

Comparison of Drug Concentrations in Blood and Oral Fluid Collected with the Intercept® Sampling Device

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Abstract

The aim of the study was to determine drug concentration ratios between oral fluid collected with the Intercept device and whole blood. Samples of blood and oral fluid were obtained from patients admitted to acute psychiatric treatment and drivers suspected of drugged driving. Samples were analyzed for illegal drugs, benzodiazepines, opioids, carisoprodol, and meprobamate. Drugs were detected in samples of both blood and oral fluid from 59 subjects; altogether, 17 different drugs were found. Concentration ratios between oral fluid and blood were determined for all cases. The distributions of drug concentration ratios were wide for most drugs and do not allow reliable estimations of drug concentrations in blood using concentrations in oral fluid. The median oral fluid/blood drug concentration ratios for the most prevalent drugs were 0.036 diazepam, 0.027 nordiazepam, 7.1 amphetamine, 2.9 methamphetamine, 5.4 codeine, 1.9 morphine, and 4.7 tetrahydrocannabinol. The correlation coefficients between drug concentrations in oral fluid and blood ranged from 0.15 to 0.96 for the six most prevalent drugs.

Introduction

Samples of oral fluid (mixed saliva) may be used to detect the use of alcohol, medicinal drugs, and illegal drugs (1–4). Although drugs may be detected in urine for several days after use and even weeks for cannabis, most drugs may be detected in oral fluid for approximately the same length of time as in blood (5). Many kits for sampling oral fluid are available. The collection may be performed by placing a collection pad between cheek and gums or under the tongue for 2–5 min, followed by transfer of the pad to a vial containing a conservation buffer. The collection pads of some brands may contain saliva-stimulating agents (6,7).

The transfer of a drug from blood to oral fluid, and hence the concentration ratio between fluid and blood (OF/B ratio), de-

pends on the compound's physicochemical properties, namely pK_a , protein binding, lipophilicity, molecular weight, and spatial configuration (5). For drugs with pK_a close to the pH of oral fluid, which normally is between pH 6.0 and 7.0 (8) (e.g., amphetamines and opioids), the pH of the individual's oral fluid may greatly affect the OF/B ratio. The route of drug administration may also affect the drug concentration in oral fluid, and in addition, the sampling procedure and the properties of the sampling device may also affect the determined drug concentration (5–7,9–11).

The Intercept collection device is often used for the collection of oral fluid. The collection pad is treated with a solution containing citric acid and sodium chloride to stimulate the production of oral fluid, which may affect the drug concentration in the sampled oral fluid. The recovery for tetrahydrocannabinol (THC) from this sampling device is low, but the recoveries for many other drugs are fairly good (6).

Data on OF/B ratios for this collection device are needed in order to better assess analytical results from single patients and large populations. However, few studies on OF/B ratios for the Intercept collection device have so far been published (12–14).

OF/B ratios for different drugs are often determined in pharmacokinetic studies. We believe that the interindividual variation between random drug users may be greater than the variation observed in well-controlled pharmacokinetic studies. The aim of this study is to investigate the variation in individual OF/B drug concentration ratios in samples from patients and suspected drugged drivers when using the Intercept collection device and to determine the average and median OF/B ratios for several drugs.

Materials and Methods

Samples of oral fluid and blood were collected from patients admitted to acute psychiatric treatment at Lovisenberg Deaconal Hospital (Oslo, Norway) or Sørlandet Hospital (Arendal, Norway) from September 2006 to May 2007 and from motor

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vehicle drivers suspected of driving under the influence of drugs apprehended by the police from August 2004 to November 2005 (15). The hospital patients volunteered in a study of drug use among acute psychiatric patients (Mordal et al., manuscript in preparation). Samples of blood and oral fluid were taken by hospital staff shortly after admission, and results were used for research purposes only. The study of motor vehicle drivers was a part of the Roadside Testing Assessment project Rosita-2 (16). Samples of whole blood were taken by police physicians for forensic purposes, while samples of oral fluid were given voluntarily for research purposes.

