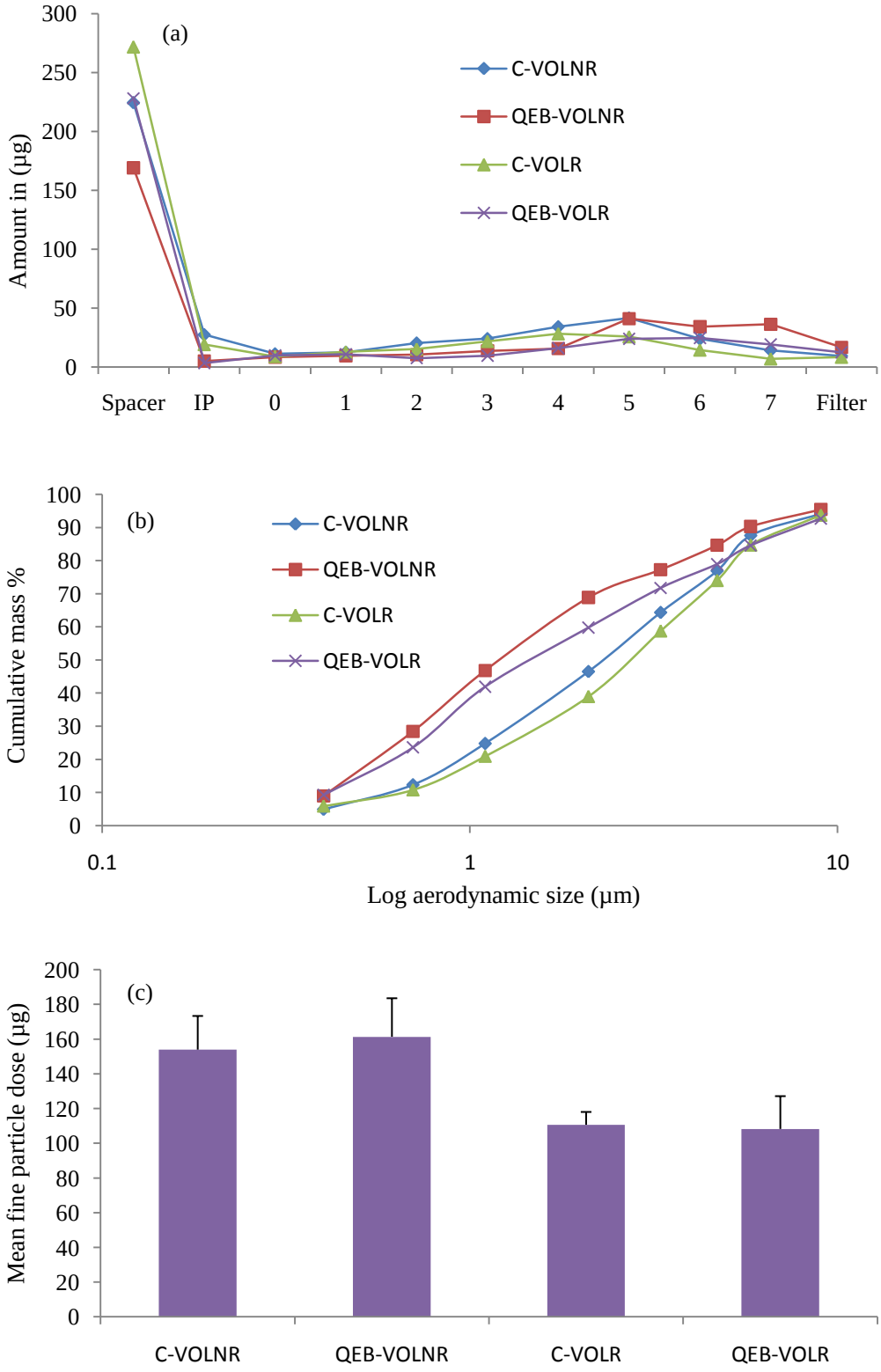


Figure 7.2: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose emitted from different inhalers with the Volumatic spacer at a flow rate 28.3 L min^{-1}, (n=5).

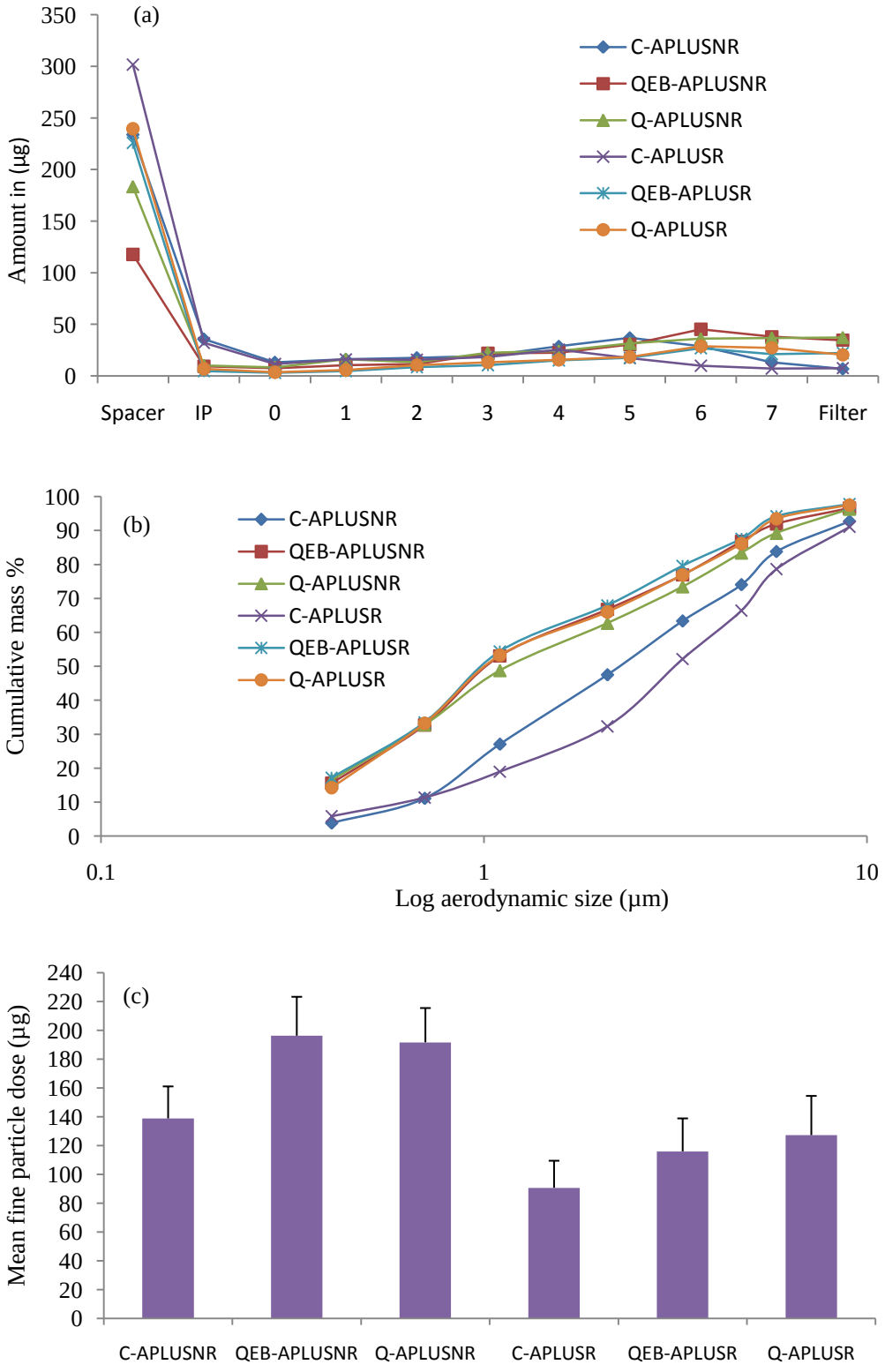


Figure 7.3: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose emitted from different inhalers with the Aerochamber Plus spacer at a flow rate 28.3 L min^{-1}, (n=5).

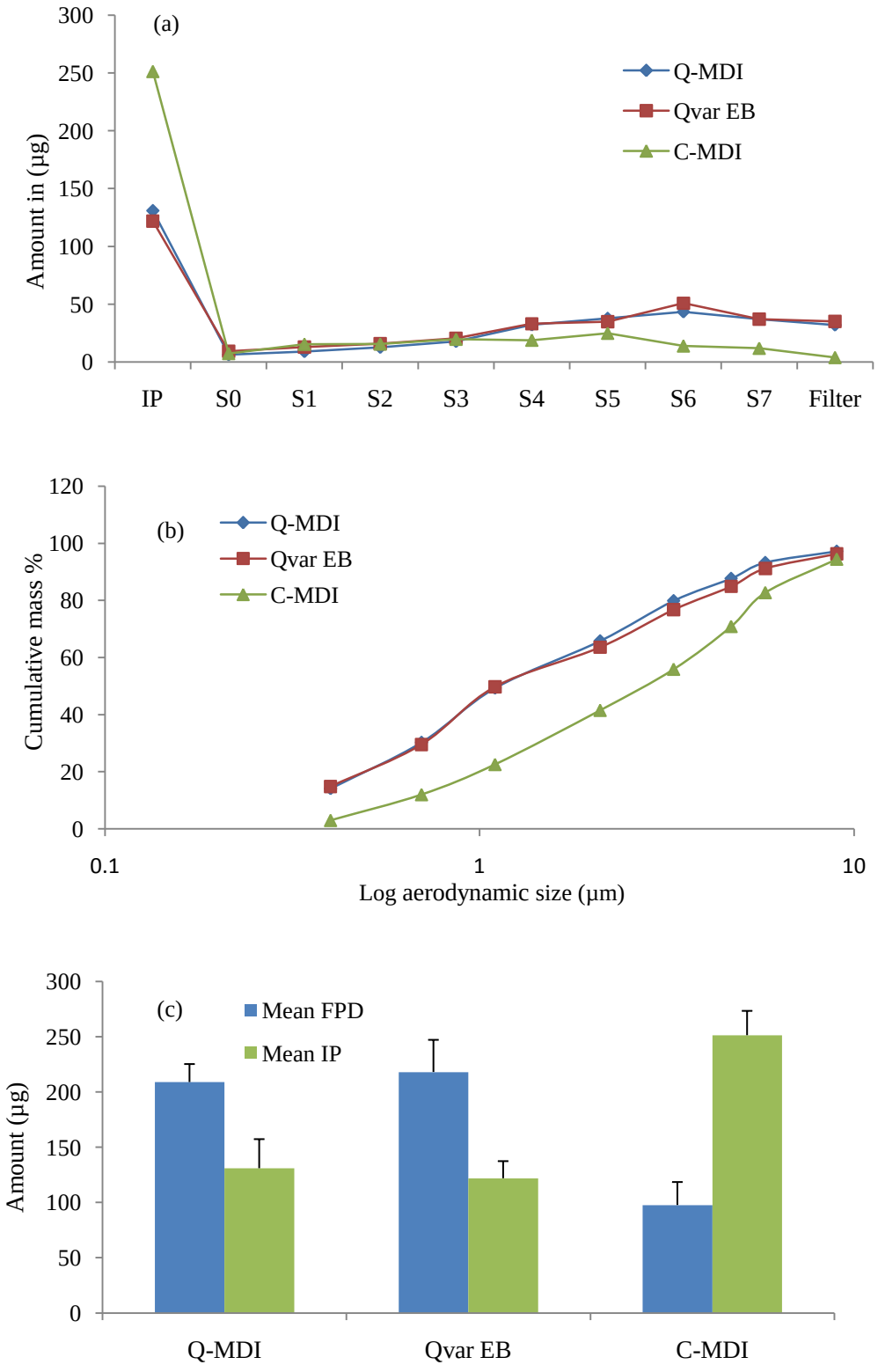


Figure 7.4: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose and induction port deposition emitted from different inhalers at a flow rate of 28.3L min^{-1}, (n=5).

