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Stability of Drugs in Stored Postmortem Femoral Blood and Vitreous Humor*

ABSTRACT: The stability of 46 drugs in postmortem femoral blood stored for one year at −20°C was investigated. The drugs included benzodiazepines, antidepressants, analgetics and hypnotics. For seven drugs we found a significant change in the concentration between the first and second analysis. Five substances; ethanol, desmethylmianserin, 7-amino-nitrazepam, THC and zopiclone showed a decrease in the concentration whereas the concentrations of two substances; ketobemidone and thioridazine increased. However, the changes observed were not of such an order that it would affect the interpretation in normal forensic casework. We also investigated the possible influence of potassium fluoride on the concentrations of the 46 drugs in vitreous humor after storage for one year. For two substances, ethanol and zopiclone, there were significantly lower concentrations in the samples without potassium fluoride. Furthermore, we also studied the correlation between the concentrations in femoral blood and vitreous humor. For 23 substances there was a significant difference between the concentrations in the vitreous humor and femoral blood. Significant correlations between the concentrations in these two specimens were found for 23 substances, indicating that vitreous humor can be an alternative specimen when blood samples are not available, provided that such correlation exists for the particular substance. Statistical analysis also revealed a correlation between the degree of protein binding of the different drugs and percentage of vitreous/femoral blood concentrations.

KEYWORDS: forensic science, postmortem toxicology, stability, stored samples, vitreous humor, femoral blood

Interpretation of postmortem forensic toxicological results is a crucial task and requires knowledge about the pharmacokinetics and pharmacodynamics of the substances involved as well as possible interactions in cases with several drugs detected. Further, postmortem reference data are important because clinical concentration data may not be applicable due to various chemical changes and drug redistribution in the body after death. The fate of a drug in the body from the time of death until the autopsy and collection of samples for toxicological analysis is for obvious reasons difficult to study, whereas changes in vitro can be, and has been, studied. Since the time between autopsy and the final toxicological analysis may in some cases be rather long, particularly if a reanalysis is requested, it is important to know the possible increase or decrease in drug concentrations that may occur during storage of the samples. Such changes in concentration may lead to problems in interpreting the analytical results. Some reports have addressed this issue concerning benzodiazepines (1–4), diltiazem (5), cocaine and metabolites (6–8), morphine (9,10) methadone (11) and ethanol (12) evaluating the effect of different storage time and different storage conditions; room temperature, +4°C and −20°C, on the drug concentrations. These studies have shown that the concentrations of nitro-benzodiazepines (1,4) and diltiazem (5) decreased after storage. However, for the majority of substances occurring in routine forensic casework, information about stability in stored samples is still lacking.

Vitreous humor (VH) has, in addition to femoral blood, urine and tissue samples, become an alternative specimen for toxicological analyses, especially in cases were blood samples are not available or suitable due to severe trauma or exsanguination. Vitreous humor is therefore routinely collected during forensic autopsies in Sweden. The blood-vitreous barrier only allows small molecules to enter the vitreous, thus the concentration of a drug in VH will only include the free fraction (not protein bound) (13). This means that drugs that are highly protein bound will have a much lower concentration in VH compared with the concentration in the blood. VH has been used as the specimen of choice for "postmortem chemistry" analyses including glucose, lactate and potassium (14–17). Several studies have also demonstrated the possible use of VH as an alternative to blood concerning analysis of ethanol (12,18–20). It has been shown for ethanol that a correlation exists between the concentration in blood and VH and that this correlation can be used to predict the blood alcohol concentration from an analysis in VH with a defined degree of certainty (21). Furthermore, the possible use of VH as an alternative specimen to blood has been evaluated even regarding some pharmaceutical drugs (3,22–28).

The advantage of VH over blood is that it contains few cells and is rarely subject to bacterial growth. The rationale for adding preservatives to VH samples such as fluoride may therefore be questioned.

The aim of the present study was to investigate the long-term stability of several different classes of commonly found drugs in routine forensic casework and also to investigate the possibility to use VH as an alternative specimen and to explore the possible correlation between the drug concentrations in VH and femoral blood.

Material and Methods

Femoral blood was collected at autopsy and potassium fluoride was added as a preservative to a concentration of about 1%. Vitreous humor was collected from the center of the eye with a needle and a syringe, avoiding the inclusion of retinal or iris epithelium. Samples from both eyes were pooled and divided into two equal parts and potassium fluoride was added to one of the samples.

