Long Title: **Analysis of Phenazepam and 3-hydroxyphenazepam in Postmortem Fluids and Tissues**

Short Title: Analysis of phenazapam in postmortem samples

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Abstract

Phenazepam is a benzodiazepine that is predominantly used clinically in the former Soviet states but throughout the wider world is being abused. This study reports the tissue distribution and concentration of both phenazepam and 3-hydroxyphenazepam in 29 cases quantitated by LC-MS/MS in a variety of postmortem fluids (subclavian blood, femoral blood, cardiac blood, urine, vitreous humor) and tissues (thalamus, liver and psoas muscle). In 27 cases the cause of death was not directly related to phenazepam (preserved (fluoride/oxalate) femoral blood phenazepam concentrations 0.007 mg/L to 0.360 mg/L (median 0.097 mg/L)). In two cases phenazepam was either a contributing factor to, or the certified cause of death (preserved (fluoride/oxalate) femoral blood 0.97 mg/L and 1.64 mg/L). The analysis of phenazepam and 3-hydroxyphenazepam in this study suggests that they are unlikely to be subject to large postmortem redistribution and that there is no direct correlation between tissues/fluid and femoral blood concentrations. Preliminary investigations of phenazepam stability comparing femoral blood phenazepam concentrations in paired preserved (2.5% fluoride/oxalate) and unpreserved blood show that unpreserved samples show on average a 14% lower concentration of phenazepam and we recommend that phenazepam quantitation is carried out using preserved samples wherever possible.

Introduction

Phenazepam is a 1,4-benzodiazepine that was originally developed in the USSR in the late 1970's [1]. It is used clinically as an anxiolytic, anticonvulsant, sedative and muscle relaxant in the former soviet countries [2] but to date has not been licenced for clinical use elsewhere. As with other benzodiazepines it acts as a positive allosteric modulator of the gamma-amino butyric acid type A ($GABA_A$) receptor [3]. Further information on phenazepam can be found in two recent reviews [1, 4] In the past few years it has been used as a drug of abuse across the world with cases being reported in the UK [5], USA [6, 7], Finland [8], New Zealand [9] and Norway [10]. The abuse of phenazepam has led to it being controlled in a number of European countries including the UK, Norway, Finland and Lithuania [6]. This paper describes the toxicological findings from 29 postmortem investigations between Jan 2011 – April 2013 where phenazepam was detected and quantitated in a variety of postmortem fluids (blood, urine and vitreous) and tissues (brain, liver and muscle) using LC-MS/MS. We have also investigated the presence and levels of 3-hydroxyphenazepam a metabolite of phenazepam [3] . This study will help with the interpretation of phenazepam and 3-hydroxyphenazepam levels in postmortem cases.

Experimental

Postmortem samples

All samples were collected and analysed as part of the routine autopsy procedure. The specific samples collected depended on the specific case circumstances. Femoral venous blood (preserved and unpreserved), cardiac blood (unpreserved), urine (preserved and unpreserved), subclavian blood (preserved and unpreserved), preserved vitreous humour, psoas muscle (deep within the abdomen), brain (thalamus) and liver (centre of the right lobe). All femoral venous samples were obtained by needle and syringe from the femoral vein after cross-clamping the external iliac vein. Urine was collected from the bladder. Unpreserved samples were collected into 20ml Sterlin® tubes and preserved samples into 2.5% ISS fluoride/oxalate (F/O) tubes.

Chemicals and reagents

Stock solutions of 1 mg/ml phenazepam, 1 mg/ml 3-hydroxyphenazepam and 100 µg/ml diazepam-D5 were purchased from LGC standards (Teddington, U.K.). Methanol (gradient grade for HPLC ≥99.9%), acetonitrile (FAR UV grade), hexane and ethyl acetate were supplied by Fisher Scientific (Loughborough, U.K.). Blank Human SAGM blood was obtained from Blood Transfusion Service (Ninewells NHS hospital, Dundee). Ethical approval from the local ethical review committee was obtained for the use of this blood. Blank human urine was supplied by members of the laboratory. Sodium carbonate, ammonium formate and formic acid were purchased from Sigma-Aldrich (Poole, UK). Deionized water was further purified through a Millipore Direct-Q 3UV system (Millipore, Watford, UK). Blank samples of the following were purchased from local butchers: porcine muscle, porcine brain, porcine vitreous and porcine liver. Blank equine plasma was obtained from TCS biosciences (Buckingham, U.K.).

Standards, calibrators, control and internal standard preparation

Phenazepam
A nine point calibration range of 0.0007, 0.0015, 0.003, 0.006, 0.0125, 0.025, 0.05, 0.1 and 0.2 mg/L was prepared in blank equine plasma, using the stock solution of 1 mg/ml phenazepam. Quality control standards of 0.005 mg/L and 0.05 mg/L were prepared in blank equine plasma using a separate stock solution to that used in the preparation of the calibration range. Preparation of the calibrator and control samples were performed prior to each extraction.

3-hydroxyphenazepam
The stock solution of 3-hydroxyphenazepam (1 mg/ml) was used to prepare a seven point calibration range of 0.016, 0.031, 0.062, 0.125, 0.25, 0.5 and 1 mg/L in blank horse plasma. A separate stock solution of 3-hydroxyphenazepam was used to prepare internal quality control standards of 0.05 mg/L and 0.5 mg/L, again prepared in blank horse plasma. Calibrator and control samples were prepared prior to each extraction.

Internal Standard
10µL of the 0.1 mg/ml diazepam-D5 stock solution (internal standard, deuterated phenazepam or 3-hydroxyphenazepam were not available at the time of this study) was diluted to 50ml with methanol to produce a working solution of internal standard of 0.02 mg/L.

When required, blood, muscle, brain and liver samples were diluted with blank equine plasma. Urine and vitreous samples were diluted with deionised water, so that the samples fitted into the linear calibration range to enable quantitation. Single negative (internal standard only) and double negative (neither spike nor internal standard) control samples were prepared in plasma and run before the analysis of each batch of samples.

Extraction method for biological specimens
Muscle, brain and liver were homogenised for extraction, adapting and applying the procedure previously published by Flanagan *et al*. [11] . In brief, tissue paper was used to dry the tissue samples, which were then accurately weighed and homogenised (Janke & Kunkel Ultra-Turrax T25) with 4 parts of de-ionised water.

The extraction method has been described previously [12] . In brief 50 µl of internal standard (0.02 mg/L) was added to 500 µl of each standard/biological sample in polypropylene tubes. To this was added 500 µl of 0.2 M Na_2CO_3 and 5 ml 70:30 hexane:ethyl acetate mixture. The mixture is briefly vortexed then mixed on a rotating mixer for 10 min followed by 5 min centrifugation at 3000 rpm. The top layer solvent was transferred to glass vials and dried down under air at 45°C using a Techne Dri-block DB-3 and reconstituted with 50 µl of a methanol:water (50:50) mix for analysis.