Oral fluid was sampled using the Intercept oral fluid sampling kit from Orasure Technologies (Bethlehem, PA). Samples of whole blood were collected using 5-mL Vacutainer® tubes containing fluoride and heparin (BD Diagnostics, Franklin Lakes, NJ). Samples of oral fluid and blood were frozen within a few days after arrival at the laboratory and analyzed for drugs within a few weeks after sampling.

Samples were excluded from the study if the time lapse between sampling of blood and oral fluid exceeded 10% of one half-life in blood (17) or if the volume of collected oral fluid was less than 0.2 mL, an amount that is regarded to be too small for accurate determination of the oral fluid dilution factor.

The Intercept oral fluid sampling kit contained 0.8 mL buffer with Flag Blue dye. The dilution of oral fluid with buffer was determined by analyzing the concentration of Flag Blue dye in the oral fluid/buffer mixture by using high-performance liquid chromatography (HPLC) with UV detection using a modification of a previously published method (18). The following modifications were implemented: the HPLC system consisted of a Waters Alliance 2695 system with a diode-array detector (Milford, MA). A Waters XTerra Phenyl HPLC column was used (150 × 2.1-mm i.d.). The mobile phase was composed of 10 mM ammonium formate pH 4 with an acetonitrile gradient. Each sample was diluted 1:4 (v/v), and 10 µL was injected. The column effluent was monitored at 629 nm. Samples were stored at about -20°C until the analysis of Flag Blue dye was performed.

All drug concentrations presented in this study are concentrations in undiluted oral fluid calculated by dividing the drug concentration found in the oral fluid/buffer mixture with the determined dilution factor.

Drug concentrations in samples of oral fluid were determined using HPLC with tandem mass spectroscopic detection (MS-MS) (19). Samples of blood were screened for amphetamines, cannabinoids, cocaine metabolites, and opiates using Enzyme Multiplied Immunoassay Technique (EMIT) (20). Screening for other drugs was performed using HPLC-MS (21). Drug findings were confirmed and quantified using gas chromatography-MS or LC-MS (21-24). The laboratory was accredited according to ISO

17025 for performing these confirmation and quantification methods for forensic toxicology purposes by the Norwegian body for accreditation of laboratories (Norsk Akkreditering, Kjeller, Norway). Cutoff thresholds are presented in Table I. The cutoff thresholds were more than the limit of quantitation (LOQ) of the analytical methods and were also set to exclude low drug concentrations without pharmacological or toxicological importance.

Correlations between drug concentrations in blood and oral fluid were determined by linear regression. Pearson's product-moment correlation coefficients (*r*) were calculated. Pearson's second coefficient of skewness was used to determine the skewness of the OF/B ratios.

Results

Altogether, 90 pairs of blood and oral fluid from patients and 22 pairs of blood and oral fluid from suspected drugged drivers were available for comparison of drug concentrations. The volume of oral fluid collected ranged from 0.2 to 1.8 mL with a median of 0.6 mL.

Drugs were found in oral fluid and/or blood from 37 of the patients and all of the suspected drugged drivers. Two obvious outliers were excluded for flunitrazepam and clonazepam; in those cases, the OF/B ratios were 35 or 137 times larger than the median OF/B ratio and might have been caused by residual tablet ingredients in the mouth due to recent tablet intake.

