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Original Citation

Waters, Laura J., Shokry, Dina and Parkes, Gareth M.B (2016) Predicting human intestinal absorption in the presence of bile salt with micellar liquid chromatography. Biomedical Chromatography. ISSN 1099-0801

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Predicting human intestinal absorption in the presence of bile salt with micellar liquid chromatography

Laura J. Waters*, Dina S. Shokry and Gareth M. B. Parkes

School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK,
e-mail: l.waters@hud.ac.uk, 01484 472190

Abstract

Understanding intestinal absorption for pharmaceutical compounds is vital to estimate bioavailability and therefore the in vivo potential of a drug. This study considers the application of micellar liquid chromatography (MLC) to predict passive intestinal absorption with a selection of model compounds. MLC is already known to aid prediction of absorption using simple surfactant systems however, with this study the focus was on the presence of a more complex, bile salt surfactant, as would be encountered in the in vivo environment. As a result, MLC using a specific bile salt has been confirmed as an ideal in vitro system to predict the intestinal permeability for a wide range of drugs, through the development of a quantitative partition-absorption relationship. MLC offers many benefits including environmental, economic, time-saving and ethical advantages compared with the traditional techniques employed to obtain passive intestinal absorption values.
Keywords

Intestinal absorption; chromatography; MLC; micellar; bile salts

Introduction

The most favourable option for drug administration is the oral route, accounting for the majority of pharmaceutical formulations on the market. A large percentage of these products are absorbed within the gastrointestinal (GI) tract thus it is essential to quantify the extent of absorption to predict bioavailability. Most new chemical entities (NCEs) intended for oral administration are within Class II or IV of the Biopharmaceutics Classification System (BCS), i.e. of low aqueous solubility (Williams, Trevaskis, Charman, Shanker, Charman, Pouton and Porter 2013). As a result these compounds tend to exhibit poor bioavailability which can be problematic for development. Facilitating the prediction of drug absorption is therefore fundamental to maximise potential bioavailability, and consequently, efficacy of an NCE.

Traditionally, in vivo performance following oral administration has been predicted using animal models. In recent years this has been less favourable for reasons including interspecies variability (Martignoni, Groothuis and de Kanter 2006), substantial economic costs and ethical considerations (Zurlo and Hutchinson 2014). For these reasons research has focused on the development of alternatives to such models. One of the most widely researched in vitro methods to simulate in vivo performance is the application of dissolution studies in biorelevant media (Berthelsen, Sjögren, Jacobsen, Kristensen, Holm, Abrahamsson and Müllertz 2014). It is believed that the use of physiologically relevant media is crucial as the components present, for example bile salts, are present in intestinal fluids allowing a closer replication of the in vivo scenario (Tomaszewska, Karki, Shur, Price and Fotaki 2013). The composition of GI fluids is well characterised with respect to pH, buffer capacity, osmolarity, surface tension and lipid concentration under fasted and fed conditions. Numerous studies have confirmed the relationship between the impact of these properties through preformulation studies, allowing an estimation of the fraction of drug absorbed in vivo for orally administered compounds (Bergström, Holm, Jørgensen, Andersson, Artursson, Beato, Borde, Box, Brewster, Dressman, Feng, Halbert, Kostewicz, McAllister, Muenster, Thinnes, Taylor and Mullertz 2014). However, some researchers have found that dissolution testing by itself may not be adequate and it may be more beneficial to undertake the
simultaneous assessment of dissolution and permeation (Sugano, Kataoka, da Costa Mathews and Yamashita 2010).

