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Popliteal Vein Blood Sampling and the Postmortem Redistribution of Diazepam, Methadone and Morphine*

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ABSTRACT: Postmortem redistribution (PMR) refers to the site- and time- related blood drug concentration variations after death. We compared central blood (cardiac and subclavian) with peripheral blood (femoral and popliteal) concentrations of diazepam, methadone and morphine. To our knowledge, popliteal blood has never been compared with other sites. Intracardiac blood (ICB), subclavian blood (SB), femoral blood (FB) and popliteal blood (PB) were sampled in 30 cases. To assess PMR, mean concentrations and ratios were compared. Influence of postmortem interval on mean ratios was also assessed. Results show that popliteal mean concentrations were lower than other sites for all three drugs, even than femoral blood, mean ratios suggested that popliteal site was less subject to PMR and estimated postmortem interval did not influence ratios except for diazepam and methadone FB/PB. In conclusion, our study is the first to explore popliteal site and suggests that popliteal blood is less prone to postmortem redistribution.

KEYWORDS: Forensic Science, Forensic Toxicology, Postmortem Redistribution, Popliteal Blood, Diazepam, Methadone, Morphine

The postmortem redistribution (PMR) of a substance is a phenomenon that describes the site- and time-dependent change in the concentration of a compound in the blood after death. It is amply described elsewhere (2,3,4,5,10,13,17). It is impossible to predict the extent to which a substance will redistribute after death, but pH, large volume of distribution, protein binding and how lipophilic the compound is all seem to play a role (2,3,7). There are other factors probably involved, such as bacterial breakdown and other putrefactive processes (2,3,21). Sampling from central sites (subclavian vessels and heart) tends to be more affected by PMR than peripheral sites (iliac and femoral vessels). The extent to which a drug is prone to postmortem redistribution is usually described by the ratio of the Central (C) to Peripheral (P) concentration of a drug, or C/P ratio (2,3,10,13,16). Higher ratios imply greater postmortem redistribution. Some authors suggest that the [C]/[P] ratio is not always a reliable indicator of postmortem redistribution for a particular substance (7,26,27). Femoral sampling, ideally done after dissection and clamping of the vessel, is currently considered the blood sampling site of choice since it is less subject to the processes that cause postmortem redistribution (2,3,4). The blindstick method of drawing femoral blood is considered equivalent (24). There are papers that suggest that subclavian sampling should not be considered a strictly central site, but rather an intermediate one (15,16). Some authors used dissection and clamping of the vein technique, others a blindstick method, and some did not mention which they used. To our

knowledge, the popliteal vein has never been described as a sampling site. Because of its distance from the trunk and a usually sterile site in life, it theoretically is a more favorable site for taking a peripheral sample.

Other than the sampling site, the postmortem interval appears to be important, especially shortly after death, which is when most postmortem redistribution is thought to occur. Although passive diffusion from reservoir organs theoretically occurs within the first few hours after death (2,3), cellular autolysis and the putrefactive action of bacteria could also result in later changes as suggested by some studies (8,25).

In this study, we sampled a number of drugs from popliteal blood and compared the results obtained there with concentrations obtained from routine sampling sites. We chose drugs more commonly abused in the jurisdiction of the Medico-Legal Institute of the University of Liège, Belgium. These were diazepam, methadone and morphine. They are also compounds subject to postmortem redistribution (1,5,6,7,8,9,12,14,18,19,21).

Methods

We included 30 cases that came to our medico-legal office in Liège between November 2012 and November 2013. A urine drug screen was done to assess the presence of the drugs of interest (*Drug-Screen®, nal von minden GmbH*).

Intracardiac blood (ICB), subclavian blood (SB), femoral blood (FB) and popliteal blood (PB) were drawn. Cardiac blood was always sampled in the right atrium, accessed via a small chest dissection. For the subclavian and femoral vein samples, transcutaneous sampling (blind stick) was done on the left, and dissection with proximal clamping was done on the right. Because the popliteal vessels are deep in the posterior aspect of the knee, dissection for access is necessary, and the popliteal vein clamped as cephalad as possible to prevent any theoretical femoral blood reflux during sampling. After popliteal vein dissection and clamping, compression of the leg was sometimes required to obtain an adequate amount of blood for testing (i.e. 2-5 ml from each side).

Diazepam, methadone and morphine were assayed. Blood samples were put into NaF tubes and frozen at -20 °C prior to analysis which was performed quickly i.e. within first weeks (4 to 6 weeks) after sampling.

The postmortem interval was estimated using routine observations from the scene of death and assessment of postmortem changes (ambient and rectal temperatures, lividity, rigidity, state of decomposition, skin slippage, eye changes, bloating, discoloration, etc). This was done for each case.

Quantitative Analysis

The quantification of morphine and methadone was performed on an ultra-high pressure liquid chromatograph Acquity® coupled to a tandem mass spectrometer Quattro Premier® (*Waters, Zellik, Belgium*). After solid phase extraction of the sample on Oasis MCX® cartridges, the separation was performed on an Acquity HSS T3 column. The mobile phase consisted in a gradient of ammonium formate (pH 3) and acidified methanol (22). Diazepam was analyzed in blood using a high performance liquid chromatography with photodiode array detection (Alliance®, Waters) based on a method described by Y. Gaillard et al. (23). After a liquid-liquid extraction using a mixture of diethyl ether, dichlormethane, hexane and n-amyl alcohol, the sample was injected on a Symmetry C8 column with phosphate buffer (pH 3.8) and acetonitrile delivered according to a gradient mode as mobile phase. Mean coefficient of variation (CV) was respectively 4.00 % for diazepam, 5.08 % for methadone and 4.24 % for morphine.