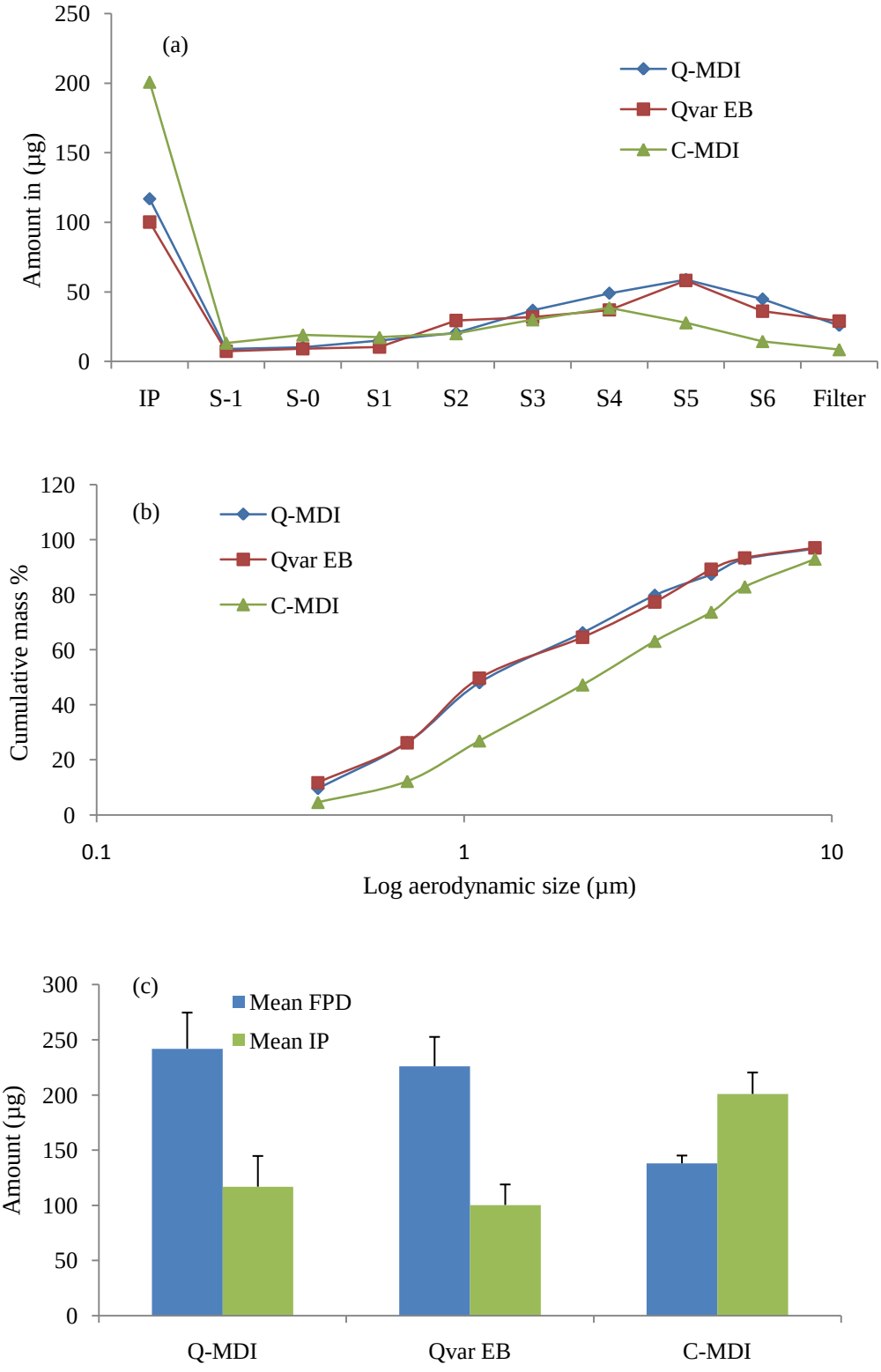


Figure 7.5: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg) (b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose and induction port deposition emitted from different inhalers at a flow rate of 60 L min^{-1}, (n=5).

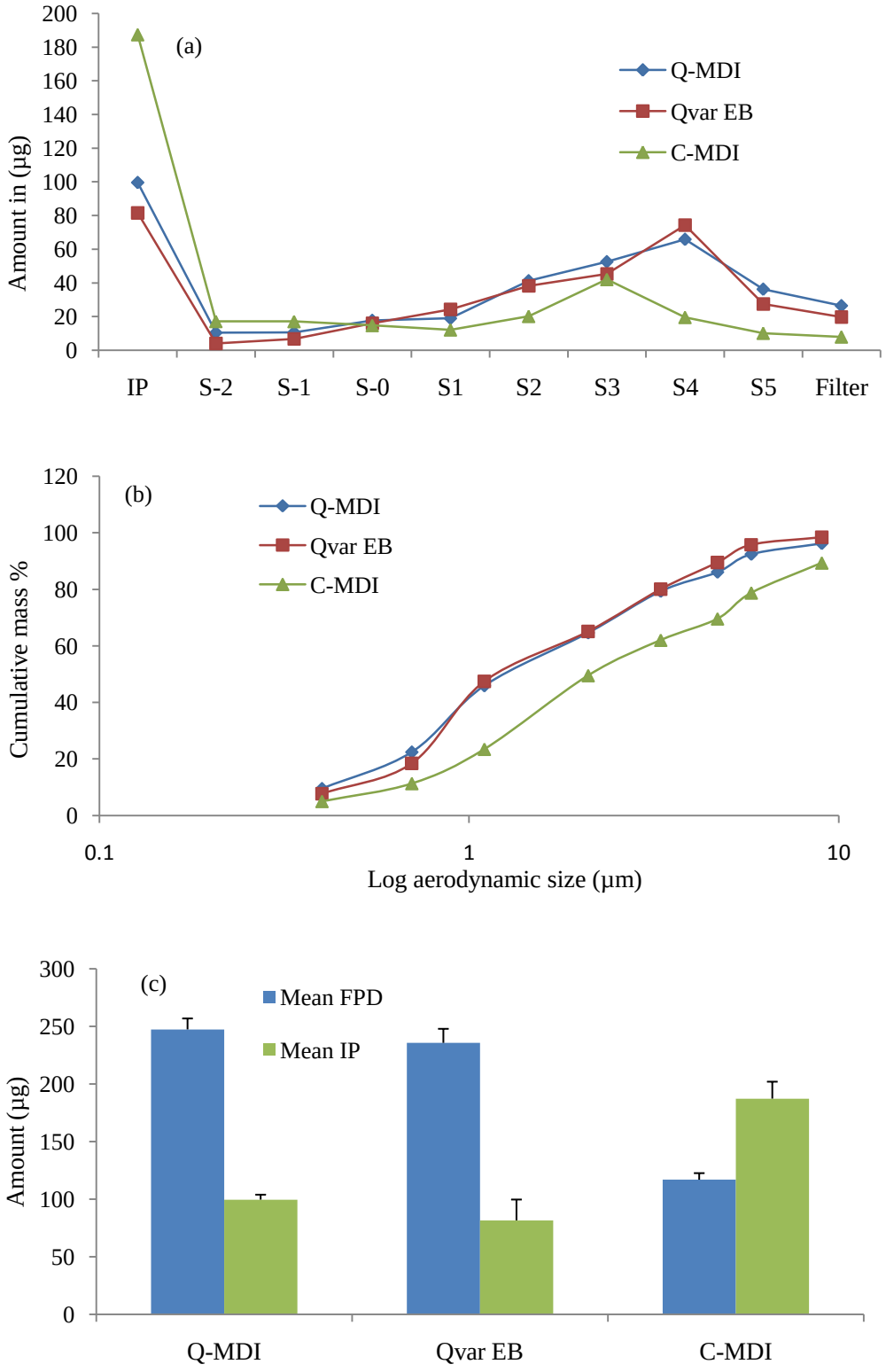


Figure 7.6: Mean beclometasone dipropionate (a) deposited in each stage of the ACI (µg)(b) aerodynamic distribution of the emitted dose (c) mean (SD) fine particle dose and induction port deposition emitted from different inhalers at a flow rate of 90 L min^{-1}, (n=5).

Table 7.3: Mean difference (95% confidence interval) for each spacer used with different inhalers at 28.3 L/min flow rate.

Comparator		%FPF of nominal dose	%TED of nominal dose
C-APLUSNR	QEB- APLUSNR	-21.3 (-29.1, -13.5)***	-15.3 9-23.1, -7.4)***
	Q-APLUSNR	-20.1 (-27.9, -12.4)***	-16.4 9-24.2, -8.6)***
C-APLUSR	QEB- APLUSR	-10.9 (-18.7, -3.1)**	-2.3 (-10.2, 5.5)
	Q-APLUSR	-13.8 (-21.5, -5.9)**	-6.5 (-14.3, 1.4)
Q-APLUSNR	QEB- APLUSNR	-1.1 (-8.9, 6.6)	1.2 (-6.7, 9.0)
Q-APLUSR	QEB- APLUSR	2.8 (-5.0, 10.6)	4.1 (-3.7, 11.9)
C-VOLNR	QEB-VOLNR	-9.5 (-17.3, -1.8)*	-4.1 (-11.9, 3.7)
C-VOLR	QEB-VOLR	-4.9 (-12.7, 2.9)	-2.1 (-9.9, 5.7)
C-OPTNR	QEB-OPTNR	-26.2 (-34.0, -18.5)***	-19.2 (-2.7, -11.4)**
C-OPTR	QEB-OPTR	-19.3 (-27.0, -11.5)**	-12.2 (-20.0, -4.4)**

Table 7.4: Mean difference (95% confidence interval) for different inhalers when used at various flow rates.