Instrumentation and chromatographic conditions

Quantitative LC-MS/MS analysis was performed using an ABSciex 3200 QTRAP coupled to an Agilent 1260 Infinity series HPLC system consisting of a quarternary pump, degasser and an autosampler (Warrington, UK). A Phenomenex Gemini column (150 X 2.0 mm, 5 µm) was protected with a 4 mm x 2 mm Phenomenex Gemini guard column and Phenomenex Krudkatcher In-Line Filter (0.5 µm X 0.004 in). The injection volume was 10

µl. The chromatographic system was run with a gradient of 95 % mobile phase A, 5 % mobile phase B down to 10%A : 90%B over 5 mins before returning to 95%A : 5%B for a further minute. Mobile phase A comprised of 0.1 % formic acid. Mobile phase B comprised of acetonitrile containing 0.1 % formic acid. The flow rate was 0.8 ml/min with a column temperature of 40°C. Ionisation was achieved with a Turbo V electrospray source. The ions produced entered the QTRAP® for multiple reaction monitoring (MRM) with information-dependant (above 1000 cps) Enhanced Product Ion (EPI) scanning (between 50 and 700 Da). The following parameters were used; source temperature, 700 ºC; curtain gas, 30.0; ION gas 1, 45 units; ION source gas 2, 50 units; ion spray voltage, 5500 V; collision gas, high; declustering potential, 20 V; entrance potential, 10 V; scan rate, 4000 Da/s (EMS) and 4000 Da/s (EPI); and linear ion trap fill-time (LIT), 50 ms. The analytical data was collected using Analyst Version 1.5.2 (AB Sciex, Warrington, UK). The following ion transitions and retention times were observed: Phenazepam (*m/z* 350.8/206.3 & 350.8/104.4); 4.75 min, 3-hydroxphenazepam (*m/z* 366.9/320.8 & 366.9/194.2), 4.21 min and the internal standard diazepam-D5 *m/z* 290.0/198.1 & 290.0/154.2 at 4.86 mins (quantitation ions underlined).

Method validation

Using guidelines previously described by Peters *et al*. [13], the LC-MS/MS method for phenazepam quantitation was validated in blank equine plasma, blank human SAGM blood:blank equine plasma (50:50), blank human urine, blank bovine muscle, blank porcine brain and blank porcine liver:blank equine plasma (50:50). The 3-hydroxyphenazepam method was validated in blank urine, blank human SAGM blood, equine plasma and porcine vitreous. Standard calibration curves for phenazepam and 3-hydroxyphenazepam in equine plasma were produced using least squares regression with 1/x weighting factor and were found to be linear in the range of 0.0007 mg/L to 0.2 mg/L (Phenazepam) and 0.016 mg/L to .1.0 mg/L (3-hydroxyphenazepam), with an $r^2 > 0.99$. As shown in Table 1 the inter-day and intra-day accuracy and precision were calculated using one way ANOVA with the varied factor (day) as the grouping variable. This showed the method to be both accurate and reproducible as both within-run and between-run accuracy and precision were within ± 15 % for all matrices tested (n=15), thus within recommended guidelines [13]. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using a calculated signal-to-noise ratio using the instrument software; the signal-to-noise ratio was greater than 3:1 and 10:1 for LOD and LOQ, respectively, producing an LOD of 0.0003 mg/L and an LOQ of 0.0007 mg/L in plasma for phenazepam (0.007 mg/L and 0.016 mg/L for 3-hydroxyphenazepam). The results from matrix matching experiments carried out between equine plasma, human SAGM blood, human urine, bovine muscle, porcine brain and porcine liver showed a good correlation between the matrices used, allowing equine plasma to be used for the calibration curves. The effects of the various matrices on phenazepam were investigated using the methods of Matuszewski *et al*. [14]. Although the matrices exhibited ion suppression/enhancement, it was less than 15 %; therefore none of the matrices used significantly affected the detection and quantitation of phenazepam. From these experiments it is possible to calculate recovery (phenazepam between 38% and 72%, 3-hydroxyphenazepam between 45% and 78%) The method was also shown to be selective as when samples of SAGM Blood, plasma and urine were spiked with 63 drugs at a concentration of 10 mg/L Anticonvulsants (gabapentin, lamotrigine) Antidepressants (amitriptyline, citalopram, fluoxetine, moclobemide, nortriptyline, sertraline, trazodone, venlafaxine. Antihistamines (chlorpheneramine, cyclizine) Antipsychotics (amisulpride, olanzapine, quetiapine) Benzodiazepines (bromazepam, carbamazepine, clonazepam, diazepam, 3-hydroxyphenazepam, lorazepam, nitrazepam, nordiazepam,

oxazepam, phenazepam, temazepam). Beta-blockers (atenolol, bisoprolol, propranolol). Calcium channel blockers (amlodipine, diltiazem, verapamil). Dissociative anaesthetics (3-MEO-PCE, 3-MEO-PCP, methoxetamine). Opiates/Opioids (6-acetylcodiene, 6-monoacetylmorphine, dihydrocodeine, methadone, morphine, norcodeine, O-desmethyltramadol, oxycodone, tramadol. Stimulants (5-ABP, 6-ABP, amphetamine, benzedrone, benzoylecgonine, cocaine, 5-IT, 4-MEC, 4-methylamphetamine, MDMA, MDPV, MPA, PMMA). Non-opiate pain killers (buprenorphine, paracetamol, nefopam) Others (noscapine, papaverine, zolpidem) No significant interference of these compounds with either phenazepam or 3-hydroxyphenazepam was observed. The validity of the phenazepam method was also given external corroboration as a satisfactory z score of 1.68 was found when an external blood sample containing an unknown concentration of phenazepam was tested using this method as part of the Quartz Forensic Blood Toxicology external proficiency testing scheme (LGC Standards, Teddington U.K.)

Results and Discussion

This study reports the quantitative results of 29 postmortem cases (between January 2011 and April 2013) in which phenazepam was detected. Table 2 shows cases in which phenazepam was detected but not mentioned as part of the certified cause of death. In two cases phenazepam toxicity was mentioned as the certified cause of death. These results are shown in table 3. Further information about the cases, such as cause of death and concomitantly detected drugs are shown in table 4. Table 5A lists the publications that have cited phenazepam blood/plasma concentrations in a variety of case types. The postmortem femoral blood concentrations in this study were 0.009 - 0.370 mg/L well within the ranges of phenazepam concentrations seen in both in driving under the influence of drugs cases (DUID) (0.004 mg/L and 3.6 mg/L) [6, 8, 15-17], and also in postmortem cases in which the deaths were not directly attributed to phenazepam (0.008 mg/L – 1.6 mg/L) [4, 7, 18]. The femoral blood concentrations in this study were generally greater than “therapeutic” levels of phenazepam where in the only human pharmacokinetic study of phenazepam doses of 3 mg and 5 mg were found to show peak plasma concentrations of 0.024 mg/L and 0.038 mg/L respectively [19]. The femoral blood levels in the study however are lower than the 1.2 ug/g (~1.2 mg/L) blood concentration of phenazepam observed 7 days post ingestion of between 300 – 600 mg of phenazepam [20] and the 0.49 mg/L serum concentration of phenazepam 16 h after the insufflation of up to 1g of “three white powders”. [21]. These two studies and other DUID studies in which only phenazepam was detected give a limited insight into the acute effects of phenazepam over the published therapeutic levels. In a 40 year old male with phenazepam blood levels of ~ 0.04 mg/L slurred speech, confusion a lack of awareness and constricted pupils were observed [6]. In a DUID case a 24 year old female with similar blood levels of phenazepam (~0.05 mg/L) appeared lethargic, with an intoxicated appearance and a heart rate of 150 BPM [6]. In another DUID case a 21 year old male was also found to be “intoxicated” and lethargic (blood phenazepam ~0.06 mg/L) [6]. Kriikku *et al.* [8] reported 5 cases of DUID where only phenazepam was detected in the blood in which clinical examinations revealed “behavioural aberrations” with only 2 cases in which moderate (or greater) functional impairment was observed. Phenazepam blood concentrations were 0.23 mg/L – 3.00 mg/L, unfortunately no more details were given on the clinical symptoms. An emergency department report on a 42 year old male with a phenazepam concentration of 0.49 mg/L on admission (16 hours after insufflation of phenazepam) showed the patient was “noted to be confused and disorientated in time, place and person”, pupils were dilated with normal heart rate and blood pressure. The