Statistical Analysis

Statistical analyses were performed by using SAS software (version 9.3 for windows) and R software. Normality of the distributions was checked by using a Shapiro-Wilk test. A logarithmic transformation of concentrations was also used to normalize the distributions.

For each substance, mean concentrations at each site were calculated and the 4 sampling sites were compared with a non-parametric Friedman test. For the comparison of concentrations in the different sampling sites and for the comparison of mean ratios, a Bonferroni's correction (0.05/6 = 0.0083) was used to consider statistically significant results ($p<0.0083$).

For each substance, drug concentrations differences between sites were calculated as follows: ICB - SB, ICB - FB, ICB - PB, SB - FB, SB - PB, and FB - PB. A non-parametric Wilcoxon signed-rank test was used to assess a significant concentration difference.

For each substance, the following ratios were calculated: ICB / SB, ICB / FB, ICB / PB, SB / FB, SB / PB and FB / PB. A non-parametric Wilcoxon signed-rank test was also utilized to assess a significant ratio, i.e. a ratio different to 1.

Quantitative variables were summarized by the mean, standard deviation (SD), median, minimum and maximum. Qualitative variables were summarized by means of number (N) and percentage (%).

To assess the influence of post-mortem interval, non-parametric Spearman correlation coefficients were calculated to assess the correlation between ratios and estimated postmortem interval. A negative coefficient showed a decreasing relation between the two parameters (when one increased, the other decreased) while a positive coefficient showed an increasing relation (when one increased, the other increased too). For assessing the influence of post-mortem interval, results were considered as statistically significant at 5% level ($p< 0.05$).

Results

Table 1 shows age, sex and average estimated postmortem interval as determined by the protocol in use by our office.

Table 2 shows targeted substances and their respective frequencies.

Table 3 shows, for each case, concentrations for each substance and by site, ratios for each substance, suspected mode of delivery for each substance as well as cause and manner of death according to external examination, toxicological findings and elements from death scene.

Concerning delivery mode, oral ingestion was always used for diazepam and methadone, whereas morphine was orally ingested in one case (case #1) and smoked or injected as heroin in respectively 14 and 2 cases (case #9 and 20).

Concerning concentrations and ratios, some interesting findings concern intracardiac blood with ICB/FB and even ICB/PB ratios found very low (ie less than 0.80) in some cases.

An ICB/FB ratio less than 1 was a common finding for diazepam in 10 of 14 cases, probably depending on benzodiazepines higher instability in postmortem blood. Methadone and morphine showed less frequently such ICB/FB and even ICB/PB ratio less than 1, probably accounting for other mechanisms.

Concerning methadone, cases #2, 10, 17, 19, 22, 29 and 30 showed ICB/FB ratios less than 0.80. According to the respective context, it suggested either rapid death with incomplete distribution and/or recent use of methadone with probably shorter accumulation of the drug in the myocardium and other reservoir organs. However, in cases #2 and 17, ICB/PB ratios were greater than 1. SB/FB ratios were less than 1 in 4 cases (#5, 11, 17 and 22) but never less than 0.86, whereas SB/PB ratios were always greater than 1. Our findings may indicate than ICB is probably more concerned with incomplete distribution and/or recent methadone use than SB.

Morphine was associated with ICB/FB ratio less than 1 in only one case (case #4) where cause of death was found to be methadone intoxication, suggesting that the victim could have smoked heroin a short time before unconsciousness and death, accounting for an incomplete distribution. In the two cases where heroin was injected and death attributed to it, there was no negative ratio. Furthermore, there was only one SB/FB ratio less than 1 (case # 13) and, except in case # 1 (0.93), all SB/PB ratios were greater than 1. This is probably because of delivery mode of morphine (smoking and injection in 16 of 17 cases) allowing distribution to occur significantly quicker than for methadone (always orally ingested).

Figure 1 (a,b,c) shows drugs blood concentrations distribution with mean concentration and standard deviation (y-axis) for each sampling site (x-axis). For methadone, ICB is shown on a separate graph from SB, FB and PB, because of one significant outlier.

ICB, SB, FB and PB mean concentrations tend to decline the further the sampling site is from the heart, except for diazepam, which shows a slightly higher femoral blood concentration. Popliteal mean concentrations are lower than other three sites for all drugs.

Figure 2 shows mean drug concentrations differences between sites. Cardiac and subclavian sites show no significant mean concentration differences for the three compounds. Cardiac and femoral sites show statistically significant mean concentrations differences for morphine ($p=0.0026$); cardiac blood concentrations are consistently higher than femoral blood. For methadone ($p=0.0051$) and morphine ($p=0.0001$), cardiac and popliteal sites show significant mean concentration differences; so do subclavian and femoral sites (methadone $p<0.0001$, morphine $p=0.0004$). Subclavian and popliteal sites show significant mean concentrations differences for the three drugs (diazepam $p=0.0006$, methadone $p<0.0001$, morphine $p<0.0001$). This is also true for the difference between femoral and popliteal sampling sites; for all three cases, the popliteal sample had the lowest concentrations (diazepam $p=0.0005$, methadone $p<0.0001$, morphine $p=0.0005$).