Another technique to predict in vivo permeability is the parallel artificial membrane permeability assay, also known as PAMPA. This assay has been used previously to predict permeability through a range of biological environments including skin (Ottaviani, Martel and Carrupt 2006), the blood brain barrier (Di, Kerns, Fan, McConnell and Carter 2003) and the GI tract (Bujard, Sol, Carrupt and Martel 2014). Although it has been shown to be useful for in vivo prediction, PAMPA does have some limitations, for example unpredictable drug retention for highly lipophilic drugs (Bendels, Tsinman, Wagner, Lipp, Parrilla, Kansy and Avdeef 2006) and a significant unstirred water layer (Avdeef, Nielsen and Tsinman 2004, Ruell, Tsinman and Avdeef 2003). Some of these challenges have been investigated in an attempt to overcome these difficulties to improve accuracy and precision with a degree of success in certain aspects (Buckley, Fischer, Fricker and Brandl 2012).

Many researchers consider the ‘gold standard’ for predicting intestinal absorption to be the Caco-2 model (Wuyts, Riethorst, Brouwers, Tack, Annaert and Augustijns 2015). This is a cell culture model consisting of monolayers cultivated on permeable growth inserts. Such models exhibit structural and biological properties similar to those in vivo with the expression of appropriate enzymes, conferring their suitability as a model system. However, cellular models are renowned for their limited reproducibility, extensive culturing requirements and economic cost (Buckley, Fischer, Fricker and Brandl 2012). Some research has attempted to enhance the biorelevance of permeability data, for example by using fasted state human intestinal fluid as the solvent system with prediction for a series of 16 model drugs (Wuyts, Riethorst, Brouwers, Tack, Annaert and Augustijns 2015).

Other less well known models have also been proposed, some of which are modifications of those previously discussed and others more novel. One such example of the latter is the application of spectrofluorimetry and derivative spectrophotometry to determine partition coefficients to assess the role of mixed (bile salt) micelles in gastrointestinal absorption (De Castro, Gameiro, Guimarães, Lima and Reis 2001). Interestingly, it was found that hydrophobic compounds are fully incorporated within mixed bile salt micelles whereas amphiphilic drugs are not. This work demonstrates the significant effect the presence of bile salt micelles can have on absorption, and therefore, bioavailability.
One particular technique that explores the effects of micelles on the behaviour of compounds is micellar liquid chromatography (MLC). Approximately thirty years since its development, MLC has become renowned for providing a variety of analytical information on a wide range of chemical samples. There are several advantages for the use of MLC including the avoidance of organic solvents, analytical rapidity, data reproducibility, sample flexibility and economic savings (El-Shaheny, El-Maghrabey and Belal 2015). MLC employs a mobile phase containing one, or more, surfactants over a range of concentrations. Ideally, the chosen surfactant will have a comparatively low critical micellar concentration (CMC) and aggregation number. Upon injection of the sample (solute), several competing phenomena occur. Each compound within the sample will partition between the bulk aqueous phase and micellar aggregates plus the bulk aqueous phase and surfactant-coated stationary phase. These interactions will dictate the retention time of the solute which can then be measured using a variety of analytical techniques, such as UV or fluorescence spectroscopy. Using well established equations it is possible to calculate a range of physicochemical partitioning properties for the solute under investigation (Waters and Kasprzyk-Hordern 2010, Waters, Shahzad and Mitchell 2012). A summary of the wide array of developments in MLC over recent years can be found in the work of (Ruiz-Ángel, Garcia-Álvarez-Coque and Berthod 2009). For example, MLC has very recently been applied in the determination of the lipophilicity of organic compounds alongside another technique, namely microemulsion liquid chromatography, as potential high-throughput screening platforms (Xu, Li, Huang, Yu, Wang and Li 2015). More specific applications for MLC have also been investigated, for example to determine blood-brain barrier penetration (Lu, Sun, Wang, Li, Liu, Fang and He 2009, Stepnik and Malinowska 2013). In one particular study drug penetration was predicted and comparisons made between MLC and immobilised artificial membrane liquid chromatography (De Vrieze, Lynen, Chen, Szucs and Sandra 2013). This study also confirms the benefits of using sodium deoxycholate, including their micellisation ability and UV transparency although other benefits are also known, such as their low economic cost. Research has also focussed on prediction of ocular tissue permeability (Martín-Biosca, Molero-Monfort, Sagrado, Villanueva-Camañas and Medina-Hernández 2003) and modelling skin permeability (Martínez-Pla, Martín-Biosca, Sagrado, Villanueva-Camañas and Medina-Hernández 2003, Waters, Shahzad and Stephenson 2013). With respect to predicting human drug absorption using MLC, research is incredibly limited to a small number of papers, employing standard surfactants as the basis of the mobile phase. For example, in 2001 Molero-Monfort et al. published a paper using Brij 35 to develop a model based on the
retention of selected compounds which showed a predictive ability for drugs absorbed by passive diffusion (Molero-Monfort, Escuder-Gilabert, Villanueva-Camañas, Sagrado and Medina-Hernández 2001). This work was then followed by research published in 2014 that considered two further surfactants within the mobile phase, namely sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) (Stępnik, Malinowska and Rój 2014).