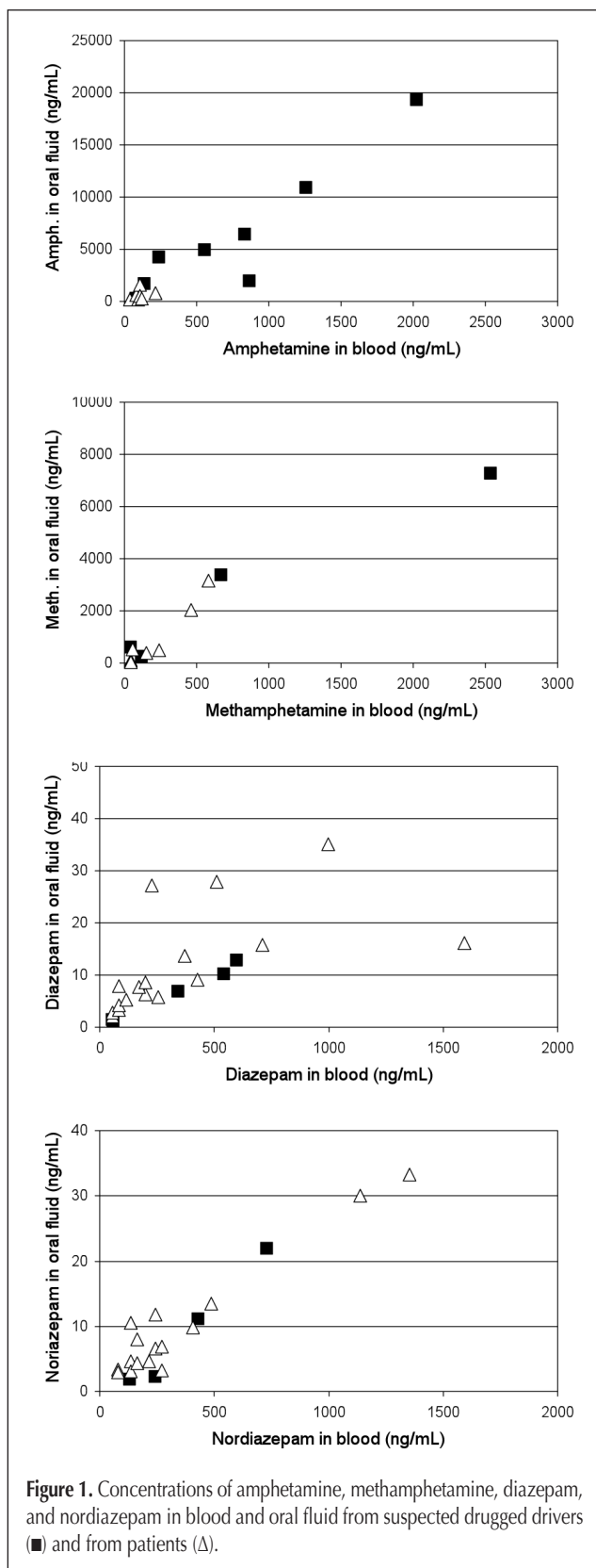
The most frequently detected drugs were diazepam, nordiazepam, amphetamine, methamphetamine, THC, and morphine. Drug concentrations in oral fluid and blood for amphetamine, methamphetamine, diazepam, and nordiazepam are

Table I. Analytical Cutoff Concentrations for Drugs in Blood and Oral Fluid as Analyzed by GC-MS or LC-MS

Drug	Cutoff Concentrations (µg/L)	
	Blood	Oral fluid/ buffer mixture
Tetrahydrocannabinol	1	1
Flunitrazepam	0.8	0.3
Clonazepam	10	0.5
Nitrazepam	10	0.5
Alprazolam	10	1
Zopiclone	10	1
Diazepam	30	1
Nordiazepam	30	1
Oxazepam	150	5
Amphetamine	40	5
Methamphetamine	40	5
3,4-Methylenedioxy-N-methylamphetamine (MDMA)	40	5
Codeine	5	2.5
Morphine	10	5
Methadone	30	5
Carisoprodol	500	10
Meprobamate	1000	20

presented in Figure 1. Only cases in which drugs were more than the cutoff thresholds in both oral fluid and blood are presented.

The correlation coefficients (r) were 0.95 for amphetamine, 0.96 for methamphetamine, 0.61 for diazepam, 0.95 for nor-diazepam, 0.15 for THC, and 0.85 for morphine.