All concentrations are expressed in microgram per liter of blood (µg/L).

To assess the occurrence of postmortem redistribution, for each substance, the following average ratios of concentrations were obtained: ICB/SB, ICB/FB, ICB/PB, SB/FB, SB/PB and FB/PB as shown in Table 4.

ICB/SB mean ratios are not statistically significant, i.e. different from 1, for any substances. ICB/FB mean ratios are not statistically significant, i.e. different from 1, for any substances. ICB/PB mean ratios are statistically greater than 1, for methadone and morphine but are not statistically significant, i.e. different from 1, for diazepam. SB/FB means ratios are statistically greater than 1, for methadone and morphine but show no signification, i.e. different from 1, for diazepam. SB/PB mean ratios are statistically greater than 1 for methadone

and morphine but are not statistically significant, i.e. different from 1, for diazepam. FB/PB mean ratios are statistically greater than 1 for all three targeted-substances.

Figure 3 illustrates the mean concentrations ratios for the 3 compounds. ICB/FB and SB/FB ratios are consistently less than the ICB/PB and SB/PB ratios. For methadone and morphine, FB/PB ratios are consistently lower than the more usual central (cardiac/subclavian) / peripheral (femoral/popliteal) ratios. For diazepam, the FB/PB diazepam ratio is greater than the central (cardiac/subclavian) / peripheral (femoral/popliteal) ratios.

To assess the influence of postmortem interval, for each substance, the correlations between ratios of concentrations obtained and the estimated postmortem interval were calculated as shown in Table 5. There is only a significant correlation between postmortem interval and F/P ratio for diazepam ($r = 0.57$, $p = 0.032$) and for methadone ($r = 0.55$, $p = 0.0057$) but no significant correlation was observed for any other ratios for any substances.

Discussion

For morphine and methadone, our results were consistent with those described in the literature, i.e., these compounds are subject to PMR (4,5,6,11,12,14,19). For diazepam, ICB/FB and SB/FB ratios were constantly lower than or equal to 1, which is not consistent with other studies (5,9,14,15). However, one study describing nordiazepam and bromazepam showed the same trend (1).

According to some authors, diazepam is stable in blood and tissues (28,35), even with putrefaction (29), unlike other benzodiazepines (21,29,30) although this can depend on specimen preservation (30,39), temperature (31,31,32,39), and other factors (32,39). In our study, a C/P ratio less than 1 was observed for diazepam when comparing femoral to central blood concentrations, suggesting that diazepam is not subject to redistribution. Because of blood was collected in NaF tube then frozen within one hour after sampling and quickly analyzed (within weeks), the diazepam mean C/P ratio less than 1 may also suggest central degradation before sampling especially as the FB/PB ratio was significantly greater than 1. Then, our results may indicate that degradation of diazepam actually occurred and was stronger in more central sites and less important in peripheral compartments, due to the much slower bacterial proliferation in extremities. However, our results may also reflect more complex changes in diazepam blood concentrations as for other benzodiazepines, and further sampling efforts are needed.

With regard to the stability of morphine and glucuronides, some authors did not see significant changes in morphine and glucuronides concentrations in patient samples and stored blood even when compared with admission and postmortem blood, in some cases for days after the sample was drawn (12,20,35,36,37). Other studies showed that increased storage time, temperature and degree of putrefaction resulted in greater free morphine generation (33) whereas morphine and its glucuronides were stable in sampled post-mortem blood only when stored at -20°C (34,38). In this study, in order to avoid pre-analysis drug degradation, blood sampled was systematically collected into a sodium fluoride/potassium oxalate vial and frozen at -20 °C prior to analysis which was performed quickly i.e. within first weeks (4 to 6 weeks) after sampling. The mean post-mortem interval was 33.3 ± 17.8 hours, which means that significant bacterial proliferation in the extremities had not taken place theoretically.

Popliteal blood mean concentrations are significantly lower than those observed in femoral blood. In most situations, a femoral or popliteal blood sample better approximates an antemortem concentration of a drug, at least for the 3 drugs studied here. Because the FB/PB ratios are all significantly greater than 1, the popliteal vein is likely a better site to approximate the antemortem concentration of a drug.

Our results also show that there no statistically significant difference between cardiac and subclavian blood concentrations for diazepam, methadone and morphine; for practical purposes, there is no difference when sampling from either site. Intracardiac morphine concentrations tended to be higher than those in subclavian samples, but in practice this probably does not make a difference, especially if the blood is obtained from a blind stick. Diazepam mean ratios tended to rise in more peripheral sampling sites, which means the drug concentration rises in the more distal samples. This may be due to decreased degradation of diazepam in compartments distal to the trunk.