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The samples were stored at +4°C until analyzed. The blood samples were in most cases analyzed for the presence of ethanol and other drugs within two days after arrival of the samples. After the analysis all samples were stored at −20°C and were re-analyzed after 12 ± 1 month. The analysis of all investigated drugs except amphetamine, morphine, codeine and tetrahydrocannabinol (THC) were carried out by gas chromatography with a nitrogen specific detector, according to methods previously described in detail (29). Amphetamine, morphine and codeine (30) and THC were all analyzed by gas chromatography-mass spectrometry in single ion monitoring mode (SIM). During the study period, no changes were made with regard to the methods used. The coefficient of variation (CV), which is a measure of the imprecision of the method for the different substances, ranged from 5–25%.

Statistical Methods

Differences between the groups, i.e., between results from the first and second analysis of femoral blood, between VH with and without potassium fluoride and between the concentrations at the second analysis of femoral blood and VH with potassium fluoride were determined using Students paired *t*-test. Correlation between VH and femoral blood result's was studied using Pearson correlation. A p value less than 0.05 was regarded as significant. All statistical analyses were performed using Statistica™ 6.0 from StatSoft Inc, Tulsa, OK.

Results

The mean concentrations, range and the number of cases for all substances analyzed in femoral blood immediately after the arrival of the samples and after one year of storage are presented in Table 1. In cases with concentrations below the limit of quantification during the second analysis the concentration were given the value 0.0. In most cases the concentration of the different substances varied over a wide range, thus reflecting a normal casework panorama. For five substances a significant decrease in the concentration was observed; ethanol declined from 1.75 to 1.61 mg/mL, desmethylmianserin from 0.15 to 0.07, 7-amino-nitrazepam from 0.15 to 0.08, THC from 0.003 to 0.001 and zopiclone from 0.19 to 0.15 μg/g femoral blood. Two substances showed an increase in the concentration; ketobemidone from 0.14 to 0.18 and thioridazine from 0.62 to 0.86 μg/g femoral blood. Fluoxetine, desmethylsertraline and phenytoin also showed some changes in concentration, but the differences were not significant. All other substances exhibited very similar mean concentrations at the first and second analysis.

The addition of potassium fluoride to VH only affected the concentration of two substances, ethanol and zopiclone; the concentrations decreased in the samples without a preservative from 2.0 to 1.21 and from 0.15 to 0.03 for ethanol and zopiclone, respectively (Table 2). Generally, the concentration in VH was lower than the corresponding concentration in femoral blood with some exceptions. Amphetamine, codeine, diltiazem, ethanol, ketamine, tramadol, and O-desmethylvenlafaxine all showing higher mean concentrations in VH, although the differences were not statistically significant. For 23 substances there were no significant differences between the concentrations in VH and femoral blood but for the remaining 23 substances significant differences were observed (Table 2). Independent of the differences between the concentrations in VH and blood significant correlations were found between the concentrations in these two specimens for 23 of the substances, (Table 2, Fig. 1). Because it can be assumed that the VH concentrations reflects the unbound fraction of the blood

TABLE 1—*Summary of the analytical results from femoral blood with the first and second analysis after 12 month and the number of cases (N) and the mean and (range) concentrations. P-value < 0.05 is regarded as significant.*