Flow rate (L/min)	Comparator		%FPF of nominal dose	%TED of nominal dose
28.3	C-MDI	Qvar-EB	-35.0 (-42.8, -27.1)***	-16.8 (-27.7, 5.9)**
		Q-MDI	-32.4 (-40.3, -24.6)***	-14.2 (-25.1, -3.3)*
	Qvar-EB	Q-MDI	2.6 (-5.3, 10.4)	2.6 (-8.3, 13.5)
60	C-MDI	Qvar-EB	-37.2 (-45.0, -29.3)***	-10.6 (-21.5, 0.3)
		Q-MDI	-32.8 (-40.7, -25.0)***	-20.2 (-31.1, -9.3)**
	Qvar-EB	Q-MDI	4.3 (-3.5, 12.2)	-9.6 (-20.5, 1.3)
90	C-MDI	Qvar-EB	-35.5 (-43.4, -27.7)***	-14.8 (-25.7, -3.9)*
		Q-MDI	-38.5 (-46.3, -30.6)***	-25.3 (-36.2, -14.4)***
	Qvar-EB	Q-MDI	-2.9 (-10.8, 4.9)	-10.5 (-21.4, 0.4)

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

7.2.3. Discussion

The comparison of two actuations of 250µg Clenil MDI to four actuations of 100µg Qvar inhalers alone without a spacer at different flow rates of 28.3, 60, and 90L/min showed significantly more % FPF of nominal dose ($p<0.001$), from either the Qvar® MDI or the Qvar® EB device than that obtained from the Clenil® MDI. Similarly, the % TED with respect to the nominal dose was higher for either the Qvar EB or the Qvar MDI when compared to the Clenil MDI at different flow rates. The results were always significant except when comparing Clenil vs Qvar-EB at a flow rate of 60 L/min where it failed to reach significance.

According to the results from this study the mean (SD) MMAD obtained from the ACI at a flow rate of 28.3L/min for Clenil® MDI, Qvar® EB, and Qvar® MDI are 2.8 (0.4), 1.1 (0.3), and 1.2 (0.2), respectively. As shown in figure 7.4, a great difference by more than half was found in the mean (SD) FPD obtained from either Qvar® EB 218.0 (29.1) or Qvar® MDI 208.9 (156.3) when compared to Clenil® MDI 97.6 (20.8).

The greater total mass of fine particles obtained from Qvar® inhalers than Clenil® MDI at equivalent dosages would be expected to greatly increase lung deposition with better delivery to the small airways. This was in agreement with a previous study by Leach et al (2002) who showed that the MMAD of Qvar® HFA-BDP is in the range of 0.9-1.1µm and offers more improved delivery to the small airways. This study suggested that the HFA-BDP extra fine aerosol would provide equivalent efficacy to existing CFC-BDP but at half the nominal dose. Several other studies gave similar findings (Busse et al., 1999b; Harrison et al., 1999b; Agertoft et al., 2003). On the other hand, several clinical trials have consistently shown no differences in lung function, asthma control or tolerability between BDP Modulite® (2.9µm MMAD) and CFC-BDP inhaler (Anderson et al., 2002; Lee et al., 2002; Woodcock et al., 2002b).

The mean (SD) amounts of drug deposited in the induction port were 251.3 (22.0), 121.8 (15.5), and 130.9 (26.3) for Clenil MDI, Qvar EB, and Qvar MDI, respectively. The greater induction port deposition associated with Clenil® MDI compared to Qvar® inhalers when used without a spacer is due to the greater particle size of Clenil® which will be expected to undergo greater impaction and sedimentation due to gravity within the induction port of the Andersen cascade Impactor (ACI). Alternatively, with the spacer addition to Clenil®, the high proportion of large particles in the formulation will be expected to deposit more on the spacer. In contrast, Qvar® formulations have higher proportion of smaller particles. These small particles escape from impaction on spacer surface and penetrate the airways more deeply. Similarly, a previous study reported a poor deposition of the small particles of Qvar® formulation in three different mouth throat models studied (Zhang et al., 2007) while aerosols with larger sized particles showed considerable mouth throat deposition due to inertial impaction (Grgic et al., 2004).

When comparing the FPF and the TED with respect to the nominal dose between Qvar® EB and Qvar® MDI either alone or when attached to the Aerochamber Plus with or without rinsing, the results were always non significant. The small drug particles in the Qvar BDP inhaler would be longer suspended in the air, thus allowing more time for inhalation after actuation and makes the inhalation technique less critical. Thus, using the Easi-breathe device may offer no extra advantage with these highly fine formulations. This was confirmed by a previous study by Leach et al (2005) whereby lung deposition results of Qvar MDI without spacers was only reduced from 59 to 37% under extreme discoordination circumstances.

It is shown from the results that the use of the not rinsed Aerochamber Plus spacer with two actuations from Clenil® MDI (250µg) resulted in significantly lower % FPF of

nominal dose ($p<0.001$) and lower %TED ($p<0.001$) than that obtained from four 100µg actuations from Qvar® EB or Qvar® MDI attached to the same spacer. Similarly, using the rinsed Aerochamber Plus spacer with the same dose of Clenil® MDI resulted in lower %FPF and %TED of nominal dose than that obtained from four actuations of Qvar inhalers (100µg).The results for the % TED and the % FPF showed a non-significant difference for the former and a significant difference for the latter ($p<0.01$).

In addition, using the Optimiser spacer either rinsed or not rinsed with Clenil® MDI, significantly reduced the %FPF of nominal dose to nearly half that obtained when the same spacer used with Qvar® EB inhaler. Similarly, the % TED for the combination between the Optimiser spacer (rinsed or not rinsed) and Clenil was significantly decreased when compared to Qvar EB attached to the same spacer ($p<0.01$).

However, when comparing the combination of the Volumatic spacer (R or NR) with Clenil® MDI vs the same spacer combination with Qvar® EB, the results for % FPF of nominal dose showed a non-significant difference for the Volumatic spacer when rinsed and a significant difference when it is not rinsed ($p<0.05$). However, the results for % TED for both inhalers with the Volumatic spacer were non-significant.

The above results revealed that the formulation particle size could greatly affect aerosol particle size distribution in the Andersen Cascade Impactor with or without a spacer device. The smaller particle size of Qvar® formulation (1.1µm MMAD) appears to be more suitable with smaller sized spacers such as the Optimiser and the Aerochamber Plus. In contrast, the large volume spacers such as the Volumatic were more suitable to the larger particle size of Clenil® MDI (2.9µm MMAD). This is may be due to that the large volume of the Volumatic spacer when attached to Clenil® MDI will create more space for this larger particle size aerosol (MMAD 2.9µm) to expand and have more sufficient time for the propellant to evaporate, thus it results in finer aerosol spray when

compared to smaller volume spacers. In addition, smaller volume spacers will be expected to undergo greater impaction of larger particles on their walls and thus reduce the amount of aerosol generated from them (Dolovich and Dhand, 2011). On the other hand, the extra-fine properties of Qvar® formulations will lead to limited or small capacity for particle size reduction or spacer impaction. Thus, the FPD will not be significantly influenced as a function of time or distance (Smyth et al., 2004). This explains why the use of the not rinsed Aerochamber Plus spacer device did not improve drug delivery from Qvar inhalers. This highly fine formulation allows the BDP aerosol particles to remain suspended in the inhaled air with better penetration to the small airways of the lung. Therefore, it decreases the need for spacers, since their main function is to improve fine particles by exclusion of larger particles.

However, the use of any of the spacers with any inhalers was always associated with a substantial reduction in the amount deposited in the induction port compared to that obtained with any inhaler alone. This is consistent with a previous study by Rahmatallah et al (2002), who reported that using an Aerochamber plus with Qvar® MDI was not associated with any significant increase in the actual dose delivered to the respiratory tract. Instead, a significant decrease in the total inhaled dose due to greater deposition on the spacer walls. The spacers become the major site of drug deposition, thus reducing particles deposition in the induction port of the ACI.

As shown in table 7.2, increasing the flow rate was associated with an increase in both the FPD and the % FPF with both Clenil and Qvar formulations. The mean (SD) FPD for Clenil were 97.6 (20.8), 138.7 (7.1), and 116.5 (5.7) at a flow rate of 28.3, 60, and 90L/min, respectively. The mean (SD) FPD for Qvar EB was 218 (29.1), 225.9 (26.6), and 235.7 (12.2) while that for Qvar MDI was 208.9 (16.3), 241.8 (32.7), and 247.4 (9.5) at a flow rate of 28.3, 60, and 90L/min, respectively. This gives the indication that

smaller particles were emitted at higher flow rates. Despite that increasing the flow rate is expected to be associated with greater impaction in the induction port and hence lower FPD and lower FPF; however, this was not the case in this study or with previous studies that gave similar findings. This may give indication that inertial impaction is not the only mechanism by which particles are deposited in the induction port. This behaviour of aerosol particles can be due to turbulence mechanism affecting particle deposition in the induction port, which is inversely proportional to inhalation flow rate. This turbulence arises from the velocity difference between the MDI aerosol plume and the inspired air, which is expected to be greater at low flow rates. Therefore, the combined effect of both mechanisms; turbulence and impaction are the major determinant for particles deposition in the induction port (Feddah et al., 2000; Rahmatalla et al., 2002).

It is apparent from previous studies with different spacers that considerable variations in spacers' size can probably lead to a unique drug delivery characteristics form each spacer. Several studies have shown that spacers could act differently when attached to the same drug formulation (Agertoft and Pedersen, 1994; Ahrens et al., 1995; Barry and O'Callaghan, 2000; Feddah et al., 2001) or when attached to different formulations of the same drug (Miller and Bright, 1995; Barry and O'Callaghan, 1997; Finlay and Zuberbuhler, 1998; Dubus et al., 2001). Miller and Bright (1995) compared the *in-vitro* drug output of three different BDP inhalers from three different manufacturers when used with the Volumatic spacer and indicated a significant difference in the drug output between inhalers of different manufacturers. Another *in-vitro* study by Barry and O'Callaghan (1997) has shown large differences in the amount of drug obtained in small particles when the conventional and CFC-free formulations of salbutamol MDIs are used with different spacer devices.