Postmortem interval did not significantly influence results except for diazepam and methadone FB/PB ratios, where longer postmortem intervals correlated with increased ratios. Our results are consistent with some authors suggesting that PMR occurs within the first few hours after death because of early passive diffusion from reservoir organs like lungs (2,3). Our results also show that, for diazepam and methadone, redistribution may still occur at femoral site while not in popliteal blood, strengthening the hypothesis that popliteal blood is less prone to PMR. Some authors have demonstrated significant changes in femoral blood concentrations with time for some drugs whereas other drugs, yet with the same pharmacological properties, did not show the same trend. Saar et al. (25) demonstrated significant changes with time for antipsychotic drugs like clozapine, olanzapine and zuclopenthixol but haloperidol did not show the same trend, even those drugs are basic and lipophilic with a large Vd and one could expect that they are likely to undergo PMR. Besides, Geroustamoulos et al. (8) found that methadone, mirtazapine and sertraline femoral blood concentrations showed statistically significant increases irrespective of the delay in the postmortem interval, whereas olanzapine and diazepam concentrations were found to be stable with postmortem interval. Those results suggest that the phenomenon is actually more complex and may involve other mechanisms as well as large interindividual variations. Furthermore, because the postmortem interval is itself more often than not an estimate, only those cases where an antemortem blood sample can be compared to a postmortem sample will resolve the question, as it can happen in cases that survive long enough to undergo resuscitation in a hospital setting. In any case, one should be aware of the potential concentration changes with time and ideally encourage sampling as soon as possible after death.

In conclusion, our study is the first to describe popliteal blood concentrations of diazepam, methadone and morphine and shows that sampling from this site results in drug concentrations lower than those in cardiac, subclavian and even femoral sampling. This suggests that popliteal blood is less prone to postmortem redistribution. The reasons for this are unclear, but distance from the trunk and isolation from many of the contributing factors to postmortem redistribution must certainly play a role. The concentrations of these three drugs obtained from femoral blood and popliteal blood are also comparable, indicating that femoral blood is still a good sample for obtaining peripheral blood. However, because popliteal blood concentrations are lower than those obtained from femoral blood, it is likely that drug concentrations obtained from this site even more closely reflect antemortem concentrations.

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TABLE 1—*Sex, age and estimated postmortem interval.*

	N	Mean +/- SD	Min-Max
Sex			
Male	23		
Female	7		
Age (y)	30	40.2 +/- 9.5	26.8-58.2
Postmortem interval (h)	30	33.3 +/- 17.8	8.5-88.0

TABLE 2—*Targeted substances.*

	N
Diazepam	14
Methadone	24
Morphine	17

TABLE 3—*Concentrations and ratios by substance, mode of delivery, cause and manner of death, for all cases.*

Case #	Substance	Site	[µg/L]	Ratios	Delivery mode	Cause and manner of death
1	Morphine	ICB	100	ICB/SB = 1.15	Oral	Polymedication Intoxication
				ICB/FB = 1.25		Suicide
				ICB/PB = 1.07		
		SB	86	SB/FB = 1.08		
				SB/PB = 0.93		
		FB	79	FB/PB = 0.85		
		PB	93			
2	Diazepam	ICB	282	ICB/SB = 0.67	Oral	Methadone Intoxication
				ICB/FB = 0.72		Accident
				ICB/PB = 1.10		
		SB	422	SB/FB = 1.05		
				SB/PB = 1.64		
		FB	390	FB/PB = 1.51		
		PB	257			
	Methadone	ICB	1020	ICB/SB = 0.70	Oral	
				ICB/FB = 0.77		
				ICB/PB = 1.43		
		SB	1455	SB/FB = 1.10		
				SB/PB = 2.04		
		FB	1325	FB/PB = 1.85		
		PB	714			
	Morphine	ICB	31	ICB/SB = 0.97	Smoke	
				ICB/FB = 1.00		

				ICB/PB = 1.63		
		SB	32	SB/FB = 1.03		
				SB/PB = 1.68		
		FB	31	FB/PB = 1.63		
		PB	19			
3	Diazepam	ICB	339	ICB/SB = 1.05	Oral	Methadone Intoxication
				ICB/FB = 0.89		Accident
				ICB/PB = 1.29		
		SB	323	SB/FB = 0.85		
				SB/PB = 1.23		
		FB	378	FB/PB = 1.44		
		PB	262			
	Methadone	ICB	375	ICB/SB = 1.12	Oral	
				ICB/FB = 2.37		
				ICB/PB = 2.95		
		SB	334	SB/FB = 2.11		
				SB/PB = 2.63		
		FB	158	FB/PB = 1.24		
		PB	127			
4	Diazepam	ICB	1299	ICB/SB = 1.08	Oral	Methadone Intoxication
				ICB/FB = 1.46		Suicide
				ICB/PB = 1.58		
		SB	1201	SB/FB = 1.35		
				SB/PB = 1.46		
		FB	888	FB/PB = 1.08		
		PB	823			
	Methadone	ICB	1754	ICB/SB = 0.96	Oral	
				ICB/FB = 1.20		
				ICB/PB = 1.68		
		SB	1825	SB/FB = 1.25		
				SB/PB = 1.75		
		FB	1462	FB/PB = 1.40		
		PB	1041			
	Morphine	ICB	10	ICB/SB = 0.50	Smoke	
				ICB/FB = 0.66		
				ICB/PB = 1.11		
		SB	20	SB/FB = 1.33		
				SB/PB = 2.22		
		FB	15	FB/PB = 1.66		
		PB	9			
5	Methadone	ICB	338	ICB/SB = 1.66	Oral	Methadone Intoxication
				ICB/FB = 1.51		Accident
				ICB/PB = 1.87		
		SB	203	SB/FB = 0.91		
				SB/PB = 1.12		
		FB	223	FB/PB = 1.24		
		PB	180			
6	Morphine	ICB	80	ICB/SB = 1.53	Smoke	Polymedication Intoxication
				ICB/FB = 2.10		Accident