Substance	*N*	Femoral Blood* 1st	Femoral Blood* 2nd	*P*-Value
Acetaminophene	27	20.0 (0.9–230)	19.2 (1–210)	
Alimemazine	14	0.29 (0.1–1.3)	0.26 (0.0–1.2)	
Desmethylalimemazine	5	0.24 (0.2–0.4)	0.16 (0.1–0.2)	
Amphetamine	5	1.1 (0.1–3.0)	1.1 (0.1–3.3)	
Amitriptyline	11	0.64 (0.1–1.8)	0.67 (0.1–2.1)	
Nortriptyline	8	0.71 (0.1–3.9)	0.62 (0.1–3.2)	
Carbamazepine	12	9.6 (0.8–30)	9.6 (0.9–33)	
Citalopram	19	0.63 (0.1–1.7)	0.64 (0.1–1.6)	
Desmethylcitalopram	16	0.16 (0.1–0.4)	0.13 (0.0–0.3)	
Clomipramine	11	0.32 (0.1–0.8)	0.33 (0.1–0.9)	
Desmethylclomipramine	8	0.86 (0.1–3.6)	0.91 (0.1–3.7)	
Clozapine	4	0.40 (0.1–0.7)	0.30 (0.1–0.6)	
Codeine	5	0.30 (0.01–0.7)	0.31 (0.01–0.8)	
Dextropropoxyphene	20	0.89 (0.1–4.5)	0.84 (0.1–3.6)	
Diazepam	19	0.26 (0.1–1.0)	0.27 (0.1–1.0)	
Desmethyldiazepam	19	0.27 (0.1–1.3)	0.30 (0.1–1.3)	
Diltiazem	6	1.77 (0.1–9.2)	2.05 (0.1–11.0)	
Ethanol	16	1.75 (0.39–3.6)	1.61 (0.30–3.4)	<0.001
Flunitrazepam	6	0.016 (0.01–0.03)	0.011 (0.0–0.02)	
7-amino-flunitrazepam	11	0.084 (0.02–0.16)	0.067 (0.0–0.2)	
Fluoxetine	6	0.90 (0.3–3.6)	0.65 (0.3–1.9)	
Ketamine	8	2.1 (0.4–5.9)	2.1 (0.5–6.1)	
Ketobemidone	13	0.14 (0.03–0.7)	0.18 (0.03–0.7)	0.015
Lidocaine	13	0.43 (0.1–2.3)	0.46 (0.1–2.8)	
Methotrimeprazine	8	0.92 (0.1–5.2)	0.86 (0.1–5.3)	
Desmethylmethotrimeprazine	5	0.93 (0.05–4.1)	0.64 (0.0–2.4)	
Mianserin	6	0.29 (0.05–0.8)	0.20 (0.06–0.4)	
Desmethylmianserin	4	0.15 (0.1–0.2)	0.07 (0.0–0.1)	0.044
Mirtazapine	10	0.15 (0.1–0.3)	0.16 (0.1–0.4)	
Morphine	12	0.15 (0.008–0.8)	0.15 (0.008–0.8)	
7-amino-nitrazepam	10	0.15 (0.06–0.2)	0.08 (0.03–0.18)	0.0002
Orphenadrine	8	2.0 (0.2–10.0)	2.0 (0.2–10.0)	
Phenytoin	10	5.5 (1.3–18)	7.1 (1.9–22)	

TABLE 1—*Continued*

Substance	*N*	Femoral Blood* 1st	Femoral Blood* 2nd	*P*-Value
Propiomazine	10	0.09 (0.03–0.3)	0.08 (0.0–0.2)	
Dihydropropiomazine	20	0.26 (0.04–1.1)	0.29 (0.04–1.1)	
Sertraline	8	0.27 (0.1–0.9)	0.23 (0.06–0.7)	
Desmethylsertraline	7	0.57 (0.1–1.8)	0.26 (0.0–0.6)	
Theophylline	9	5.6 (2–16)	5.0 (2–14)	
THC	6	0.003 (0.002–0.006)	0.001 (0.0–0.004)	0.002
Thioridazine	5	0.62 (0.3–1.1)	0.86 (0.4–1.5)	0.009
Tramadol	4	0.67 (0.2–1.3)	0.62 (0.2–1.3)	
Venlafaxine	7	6.7 (0.1–43)	7.0 (0.1–44)	
O-desmethylvenlafaxine	8	0.68 (0.1–2.3)	0.80 (0.1–2.8)	
Verapamil	6	0.28 (0.1–0.5)	0.33 (0.1–0.7)	
Zolpidem	11	0.55 (0.08–2.3)	0.56 (0.04–3.1)	
Zopiclone	13	0.19 (0.03–0.5)	0.15 (0.0–0.45)	0.033

* Values in μg/g.

concentrations, the percentage of VH concentration of the corresponding femoral blood concentrations were plotted against the known protein binding for each substance, and significant correlation was found, Fig. 2. In one of the morphine-positive cases, 6-acetyl-morphine was detected in femoral blood and at a concentration ten times higher in VH with fluoride added. In addition, in another four cases 6-acetyl-morphine was detected exclusively in VH with fluoride added. This is in agreement with Pragst et al. (25) who suggested that VH could be an alternative specimen to prove heroin intake.