However, the application of MLC with micellar systems that are actually encountered within the GI tract, i.e. bile salt micelles, has not been investigated prior to this study. Through combining the advantages of MLC with the incorporation of micelles that are known to exist in vivo will aid the development of an in vitro model that is simple, reproducible, inexpensive, accurate and moreover, precise, for the prediction of human absorption data.

In summary, the main aim of this study was to investigate the use of MLC with bile salt to develop a quantitative partition-absorption relationship, thus creating an alternative in vitro system to predict intestinal permeability for pharmaceutical compounds.

Micellar Liquid Chromatography Method and Instrumentation

A detailed description of the basic MLC methodology employed in this study has been published previously (Waters, Shahzad and Stephenson 2013). In summary, each mobile phase containing the bile salt surfactant was pumped through the chromatographic system (Severn Analytical SA 6410B) with a reversed phase cyanopropyl column (Spherisorb 5μm, 15cm x 4.6 mm i.d.) and a flow rate of 1.34 mL per minute. The selection of this column and flow rate was decided based on previous work that proves the superiority of using a cyanopropyl column over C18 due to its tendency to adsorb less surfactant and the binding of the surfactant to the bonded phase through electrostatic interaction decreasing the charged layer on the surface of the column (Waters, Shahzad and Stephenson 2013). Drug samples (0.2 mM) in a solvent identical to that of the mobile phase were injected via a Rheodyne injector. A UV detector (Waters 2487), set at a wavelength appropriate for each drug (221 – 360 nm), identified retention of the solute within the column as a function of time. Data were recorded and then analysed to obtain retention factors and each run was repeated a minimum of three times to ensure reasonable accuracy and precision were achieved. A high level of
reproducibility was attained for the retention data with a maximum standard deviation of 0.975 for all assays.

**Materials and Reagents**

An aqueous solution containing a bile salt surfactant, namely sodium deoxycholate (NaDC, Figure 1) (log Po/w: 1.24, CMC: 4.3-5.5 mM at 291-298 K, pKa: 6.2, Aggregation number: 5.1-7.1) (Olesen, Westh and Holm 2015) and (Vadnere and Lindenbaum 1982), was considered as a mobile phase for this study, used as purchased from Sigma Aldrich, Dorset, UK (97 %). NaDC was diluted with distilled water as necessary to achieve concentrations from 5 to 20 mM. The 16 compounds considered in this work were: acetaminophen (99 %, Sigma Aldrich, Dorset, UK), fluconazole (98 %, Sigma Aldrich, Dorset, UK), indomethacin (99 %, Sigma Aldrich, Dorset, UK), ketoprofen (98 %, Sigma Aldrich, Dorset, UK), lidocaine (98 %, Sigma Aldrich, Dorset, UK), nicotinic acid (99.5 %, Sigma Aldrich, Dorset, UK), phenylbutazone (98.5 %, Sigma Aldrich, Dorset, UK), piroxicam (98 %, Sigma Aldrich, Dorset, UK), propranolol (99 %, Sigma Aldrich, Dorset, UK), acetyl salicylic acid (99 %, Acros organics, Geel, Belgium), diclofenac (98 %, TCI, Europe), diphenhydramine (98 %, TCI, Europe), theophylline (98 %, TCI, Oxford, UK), fenoprofen (97 %, Fluka, Dorset, UK), gemfibrozil (98 %, TCI, Europe), ibuprofen (98 %, BASF, Cheshire, UK), used as purchased with each at a concentration of 0.2 mM. All experimental work was conducted with no organic modifier or buffer present due to the formation of polymer like aggregates or gels upon adjusting the medium to any pH lower than 8. Retention data are averages of triplicates.