				ICB/PB = 2.35		
		SB	52	SB/FB = 1.37		
				SB/PB = 1.53		
		FB	38	FB/PB = 1.12		
		PB	34			
7	Diazepam	ICB	232	ICB/SB = 0.71	Oral	Methadone Intoxication
				ICB/FB = 0.91		Accident
				ICB/PB = 0.93		
		SB	328	SB/FB = 1.29		
				SB/PB = 1.32		
		FB	255	FB/PB = 1.02		
		PB	249			
	Methadone	ICB	685	ICB/SB = 1.06	Oral	
				ICB/FB = 2.23		
				ICB/PB = 2.44		
		SB	647	SB/FB = 2.11		
				SB/PB = 2.30		
		FB	307	FB/PB = 1.09		
		PB	281			
8	Morphine	ICB	38	ICB/SB = 1.53	Smoke	Pulmonary Infection
				ICB/FB = 2.10		Natural
				ICB/PB = 2.35		
		SB	67	SB/FB = 1.37		
				SB/PB = 1.53		
		FB	58	FB/PB = 1.12		
		PB	46			
9	Diazepam	ICB	149	ICB/SB = 1.01	Oral	Heroin Intoxication
				ICB/FB = 1.27		Accident
				ICB/PB = 1.23		
		SB	147	SB/FB = 1.26		
				SB/PB = 1.21		
		FB	117	FB/PB = 0.97		
		PB	121			
	Methadone	ICB	424	ICB/SB = 1.64	Oral	
				ICB/FB = 3.75		
				ICB/PB = 4.12		
		SB	259	SB/FB = 2.29		
				SB/PB = 2.51		
		FB	113	FB/PB = 1.10		
		PB	103			
	Morphine	ICB	252	ICB/SB = 0.73	Injection	
				ICB/FB = 1.27		
				ICB/PB = 1.79		
		SB	346	SB/FB = 1.75		
				SB/PB = 2.45		
		FB	198	FB/PB = 1.40		
		PB	141			
10	Methadone	ICB	536	ICB/SB = 0.52	Oral	Methadone Intoxication
				ICB/FB = 0.71		Accident

				ICB/PB = 0.81		
		SB	1026	SB/FB = 1.37		
				SB/PB = 1.55		
		FB	749	FB/PB = 1.13		
		PB	663			
11	Methadone	ICB	1216	ICB/SB = 1.31	Oral	Methadone Intoxication
				ICB/FB = 1.12		Accident
				ICB/PB = 1.57		
		SB	926	SB/FB = 0.86		
				SB/PB = 1.20		
		FB	1080	FB/PB = 1.40		
		PB	772			
12	Diazepam	ICB	936	ICB/SB = 1.03	Oral	Methadone Intoxication
				ICB/FB = 0.92		Accident
				ICB/PB = 1.02		
		SB	909	SB/FB = 0.89		
				SB/PB = 0.99		
		FB	1019	FB/PB = 1.10		
		PB	921			
	Methadone	ICB	635	ICB/SB = 0.83	Oral	
				ICB/FB = 0.84		
				ICB/PB = 1.20		
		SB	761	SB/FB = 1.00		
				SB/PB = 1.44		
		FB	759	FB/PB = 1.43		
		PB	528			
	Morphine	ICB	31	ICB/SB = 1.00	Smoke	
				ICB/FB = 1.15		
				ICB/PB = 1.29		
		SB	31	SB/FB = 1.15		
				SB/PB = 1.29		
		FB	27	FB/PB = 1.29		
		PB	24			
13	Diazepam	ICB	1539	ICB/SB = 1.29	Oral	Polymedication Intoxication
				ICB/FB = 1.04		Accident
				ICB/PB = 1.55		
		SB	1194	SB/FB = 0.81		
				SB/PB = 1.20		
		FB	1475	FB/PB = 1.49		
		PB	990			
	Methadone	ICB	868	ICB/SB = 1.92	Oral	
				ICB/FB = 2.12		
				ICB/PB = 2.81		
		SB	453	SB/FB = 1.11		
				SB/PB = 1.46		
		FB	409	FB/PB = 1.32		
		PB	309			
	Morphine	ICB	19	ICB/SB = 1.35	Smoke	
				ICB/FB = 1.19		

				ICB/PB = 1.46		
		SB	14	SB/FB = 0.87		
				SB/PB = 1.08		
		FB	16	FB/PB = 1.23		
		PB	13			
14	Methadone	ICB	182	ICB/SB = 0.71	Oral	Methadone Intoxication
				ICB/FB = 0.91		Accident
				ICB/PB = 0.93		
		SB	449	SB/FB = 1.29		
				SB/PB = 1.32		
		FB	310	FB/PB = 1.02		
		PB	195			
	Morphine	ICB	11	ICB/SB = 1.06	Smoke	
				ICB/FB = 2.23		
				ICB/PB = 2.44		
		SB	20	SB/FB = 2.11		
				SB/PB = 2.30		
		FB	15	FB/PB = 1.09		
		PB	9			
15	Methadone	ICB	138	ICB/SB = 1.02	Oral	Polymedication Intoxication
				ICB/FB = 1.79		Accident
				ICB/PB = 1.97		
		SB	135	SB/FB = 1.75		
				SB/PB = 1.92		
		FB	77	FB/PB = 1.10		
		PB	70			
16	Diazepam	ICB	61	ICB/SB = 0.83	Oral	Methadone Intoxication
				ICB/FB = 0.73		Accident
				ICB/PB = 0.73		
		SB	73	SB/FB = 0.88		
				SB/PB = 0.88		
		FB	83	FB/PB = 1.00		
		PB	83			
	Methadone	ICB	822	ICB/SB = 2.38	Oral	
				ICB/FB = 2.73		
				ICB/PB = 3.22		
		SB	345	SB/FB = 1.15		
				SB/PB = 1.35		
		FB	301	FB/PB = 1.18		
		PB	255			
17	Methadone	ICB	92	ICB/SB = 0.76	Oral	Methadone and Heroin Intoxication
				ICB/FB = 0.66		Accident
				ICB/PB = 1.13		
		SB	121	SB/FB = 0.88		
				SB/PB = 1.49		
		FB	138	FB/PB = 1.70		
		PB	81			
	Morphine	ICB	61	ICB/SB = 1.03	Injection	
				ICB/FB = 1.13		