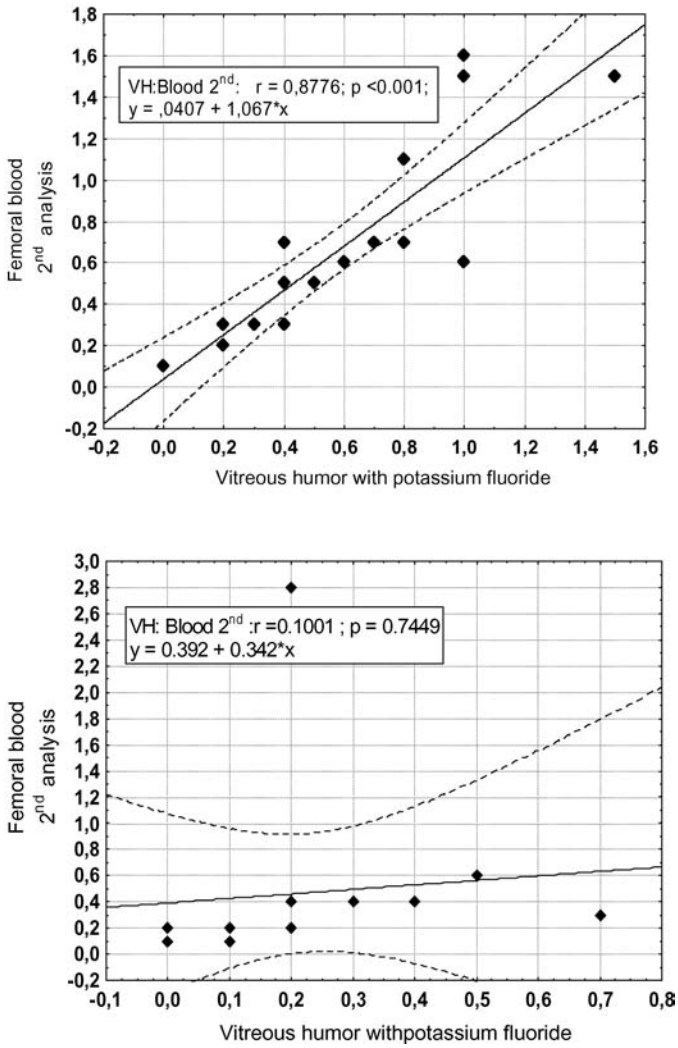

FIG. 1—*Examples of correlation between concentrations in vitreous humor and femoral blood; citalopram (upper) and lidocaine (lower) with 95% confidence limits (dotted lines). r is the correlation coefficient.*

Discussion

We have shown that most of the drugs studied were stable in fluorinated femoral blood samples after one year of storage at −20°C.

Knowledge of the stability of drugs is an important factor when interpreting forensic toxicological results. Probably, most changes in the concentration occur during the time from death to the collection of a sample and in this respect the postmortem redistribution and degradation constitute the most important problems. However, the stability of drugs in stored samples is also a question of importance, because several factors might influence the stability, such as the storing temperature, storing time, addition of preservatives, and initial condition of the collected sample.

Differences in the concentration between two analytical results are not only a question of the stability but also a question of the precision of the analytical method used. Since the CV varies depending on the concentration with higher CV at higher concentrations, we chose an arbitrary difference of ±15% of the original result to represent an actual change in the concentration. Applying this limit to the substances were a statistically significant change in the concentration in femoral blood between the first and second analysis were found, the differences were greater and probably not depending on

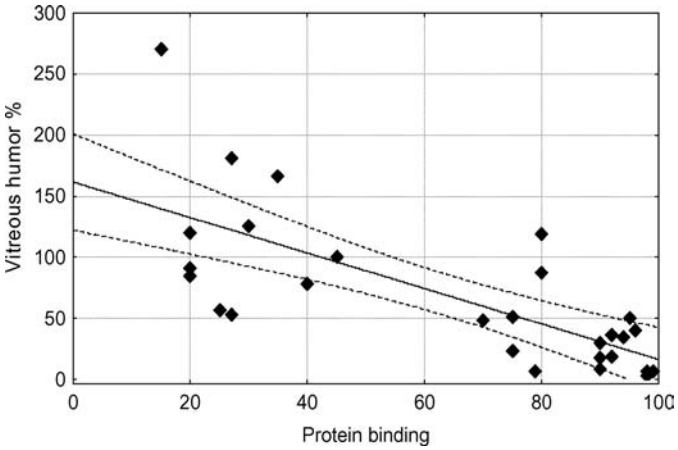

FIG. 2—*Correlation between the degree of protein binding and the percentage of concentration in vitreous humor the femoral blood concentration for all drugs studied with a known degree of protein binding; 95% confidence limits are shown as dotted lines. The correlation coefficient was* $r = -0.7260$.