**Data Analysis**

In agreement with previous work, it was possible to calculate a value for the micelle/water partition coefficient (logP_{mw}) by plotting the surfactant concentration (after subtraction of the CMC, i.e. 5 mM) with the inverse of the capacity factor. A representative chromatogram can be seen in Figure 2. It should be noted that pH and ionic strength were not maintained at a constant value during this work as it was thought the addition of further compounds to the mobile phase may interfere with the partitioning process.
Results from the MLC technique permitted the development of a dataset for the bile salt solutions that contained logP\textsubscript{mw} values for 14 different compounds along with their physicochemical parameters, such as molecular weight (MW) and published human intestinal absorption values (HIA %). Multiple linear regression (MLR) analysis focussed on the development of quantitative partition-absorption relationships to correlate the MLC data for each drug with published \textit{in vivo} data using Minitab 17\textsuperscript{®}, Minitab Inc., State College, Pennsylvania (licensed to the University of Huddersfield). Once established, the equation relating logP\textsubscript{mw} with intestinal absorption was used to investigate two final compounds (indomethacin and piroxicam) to determine the applicability of the equation.

Results and discussion

**Determination of log P\textsubscript{mw}**

Sodium deoxycholate (NaDC) is a well-known bile salt surfactant present in intestinal fluids in the GI tract. Moreover, NaDC forms micellar structures that aid the passive absorption of compounds \textit{in vivo} (Holm, Mullertz and Mu 2013). The ability of NaDC-based MLC to predict human oral drug absorption was evaluated using a set of 14 drugs of variable structure and physicochemical properties. All of the compounds analysed were known to undergo passive absorption with reliable \textit{in vivo} data available. Retention data for each compound led to the calculation of micelle-water partition coefficient (P\textsubscript{mw}) values which were then analysed alongside oral absorption data from published literature data. A summary of the calculated P\textsubscript{mw} values and percentages of oral absorption (from previously published data) can be found in Table 1. Also the determination coefficients (R\textsuperscript{2}) for the linear equations relating the inverse of the capacity factor for all drugs against the micellar concentrations of NaDC used are stated in Table 2.

**Prediction of HIA values**

Although there was a linear relationship between the literature % absorption values and experimental logP\textsubscript{mw} values, it was decided to instead use logit(Abs) to improve this relation as is often used in published studies of a similar nature (Norinder, Österberg and Artursson 1999, Raevsky, Fetisov, Trepalina, McFarland and Schaper 2000, Zhao, Abraham,
Le, Hersey, Luscombe, Beck, Sherborne and Cooper 2002, summed in Equation 1 where \( FA \) = fraction absorbed.

\[
\text{Logit}(\text{Abs}) = \log(\frac{\text{FA}}{1-\text{FA}}) \quad (1)
\]

As a result, all drugs with percentages of absorption of 100% or 0% were excluded from the training set for simplification.