				ICB/PB = 1.45		
		SB	59	SB/FB = 1.09		
				SB/PB = 1.40		
		FB	54	FB/PB = 1.28		
		PB	42			
18	Methadone	ICB	527	ICB/SB = 0.60	Oral	Methadone Intoxication
				ICB/FB = 0.93		Suicide
				ICB/PB = 1.02		
		SB	881	SB/FB = 1.55		
				SB/PB = 1.71		
		FB	566	FB/PB = 1.10		
		PB	515			
	Morphine	ICB	8	ICB/SB = 1.23	Smoke	
				ICB/FB = 1.60		
				ICB/PB = 2.28		
		SB	6.5	SB/FB = 1.30		
				SB/PB = 1.86		
		FB	5	FB/PB = 1.43		
		PB	3.5			
19	Diazepam	ICB	97	ICB/SB = 0.45	Oral	Methadone Intoxication
				ICB/FB = 0.21		Accident
				ICB/PB = 0.90		
		SB	217	SB/FB = 0.46		
				SB/PB = 2.01		
		FB	467	FB/PB = 4.34		
		PB	107			
	Methadone	ICB	204	ICB/SB = 0.44	Oral	
				ICB/FB = 0.62		
				ICB/PB = 0.84		
		SB	466	SB/FB = 1.42		
				SB/PB = 1.91		
		FB	328	FB/PB = 1.34		
		PB	244			
20	Morphine	ICB	481	ICB/SB = 1.33	Injection	Heroin Intoxication
				ICB/FB = 2.32		Accident
				ICB/PB = 2.15		
		SB	359	SB/FB = 1.73		
				SB/PB = 1.61		
		FB	207	FB/PB = 0.92		
		PB	223			
21	Diazepam	ICB	187	ICB/SB = 0.60	Oral	Polymedication Intoxication
				ICB/FB = 0.40		Accident
				ICB/PB = 1.06		
		SB	309	SB/FB = 0.67		
				SB/PB = 1.74		
		FB	463	FB/PB = 2.61		
		PB	177			
	Morphine	ICB	43	ICB/SB = 2.61	Smoke	
				ICB/FB = 5.37		

				ICB/PB = 5.37		
		SB	16	SB/FB = 2.00		
				SB/PB = 2.00		
		FB	8	FB/PB = 1.00		
		PB	8			
22	Diazepam	ICB	9	ICB/SB = 0.50	Oral	Methadone and Cocaine Intoxication
				ICB/FB = 0.47		Accident
				ICB/PB = 0.62		
		SB	18	SB/FB = 0.95		
				SB/PB = 1.24		
		FB	19	FB/PB = 1.31		
		PB	14			
	Methadone	ICB	422	ICB/SB = 0.81	Oral	
				ICB/FB = 0.74		
				ICB/PB = 0.86		
		SB	519	SB/FB = 0.91		
				SB/PB = 1.06		
		FB	571	FB/PB = 1.16		
		PB	490			
	Morphine	ICB	10	ICB/SB = 1.00	Smoke	
				ICB/FB = 1.00		
				ICB/PB = 1.18		
		SB	10	SB/FB = 1.00		
				SB/PB = 1.18		
		FB	10	FB/PB = 1.18		
		PB	8.5			
23	Methadone	ICB	848	ICB/SB = 0.63	Oral	Methadone and Heroin Intoxication
				ICB/FB = 0.99		Suicide
				ICB/PB = 0.87		
		SB	1356	SB/FB = 1.59		
				SB/PB = 1.39		
		FB	852	FB/PB = 0.88		
		PB	969			
	Morphine	ICB	143	ICB/SB = 0.94	Smoke	
				ICB/FB = 1.36		
				ICB/PB = 1.54		
		SB	152	SB/FB = 1.45		
				SB/PB = 1.64		
		FB	105	FB/PB = 1.13		
		PB	92			
24	Methadone	ICB	703	ICB/SB = 0.81	Oral	Methadone Intoxication
				ICB/FB = 1.11		Digestive hemorrhage
				ICB/PB = 1.63		Accident vs Natural
		SB	870	SB/FB = 1.37		
				SB/PB = 2.02		
		FB	633	FB/PB = 1.47		
		PB	431			
25	Diazepam	ICB	130	ICB/SB = 1.37	Oral	Polymedication and Methadone Intoxication
				ICB/FB = 1.16		Suicide