TABLE 2—*Summary of the results from vitreous humor (VH) and femoral blood (FB) after 12 month given as mean and (range) together with the degree of protein binding (VH%), correlation between vitreous humor and femoral blood concentrations and VH concentration with KF as percent of the FB concentration (VH%).*

Substance	*N*	Femoral Blood 12 month	Vitreous Humor		P^2	P^3	Correlation	P^4	Protein[5]	
			Without KF[1]	With KF	VH	VH-FB	VH-FB		Binding	VH %
Acetaminophene	27	19.2 (1–210)	6.0 (1–16) 26	10.9 (1–120)			0.979	0.000	25	57
Alimemazine	14	0.26 (0.0–1.2)	0.04 (0.0–0.3)	0.06 (0.0–0.4)		0.04	0.916	0.000		23
Desmethylalimemazine	5	0.16 (0.1–0.2)	0	0.02 (0.0–0.1)		0.02				12
Amphetamine	5	1.1 (0.1–3.3)	2.0 (0.2–5.3)	2.0 (0.2–5.6)			0.993	0.001	27	181
Amitriptyline	11	0.67 (0.1–2.1)	0.24 (0.0–1.1)	0.23 (0.0–0.9)		0.01	0.919	0.000	94	34
Nortriptyline	8	0.62 (0.1–3.2)	0.21 (0.0–1.3)	0.12 (0.0–0.8)			0.995	0.000	92	19
Carbamazepine	12	9.6 (0.9–33)	2.3 (0.0–8)	2.2 (0.0–8)		0.004	0.926	0.000	75	23
Citalopram	19	0.64 (0.1–1.6)	0.60 (0.2–1.4)	0.56 (0.0–1.5)			0.877	0.000	80	87
Desmethylcitalopram	16	0.13 (0.0–0.3)	0.18 (0.0–0.4)	0.15 (0.0–0.4)			0.768	0.000		115
Clomipramine	11	0.33 (0.1–0.9)	0.01 (0.0–0.1)	0.01 (0.0–0.1)		0.001			98	3
Desmethylclomipramine	8	0.91 (0.1–3.7)	0.06 (0.0–0.3)	0.06 (0.0–0.2)						6
Clozapine	4	0.30 (0.1–0.6)	0.15 (0.1–0.2)	0.15 (0.1–0.2)					95	50
Codeine	5	0.31 (0.01–0.8)	0.73 (0.009–2.0)	0.84 (0.008–2.5)					15	270
Dextropropoxyphene	20	0.84 (0.1–3.6)	0.39 (0.0.0–1.5)	0.43 (0.0–2.2)		0.001	0.863	0.000	75	51
Diazepam	19	0.27 (0.1–1.0)	0.031 (0.0–0.2) 11	0.018 (0.0–0.1) 13		0.0005	0.887	0.000	98	7
Desmethyldiazepam	19	0.30 (0.1–1.3)	0.03 (0.0–0.3) 16	0.02 (0.0–0.2) 17		0.0006			79	7
Diltiazem	6	2.05 (0.1–11.0)	2.58 (0.0–14.0)	2.45 (0.0–13.0)			0.999	0.000	80	119
Ethanol	16	1.61 (0.30–3.4)	1.21 (0.0–3.1)	2.0 (0.42–4.4)	0.002	0.001	0.969	0.000		
Flunitrazepam	6	0.011 (0.0–0.2)	0	0		0.000			78	
7-amino-flunitrazepam	11	0.07 (0.0–0.2)	0.006 (0.0–0.03)	0.005 (0.0–0.02)		0.002				
Fluoxetine	6	0.65 (0.3–1.9)	0.03 (0.0–0.2)	0.05 (0.0–0.3)		0.03			90	8
Ketamine	8	2.1 (0.5–6.1)	3.9 (0.4–16.5)	3.5 (0.6–13.8)			0.964	0.000	35	166
Ketobemidone	13	0.18 (0.03–0.7)	0.15 (0.0–0.5)	0.15 (0.03–0.5)			0.944	0.000		
Lidocaine	13	0.46 (0.1–2.8)	0.18 (0.0–0.6) 12	0.22 (0.0–0.7)					70	48
Methotrimeprazine	8	0.86 (0.1–5.3)	0.17 (0.0–0.5) 7	0.14 (0.0–0.3)						
Desmethylmethotrimeprazine	5	0.64 (0.0–2.4)	0.16 (0.0–0.5)	0.10 (0.0–0.3)						
Mianserin	6	0.20 (0.06–0.4)	0.08 (0.0–0.4)	0.08 (0.0–0.4)		0.02			96	40
Desmethylmianserin	4	0.07 (0.0–0.1)	0.01 (0.0–0.05)	0						
Mirtazapine	10	0.16 (0.1–0.4)	0.07 (0.0–0.3) 9	0.07 (0.0–0.3)		0.03				
Morphine	12	0.15 (0.008–0.8)	0.10 (0.007–0.3)	0.08 (0.007–0.3)			0.858	0.000	27	53
7-amino-nitrazepam	10	0.08 (0.03–0.18)	0.02 (9.0–0.06) 8	0.02 (0.0–0.07)		0.002				
Orphenadrine	8	2.0 (0.2–10.0)	1.6 (0.1–6.9)	1.7 (0.1–8.0) 7			0.995	0.000	20	85
Phenytoin	10	7.1 (1.9–22)	1.3 (0.0–3.7) 9	1.3 (0.0–3.8)		0.01	0.906	0.000	90	18
Propiomazine	10	0.08 (0.0–0.2)	0.01 (0.0–0.1)	0.02 (0.0–0.06) 9		0.002				