Individual drug concentration ratios between oral fluid and blood are listed in Table II. The median OF/B ratios were less than 0.2 for carisoprodol and all benzodiazepines except alprazolam, in the intermediate range of 0.2–2 for meprobamate, alprazolam, methadone, and morphine, and more than 2 for amphetamines, zopiclone, THC, and codeine.

The distribution of OF/B ratios was wide for most compounds. Pearson's coefficient of skewness was larger than 0.5 for nine of the drugs detected. The distributions of OF/B ratios for codeine, methadone, and morphine were much skewed with coefficients of skewness larger than 1.3.

Discussion

The results of our study show large interindividual variations in drug concentration ratios between oral fluid and blood. This wide range of OF/B ratios does not allow reliable estimations of drug concentrations in blood based on drug concentrations in oral fluid. Similar variations have also been found in the Rosita-2 study (12); however, in that study the actual oral fluid dilution was determined only by a few of the participating laboratories. In most cases, the average dilution factor was used. The observed distribution of OF/B ratios for some drugs were skewed, particularly for opiates. The wide variation of OF/B ratios caused a number of samples to be positive for a drug in either blood or oral fluid and negative in the other type of specimen (results not shown).

The OF/B ratio for zopiclone was fairly high with a median of 3.8. An oral fluid/plasma (OF/P) ratio of 2.2 has previously been reported (25). As the reported blood/plasma ratio for zopiclone is 1.0 (26), the OF/B ratio would also be 2.2, which is within the range observed in our study. However, a different sampling procedure for collecting oral fluid was used in our study; this may have affected the OF/B ratio.

A high OF/B ratio was observed for amphetamine with a median ratio of 7.1. This is significantly higher than the theoretical ratio of 3.33 (5) but lower than the median ratio of 13.4 found in the Rosita-2 study (11). A low drug concentration ratio between oral fluid and plasma of 2.76 has also been reported (27). These large differences may reflect differences in drug administration and sampling of oral fluid in those studies in addition to variations between individuals.

The median OF/B ratio for THC of 4.7 was fairly low compared to the overall result for the Rosita-2 study in which a median OF/B ratio of 15 was found (12). However, OF/P ratios for THC as low as 0.5–2.2 have also been reported in a pharmacokinetic study (28).

It has been claimed that the concentration of THC in oral fluid is primarily a result of contamination of the oral cavity by THC when smoking cannabis (5,29). The decline in THC concentration may therefore be primarily due to removal of THC from the oral cavity (e.g., by eating, drinking, or swallowing oral fluid) and, to a lesser extent, absorption. The THC concentration in oral fluid therefore does not depend on the THC concentration in blood or serum. The observation of similar elimination rates for THC in oral fluid and serum is

probably accidental (30). Large interindividual variations in OF/B ratio have been also been reported previously (12,30), and a poor correlation between the magnitude of performance impairment and THC concentration in oral fluid has been found (31).

The THC concentrations observed in blood and oral fluid in our study were in most cases fairly low, indicating that cannabis was smoked a number of hours ago. This may be one reason for the low ratios observed in our study. In addition, the recovery for THC from the Intercept collection kit is low (6); however, the same type of oral fluid sampling device was used in the Rosita-2 project, so the type of sampling kit does not explain the difference between our result and the overall results for Rosita-2.

Other drugs with high OF/B ratios were MDMA (4.6), codeine (5.4), and methamphetamine (2.9). Previously published OF/B and OF/P ratios for these drugs also vary significantly (10–12,32–35).

Benzodiazepines had low OF/B ratios, the median ratios were 0.036 for diazepam, 0.027 for nordiazepam, 0.056 for oxazepam, 0.090 for nitrazepam, 0.16 for flunitrazepam, and

0.17 for clonazepam; some median ratios are based on few individual determinations and therefore may not be representative for larger populations. For alprazolam, a single ratio of 0.36 was observed.