A series of molecular descriptors (polar surface area, freely rotating bonds, number of hydrogen bond donors/acceptors, molecular weight, molar volume and solubility) permitted the development of an appropriate equation using multiple regression analysis. Descriptors present in the final model were assessed for significance and relative importance using standardised coefficients and associated P-values where the standardised coefficients for log \( P_{\text{mw}} \) (micelle/water partition coefficient), Mwt (molecular weight) and Sw (solubility in water) were found to be -0.431, 1.050 and 0.761 respectively while their P values at 95% confidence level were found to be 0.007, 0.000 and 0.001 respectively which proves their statistical significance. Through combining the data in Table 1 and Table 2 a residual plot for optimal regression and partial regression plots of experimental logit(Abs) values against log\( P_{\text{mw}} \), Mwt and Sw (Figures 3 and 4) facilitated the development of an equation to utilise experimental MLC data to predict the percentage of human intestinal absorption in vivo.

Overall, the optimal model obtained incorporated 3 descriptors and is as follows (Equation 2):

\[
\text{logit}(\text{Abs}) = -0.410 - 0.482 \log P_{\text{mw}} + 0.00852 \text{Mwt} + 0.04799 \text{Sw} \quad (2)
\]

Where \( \log P_{\text{mw}} \) is the partition coefficient experimentally determined by MLC, Mwt is the molecular weight and Sw is the solubility in water (Table 2). The value obtained using Equation 2 can then be converted into a percentage of absorption using Equation 1. Standard error (S.E.) = 0.195, \( R^2 = 0.86 \), \( R^2_{\text{adj}} = 0.82 \), \( R^2_{\text{pred}} = 0.75 \), \( F=20.994 \), PRESS=0.7, Mallows' Cp=4 where \( R^2_{\text{pred}} \) is the predicted coefficient of determination that determines the predictive power of the model, PRESS is the predicted residual sum of squares to determine the predictive ability of the model when compared with other models of an identical dataset and Mallows' Cp is used in choosing between multiple regression models. The P-values obtained for this model indicate that the relationship between % HIA and \( P_{\text{mw}} \) values was statistically significant at the 95% confidence level where they were < 0.05. The unadjusted \( R^2 \) of 0.86 derived from the current data indicates that the fit of the sampled drugs to the model is good,
with about 86% of the variance in the outcome measure being accounted for by logP_{mw} and other included descriptor values. The R^2_{pred.} value of 0.75 indicates that the fit of the drugs to the model is suitable and confirms the potential suitability of MLC using NaDC to predict oral drug absorption in the human GI tract. Furthermore, if the experimental logP_{mw} values were replaced with published octanol-water values then it was found there was no predictability of HIA % possible, i.e. logP_{mw} was a significant contributor to the predictive ability. Mallows' Cp value of (4.0) is the same as the number of predictors plus the constant (4,0), indicating that the model is relatively precise and unbiased in estimating the true regression coefficients and predicting future response. A 95% confidence interval for the P_{mw} parameter is given by (-0.796, -0.167); a 95% confidence interval for the Mwt parameter is given by (0.006, 0.011) and a 95% confidence interval for the Sw parameter is given by (0.026, 0.070). A residual analysis did not detect any marked relationship between residuals and predicted values as illustrated.

All covariates were statistically significant (t_1=-3.42, p<0.05 for log P_{mw}; t_1=-6.93, p<0.05 for molecular weight; t_1=4.84, p<0.05 for aqueous solubility), with the model F-ratio also being statistically significant (F=20.99, p<0.05). None of the drugs used in the current dataset used in development of the previous model were found to have high residuals or to be influential according to studentised residuals or Cook's distance. Also the consistency of the R^2_{adj} and R^2_{pred.} does not suggest any evidence of model or data inadequacies in the current model.

In summary, the values presented in Table 1 for HIA % predicted versus those from literature show a remarkably similar trend. For example, differences between the two values are 0 % (theophylline) through to a maximum of only 7.6 % (acetylsalicylic acid) with the vast majority successfully predicting within 4 % of the literature value.