TABLE 2—*Continued.*

Substance	*N*	Femoral Blood 12 month	Vitreous Humor Without KF[1]	Vitreous Humor With KF	P^2 VH	P^3 VH-FB	Correlation VH-FB	P^4	Protein[5] Binding	VH %
Dihydropropiomazine	20	0.29 (0.04–1.1)	0.06 (0.0–0.3) 18	0.05 (0.0–0.3) 19		0.000	0.770	0.000		
Sertraline	8	0.23 (0.06–0.7)	0	0		0				
Desmethylsertraline	7	0.26 (0.0–0.6)	0	0		0				
Theophylline	9	5.0 (2–14)	3.4 (0.8–10)	3.9 (0.9–12)		0.001	0.984	0.000	40	78
THC	6	0.001 (0.0–0.004)	0	0		0				
Thioridazine	5	0.86 (0.4–1.5)	0.08 (0.0–0.3)	0.06 (0.0–0.2)		0.009			99	7
Tramadol	4	0.62 (0.2–1.3)	0.85 (0.2–2.1)	0.75 (0.2–1.9)			0.973	0.026	20	120
Venlafaxine	7	7.0 (0.1–44)	5.6 (0.1–32)	6.4 (0.1–30)			0.925	0.000	20	91
O-desmethylvenlafaxine	8	0.80 (0.1–2.8)	0.80 (0.1–2.6)	1.0 (0.2–4.3)			0.926	0.000	30	125
Verapamil	6	0.33 (0.1–0.7)	0.2 (0.1–0.4) 5	0.1 (0.1–0.3) 5					90	30
Zolpidem	11	0.56 (0.04–3.1)	0.2 (0.04–0.7) 9	0.2 (0.0–0.8) 10					92	36
Zopiclone	13	0.15 (0.0–0.45)	0.03 (0.0–0.16) 9	0.15 (0.0–0.4) 12	0.007		0.794	0.002	45	100

[1] KF = potassium fluoride.
[2] *P* VH is the *p*-value for the statistical significance test between the concentration in VH with and without the addition of a preservative.
[3] *P* VH-FB is the *p*-value for the statistical significance test between the concentration in VH with the addition of preservative and the concentration in FB.
[4] *P* is the *p*-value for the correlation between the concentrations in VH and FB.
[5] Mainly from Hardman, Limbird, Molinoff, Ruddon, Gillman (eds): Goodman and Gillman's The pharmacological basis of therapeutics, 9th ed. McGraw-Hill, 1996 and Drummer OH (ed). The forensic pharmacology of drugs of abuse. Arnold, London, 2001.

the uncertainty of the analytical methods. Further, such analytical imprecision should rather be expected to reduce the likelihood of achieving significant differences, since the analytical variation can be assumed to be randomly distributed.