confusion and disorientation lasted for around 48 h post admission [21]. In Sweden acute phenazepam intoxication was observed in a 24 year old male, on admission he had balance problems, confusion, memory disturbances and slurred speech. One day post admission (7 days post ingestion) his blood phenazepam level was 1.2 ug/g (~1.2 mg/L), auditory and visual hallucinations were observed post admission. It was estimated he ingested 300 – 600mg of phenazepam. These studies and a detailed study into the clinical features of toxicity and respective phenazepam in children aged 11-14 [22] have shown that phenazepam exhibits prolonged side effects including CNS depression, impaired balance, slurred speech, confusion, memory loss, ataxia, muscle hypotonia, hallucinations and tachy/bradycardia [1] and in a series of cases in Sweden were observed in 14 of 61 cases for more than 5 days (up to 3 weeks for CNS symptoms) and are thought to be related to the long (up to 60 h) half-life of phenazepam [23].

There is limited information in the literature about deaths that have involved phenazepam. Table 5B outlines the 4 previous published cases where phenazepam was included in the cause of death (phenazepam blood concentrations of 0.22 mg/L – 2.52 mg/L were recorded) [4, 7, 18]. In all of these cases multidrug toxicity was included in the cause of death. In two of the cases in the study phenazepam was implicated in the deaths. Table 3A/3B gives the full analytical results and further information for the two cases. In case 29 the cause of death was given as 'phenazepam toxicity' with a femoral blood level of 0.96 mg/L, although nordiazepam, diazepam and dihydrocodeine were detected as well (<0.16mg/L). This is the only case published to date in which phenazepam was the sole cause of death and phenazepam blood concentrations have been given. A case has previously been published in which the death was attributed to phenazepam toxicity but only the levels for 5-bromo-(2-chlorophenyl)-2-aminobenzophenone (ABPH), a purported phenazepam metabolite was reported [24]. In case 28 the cause of death was attributed to 'phenazepam and opiate toxicity' with an unpreserved femoral blood level of 0.96 mg/L (the other drug levels detected are given in table 3A). In both of these cases the phenazepam levels are within the concentrations of phenazepam that have been observed in DUID and postmortem cases with deaths not attributed to phenazepam (0.004 mg/L and 3.6 mg/L and 0.008 mg/L – 1.6 mg/L respectively), it is well known that benzodiazepines rarely cause death alone even at higher concentrations [25]. It is unfortunate that the source of blood is not given in the published cases as it is well known that differences in postmortem drug level can occur between various sources of blood due to postmortem redistribution greatly increasing the complexity of interpretation and also complicating direct comparisons between different cases. Femoral blood has been shown to be the best sample for interpretation of postmortem drug levels [26]. Without further knowledge of case 29 it is unclear why the cause of death was given as phenazepam toxicity and not multidrug toxicity as both dihydrocodeine and other benzodiazepines were detected within ranges that have previously been ascribed to multidrug toxicity and it is well known that benzodiazepines can potentiate the effects of opiates [27].

Apart from femoral blood this study reports the concentration of phenazepam in a range of other matrices where phenazepam was not mentioned as part of the case of death cardiac blood 0.086 mg/L (0.014 - 0.310 mg/L), subclavian blood 0.039 mg/L (0.016 - 0.270 mg/L), F/O vitreous humour 0.013 mg/L (0.007-0.054 mg/L), urine 0.016 mg/L (0.007 - 0.049 mg/L), muscle 0.166 mg/Kg (0.034 - 0.469 mg/Kg) brain 0.325 mg/Kg (0.065 -1.013 mg/Kg) and liver 0.584 mg/Kg (0.099 - 2.125 mg/Kg). Only one other study has published data of other phenazepam matrices in 10 postmortem cases [28]. They give similar results to those published in our study; subclavian blood 0.024 – 0.171 mg/L; vitreous humour 0.001 –

0.016 mg/L; skeletal muscle 0.023 – 0.522 mg/Kg in both studies the concentration of phenazepam in muscle was greater than blood and the lowest concentrations were seen in vitreous. Although urine is of limited use in determining the cause of death in postmortem investigations it can confirm the use of phenazepam in both postmortem, DUID and workplace drug testing investigations. In living subjects the median concentration of phenazepam in urine was 0.029 mg/L (0.012 - 0.060 mg/L n=7) [8], this was slightly higher than the median of 0.016 mg/L (0.007-0.049 mg/L) found in our investigations but similar to a previous study of 10 postmortem cases 0.001 – 0.030 mg/L [28]

The metabolism of phenazepam in humans is unclear and no comprehensive studies have been carried out that are available in the English literature. In a fatal phenazepam overdose, a metabolite of 3-hydroxyphenazepam, namely ABPH (also known as 2-amino-5-bromo, chloro-aminobenzophenone (ABCB)) was detected [24]. In a human study in which subjects were given 7 mg of phenazepam the metabolites 3-hydroxyphenazepam, ABPH and another unnamed metabolite, (see figure 1) were detected in urine in living subjects [29] and in a more recent study of phenazepam abuse in Finland 3-hydroxyphenazepam was detected in blood and urine again in living subjects [8]. Another metabolite (6-bromo-(2-chlorophenyl) quinazoline-2-one (QNZ) has been suggested based on animal studies [30] but as yet has not been observed in human studies. The quantitation of 3-hydroxyphenazepam appears problematic as to date no validated methods have appeared in the western literature and the only previous paper mentioned that 3-hydroxyphenazepam may be thermally unstable undergoing thermal dehydration [31]. During the validation of our 3-hydroxyphenazepam method we observed that degradation occurred as phenazepam was detected in the presence of blank samples which had only been spiked with 3-hydroxyphenazepam, (<0.0016 mg/L Phenazepam detected with 0.1 mg/L 3-hydroxyphenazepam) confirming some thermal instability. Although we were able to detect 3-hydroxyphenazepam at a concentration of 0.016 mg/L, we are as yet unable to develop a more sensitive method due the thermal instability of 3-hydroxyphenazepam.

The putative metabolites of phenazepam have been shown to be pharmacologically active, with 3-hydroxyphenazepam (at least *in vitro*) being shown to be more potent than phenazepam [3, 32]. For the first time we have been able to quantitate 3-hydroxyphenazepam in blood, urine and vitreous. In non-fatal phenazepam cases the median concentration of 3-hydroxyphenazepam in femoral blood was 0.052 mg/L (range 0.019 - 0.159 mg/L), cardiac blood 0.054 mg/L (0.019 - 0.161 mg/L), subclavian blood 0.030 mg/L, F/O vitreous humour 0.050 mg/L, urine 0.081 mg/L (0.017 - 0.274 mg/L). Semi-quantitative values of 3-hydroxyphenazepam were obtained for muscle, brain and liver and are shown in table 5. These results are semi-quantitative as these matrices could not be validated according to recommended guidelines, with accuracy and precision <> 15% [13].