As a final aspect of investigation to assess the success of the model an additional two compounds were investigated, namely indomethacin and piroxicam. For indomethacin, with an experimental MLC logP_{mw} measurement of 1.74 and applying Equation 2, the percentage of human intestinal absorption was calculated (i.e. predicted) to be 98.4 %. With a literature percentage of human intestinal absorption value of 100 % (Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014) then the model was deemed to be a successful method for prediction of in vivo behaviour, i.e. with less than 2 % difference between the calculated and literature values. A similar result was obtained for piroxicam with an experimental MLC logP_{mw} measurement of 3.37 whereby the literature percentage of
human intestinal absorption value is known to be 100 % (Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014) and the predicted value was 98.7 %.

**Comparison with other methods to predict HIA**

Predicting the extent to which drugs may permeate the intestinal barrier is a key factor in predicting subsequent bioavailability. As previously discussed, there are several methods to predict intestinal absorption with data publicly available which allows comparisons to be made between this MLC-based method and others. Firstly, compared with Caco-2 absorption data, it can be said that the MLC method is comparable in predictive ability yet simpler, faster and cheaper to undertake. A variety of values have been published for Caco-2 absorption, such as the work of Stepnik et al. (Stępnik, Malinowska and Rój 2014), with published data ranging in predictive ability where, in general, the level of prediction is similar to that published in this work. Based on the comparable ability of MLC for prediction along with the variety of advantages it appears to confirm its potential as a method of choice.

Secondly, artificial membrane based techniques, such as PAMPA, have been applied to the prediction of intestinal absorption. As with published data for Caco-2 absorption models, predictions have been made using PAMPA and their success rate have been generally high, for example the work of (Bujard, Sol, Carrupt and Martel 2014). Again, the predictive ability of the MLC method presented in this paper is generally comparable with that using artificial membranes yet does not pose the same limitations as discussed earlier.

Thirdly, MLC has been considered prior to this work in a limited manner with the use of simpler surfactant systems, such as SDS and Brij 35. In this case there are no advantages related to the method employed as both require a similar set-up and therefore time and economic requirements. However, the benefits of using a bile salt surfactant are clearly evident based on the increased similarity to the *in vivo* scenario which is reflected in the enhanced predictive ability. For example, compared with the work of Molero-Monfort et al. (Molero-Monfort, Escuder-Gilabert, Villanueva-Camañas, Sagrado and Medina-Hernández 2001) and Stepnik et al. (Stępnik, Malinowska and Rój 2014), the data presented in this research displays a closer fit with human intestinal absorption data. Also other work has been undertaken using sodium cholate and sodium taurodeoxycholate which were also used in prediction of intestinal absorption and the obtained models support the closer fit of the data in this paper to human intestinal absorption. These comparisons highlight the importance of surfactant choice when designing MLC experiments as it is very clear that the composition of
the mobile phase can significantly affect drug retention which will ultimately affect absorption prediction.

Conclusions

Determining a good prediction of intestinal absorption chromatographically is an exciting advance in analysis for many reasons, not only for the replacement of using animal models but also to save time and money which will consequently help enhance the development of new drugs. Based on analysis for these model compounds it has been found that NaDC is suited to intestinal absorption prediction and appears to provide superior prediction compared with alternative methods. Furthermore, data presented in this study could be expanded to display the more general predictive ability of MLC for a wider set of compounds thus confirming its potential as a general method for predicting human intestinal absorption.

References


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Olesen NE, Westh P and Holm R Determination of thermodynamic potentials and the aggregation number for micelles with the mass-action model by isothermal titration calorimetry: A case study on bile salts. Journal of Colloid and Interface Science 2015; 453: 79-89. DOI: 10.1016/j.jcis.2015.03.069.