				ICB/PB = 1.15		
		SB	95	SB/FB = 0.85		
				SB/PB = 0.84		
		FB	111	FB/PB = 0.99		
		PB	113			
	Methadone	ICB	808	ICB/SB = 1.09	Oral	
				ICB/FB = 5.98		
				ICB/PB = 6.23		
		SB	740	SB/FB = 5.48		
				SB/PB = 5.72		
		FB	135	FB/PB = 1.04		
		PB	129			
26	Diazepam	ICB	1345	ICB/SB = 1.41	Oral	Methadone Intoxication
				ICB/FB = 1.06		Suicide
				ICB/PB = 1.45		
		SB	956	SB/FB = 0.75		
				SB/PB = 1.03		
		FB	1267	FB/PB = 1.37		
		PB	925			
	Methadone	ICB	32473	ICB/SB = 2.27	Oral	
				ICB/FB = 2.96		
				ICB/PB = 4.04		
		SB	14302	SB/FB = 1.30		
				SB/PB = 1.79		
		FB	10944	FB/PB = 1.36		
		PB	8040			
	Morphine	ICB	124	ICB/SB = 2.69	Smoke	
				ICB/FB = 3.54		
				ICB/PB = 4.51		
		SB	46	SB/FB = 1.31		
				SB/PB = 1.67		
		FB	35	FB/PB = 1.27		
		PB	27			
27	Diazepam	ICB	362	ICB/SB = 0.73	Oral	Polymedication and Heroin Intoxication
				ICB/FB = 0.96		Accident
				ICB/PB = 0.98		
		SB	495	SB/FB = 1.31		
				SB/PB = 1.33		
		FB	377	FB/PB = 1.01		
		PB	370			
	Morphine	ICB	164	ICB/SB = 0.88	Smoke	
				ICB/FB = 1.72		
				ICB/PB = 2.20		
		SB	185	SB/FB = 1.95		
				SB/PB = 2.49		
		FB	95	FB/PB = 1.27		
		PB	74			
28	Methadone	ICB	185	ICB/SB = 0.54	Oral	Methadone Intoxication
				ICB/FB = 0.85		Accident

				ICB/PB = 1.06		
		SB	340	SB/FB = 1.57		
				SB/PB = 1.95		
		FB	216	FB/PB = 1.24		
		PB	174			
29	Diazepam	ICB	13	ICB/SB = 0.48	Oral	Methadone Intoxication
				ICB/FB = 0.39		Accident
				ICB/PB = 0.46		
		SB	27	SB/FB = 0.81		
				SB/PB = 0.96		
		FB	33	FB/PB = 1.18		
		PB	28			
	Methadone	ICB	134	ICB/SB = 0.42	Oral	
				ICB/FB = 0.49		
				ICB/PB = 0.51		
		SB	322	SB/FB = 1.17		
				SB/PB = 1.23		
		FB	275	FB/PB = 1.05		
		PB	261			
30	Methadone	ICB	276	ICB/SB = 0.28	Oral	Methadone Intoxication
				ICB/FB = 0.60		Suicide
				ICB/PB = 0.66		
		SB	978	SB/FB = 2.13		
				SB/PB = 2.35		
		FB	459	FB/PB = 1.10		
		PB	415			

TABLE 4—*Mean concentrations ratios according to targeted substances.*

Substance	Ratios	N	Mean +/- SD	Min	Median	Max	Wilcoxon p-value

Diazepam	ICB/SB	14	0.87 +/-0.33	0.45	0.79	1.41	0.22
	ICB/FB	14	0.81 +/-0.35	0.21	0.90	1.46	0.058
	ICB/PB	14	1.06 +/-0.33	0.46	1.04	1.58	0.54
	SB/FB	14	0.93 +/-0.25	0.46	0.86	1.35	0.33
	SB/PB	14	1.28 +/-0.34	0.84	1.24	2.02	0.011
	FB/PB	14	1.53 +/-0.91	0.97	1.24	4.35	**0.005***
Methadone	ICB/SB	24	1.01 +/-0.58	0.28	0.82	2.38	0.62
	ICB/FB	24	1.57 +/-1.29	0.49	1.05	5.99	0.22
	ICB/PB	24	1.91 +/-1.38	0.51	1.50	6.24	**0.0022***
	SB/FB	24	1.58 +/-0.93	0.86	1.37	5.49	**<0.0001***
	SB/PB	24	1.93 +/-0.92	1.06	1.77	5.72	**<0.0001***
	FB/PB	24	1.27 +/-0.23	0.88	1.24	1.85	**<0.0001***
Morphine	ICB/SB	17	1.22 +/-0.62	0.50	0.91	2.70	0.35
	ICB/FB	17	1.73 +/-1.27	0.66	1.26	5.73	0.015
	ICB/PB	17	2.05 +/-1.25	0.83	1.55	5.38	**0.0001***
	SB/FB	17	1.36 +/-0.36	0.85	1.31	2.20	**0.0003***
	SB/PB	17	1.69 +/-0.45	0.93	1.65	2.49	**<0.0001***
	FB/PB	17	1.26 +/-0.23	0.85	1.27	1.63	**0.0005***