The investigation of metabolites is also important in forensic investigation of death as the concentration ratio of parent to metabolite in blood may give information on the possible chronic or acute overdosing of the drug concerned with high ratios (>3) suggestive of acute overdose and low ratios (<1) indicative of chronic dosage [33] . However, it is possible that the parent to drug metabolite ratio may give misleading information, e.g. altered metabolic capacity or drug interactions [34] and should be interpreted with care. The ratios of phenazepam to 3-hydroxyphenazepam for each of the cases are given in table 6. In all cases (apart from case 15 (0.9)) the parent to metabolite ratio is >1, the only matrix that

was different to this is urine where in all cases (except case 18, (1.2)) the phenazepam to 3-hydroxyphenazepam ratio was <1. These results suggest that 3-hydroxyphenazepam does not accumulate as is the case with metabolites of diazepam where daily use of the diazepam causes accumulation of the metabolite and parent to drug ratios of ~1 [35]. The urinary results suggest that phenazepam is mainly excreted in the urine as 3-hydroxyphenazepam.

Due to postmortem redistribution the postmortem concentration of a drug may not be the same as that of the drug at the time of death. Postmortem redistribution is the movement of drugs in the body after death, thought mainly to occur by passive diffusion down a concentration gradient resulting in drug concentrations which do not represent those present at the time of death and thus confusing the interpretation of toxicological results [26]. It is important to be able to identify if a drug is likely to undergo postmortem redistribution. A drug is considered more likely to undergo postmortem redistribution if it has a volume of distribution (Vd) greater than 3 L/kg [36]. The only published value of Vd for phenazepam is given in L not L/kg (4.7 – 6.0 L) [19, 37] but would be expected to have a similar Vd as other benzodiazepines of between 0.6 - 2.6 L/kg [27] and like other benzodiazepine would exhibit very little postmortem redistribution [27]. It is a common assumption that if the ratio of the concentration of a drug in cardiac blood/concentration of a drug in peripheral (usually femoral) blood is >1 then the drug will undergo postmortem redistribution, however this difference may just represent a differing distribution of the drug during life [38]. Postmortem redistribution can only be definitively proved if changes in drug concentration are observed over time in the postmortem period. The differences in tissue concentration we observed are shown in table 7, and as the concentrations in the liver and muscle are higher than blood a concentration gradient would exist for possible postmortem redistribution. The results present here suggest that due to the differing concentrations observed in various tissues after death it is possible postmortem redistribution may occur. However further studies would need to be carried out in order to confirm temporal changes in phenazepam in various matrices over time and to investigate other tissue concentrations of phenazepam in postmortem samples (such as kidney, lung, heart and stomach contents).

Sometimes in postmortem cases it is not possible to obtain blood samples and other samples need to be analysed in order to investigate the possible role of drugs in the death. Vitreous humor is often used in toxicological analysis due to its low susceptibility to post mortem effects, such as drug redistribution and its protection from bacterial drug degredation. Vitreous is commonly used for confirmation of postmortem alcohol levels [39], biochemical analysis [40], determination of heroin use [41] and for drug screening when minimal blood samples are available, such as in decomposition, exsanguination or burning cases [42]. Femoral blood is known to be the postmortem sample to be least affected by postmortem redistribution and thus most likely to give similar concentrations to the antemortem blood sample [26]. It has been suggested that vitreous may be a suitable alternative to femoral blood for the estimation of the antemortem concentration of benzodiazepines [43] as previously a correlation between postmortem blood concentrations of diazepam and temazepam with vitreous concentrations have been observed. Figure 2 shows the result of the F/O femoral blood samples vs F/O vitreous concentrations of phenazepam. The correlation coefficient (R^2 = 0.92) on face value would suggest that if only vitreous is available the regression equation (y=mx+c) could be used to calculate the femoral blood concentration, however when back calculations were carried out using the result from this study it was found that the calculated results produced from the vitreous

showed a greater than 30% variation from the known value, with 20% variation being the acceptable variation for postmortem samples [44]. The error from these results is too great to be used to calculate postmortem femoral concentrations when only the postmortem vitreous is available.

The postmortem concentrations of drugs may be affected by degradation for this reason blood, vitreous and urine are commonly collected into tubes containing preservatives, such as Sodium fluoride / Potassium Oxalate to reduce possible degradation of sample by bacteria. This is especially important for drugs such as ethanol, which can be formed postmortem by bacterial action [39] and cocaine, which is known to be particularly unstable postmortem due to various enzymes [45]. Benzodiazepines have previously been found to have differing stability in blood. Diazepam has been found to be stable when stored at room temperature or when refrigerated for a 5 month period, however chloridiazepoxide and nordiazepam were found to be unstable in similar conditions [46] and nitrobenzodiazepines, such as nitrazepam have been found to be particularly unstable when in the presence of bacteria [47]. Gastineau *et al.* investigated the stability of 0.25 mg/L phenazepam over 2 months in whole blood and urine at both 20℃ and 4℃ they observed a decrease in phenazepam concentration of between 16 – 30% from the initial concentration [28] as there may have been concentration changes in phenazepam we briefly investigated differences between samples stored in different preservatives. Phenazepam femoral blood concentration in unpreserved blood were found to be on average 14% lower than the paired F/O femoral samples mean (SD) 0.10 ± 0.09 mg/L and 0.12 ± 0.10 mg/L respectively (n=20). The unpreserved blood concentrations of 3-hydroxyphenazepam were also found to be lower than the preserved paired blood samples (0.055 ± 0.049 mg/L and 0.074 ± 0.077 mg/L respectively (n=8)). In urine, which would not be expected to contain endogenous bacteria, there is no significant difference between the phenazepam concentration in preserved and unpreserved collection (unpreserved concentration on average only 1.4% lower than preserved)). For 3-hydroxyphenazepam, the concentration in unpreserved urine was higher than that in the preserved urine (0.096 ± 0.083 mg/L and 0.074 ± 0.074 mg/L respectively). These results may be due to artefacts from the storage materials however this is not thought to be likely due to the differences between whole blood results (in which changes were observed) and urine (in which no changes were observed). Based on these results it is recommended that where possible, preserved samples are used for quantitation, however more work needs to be carried out in order to conclusively determine the stability of both phenazepam and 3-hydroxyphenazepam in not only blood and urine, but also various other matrices that may be obtained for postmortem investigation.

This study reports for the first time postmortem concentrations of both phenazepam and 3-hydroxyphenazepam in a range of matrices. The results here suggest that 3-hydroxyphenzepam does not accumulate in blood and that there is a possibility that phenazepam may redistribute after death and that further studies need to be carried out in order to confirm the stability of both phenazepam and 3-hydroxyphenazepam in a range of biological sample. The results of this study will help forensic toxicologists and pathologists with the detection and interpretation of both phenazepam and 3-hydroxyphenazepam in postmortem cases.