Among the substances investigated, the change in mean concentrations between the first and second analysis was in general small. However, for seven drugs a significant difference between the first and second analysis in femoral blood was found with lower concentrations in the second analysis for five of these. The decrease in concentrations for ethanol, flunitrazepam, although not significant, and 7-amino-nitrazepam corroborate previous findings (1,12). We did not notice any decrease in the concentration for diltiazem like Koves et al. (5) previously have reported but in their paper the sample site of the postmortem blood was not stated and addition of a preservative was not always performed, factors that may influence the outcome. Although based on a small number of cases desmethylmianserin and desmethylsertraline showed a decrease in the concentration, whereas the parent drugs, mianserin and sertraline, did not show the same pronounced decrease. This finding is important since the ratio between the parent drug and metabolite may be used as a tool to differentiate between a chronic or acute intake. Hence, differences in the stability may influence the interpretation, an issue that has been addressed previously concerning diazepam (4).

Two substances, ketobemidone and thioridazine showed a significant increase in the concentration in femoral blood, more marked for thioridazine than for ketobemidone where the increase probably is of little importance. A possible explanation for the increase in concentration for thioridazine could be conversion from the metabolite thioridazine-sulphoxide. However, since we did not measure this metabolite, this possibility remains a hypothesis.

Vitreous humor is considered to be a specimen relatively unaffected by bacterial influence after death and is situated well isolated from other organs and body fluids and would therefore be suitable for toxicological analysis since the conditions for change in drug concentrations postmortem are less pronounced than in blood. If this hypothesis is true, addition of a preservative to VH would not be necessary. This is an important strategic question because VH is also an important sample for analysis of various biochemical compounds, including salts. Thus, addition of a preservative to the VH completely prevents analysis of sodium and/or potassium. Among the 46 substances that were investigated, we only found two drugs that showed a difference between the samples with and without addition of potassium fluoride as a preservative; ethanol and zopiclone which both showed significantly lower concentrations in the sample without preservative. Further, 6-acetyl-morphine was only detected in VH with a preservative. Since ethanol is one of the most frequently found drug in forensic cases and since analysis of ethanol in VH is well established we suggest that addition of a preservative even to VH is necessary and that samples for electrolytes should be taken separately.

Generally, the concentrations found in VH with a preservative added were lower than in femoral blood. However, for some drugs we found the opposite situation. The concentration in VH is dependent on the time between intake and death, leading to lower VH to blood ratios before equilibrium has been reached. The time to achieve equilibrium probably depends on several causes like degree of protein binding, lipophilicity and hydrophilicity of the drug. For example, De Letter et al. (26) found that the equilibrium time for MDMA given to rabbits was between 30 and 120 min after administration of the drug. The route of administration of the substance might also affect the concentration ratios between VH and blood.

For instance, lidocaine is often given by intravenous or intracardial injections in connection with resuscitation and for this substance we found very large variations in the concentrations in VH between cases with the same concentration in blood (Fig. 1).

Half of the substances investigated showed a significant correlation between the concentrations in VH and femoral blood but for half of the substances no correlation existed (Table 2). The concentration of drugs in VH represents the free unbound fraction since only small molecule are able to cross the blood-vitreous-barrier (13) and therefore one could expect to find a correlation between the degree of protein binding and the concentration in VH expressed as the percent of the concentration in the blood. We found a significant correlation but with a higher intercept of the linear regression line than theoretical. However, the degree of protein binding is affected by several factors such as the concentration of the drug, diseases as well as intake of several drugs, which makes it difficult to know the degree of protein binding in the individual case.

In summary, with the addition of a preservative and storage of postmortem femoral blood samples at −20°C, most substances in this study were stable for one year but a few exceptions were noted. Such changes during storage are important to be aware of when interpreting analytical results carried out a long time after the collection of the sample. Our results also support the opinion that VH is a useful alternative specimen to blood for drug analysis, particularly regarding the substances where we were able to establish a significant correlation between femoral blood and VH concentrations. Combined analysis of VH and femoral blood may also assist in the differentiation between acute and chronic intake. However, interpretation of concentrations in VH must be done with caution and should only be made regarding substances with known femoral blood-VH correlations.

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