Besides, the drug delivery from each plastic spacer can be greatly affected by different levels of electrostatic charge accumulated on its surfaces during handling. This may lead to significant reduction in the respirable dose (O'Callaghan et al., 1993), lung deposition (Kenyon et al., 1998) and clinical response (Wildhaber et al., 2000b). The presence of electrostatic charge on spacers' surfaces leads to continuous and rapid loss of the aerosol to spacers' walls, thus significantly reducing the aerosol half-life (the normal aerosol half-life may decrease from 30 sec to 10 sec in presence of electrostatic charge). The short half-life of the aerosol increases the need for coordination between actuation and inhalation as patients will not be able to empty the aerosol before a considerable fraction is lost within the spacer. Consequently, the full predictable dose will not be delivered (Mitchell et al., 2007b). According to the results from this study, which agreed with previous findings (Pierart et al., 1999; Wildhaber et al., 2000a), conditioning spacer devices by washing with a conductive surfactant (detergent) without subsequent water rinsing presented a simple solution to this problem and improved drug delivery from spacers with both inhalers used.

7.3. In- vivo comparison of relative lung and systemic bioavailability of beclometasone dipropionate inhaled from Qvar® and Clenil® Modulite inhalers

The aim of this section is to compare the relative lung and systemic bioavailability of beclometasone dipropionate post-inhalation from Qvar® EB, Qvar® MDI, and Clenil® inhalers with spacers by using the previous results illustrated in chapter 5.3 and 6.3 of this thesis.

7.3.1. Statistical Analysis

Statistical analysis of the 0.5hr and the 24 hours urinary excretion expressed as percent of nominal dose of beclometasone dipropionate and its metabolites following administration of C-MDI, Qvar EB and Q-MDI with different spacers were carried out using a one way

analysis of variance (ANOVA) test using SPSS V17.0 (SPSS Inc., Chicago, USA). In addition, one-way analysis of variance with the application of Bonferroni correction was used to determine any difference between the urinary excretions of different inhalers. To identify equivalence of the urinary excretions between the inhalation methods, the 30 minutes and cumulative 24hr amounts, excreted for each inhalation method, were normalised for the nominal dose and then log transformed. From the mean square error of the analysis of variance, using patients and inhalation method as the main factors, the mean ratio (90% confidence interval) was calculated.

7.3.2. Results

The mean (SD) urinary amounts of BDP and its metabolites excreted 0.5hr, and 24hr post eight inhalations from Clenil® MDI (250μg), Qvar® EB (100μg), and Qvar® MDI (100μg) with and without spacers for the twelve subject studied are summarized in table 7.5. Figures 7.7-7.10 represent the 0.5hr, the 24hr mean (SD) urinary amounts of BDP, and its metabolites excreted post different inhalation methods, expressed as percentage of nominal dose. A summary of the statistical data for the 30 minutes and the 24hr urinary excretion of BOH, 17-BMP, and BDP are represented in table 7.6. A summary of the mean ratio (90% confidence limits) between Qvar and Clenil inhalers with respect to the nominal dose is shown in table 7.7. These values are presented separately for BDP, 17 BMP, and BOH, as well as for all three metabolites combined.

Table 7.5: Mean (SD) amount of beclometasone dipropionate and its metabolites excreted 0.5hr, and 24hr post inhalation of different study doses with and without spacers, expressed in µg, n=12.

Inhaler	Device	Amount left in spacer (µg)	17-BMP (µg)		BOH (µg)		BDP (µg)	
			0.5hr	24hr	0.5hr	24hr	0.5hr	24hr
Clenil	MDI	---	5.0 (1.8)	28.9 (6.0)	7.4 (1.9)	88.5 (17.3)	3.7 (0.6)	30.2 (6.6)
	VOLNR	670.8 (74.4)	6.3 (2.2)	21.0 (3.1)	10.0 (2.8)	67.7 (14.4)	4.8 (0.9)	19.8 (2.6)
	APLUSNR	758.0 (136.5)	5.6 (2.0)	16.3 (2.4)	8.6 (1.6)	57.4 (12.3)	4.0 (0.8)	19.4 (2.7)
	OPTNR	705.4 (84.4)	4.8 (1.6)	16.4 (2.7)	7.1 (1.4)	50.8 (13.7)	3.6 (0.6)	17.4 (2.3)
	VOLR	732.9 (74.9)	4.6 (1.2)	16.1(2.8)	6.7 (1.1)	48.0 (10.4)	3.6 (0.6)	15.9 (1.9)
	APLUSR	784.8 (46.9)	4.2 (1.4)	14.7(3.3)	5.7 (1.1)	44.8 (14.0)	3.5 (0.8)	14.2 (2.1)
	OPTR	807.0 (120.5)	4.1 (1.6)	13.6 (2.9)	6.2 (1.6)	44.9 (12.3)	3.3 (0.6)	14.7 (1.8)
Qvar EB	EB	---	4.5 (0.8)	27.3 (3.9)	6.9 (1.4)	80.8 (14.6)	3.5 (0.5)	23.4 (3.9)
	VOLNR	355.5 (52.6)	3.4 (0.9)	13.7 (2.9)	6.5 (1.2)	47.2 (9.5)	3.1 (0.6)	11.7 (2.8)
	APLUSNR	336.9 (89.1)	4.3 (1.0)	18.6 (3.4)	7.2 (1.2)	60.5 (9.8)	3.7 (0.7)	17.4 (3.5)
	OPTNR	455.5 (76.8)	4.1 (1.0)	17.1 (2.6)	6.8 (1.3)	53.6 (10.0)	3.4 (0.8)	15.3 (3.5)
	VOLR	428.4 (52.5)	2.9 (0.7)	12.1(2.3)	5.5 (1.0)	37.2 (4.8)	2.8 (0.5)	10.3 (2.3)
	APLUSR	403.4 (97.8)	3.3 (0.6)	15.1 (2.8)	6.1 (1.0)	51.6 (7.0)	3.2 (0.7)	11.6 (3.9)
	OPTR	513.6 (101.8)	3.0 (0.7)	15.4 (2.9)	5.7 (1.2)	47.3 (7.3)	3.0 (0.6)	11.0 (2.5)
Qvar MDI	MDI	---	4.7 (1.1)	25.8 (7.0)	6.1 (1.4)	77.7 (15.1)	3.3 (0.8)	23.1 (4.3)
	APLUSNR	370.1(67.5)	4.4 (0.8)	17.3 (3.8)	6.8 (2.1)	53.3 (10.7)	3.9 (0.7)	16.1 (3.0)
	APLUSR	431.4 (76.3)	3.5 (0.7)	15.0 (3.0)	5.0 (1.4)	43.0 (9.8)	2.8 (0.7)	11.8 (2.5)

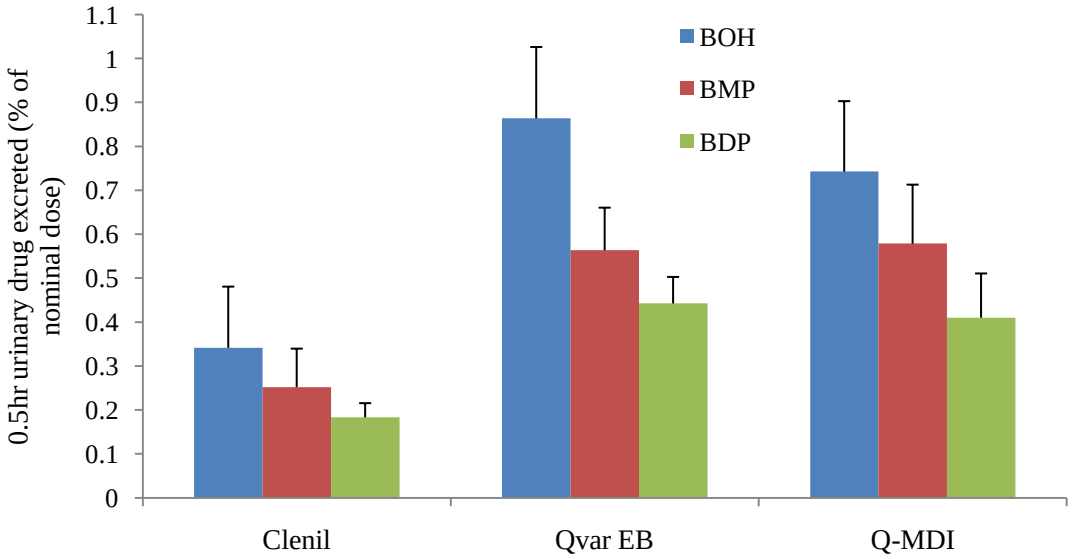


Figure 7.7: The 0.5hr mean (SD) urinary amounts of BDP and its metabolites excreted post- inhalation, expressed as percentage of nominal dose, (n=12).

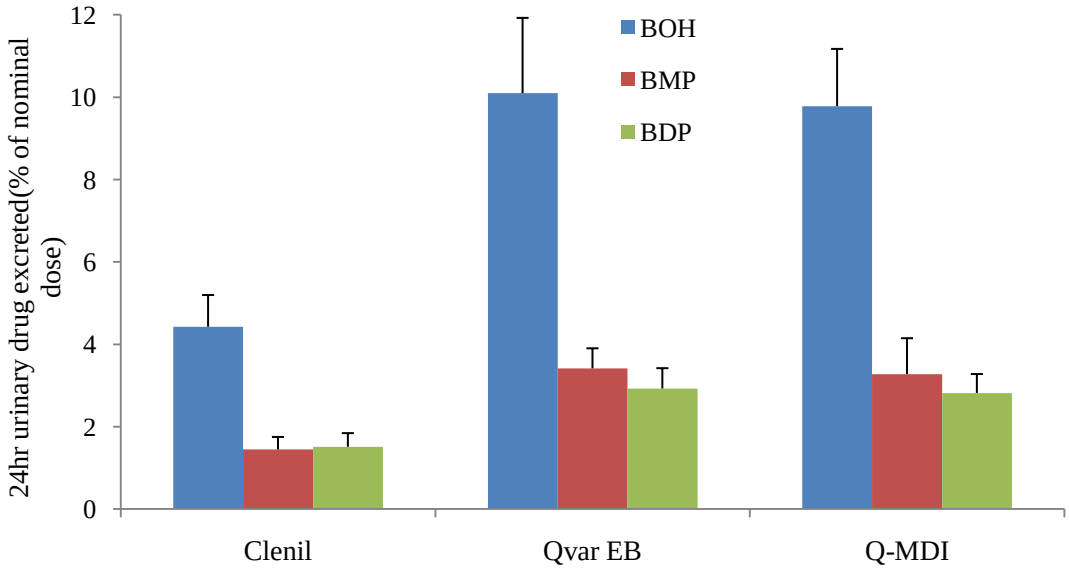


Figure 7.8: The 24hr mean (SD) urinary amounts of BDP and its metabolites excreted post-inhalation, expressed as percentage of nominal dose, (n=12).