Figure 1. Structure of sodium deoxycholate.
Figure 2. A representative chromatogram to permit calculation of logP_{mw}, in this particular case for caffeine over a series of sodium deoxycholate concentrations, highlighting the shift in retention time with bile salt concentration.
Figure 3. Residual plot for optimal regression model.
Figure 4. Partial regression plots of experimental logit(Abs) values against log $P_{mw}$, $M_{wt}$ and $S_{w}$.
Table 1. Experimentally determined P_{mw} values (P_{mw} (Expt)), published literature absorption values (HIA % (Lit)), calculated and predicted human oral absorption data (% HIA Pred.)

<table>
<thead>
<tr>
<th>Drug</th>
<th>P_{mw} (Expt)</th>
<th>HIA % (Lit)</th>
<th>HIA % Pred.</th>
<th>(Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>1.26±0.149</td>
<td>80</td>
<td>74.7</td>
<td>(Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>1.52±0.293</td>
<td>82</td>
<td>74.4</td>
<td>(Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.56±0.027</td>
<td>97</td>
<td>95.9</td>
<td>(Hou, Wang, Zhang and Xu 2007)</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>2.47±0.415</td>
<td>72</td>
<td>79.2</td>
<td>(Paixão, Gouveia and Morais 2012)</td>
</tr>
<tr>
<td>Fenoprofen</td>
<td>1.22±0.696</td>
<td>85</td>
<td>92.2</td>
<td>(Hou, Wang, Zhang and Xu 2007)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>1.44±0.071</td>
<td>97.5</td>
<td>97.4</td>
<td>(Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>1.48±0.035</td>
<td>95</td>
<td>91.1</td>
<td>(Paixão, Gouveia and Morais 2012)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.46±0.032</td>
<td>80</td>
<td>81.7</td>
<td>(Balon, Riebesehl and Müller 1999)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.74±0.019</td>
<td>100</td>
<td>98.4</td>
<td>(Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014)</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>0.91±0.01</td>
<td>92</td>
<td>95.4</td>
<td>(Castillo-Garit, Cañizares-Carmenate, Marrero-Ponce, Torrens and Abad 2014)</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>2.18±0.163</td>
<td>75</td>
<td>78.6</td>
<td>(Varma, Sateesh and Panchagnula 2005) (Molero-Monfort, Escuder-Gilabert, Villanueva-Camañás, Sagrado and Medina-Hernández 2001),</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1.22±0.069</td>
<td>88</td>
<td>89.2</td>
<td>(Castillo-Garit, Cañizares-Carmenate,</td>
</tr>
<tr>
<td>Drug</td>
<td>Mwt</td>
<td>Sw</td>
<td>HBD</td>
<td>HBA</td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>151.20</td>
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<td>2</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
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<tr>
<td>Diclofenac</td>
<td>296.15</td>
<td>0.00447</td>
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<td>3</td>
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<tr>
<td>Diphenhydramine</td>
<td>255.35</td>
<td>0.0752</td>
<td>0</td>
<td>2</td>
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<td>Fenoprofen</td>
<td>242.27</td>
<td>0.0811</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>306.27</td>
<td>1.39</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>250.33</td>
<td>0.0278</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>206.28</td>
<td>0.0684</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>357.79</td>
<td>0.0024</td>
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<td>4</td>
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<tr>
<td>Ketoprofen</td>
<td>254.28</td>
<td>0.0213</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>234.34</td>
<td>0.593</td>
<td>1</td>
<td>2</td>
</tr>
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<td>Nicotinic acid</td>
<td>123.11</td>
<td>18.00</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>308.37</td>
<td>0.144</td>
<td>0</td>
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<tr>
<td>Piroxicam</td>
<td>331.35</td>
<td>23.00</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Propranolol</td>
<td>259.34</td>
<td>0.0794</td>
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<tr>
<td>Theophylline</td>
<td>180.16</td>
<td>22.90</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Data obtained from: 

- Drugbank (http://www.drugbank.ca/), 
- Chemsper (ACD/Labs) (http://www.chemspider.com/).


Table 2 Relevant physicochemical parameters of the selected drugs analysed for development of the quantitative partition-absorption relationship.