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Table 1A: Validation Data for Quantitation of Phenazepam in Various Matrices by Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

	Plasma		***Blood:Plasma***		***Urine***		***Vitreous***		***Muscle***		***Liver:Plasma***		***Brain***	
QC Concentration (mg/L)	**0.005**	**0.05**	**0.005**	**0.05**	**0.005**	**0.05**	**0.005**	**0.05**	**0.005**	**0.05**	**0.005**	**0.05**	**0.005**	**0.05**
Accuracy (%)	-5.37	3.08	-12.41	-9.05	-8.33	-1.93	5.65	4.81	10.63	2.47	11.1	14.24	-11.93	-2.13
Precision (Within-run) (%CV)	8.32	4.61	7.81	12.09	5.51	10.44	11.87	10.81	9.80	12.2	6.89	5.43	10.70	10.03
Precision (Between-run) (%CV)	6.30	5.04	10.15	14.9	8.30	12.89	7.93	10.27	7.87	7.98	7.77	7.01	11.65	9.08
Ion suppression/Enhancement (%)	-5.20	6.60	14.2	14.8	8.10	-6.20	-0.20	13.70	-3.10	14.74	2.60	0.60	12.30	1.60
Recovery (%)	67.50	63.40	71.80	66.90	45.20	45.60	57.50	38.30	45.60	43.60	45.60	55.80	45.80	45.50

Table 1B: Validation Data for Quantitation of 3-hydroxyphenazepam in Various Matrices by Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

	Plasma		***Blood:Plasma***		***Urine***		***Vitreous***	
QC Concentration (mg/L)	**0.05**	**0.5**	**0.05**	**0.5**	**0.05**	**0.5**	**0.05**	**0.5**
Accuracy (%)	4.55	-9.16	-5.12	-12.43	-11.15	-7.92	14.75	3.92
Precision (Within-run) (%CV)	5.57	7.88	4.3	5.57	8.37	7.83	14.55	11.57
Precision (Between-run) (%CV)	6.56	6.18	10.38	3.93	11.71	5.25	11.79	14.80
Ion suppression/Enhancement (%)	18.9	-3.0	-0.51	-5.31	19.7	4.3	7.3	9.7
Recovery (%)	72.44	77.72	70.49	78.09	67.6	56.5	48.6	45.0

Table 2 Phenazepam concentrations from twenty seven fatalities

Case	*Femoral Blood*	*F/O Femoral Blood*	*Cardiac Blood*	*Subclavian Blood*	*F/O Subclavian Blood*	*F/O Vitreous Humour*	*Urine*	*F/O Urine*	*Muscle*	*Brain*	*Liver*
1	0.087	0.097	-	0.109	0.174	0.014	-	-	-	-	-
2	0.057	-	0.100	-	-	0.011	0.007	0.007	-	-	-
3	0.148	0.181	0.310	-	-	0.010	0.016	-	0.176	0.573	0.948
4	-	-	-	0.027	-	0.007	-	-	-	-	-
5	0.038	0.046	0.041	-	-	0.007	0.008	BLOQ	0.054	0.067	0.382
6	0.090	0.131	0.138	-	-	0.013	0.015	0.014	-	-	-
7	0.009	0.018	0.014	-	-	BLOQ	BLOQ	BLOQ	0.034	0.077	0.125
8	-	-	-	-	-	-	-	-	0.155	-	0.584
9	0.031	0.027	0.039	-	-	BLOQ	BLOQ	BLOQ	-	-	-
10	0.164	0.179	0.132	-	-	0.015	0.018	0.020	-	-	-
11	0.043	-	-	0.042	0.064	0.012	0.009	0.010	-	-	-
12	0.176	0.282	0.194	-	-	0.054	0.026	0.027	-	-	-
13	0.071	0.120	0.095	-	-	0.010	0.012	0.011	-	-	-
14	0.232	0.244	0.191	-	-	0.019	0.049	0.046	-	-	-
15	0.080	0.047	0.037	-	-	BLOQ	BLOQ	0.007	-	-	-
16	0.370	0.360	0.287	-	-	0.031	0.047	0.039	0.351	1.013	0.807
17	0.097	0.103	0.074	-	-	0.008	0.009	0.009	-	-	-
18	0.109	0.148	-	-	-	0.030	0.030	0.030	-	-	-
19	0.018	0.028	0.030	-	-	BLOQ	BLOQ	BLOQ	BLOQ	0.065	0.099
20	BLOQ	0.007	-	-	-	ND	BLOQ	BLOQ	-	-	-
21	-	0.032	-	0.036	-	-	BLOQ	BLOQ	BLOQ	-	-
22	-	0.224	-	0.270	-	0.019	0.020	0.022	-	-	-
23	0.126	0.167	0.181	-	-	-	0.028	0.026	0.469	0.963	2.125
24	0.014	0.011	-	0.016	-	ND	ND	BLOQ	-	-	-
25	0.019	0.045	0.015	-	-	BLOQ	BLOQ	BLOQ	-	-	-
26	0.028	0.032	0.077	-	-	BLOQ	BLOQ	BLOQ	-	-	-
27	0.054	0.059	0.067	-	-	BLOQ	0.010	0.010	-	-	-
Mean	0.094	0.113	0.112	0.083	0.119	0.017	0.020	0.020	0.207	0.460	0.724
Median	0.076	0.097	0.086	0.039	0.119	0.013	0.016	0.017	0.166	0.325	0.584
Range	0.009–0.370	0.007–0.360	0.014–0.310	0.016-0.270	0.064-0.174	0.007-0.054	0.007-0.049	0.007-0.046	0.034-0.469	0.065-1.013	0.099-2.125

*Note: All concentrations shown in units of mg/L or mg/Kg. A dash indicates that the sample type was not available for analysis, F/O = Fluoride Oxalate, BLOQ = Below limit of quantitation (0.007mg/L), ND = Not detected.

Table 3A: Concentrations of Phenazepam and 3-OH Phenazepam in various postmortem fluids.

	Case 28			*Case 29*		
	Phenazepam Concentration (mg/L)	3-hydroxyphenazepam concentration (mg/L)	Phenazepam: 3-hydroxyphenazepam Ratio	Phenazepam Concentration (mg/L)	3-hydroxyphenazepam concentration (mg/L)	Phenazepam: 3-hydroxyphenazepam Ratio
Unpreserved Femoral Blood	0.96	0.23	4.2	0.96	0.27	3.6
Preserved Femoral Blood	0.97	0.17	5.7	1.64	0.43	3.8
Unpreserved Cardiac Blood	1.43	0.28	5.1	1.24	0.321	3.7
Unpreserved Urine	0.58	2.4	0.24	0.11	0.29	0.38
Preserved Urine	0.55	3.52	0.16	0.08	0.19	0.42
Unpreserved Cardiac:Femoral Ratio	1.5	1.2		1.3	1.2	

Table3B: Certified causes of death and other drugs detected in fatal phenazepam cases

Case	*Cause of Death*	*Other Drugs Detected*
28	Phenazepam and opiate toxicity	Toxicological analysis of femoral blood revealed the presence of low levels of nicotine and naloxone (from resuscitation attempts), prescribed promazine (0.5mg/L) & metabolites together with free morphine (0.01mg/L), morphine-3-glucuronide (0.03mg/L) morphine-6-glucuronide (0.01mg/L), diazepam (<0.16mg/L) nordiazepam (<0.5mg/L) and temazepam (<0.6mg/L)
29	Phenazepam toxicity	Analysis of femoral blood revealed low levels of diazepam, nordiazepam, dihydrocodeine (all <0.16mg/L), nicotine and dihydrocodeine-6-glucuronide.