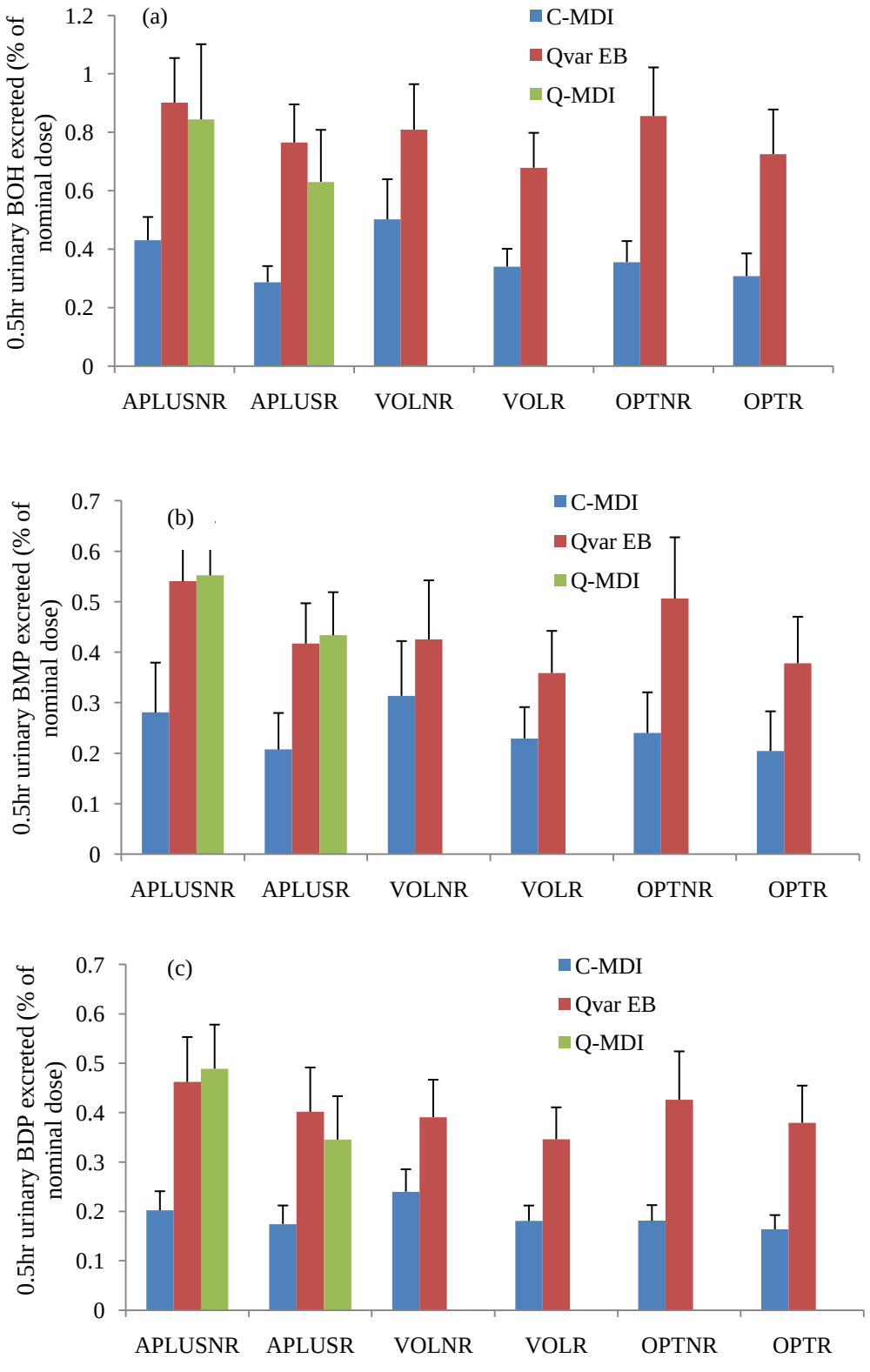


Figure 7.9: The 0.5hr mean (SD) urinary excretion of (a) BOH (b) 17-BMP (c) BDP post eight inhalation from Qvar® EB (100µg), Qvar® MDI(100µg) and Clenil® MDI (250µg) attached to either rinsed or not rinsed spacers, expressed as percentage of nominal dose, (n=12).

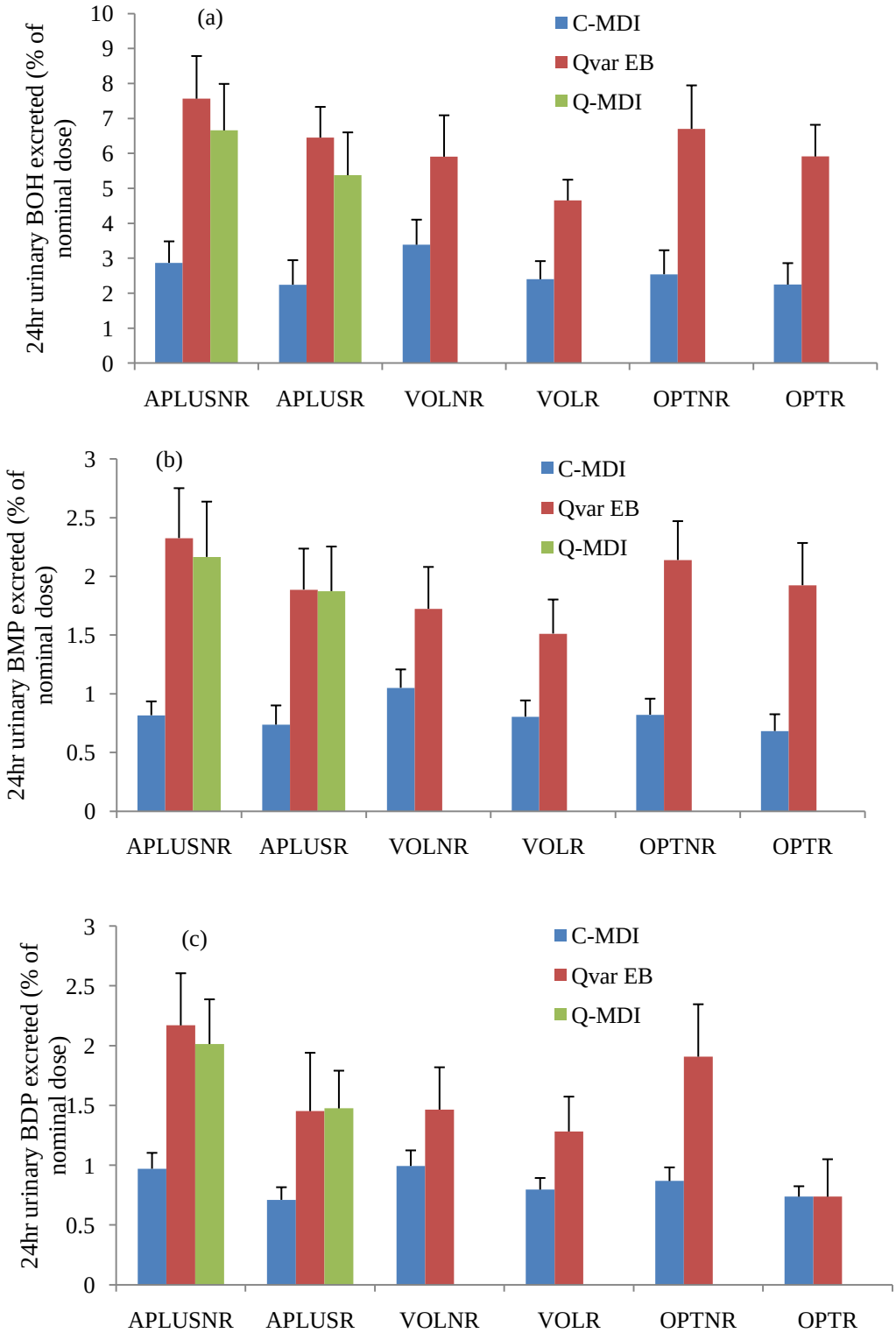


Figure 7.10: The 24hr mean (SD) urinary excretion of (a) BOH (b) 17-BMP (c) BDP post eight inhalation from Qvar® EB (100µg), Qvar® MDI (100µg) and Clenil® MDI (250µg) attached to either rinsed or not rinsed spacer, expressed as percentage of nominal dose, (n=12).

Table 7.6: Mean difference (95% confidence interval) for the percent of nominal dose of BOH, 17-BMP, and BDP excreted 0.5hr and 24hr post study doses.

Comparator		BOH		17-BMP		BDP	
		0.5hr	24hr	0.5hr	24hr	0.5hr	24hr
Qvar EB	Clenil	0.5 (0.4, 0.6)***	5.7 (4.9, 6.4)***	0.3 (0.2, 0.4)***	2.0 (1.7, 2.3)***	0.3 (0.2, 0.3)***	1.4 (1.2, 1.7)***
	Q-MDI	0.1 (0.0, 0.2)*	0.4 (-0.4, 1.1)	-0.02 (-0.09, 0.05)	0.2 (-0.1, 0.5)	0.03 (-0.02, 0.1)	0.03 (-0.2, 0.3)
Q-MDI	Clenil	0.4 (0.3, 0.5)***	5.3 (4.5, 6.0)***	0.3 (0.3, 0.4)***	1.8 (1.5, 2.1)***	0.2 (0.2, 0.3)***	1.4 (1.1, 1.6)***
C-APLUSNR	QEB- APLUSNR	-0.5 (-0.6, -0.4)***	-4.7 (-5.4, -4.0)***	-0.3 (-0.3, -0.2)***	-1.5 (-1.8, -1.2)***	-0.3 (-0.3, -0.2)***	-1.2 (-1.5, -0.9)***
	Q-APLUSNR	-0.4 (-0.5, -0.3)***	-3.8 (-4.5, -3.0)***	-0.3 (-0.3, -0.2)***	-1.3 (-1.6, -1.1)***	-0.2 (-0.2, -0.1)***	-1.0 (-1.3, -0.8)***
C-APLUSR	QEB-APLUSR	-0.5 (-0.6, -0.4)***	-4.2 (-5.0, -3.5)***	-0.2 (-0.3, -0.1)***	-1.2 (-1.4, -0.9)***	-0.2 (-0.3, -0.2)***	-0.7 (-1.0, -0.5)***
	Q-APLUSR	-0.3 (-0.4, -0.3)***	-3.1 (-3.9, -2.4)***	-0.3 (-0.3, -0.2)***	-1.1 (-1.4, -0.8)***	-0.1 (0.2, -0.1)***	-0.8 (-1.0, -0.5)***
Q-APLUSNR	QEB- APLUSNR	-0.1 (-0.1, 0.0)	-0.9 (-1.7, -0.2)*	0.01 (-0.06, 0.08)	-0.2 (-0.5, 0.1)	0.03 (-0.02, 0.07)	-0.2 (-0.4, 0.1)
Q-APLUSR	QEB-APLUSR	-0.1 (-0.2, -0.04)**	-1.1 (-1.8, -0.4)	0.02 (-0.06, 0.09)	0.3 (0.0, 0.6)	-0.06 (-0.1, -0.01)*	0.02 (-0.2, 0.3)
C-VOLNR	QEB-VOLNR	-0.3 (-0.4, -0.2)***	-2.5 (-3.3, -1.8)***	-0.1 (-0.2, -0.04)**	-0.7 (-1.0, -0.4)***	-0.2 (-0.2, -0.1)***	-0.5 (-0.7, -0.2)***
C-VOLR	QEB-VOLR	-0.3 (-0.4, -0.3)***	-2.3 (-3.0, -1.5)***	-0.1 (-0.2, -0.1)***	-0.6 (-1.0, -0.4)***	-0.2 (-0.2, -0.1)***	-0.5 (-0.7, -0.3)***
C-OPTNR	QEB-OPTNR	-0.5 (-0.6, -0.4)***	-4.2 (-4.9, -3.4)***	-0.3 (-0.3, -0.2)***	-1.3 (-1.6, -1.0)***	-0.2 (-0.3, -0.2)***	-1.0 (-1.3, -0.8)***
C-OPTR	QEB-OPTR	-0.4 (-0.5, -0.3)***	-3.7 (-4.4, -2.9)***	-0.2 (-0.2, 0.1)***	-1.2 (-1.5, -1.0)***	-0.2 9-0.3, -0.2)***	-0.6 (-0.9, -0.4)***