TABLE 5—*Correlations between estimated postmortem interval and ratios.*

Substance	Ratios	N	Mean PMI (h) +/- SD	Correlation	Spearman p-value
Diazepam	ICB/SB	14	31.30 +/-20.93	0.15	0.60
	ICB/FB	14		-0.0015	0.96
	ICB/PB	14		0.42	0.14
	SB/FB	14		-0.40	0.16
	SB/PB	14		0.33	0.25
	FB/PB	14		0.57	**0.032***
Methadone	ICB/SB	24	31.13 +/-15.23	-0.18	0.39
	ICB/FB	24		-0.21	0.33
	ICB/PB	24		-0.10	0.64
	SB/FB	24		-0.023	0.92
	SB/PB	24		0.29	0.18
	FB/PB	24		0.55	**0.0057***
Morphine	ICB/SB	17	35.04 +/-18.42	0.32	0.21
	ICB/FB	17		0.16	0.55
	ICB/PB	17		0.051	0.84
	SB/FB	17		0.069	0.79
	SB/PB	17		-0.21	0.42
	FB/PB	17		-0.31	0.22

Figure Legends

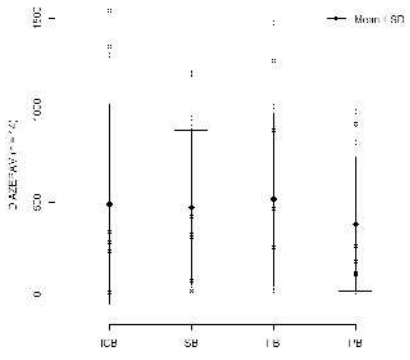
FIG. 1a—*Diazepam concentrations distribution.*

FIG. 1b—*Methadone concentrations distribution.*

FIG. 1c—*Morphine concentrations distribution.*

FIG. 2—*Diazepam, methadone and morphine mean concentrations difference.s*

FIG. 3—*Diazepam, methadone and morphine mean ratios.*



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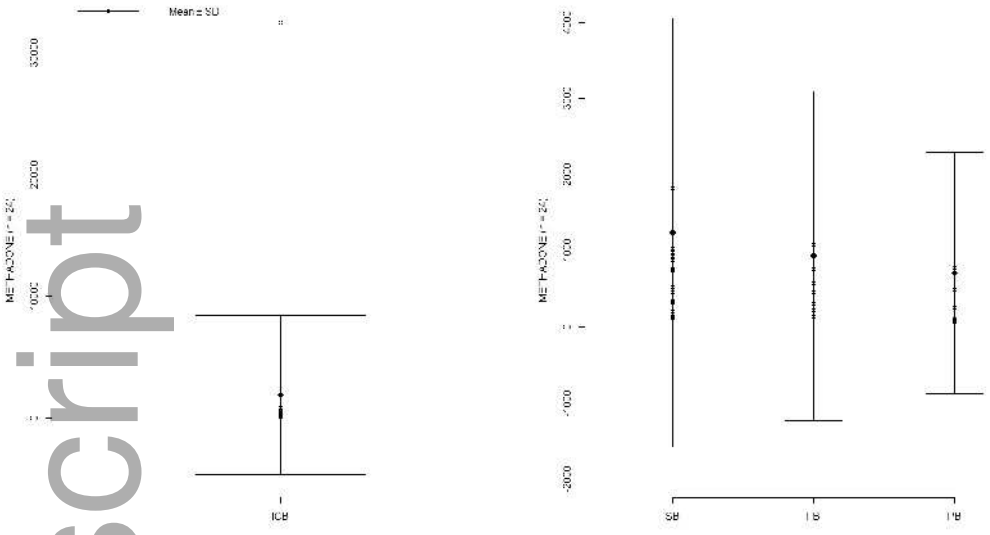

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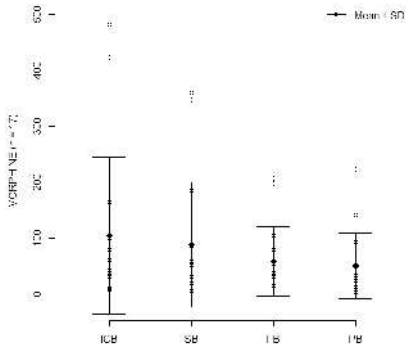

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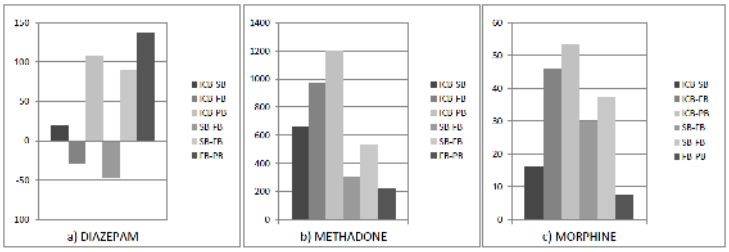

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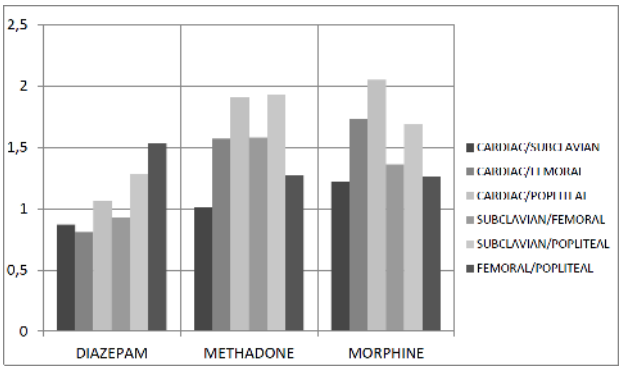

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