Table 4: Certified causes of death and other drugs detected in phenazepam cases

Case	*Cause of Death*	*Other Drugs Detected*
1	Adverse effects of Methadone & Amphetamine	Paracetamol, Amphetamine, Mirtazapine, Desmethylmirtazapine, Methadone, EDDP, Desmethylcitalopram, Nordiazepam, Diazepam, Temazepam.
2	Acute & Chronic Drug & Alcohol Abuse.	Mirtazapine, Desmethylmirtazapine, EDDP, Methadone, Naloxone, Atropine, Acetone.
3	Adverse effects of Heroin & Amphetamine.	Paracetamol, Nicotine, Nordiazepam, Diazepam, Oxazepam, Temazepam, Codeine, Morphine, Morphine-3-glucuronide, Morphine-6-glucuronide, Noscapine, Papaverine.
4	Methadone Toxicity.	EDDP, Methadone, Nordiazepam, Diazepam.
5	Chronic Alcoholism.	Dihydrocodeine, M3G, M6G, Ethanol.
6	Hanging.	Citalopram, Desmethylcitalopram, Ethanol.
7	Adverse effects of heroin, methadone and buprenorphine	Nicotine, Cotinine, Levamisole, Cocaine, Benzoylecgonine, Codeine, Paracetamol, Lignocaine, Mirtazapine & metabolite, Caffeine, Noscapine, Papaverine, EDDP, Methadone, Fluoxetine, Nordiazepam, Diazepam, Morphine, Buprenorphine, Morphine-3-glucuronide and Morphine-6-glucuronide
8	Fresh water drowning	Caffeine, Nicotine, Cotinine, Codeine, Paracetamol, Propranolol, Quetiapine, Methadone, EDDP, Citalopram, Nordiazepam, Diazepam
9	No Anatomical Cause (Possible Drug Related).	Naloxone, EDDP, Methadone, Temazepam, Nordiazepam, Diazepam, Ibuprofen metabolite.
10	No Anatomical Cause (Possible choking on Food Bolus).	Mirtazapine, Desmethylmirtazapine, Nordiazepam, Diazepam, Temazepam, Ethanol.
11	Quetiapine toxicity.	Quetiapine & metabolites, Mirtazapine, Desmethylmirtazapine, Cyclizine, Sildenafil, Nordiazepam, Diazepam, Oxazepam, Temazepam, Tramadol, Ethanol.
12	Adverse effects of Methadone & Aspiration Pneumonia.	Nordiazepam, Diazepam, Temazepam, Oxazepam, Methadone, EDDP, Mirtazapine, Desmethylmirtazapine, Gabapentin.
13	Adverse effects of Methadone.	Citalopram, Desmethylcitalopram, Methadone, EDDP, Nordiazepam, Diazepam.
14	Acute adverse effects of Methadone.	Paracetamol, Methadone, EDDP, Nordiazepam, Diazepam, Temazepam, Citalopram, Desmethylcitalopram.
15	Cor Pulmonale & adverse effects of Methadone.	Trazadone, EDDP, Methadone, Nordiazepam, Diazepam, Oxazepam, Temazepam.
16	Acute & chronic adverse effects of Heroin.	Paracetamol, Noscapine, Papaverine, Methadone, EDDP, Nordiazepam, Diazepam, Temazepam, 4-Methyl-N-Ethylcathinone (4-MEC), Codeine, Morphine, M3G, M6G, Codeine.
17	Pulmonary Thromboembolism.	Naloxone, DHC, Mirtazapine, Desmethylmirtazapine, EDDP, Methadone, Temazepam, Oxazepam, Nordiazepam, Diazepam, Morphine, M3G, M6G.
18	Acute & chronic adverse effects of Morphine (Heroin).	Morphine, M3G, M6G, 6-MAM, Papaverine, Noscapine, DHC, Codeine, Citalopram, Desmethylcitalopram, Paracetamol.
19	Adverse effects of Morphine & Diazepam.	Mirtazapine, Desmethylmirtazapine, Oxazepam, Temazepam, Nordiazepam, Diazepam, Morphine, M3G, M6G, Codeine.
20	Hanging.	Paracetamol, Mirtazapine, Methadone, EDDP, M3G, Nordiazepam, Diazepam, Temazepam, Ethanol.
21	Adverse effects of Methadone & Diazepam; Chronic Drug Abuse.	Alcohol, Olanzapine, Cyclazine, Methadone, EDDP, Nordiazepam, M3G.
22	Adverse effects of Methadone & Diazepam.	Methadone, EDDP, Nordiazepam, Diazepam, Oxazepam, Temazepam, DHC, M3G.
23	Adverse effects of Methadone.	Acetone, EDDP, Methadone, Nordiazepam, Diazepam.
24	Adverse effects of Ethanol, Methadone & Diazepam.	Ethanol, Mirtazapine, Desmethylmirtazapine, Methadone, EDDP, Nordiazepam, Diazepam, Oxazepam, Temazepam, Gabapentin.
25	Methadone & Morphine Intoxication associated with aspiration bronchopneumonia.	Morphine, M3G, M6G, Methadone, Diazepam, Nordiazepam, EDDP.
26	Adverse effects of Heroin, Diazepam & Amitriptyline.	Paracetamol, Amitriptyline, Nortriptyline, 6-Acetylcodeine, Mirtazapine, Noscapine, Papaverine, Nordiazepam, Diazepam, Temazepam, Oxazepam, Naloxone, Codeine, Morphine, M3G, M6G.
27	Tramadol Toxicity.	Paracetamol, Tramadol, O-Desmethyltramadol, Quetiapine & metabolites, Mirtazapine.

Figure 1: Metabolism of Phenazepam including 5-bromo-(2-chlorophenyl)-2-aminobenzophenone (ABPH) also known as 2-amino-5-bromo chloro-aminobenzophenone (ABCB).