* $p < 0.05$, ** $p < 0.01$, *** < 0.001 otherwise no significant difference.

Table 7.7: Mean ratio (90% confidence interval) for Qvar compared to Clenil (when normalised for the nominal dose).

Cumulative urinary excretion		Qvar EB		Q-MDI
		Clenil	Q-MDI	Clenil
BOH	0.5hr	234.4 (204.8, 268.3)	114.1 (99.8, 130.6)	205.2 (179.3, 234.9)
	24hr	227.7 (201.8, 256.8)	103.4 (91.7, 116.6)	220.1 (195.2, 248.4)
17-BMP	0.5hr	234.4 (192.5, 285.8)	97.7 (80.2, 119)	240.1 (197.0, 292.4)
	24hr	238.5 (201.0, 282.9)	108.1 (91.1, 128.3)	220.6 (186.1, 282.9)
BDP	0.5hr	242.5 (212.5, 276.8)	109.3 (95.8, 124.7)	221.9 (194.8, 252.9)
	24hr	196.0 (171.8, 223.7)	101.4 (88., 115.7)	193.3 (169.7, 220.1)
All 3 combined	0.5hr	237.0 (217.2, 258.6)	107.0 (98.0, 116.5)	221.9 (203.4, 242.1)
	24hr	222.3 (203.2, 243.5)	104.3 (95.2, 114.2)	231.2 (194.8, 233.5)

7.3.3. Discussion

As shown in tables 7.5 - 7.6 and figures 7.7 - 7.8, comparable urinary drug excretion results were obtained post eight inhalations of 250µg Clenil, 100µg Qvar EB, and 100µg Qvar MDI. Table 7.7 shows that when combining all the data of the 0.5hr urinary excretion of BDP and its metabolites for Qvar EB vs Clenil and Q-MDI vs Clenil, the overall mean ratio was 237.0%, and 221.9% with 90% confidence interval of 217.2 - 258.6, and 203.4 - 242.1, respectively. While, the overall mean ratio for Qvar EB vs Clenil and Q-MDI vs Clenil was 222.3%, and 231.2% with 90% confidence interval of 203.2 - 243.5, and 194.8 - 233.5, respectively, for the cumulative 24hr urinary excretion. This is consistent with previous results reported in Chapter four of this thesis that showed that the overall mean ratio (90% confidence limits) between Qvar EB and Clenil with respect to the nominal dose were 231.4 (209.6 - 255.7)%, and 204.6 (189.6, 220.6) % for the 30 minutes, and 24hr urinary excretion, respectively. The above results also agrees with several previous studies that confirmed that a given dose of Qvar HFA–BDP would result in approximately 2-2.5 fold greater potency compared with other CFC-containing beclometasone MDIs (Leach et al., 1998a; Busse et al., 2000). Woodcock et al (2002)

measured the area under the concentration time curve (AUC) for BDP and its metabolite 17-BMP post-inhalation of a single 1000µg dose from three different BDP formulations; BDP Modulite® with spacer Jet® (Beclojet® 250, MMAD 2.6µm), extrafine HFA-BDP (Qvar® 100, MMAD 1.2µm), and CFC-BDP with spacer Jet® (Clenil Forte® 250, MMAD 4.7µm). This study reported that the AUCs for both BDP and 17-BMP were significantly greater with the extrafine formulation than the CFC-BDP and BDP Modulite Jet formulations. The reduction of MMAD from 4.7 or 2.6 to 1.2µm markedly increased drug absorption, however, a negligible influence on drug absorption was observed when changing the MMAD from 4.7 to 2.6µm. The total systemic exposure of 17-BMP post administration of similar doses from either BDP Modulite® or CFC-BDP was comparable in asthmatic patients (Woodcock et al., 2002a), while that for Qvar formulation has been reported to be between 2-2.5 times higher than the CFC product (Harrison et al., 1999b; Agertoft et al., 2003). This explains the need to halve the dose when switching from Clenil® or from conventional CFC-beclometasone. Clenil® Modulite has been formulated with Glycerol in order to increase its particle size to closely match that of the older CFC-BDP inhalers (Chaplin and Head, 2007).

When comparing the urinary excretions of BDP and its metabolites for Qvar EB and Qvar MDI, no significant difference was found. As shown from the results, when combining all the data of the 0.5hr urinary excretion of BDP and its metabolites for Qvar EB vs Q-MDI, the overall mean ratio was 107.0% with 90% confidence interval of 98.0 - 116.5. The overall mean ratio for the 24hr urinary excretion of Qvar EB vs Q-MDI was 104.3% with 90% confidence interval of 95.2 - 114.2. These similar urinary drug excretions from Qvar® MDI and Qvar EB highlight the good inhalation technique achieved by the highly trained volunteers in this study. Several studies have shown that the HFA-BDP formulation in the Autohaler device has a similar lung deposition pattern

(Leach, 1999) and clinically equivalent (Woodman et al., 1993) to drug delivered from the MDI when used correctly. Furthermore, the extrafine properties of Qvar® were found to place less demand on the patient inhalation technique. The gentler and small particle size of this HFA-BDP formulation will be suspended longer in the airways and its lung deposition will not be greatly affected if there is a delay between dose actuation and inhalation, thus it produces an easy to use MDI. These previous findings confirm the equivalency of the Qvar EB and the Qvar MDI and agree with previous findings that reported similar deposition patterns of such extra-fine particles when inhaled with fast and a slow inhalation rate or without a breath hold (Janssens et al., 2003; Usmani et al., 2005). A previous study also showed an optimal and comparable BDP lung deposition from the breath activated Autohaler (60%) and patients with good P&B MDI technique (59%). Nevertheless, the degree of lung deposition was decreased as patients demonstrated poor inhaler technique, however, those patients still received high BDP doses (≥37%) (Leach et al., 2005).

As shown in table 7.5, the use of any detergent treated not water rinsed spacer with eight inhalations of Clenil® MDI (250µg) would increase the 30 minutes urinary excretion of BDP and metabolites than that obtained when adding the same spacer to eight inhalations of Qvar® formulation (100µg). This may be due to the differences in the particle size of the aerosol emitted from these two formulations. Qvar® inhalers have been formulated as an HFA-BDP solution system that are designed to generate an aerosol of smaller particle size (1.1µm MMAD), thus it is expected to achieve better penetration and lung deposition than CFC-BDP inhalers. In contrast, Clenil® inhaler was originally designed to deliver an aerosol with properties that more closely resembles that of the CFC-BDP inhaler. Therefore, in particular glycerol is added as a non-volatile solvent to the HFA-BDP solution as a mass mean aerodynamic modulator to modify and increase the particle

size to 2.9μm MMAD (Chaplin and Head, 2007). The larger particle size of Clenil® MDI (2.9μm MMAD) would more benefit from the spacer presence that enhances its adequate evaporation and further particle size reduction before inhalation. However, Qvar® formulation is already emitted from the inhaler device as an extrafine aerosol spray with smaller MMAD of 1.1μm. In other words, the results showed that the non-extrafine formulation of Clenil was more dependent on the spacer presence than the extrafine Qvar formulation.

Smyth et al (2004) investigated the effect of two formulations that have different proportions of the HFA propellant and different percentages of the non-volatile component added. This study reported that the formulation behaviour with larger MMAD due to greater non-volatile component (19.9 vs 2.5%) and lower HFA- propellant used (80 vs 97.5%), was more dependent on the type of spacer or whether a spacer is used.

Another recent study by Leach et al (2010), also investigated the effect of using spacers on the *in-vivo* drug delivery from Qvar® HFA-BDP formulation and Becoforte® CFC-BDP formulation radiolabeled with technetium-99m. This study reported that the smaller particle size formulation of Qvar® showed a very efficient lung deposition that averaged 52% compared to 3-7% for the larger particle size formulation of the CFC-BDP. Furthermore, the use of Aerochamber or Volumatic spacers with HFA-BDP did not alter lung deposition but it did reduce oropharyngeal deposition. Several other studies reported similar findings confirming the effect of different particle sized formulations on lung deposition with and without spacers (Leach, 1998b; Leach, 1999).

The effect of spacers on the 30 minutes urinary drug excretion, which is representative of lung dose, was different for both inhalers. For Clenil MDI, the not rinsed Volumatic showed the highest lung deposition followed by the not rinsed Aerochamber Plus, then the not rinsed Optimiser. This is in agreement with several studies that confirmed the

superior effect of large volume spacers on lung deposition compared to smaller ones (Barry and O'Callaghan, 1996; O'Callaghan, 1997; Aswania and Chrystyn, 2001). Aswania et al (2001) reported a much greater relative lung deposition obtained from Cromogen® MDI attached to the Volumatic than when the Cromogen® EB attached to the Optimiser spacer and attributed that to the large volume of the Volumatic spacer (750ml) compared to that of the Optimiser spacer (50ml). However, for Qvar MDI and Qvar EB, the highest lung deposition was with the not rinsed Aerochamber Plus and the not rinsed Optimiser, while the not rinsed Volumatic showed the least lung deposition. This indicates that these small volume spacers are more suitable for such extrafine formulations.