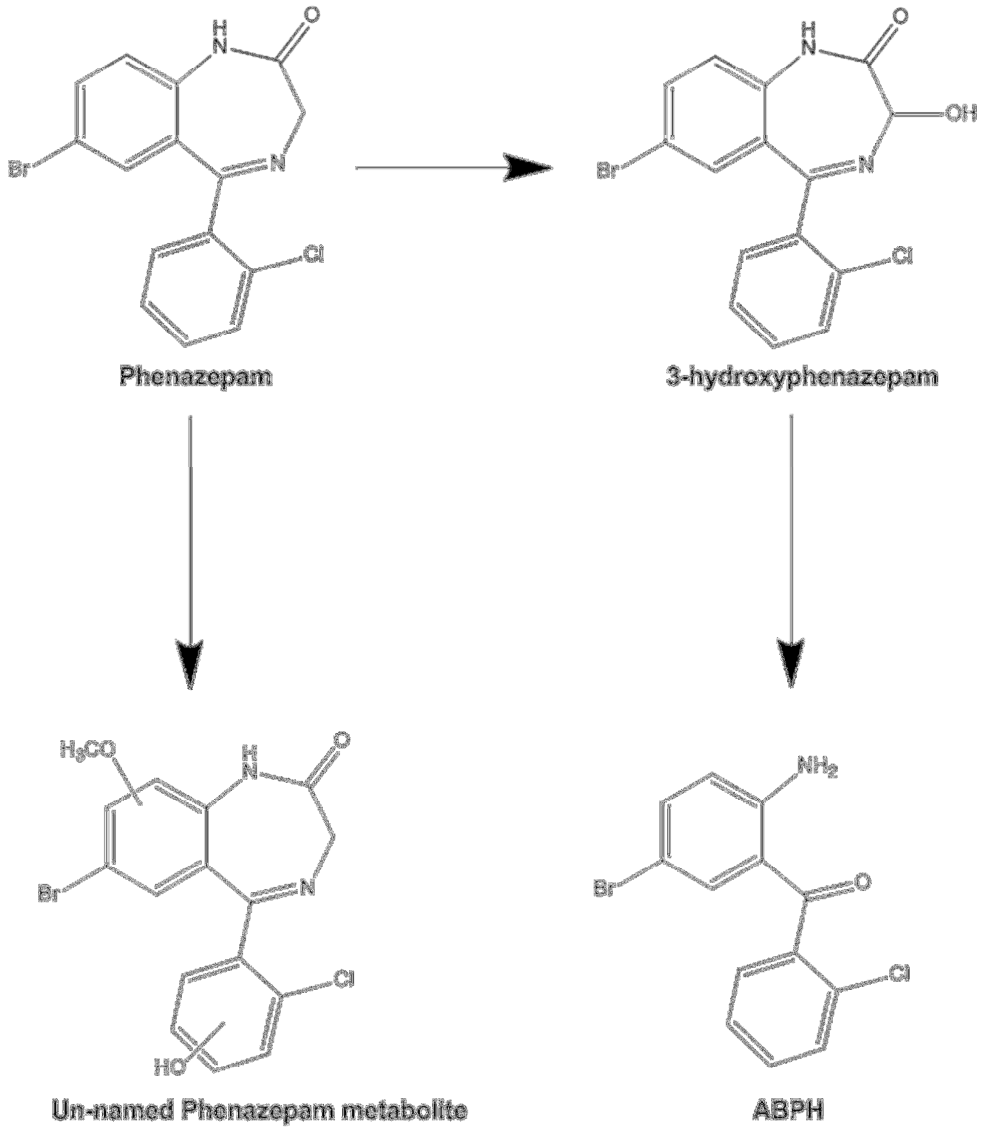


Table 5A: Blood/Plasma Concentrations of phenazepam found in a variety of case types (DUID, Deaths not directly attributed to phenazepam and clinical cases)

Author	Case Type	Number of Cases	Phenazepam Concentration
Burch *et al. [16]*	DUID	3	0.120 mg/L, 0.180 mg/L, 0.8700 mg/L
Johnson [17]	DUID	4	0.280 mg/L – 0.55 mg/L
Kerrigan *et al.,* [15]	DUID	1	0.076 mg/L
Kriikku *et al.,* [8]	DUID	141	0.004 mg/L – 3.600 mg/L (median 0.17mg/L)
Stephenson *et al.,*[6]	DUID	11	0.04 mg/L – 3.2 mg/L (median 0.17 mg/L)
Dargan *et al., [*21]	Clinical Overdose	1	0.49 mg/L ~16h post insufflation
Mrozkowska *et al.,*[20]	Clinical Overdose	1	1.2 ug/g (~1.2 mg/L)7 days post-ingestion
Gastineau *et al.* [28]	Postmortem	10	0.010 – 0.272 mg/L
Kriikku *et al.,*[8]	Postmortem	17	0.007 mg/L – 1.600 mg/L (median 0.048 mg/L)
Yip *et al.,* [48]	Postmortem	19	0.008 mg/L 1.2 mg/L (1 case phenazepam sole cause of death)

Comment [LNS1]: Check paper again median is 0.061 mg/L

Table 5B: Previous cases in deaths that were directly attributed to phenazepam

Author	Phenazepam Blood Levels	Other Drugs Detected	Certified cause of death
Bailey *et al.* [7]	0.386 mg/L	morphine (0.116 mg/L) , codeine (0.085 mg/L), thebaine (0.072 mg/L)	Multidrug toxicity
Corkery *et al.* [4]	0.22 mg/L	methadone (0.65mg/L), diazepam (0.1mg/L), nordiazepam (0.21mg/L) oxazepam, temazepam ibuprofen and EDDP were also detected	Bronchopneumonia due to the effects of a combination of methadone and phenazepam
Corkery *et al.* [4]	2.52 mg/L	Ethanol 6 mg/dl, morphine 0.36mg/L, codeine 0.38 mg/L, paracetamol detected	Phenazepam, Opiate and codeine toxicity
Karinen *et al.* [18]	1.33 mg/L	AH-7921 (0.33 mg/L), methoxetamine (0.064 mg/L), etazolam (0.27 mg/L), 7-aminonitrazepam (0.043mg/L), diazepam (0.046mg/L), nordiazepam (0.073mg/L), Oxazepam (0.018mg/L)	Multidrug Toxicity

Table 6: 3-Hydroxyphenazepam concentrations from twenty seven fatalities (Tissue values are semiquantitative)

Case	*Femoral Blood*	*F/O Femoral Blood*	*Cardiac Blood*	*Subclavian Blood*	*F/O Subclavian Blood*	*F/O Vitreous Humour*	*Urine*	*F/O Urine*	*Muscle*	*Brain*	*Liver*
1	BLOQ	0.020	-	BLOQ	0.031	BLOQ	-	-	-	-	-
2	BLOQ	-	BLOQ	-	-	BLOQ	0.036	0.041	-	-	-
3	0.066	0.074	0.161	-	-	BLOQ	0.120	-	0.053	0.087	0.389
4	-	-	-	BLOQ	-	ND	-	-	-	-	-
5	BLOQ	ND	BLOQ	-	-	ND	BLOQ	BLOQ	ND	ND	ND
6	BLOQ	BLOQ	BLOQ	-	-	BLOQ	0.017	BLOQ	-	-	-
7	ND	ND	ND	-	-	ND	BLOQ	BLOQ	ND	ND	ND
8	-	-	-	-	-	-	-	-	0.040	-	0.144
9	BLOQ	BLOQ	0.026	-	-	ND	0.034	0.022	-	-	-
10	0.020	0.025	0.020	-	-	ND	0.133	0.031	-	-	-
11	BLOQ	-	-	BLOQ	BLOQ	ND	0.032	0.031	-	-	-
12	0.159	0.246	0.149	-	-	0.050	0.140	0.214	-	-	-
13	BLOQ	0.049	BLOQ	-	-	ND	BLOQ	BLOQ	-	-	-
14	0.028	0.122	0.042	-	-	BLOQ	BLOQ	BLOQ	-	-	-
15	0.093	0.022	BLOQ	-	-	ND	BLOQ	BLOQ	-	-	-
16	0.028	0.023	0.026	-	-	BLOQ	0.274	0.169	0.351	1.013	0.807
17	0.019	0.022	0.020	-	-	BLOQ	0.062	0.062	-	-	-
18	BLOQ	0.037	-	-	-	BLOQ	0.060	0.025	-	-	-
19	BLOQ	ND	BLOQ	-	-	ND	BLOQ	BLOQ	ND	0.015	ND
20	ND	ND	-	-	-	ND	ND	ND	-	-	-
21	-	ND	-	ND	-	-	ND	ND	ND	-	-
22	-	0.086	-	0.030	-	BLOQ	ND	0.027	-	-	-
23	0.032	0.064	0.027	-	-	-	0.045	0.112	0.078	0.105	0.462
24	BLOQ	BLOQ	-	BLOQ	-	ND	BLOQ	BLOQ	-	-	-
25	BLOQ	BLOQ	BLOQ	-	-	ND	ND	ND	-	-	-
26	0.019	BLOQ	0.019	-	-	ND	0.021	BLOQ	-	-	-
27	BLOQ	BLOQ	BLOQ	-	-	ND	BLOQ	BLOQ	-	-	-
Mean	0.052	0.066	0.054	0.030	0.031	0.050	0.081	0.073	0.131	0.305	0.451
Median	0.028	0.043	0.026	0.030	0.031	0.050	0.053	0.036	0.066	0.096	0.426
Range	0.019-0.159	0.020-0.246	0.019-0.161	0.030-0.030	0.031-0.031	0.050-0.050	0.017-0.274	0.022-0.214	0.040-0.351	0.015-1.013	0.144-0.807