These *in-vivo* results confirm that regardless of the spacer/inhaler combination used, the use of the spacer always substantially reduced the 24hr urinary excretion compared to the use of either inhaler alone. This is consistent with previous studies that illustrated that the use of spacers with steroid pressurized metered dose inhaler greatly reduced the oropharyngeal deposition, and hence the total body dose without much affecting the dose delivered (Selroos and Halme, 1991). Other studies have even documented their beneficial effect in reducing hypothalamic-pituitary axis suppression by beclometasone dipropionate (Brown et al., 1990).

7.4. Conclusion

Inhaled corticosteroids are the most effective anti-inflammatory drugs available to clinicians for the control of inflammation in asthma. Inhaled corticosteroids (ICS) have a positive effect on lung function, symptoms, exercise capacity, and may decrease disease exacerbations. However, gaining these beneficial effects is greatly dependent on the aerosol generating system and its particle size distribution. Despite the fact that MDIs appear to be simple in design, several interfering factors can influence its drug delivery to

the patient. Variations in aerosol particle size, spacer size, and washing methods were found to potentially influence drug delivery.

The previous *in-vitro* and *in-vivo* results demonstrated appreciable differences in the urinary drug excretion and the aerodynamic particle size distribution of different HFA formulations of the same drug when used with or without spacers. The difference in the particle size of these formulations (Qvar®, 1.1µm vs Clenil®, 2.9µm) greatly affected drug deposition in different regions of the respiratory tract with or without a spacer device. Indeed, formulations rich in superfine particles such as Qvar® provided higher lung deposition and lower oropharyngeal impaction, thus reducing the need to use a spacer. In contrast, although the dose of Clenil does not have to be halved when switching from CFC-BDP inhalers, this products was associated with lower lung deposition and higher oropharyngeal impaction, and hence the need to use a spacer.

This implies that particle size is one of the most important design variables in an aerosol formulation that can greatly affect drug delivery. When using spacers, the aerosol impaction and fine to coarse particle ratio largely depends on spacer size and the level of the electrostatic charge on its surface. The proper choice and treatment of spacers is therefore important for optimal drug delivery. The common rule that the larger the spacer, the greater the amount of drug that remains airborne and eventually delivered, does not apply to all MDIs. The optimal spacer length is specific to a particular MDI and cannot be assumed to others. Therefore, it is inappropriate to use any formulation with any spacer device just because it fits the mouthpiece adapter without first considering the aerosol characteristics. No doubt that each MDI formulation/spacer combination need to be fully assessed even if it contains the same drug in order to guide the optimum device selection. Nevertheless, all spacers used with MDIs in this study have been always found to significantly reduce the impaction of the larger drug particles in the oropharynx and

minimize gastrointestinal tract drug deposition. Therefore, the use of spacers is always associated with a more favourable therapeutic ratio, since it has little effect on lung dose but significantly reduces throat deposition.

Indeed, the future of development in respiratory disease control will be more based on improvements in drug delivery to the lung rather than introduction of new inhaled therapies. Concerning the low therapeutic index and the high cost of inhaled corticosteroids, it is more demanding to optimise their drug delivery to the respiratory tract as variations in dose deposited in the lung could significantly influence their treatment outcomes. Based on these considerations, the presence of spacers routinely attached to BDP MDIs are of great value, especially in situations of administering high doses of ICS or when the correct use of a MDI is unlikely. This is particularly important for beclometasone dipropionate inhaled therapy compared to other inhaled corticosteroids, which is due to its lower first pass metabolism; its high oropharyngeal deposition would be expected to significantly contribute to its systemic effects without an additional increase in clinical benefit. Therefore, the addition of a spacer to an HFA-BDP MDI even in the ultra-fine formulation was found to somewhat improve the therapeutic ratio of beclometasone. Generally, using the appropriate spacer with beclometasone dipropionate inhaler was found to reduce the oropharyngeal deposition and hence the total body dose without much affecting the dose delivered to the airways. Furthermore, unlike bronchodilators frequent dosing, inhaled corticosteroids are dosed once or twice daily, thus reducing the spacers' portability issue.

Overall, whether using Qvar or Clenil inhalers, spacers should not be rinsed with water. For Qvar, the only advantage is the further reduction of the oropharyngeal deposition. For Clenil, lung deposition is improved but the more pronounced effect is in the reduction of its systemic bioavailability.

Chapter 8: Summary and Future Work

8.1. Summary

Inhaled corticosteroids (ICS) are the standard first line anti inflammatory therapy for the management of persistent asthma in all current national and international guidelines (BTS/SIGN, 2008; GINA, 2010). Beclometasone dipropionate (BDP) was the first available inhaled corticosteroid used for the treatment of asthma. It was first introduced in 1972 in a pressurized metered dose inhaler and later in a dry powder inhaler. Beclometasone dipropionate (BDP) is a prodrug that is metabolized by esterases in the human lung, liver and other parts of the body to three different metabolites, 17-beclometasone monopropionate (17-BMP), 21-beclometasone monopropionate (21-BMP) and beclometasone (BOH) (Foe et al., 2000; Derendorf et al., 2006; Rossi et al., 2007)

The mandatory replacement of the ozone damaging CFC-propellants in MDIs by the safer HFA alternatives led to vast developments in aerosol technology to accommodate these new propellants. There are now two brands of CFC-free beclometasone MDIs in the UK (Clenil Modulite® and Qvar®). These devices are not equipotent, and in order to limit prescribing errors and avoid confusion, the MHRA advises that CFC-free beclometasone MDIs should be prescribed by brand name. Clenil Modulite® is equipotent to the CFC-innovator product (Becotide®), therefore, a straightforward substitution of doses can be performed (Chaplin and Head, 2007). Qvar® contains beclometasone in solution and has been shown to deliver the drug as an extra-fine aerosol that results in a 2-2.5 fold greater potency compared with other CFC-containing beclometasone MDIs (Leach et al., 2002).

Aerosol deposition in the lung depends on several factors, including the aerosol generating system, the particle size distribution of the emitted dose, the inhalation manoeuvre, airflow obstruction and severity of lung disease (Dolovich and Dhand,

2011). These factors can be studied using simple pharmacokinetic methodology post inhalation and by in vitro characterisation of the emitted dose.

Hindle and Chrystyn (1992) reported that measurements of the 30 minutes urinary drug amounts post inhalation represent the absorption lag time of the orally swallowed portion and would account mainly for the drug absorbed from the lung, while the 24 urinary drug amounts post inhalation is an index of systemic delivery. This pharmacokinetic method was found to be simple, non-invasive and has been extended to determine the relative bioavailability of different drugs, e.g, inhaled sodium cromoglycate (Aswania et al., 1999; Chrystyn, 2000; Aswania and Chrystyn, 2001; Aswania and Chrystyn, 2002), nedocromil (Aswania et al., 1998) , gentamycin (Nasr and Chrystsyn, 1997; Al-Amoud et al., 2002; Al-Amoud et al., 2005) and formeterol (Nadarassan et al., 2007). However, the methodology has not been extended to inhaled corticosteroids.

The plasma concentrations of drugs such as inhaled corticosteroids are very low, because of the small doses used and their very large volume of distribution.(Derendorf et al., 2006) The analysis of these drugs in plasma requires highly sensitive analytical methods, whereas these drugs in urine are more concentrated.

The main aim of this work was to identify, validate, and apply a urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of inhaled beclometasone following different inhalation methods from a metered dose inhaler using two different formulations of BDP (Qvar and Clenil) and to measure the *in-vitro* aerodynamic particle size distribution of the same inhalation methods.

First, a simple, sensitive and selective LC-(ESI+)-MS method using a solid phase extraction procedure for simultaneously quantifying beclometasone dipropionate (BDP) and its two metabolites 17-beclometasone monopropionate (17-BMP) and beclometasone (BOH) in human urine samples and methanol samples after *in-vivo* inhalation and *in-*

vitro dose emission of the drug, respectively was developed and presented in chapter three. The method validation results according to the FDA and ICH guidelines have shown that it has acceptable limits for both accuracy and precision (±15%) and has been successfully used to analyze samples from this study. In addition, the preparation, separation, and identification of BDP metabolites was carried out and the final product was purified by preparative HPLC and the resulting NMR spectrum was recorded. The NMR results confirmed the rapid hydrolysis of BDP to 17-BMP (the major metabolite) via esterase enzyme.

Second, in chapter four we have used the original methodology reported by Hindle and Chrystyn (1992) to identify the feasibility of using this urinary pharmacokinetic method for inhaled BDP. The application of this approach has been determined by comparing urinary excretions of BDP and its metabolite post Qvar and Clenil inhalations. Twelve healthy, non-smoking volunteers completed an *in-vivo* urinary pharmacokinetic study to determine the relative lung bioavailability of beclometasone following inhalation. The urinary amounts excreted following an oral dose of a 20ml 20 % alcoholic solution of 2000µg beclometasone dipropionate , an oral dose (2000µg) plus oral charcoal, ten 100µg inhalations from a Qvar® EB inhaler, ten 100µg inhalations from a Qvar® EB inhaler plus oral charcoal, and eight 250µg inhalations from a Clenil® MDI were studied. No BDP, 17-BMP, or BOH was detected in any samples post oral with charcoal dosing or following the 0.5hr post the oral dose. In addition, there was no BDP detected up to 24hr following the oral dose administration. Significantly more ($p<0.001$) BDP, 17-BMP and BOH were excreted in the first 30 minutes and cumulative 24 urinary excretion post inhalation of either Clenil or Qvar compared to oral administration. This suggests that the amount of drug and metabolites excreted 30 minutes and 24hrs post dosing can be used as an index of lung deposition and relative systemic bioavailability, respectively. No significant difference was found between the amount of drug or metabolites excreted in

the urine over the 24hr collection periods post dose following inhaled Clenil® and inhaled Qvar® administration. The urinary pharmacokinetic methodology to determine the relative lung and systemic bioavailability post inhalation applies to BDP. The inhaled Qvar to inhaled Clenil ratio is consistent with related clinical equivalence and pharmacokinetic data. The overall mean ratio (90% confidence limits) between Qvar and Clenil with respect to the nominal was 231 (209.6 - 255.7) %, and 204.6 (189.6-220.6) for the 30 minute, and the 24hr urinary excretion. The low inter- and intra- subject variability of the study confirms the reproducibility of this method. These results confirm that this method can be used to study the relative lung and systemic bioavailability of BDP after an inhalation.

The use of various spacers attached to MDIs has been found to compensate for many of its problems. The role of spacers is to slow the velocity of the aerosol spray, allowing time for the propellants to evaporate and large drug particles to settle. Spacers decrease the oropharyngeal deposition and the need for coordination between actuation and inhalation while they may increase lung deposition (McFadden, 1995; Terzano, 2001). It is well documented that the type of spacer as well as the method of its handling can greatly affect the delivery of asthma medication (GINA, 2010). Chapter five, six, and seven of this thesis have focused on investigating and comparing the *in-vitro* aerodynamic characteristics as well as the *in-vivo* drug delivery from two formulations of HFA-BDP (Clenil and Qvar) with and without spacers.

The *in-vitro* dose emission characteristics of beclometasone dipropionate from two actuations of Clenil Modulite® MDI (250µg) alone and with different spacers were measured using the Andersen Cascade Impactor (ACI) according to the standard compendial methodology at a flow rate of 28.3 L/min using a 4L inhalation volume. The spacers used were the Volumatic (VOL), the Aerochamber Plus (APLUS), and the

Optimiser (OPT). Each spacer was tested after adequate prewashing in detergent solution followed by either rinsing (R) or not rinsed with water (NR), then allowed to drip dry. The TED from the MDI alone was significantly higher than all MDI + spacers. The not rinsed Volumatic (VOLNR) spacer showed the highest FPD, and % FPF. The use of water rinsed spacers significantly decreased the FPD when compared to the not water rinsed spacers.

The influence of higher inspiratory flow rates (60 and 90L/min) on the aerosol particle size distribution of Clenil Modulite® MDI alone was evaluated and compared to that at 28.3L/min. Increasing flow rate from 28.3 L/min to 60 L/min lowered the MMAD and led to a small significant increase in the FPD and the % FPF. Increasing flow rate from 28.3L/min to 90 L/min were associated with lower MMAD and higher FPD for the 90L/min flow rate compared to the 28.3L/min flow rate, however, the results were non-significant.

The previous urinary pharmacokinetic method was then applied to highlight and compare the effect of different spacers on the *in-vivo* drug delivery of inhaled beclometasone from Clenil Modulite® MDI in twelve healthy volunteers. In addition, the study aimed to determine the effect of different spacer handling procedures on drug delivery by comparing drug output from either water rinsed or not rinsed detergent coated spacers. Each spacer was adequately prewashed in detergent solution followed by either rinsing (R) or not rinsing (NR) with water, then allowed to drip dry. Subjects inhaled eight doses from Clenil Modulite® MDI (250μg) either alone or when attached to one of the following spacers; the Volumatic, the Aerochamber Plus or the Optimiser with and without rinsing. Subjects emptied their bladder prior to each study dose and then urine samples were collected at 30 minutes, and cumulatively for 24 hours post dosing of each study dose. The volume of urine excreted was recorded and aliquots of each sample were

frozen at -20°C prior to analysis. The amount of drug left in each spacer device was also determined. The use of Clenil Modulite® MDI alone resulted in significantly higher amounts of drug excreted 24hrs post dosing than that when using the MDI + spacers. The use of the spacers had a little effect on the amount of drug excreted 30 minutes post dosing. The VOLNR spacer provided significantly greater amount of BDP and metabolites than the MDI alone or the MDI + any other spacer. Rinsing spacers with water markedly decreased drug output from spacers than not rinsed spacers and should not be used. The results were consistent with the previous *in-vitro* study. For Clenil, lung deposition was improved but the more pronounced effect is in the reduction of its systemic bioavailability.

The Andersen Cascade Impactor (ACI) was used to determine the *in-vitro* particle size distribution of beclometasone dipropionate obtained from four actuations of Qvar® MDI (100µg) and Qvar® EB (100µg) alone and with different spacers at a flow rate of 28.3 L/min using a 4L inhalation volume. The spacers used were the same as mentioned with the Clenil® study. The TED from the MDI alone was significantly higher than all MDI + spacers. The use of the spacers with Qvar® inhalers significantly reduced the oropharyngeal deposition; however, the FPD was not affected. In addition, the presence of the electrostatic charge on the surface of the water rinsed spacers contributed to significant loss of drug output from the spacer compared to the not rinsed spacers.

The influence of higher inspiratory flow rates (60 and 90L/min) on the aerosol particle size distribution for Qvar® EB and Qvar® MDI was evaluated and compared to that at 28.3L/min. For the Qvar® EB , the amount of drug deposited in the induction port of the ACI was found to decrease significantly ($p<0.05$) when increasing the flow rate from 28.3 to 90L/min, while the induction port deposition decrease was non-significant when increasing the flow rate from 28.3 to 60L/min and from 60 to 90L/min. For the Qvar®

MDI, a similar reduction in the induction port deposition was indicated when increasing the flow rate; however, the results were not significant.

The same urinary pharmacokinetic method was used to determine the effect of different spacers and different spacers' washing procedures on the *in-vivo* drug delivery from eight inhalations from Qvar® MDI (100µg) and Qvar® EB (100µg) in healthy volunteers. Again subjects emptied their bladder prior to each study dose and then urine samples was collected at 30 minutes, and cumulatively for 24 hours post dosing of each study dose. The volume of urine excreted was recorded and aliquots of each sample were frozen at -20°C prior to analysis. The amount of drug left in each spacer device was also determined. The use of Qvar® MDI (100µg) and Qvar EB (100µg) alone resulted in significantly higher amounts of drug excreted 24hrs post dosing than that when using the MDI + spacers. The use of spacers did not increase the amount of drug excreted 30 minutes post dosing. The not rinsed Aerochamber Plus spacer provided greater amount of BDP and metabolites than other spacers did. Rinsing spacers with water had an obvious effect on reducing drug delivery compared to the not water rinsed spacers. Overall, the only advantage of using spacers with Qvar is the reduction in the systemic bioavailability.

The previous *in-vivo* and *in-vitro* results demonstrated appreciable differences in the urinary drug excretion and the aerodynamic particle size distribution of different HFA formulations of the same drug. It was found that using eight inhalations of either 250µg of Clenil® or 100µg of Qvar® led to comparable urinary drug excretion. The fine particle dose emitted from two actuations of Clenil® (250µg) inhaler was approximately half that obtained from four actuations of Qvar® (100µg) inhalers. This Qvar: Clenil ratio is consistent with clinical equivalence data and explains the need to halve the dose when switching from Clenil® to Qvar® inhaled therapy. The use of the not rinsed spacers with

Clenil® inhaler may increase lung deposition; however, adding spacers to Qvar® inhalers will not affect it. Small volume spacers were found to be more suitable in maintaining the extrafine particle fraction for Qvar® EB and Qvar® MDI. While large volume spacers were more suitable for the larger particle size formulation such as Clenil® as it will create more space for more efficient particle size reduction.

In conclusion, the urinary pharmacokinetic method originally pioneered for salbutamol can also be applied to inhaled beclometasone. The ratio between Qvar and Clenil is consistent with related clinical and pharmacokinetic lung deposition studies. Using this method, we found that there are several factors affecting drug delivery. These include; drug formulation, particle size, spacer size, as well as the method of handling spacers. This work confirms the concept that the efficacy of a particular spacer with one formulation cannot be assumed for another formulation, even for the same drug. Therefore, each drug formulation MDI/spacer combination should be first properly evaluated prior to use (GINA, 2010). This simple non-invasive methodology was found to be reproducible and can now be applied in clinical studies to study different formulations and products as well as inhalation methods.

8.2. Future work

Asthmatics are normally prescribed a salbutamol inhaler and an inhaled corticosteroid. Theoretically, salbutamol will open the airways and allow more inhaled corticosteroid to be deposited into the lungs. There has always been a debate that salbutamol should be given first but this has never been studied due to the unavailability of a simple method to identify lung deposition of inhaled corticosteroids. There are two different HFA-BDP formulations with different dosage recommendations, which is why the MHRA has recommended that inhaled beclometasone inhalers should be prescribed by brand. One of these formulations (Qvar; Teva Pharmaceuticals) has ultrafine particles with high lung

deposition, which may not be improved by prior inhalation of a bronchodilator. The other (Clenil®, Chiesi) has bigger particles because it was formulated to mimic Becotide (GlaxoSmithKline), the innovator product. Theoretically, inhalation of this after a bronchodilator should result in higher lung deposition. Using the urinary beclometasone method, we have designed a study to address these points. Measurement of the urinary excretion of beclometasone dipropionate, 17-beclometasone monopropionate, and beclometasone in the first 30 minutes after an inhalation of beclometasone dipropionate by adult asthmatics can be used to:

- To determine if the bronchodilator effects of salbutamol, in asthmatic patients, affects lung deposition of inhaled beclometasone.
- To compare the effect of salbutamol on the lung deposition of two different beclometasone formulations (Qvar® and Clenil®) to identify the influence of the particle size of a formulation that is inhaled following a bronchodilator.

Local hospital research ethics committee approval was obtained for this study and presented in APPENDIX C (refer to the enclosed DVD); however, there was a difficulty in recruiting patients from Huddersfield Royal Infirmary. The study was designed as follows: Patients will be enrolled into the study from the outpatient clinic seven days before Study Day 1. Each patient's metered dose inhaler technique will be checked and corrected if required.

The study doses will be

- Four doses of Qvar® Easi-Breathe (100µg), Salbutamol inhalation will be allowed after the urine sample and spirometry test at 30 minutes post inhalation
- Two salbutamol 100µg doses 15 minutes before four doses of Qvar® Easi-Breathe (100µg).

- Four doses of Clenil® MDI (250µg), Salbutamol inhalation will be allowed after the urine sample and spirometry test at 30 minutes post inhalation
- Two salbutamol 100µg doses 15 minutes before four doses of Clenil® MDI (250µg).

The order of study dose administration will be randomised and there will be a minimum wash out period of seven days between each study day. On study days, patients will withhold all their inhaled doses for 12 hours except for salbutamol, which will be required to be withheld for 6 hours. All study doses will be administered in the morning so their last inhaled dose (except salbutamol) will be the previous night. Those that need rescue medication from their salbutamol within 6 hours of their planned attendance will be allowed to continue their medication and their study day will be re-scheduled. On study day1, spirometry will be measured and each patient's inhalation technique will be checked with correction as required. Patients will void their urine 15 minutes before the inhalation of the first beclometasone dose. There will be 30 seconds between each inhaled dose. Thirty minutes after the inhalation of the first beclometasone dose patients will provide a urine sample and their spirometry will be measured. The volume of their urine will be recorded and an aliquot will be retained and frozen at minus 20^{o}C prior to analysis. Our LC-(ESI+)-MS method with solid phase extraction assay that we have developed and validated for beclometasone dipropionate and its metabolites will be used to identify amounts excreted in the urine samples.

Since, it has been shown that lung deposition of patients is affected by the inhalation flow and that coordination is not controlled. This simple non-invasive methodology can be used in patient studies to investigate the effect of coordination and inhalation flow. Besides, the methodology can be extended to budesonide and fluticasone propionate.

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