*Note: All concentrations shown in units of mg/L or mg/Kg. A dash indicates that the sample type was not available for analysis, F/O = Fluoride Oxalate, BLOQ = Below limit of quantitation (0.016 mg/L), ND = Not detected.

Table 7: Ratio of Phenazepam to 3-Hydroxyphenazepam in Postmortem Specimens

Case	*Femoral Blood*	*F/O Femoral Blood*	*Cardiac Blood*	*Subclavian Blood*	*F/O Subclavian Blood*	*F/O Vitreous Humour*	*Urine*	*F/O Urine*
1	UC	4.9	-	10.9	5.6	UC	-	-
2	UC	-	UC	-	-	UC	0.2	0.2
3	2.2	2.4	1.9	-	-	UC	0.1	-
4	-	-	-	-	-	UC	UC	-
5	UC	UC	UC	-	-	UC	UC	UC
6	UC	UC	UC	--	-	UC	0.9	UC
7	UC	UC	UC	-	-	UC	UC	UC
8	-	--	-	-	-	-	-	-
9	UC	UC	1.5	-	-	UC	UC	UC
10	8.2	7.2	6.6	-	-	UC	0.1	0.6
11	UC	-	-	UC	UC	UC	0.3	0.3
12	1.1	1.1	1.3	-	-	1.1	0.2	0.1
13	UC	2.4	UC	-	-	UC	UC	UC
14	8.3	2.0	4.5	-	-	UC	UC	UC
15	0.9	2.1	UC	-	-	UC	UC	UC
16	13.2	15.7	11.0	-	-	UC	0.2	0.2
17	5.1	4.7	3.7	-	-	UC	0.1	0.1
18	UC	4.0	-	-	-	UC	0.5	1.2
19	UC	UC	UC	-	-	UC	UC	UC
20	UC	UC	-	-	-	UC	UC	UC
21	-	UC	-	-	-	-	UC	UC
22	-	2.6	-	9.0	-	UC	UC	0.8
23	3.9	2.6	6.7	-	-	-	0.6	UC
24	UC	UC	-	-	-	UC	UC	UC
25	UC	UC	UC	-	-	UC	UC	UC
26	1.5	UC	4.1	-	-	UC	UC	UC
27	UC	UC	UC	-	-	UC	UC	UC
Mean	4.9	4.3	4.6	10.0	5.6	1.1	0.3	0.5
Median	3.9	2.6	4.1	10.0	5.6	1.1	0.2	0.3
Range	0.9 – 13.2	1.1 - 15.7	1.3 - 11.0	9.0 - 10.9	5.6 - 5.6	1.1 – 1.1	0.1 – 0.9	0.1 – 1.2

*Note: A dash indicates that the sample type was not available for analysis, F/O = Fluoride Oxalate, UC =Unable to Calculate.

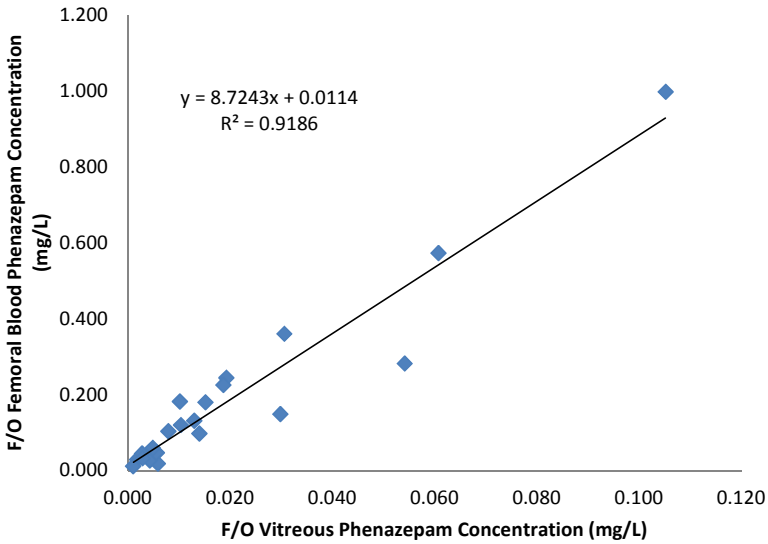
Table 8A: Postmortem Tissue Distribution Coefficients for Phenazepam

	F/O Fem/Fem Blood	*Cardiac/Fem*	*Subclavian/Fem*	*F/O SCV/F/O Fem*	*F/O VH/F/O Fem*	*Urine/Fem*	*F/O Urine/F/O Fem*	*Muscle/Fem*	*Brain/Fem*	*Liver/Fem*
N	20	18	3	1	12	14	12	5	6	6
Mean	1.3	1.3	1.1	1.8	0.1	0.2	0.1	2.2	4.7	9.1
Median	1.2	1.2	1.1	1.8	0.1	0.2	0.1	1.4	3.7	8.2
Range	0.6 - 2.4	0.5 – 2.8	1.0 – 1.3	1.8 – 1.8	0.1 – 0.2	0.1 – 0.3	0.1 – 0.2	0.9 -3.8	1.8 – 8.6	2.2 – 16.9

Table 8B: Postmortem Tissue Distribution Coefficients for 3 Hydroxyphenazepam

	F/O Fem/Fem Blood	*Cardiac/Fem*	*F/O SCV/F/O Fem*	*F/O VH/F/O Fem*	*Urine/Fem*	*F/O Urine/F/O Fem*	*Muscle/Fem*	*Brain/Fem*	*Liver/Fem*
n	8	8	1	1	7	6	3	3	3
Mean	1.6	1.2	1.6	0.2	3.6	2.2	5.3	13.6	16.4
Median	1.2	1.0	1.6	0.2	1.8	1.1	2.4	3.3	14.4
Range	0.2 – 4.4	0.8 – 2.4	1.6 – 1.6	0.2 – 0.2	0.9 – 9.8	0.3 – 7.3	0.8 - 12.5	1.3 – 36.2	5.9 – 28.8

Figure 2: Correlation between Phenazepam Fluoride Oxalate Femoral blood concentration (n=22) and Phenazepam Fluoride Oxalate Vitreous Humour concentration (n=22)



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