Our result for diazepam is similar to findings in several other studies. A previously reported median OF/B ratio was 0.02 (12), and previously reported average OF/P ratios for diazepam were 0.016–0.029 (36–39). With a blood/plasma ratio of 0.6 (17), the average OF/B ratio for these four studies is 0.035. Oral fluid was in these latter studies collected by spitting with or without stimulation by chewing Parafilm® or Teflon®. In another study, a lower OF/B ratio of 0.016 ± 0.002 was reported using free-flowing oral fluid (40).

In a previous study of nitrazepam, OF/serum ratios of 0.04–0.08 were observed (41); data on the serum/blood ratio or OF/B ratio have not been published. In a study of oxazepam, an average OF/B ratio of 0.05 was observed when oral fluid was collected by spitting (42), which is lower than the average observed in our study but similar to the median.

The low OF/B ratios for benzodiazepines is primarily due to the fact that these drugs have high protein binding, and the

Table II. Drug Concentration Ratios Between Oral Fluid and Blood

	Amphetamine	Methamphetamine	MDMA	Alprazolam	Clonazepam	Diazepam	Nordiazepam	Oxazepam	Nitrazepam	Flunitrazepam	Zopiclone	Morphine	Codeine	Methadone	Carisoprodol	Meprobamate	THC
Individual ratios	1.8	0.6	2.7	0.36	0.15	0.010	0.009	0.027	0.01	0.10	1.7	0.3	1.9	0.4	0.07	0.2	0.4
	2.2	2.0	4.2		0.16	0.019	0.012	0.043	0.05	0.10	2.7	1.4	2.4	0.5			0.6
	2.2	2.1	5.1		0.17	0.020	0.014	0.053	0.07	0.15	5.0	1.6	4.4	0.6			1.4
	3.7	2.1	8.3		0.18	0.021	0.022	0.056	0.09	0.16	5.2	1.6	5.4	0.7			1.8
	3.9	2.6			0.27	0.021	0.023	0.058	0.13	0.17		1.7	7.8	3.6			3.0
	4.6	2.9				0.022	0.024	0.065	0.13	0.73		2.0	22.4	7.2			4.7
	4.7	4.4				0.022	0.025	0.254	0.13			7.0	35.7				5.2
	7.1	5.0				0.023	0.025					14.8					6.1
	7.6	5.5				0.025	0.026					17.0					6.9
	8.7	9.1				0.032	0.026					20.2					18.3
	8.9	13.6				0.035	0.027										41.5
	9.5					0.037	0.027										
	12.2					0.038	0.028										
	14.2					0.039	0.030										
	17.5					0.043	0.034										
						0.045	0.036										
						0.046	0.041										
						0.049	0.048										
						0.049	0.049										
						0.054	0.078										
						0.093											
						0.119											
Mean	7.2	4.5	5.1	0.36	0.19	0.039	0.030	0.080	0.087	0.23	3.7	6.8	11.4	2.2	0.1	0.2	8.2
RSD (%)	65	84	47		26	64	51	98	52	105	46	113	112	127			148
Median	7.1	2.9	4.6		0.17	0.036	0.027	0.056	0.090	0.16	3.8	1.9	5.4	0.7			4.7
Skewness	0.1	1.3	0.5		1.2	0.4	0.7	0.9	-0.2	1.0	-0.3	1.9	1.4	1.6			0.9
N	15	11	4	1	5	22	20	7	7	6	4	10	7	6	1	1	11

free fractions in blood therefore are low. Differences in OF/B ratios for different benzodiazepines may be related to differences in protein binding (43).

Almost all individual OF/B ratios observed in this study fall within the ranges observed in the Rosita-2 study (12). For that report, the actual oral fluid volume was used to calculate drug concentrations in undiluted oral fluid only by a few laboratories not including the Norwegian data. In our study, we have determined the dilution of oral fluid by analyzing the concentration of the blue coloring agent in the oral fluid-buffer mixture, and the Rosita-2 results presented here therefore are better reflecting the actual concentrations in undiluted oral fluid.

Some drugs may lower the secretion of oral fluid (e.g., cannabis, stimulants, and some medicinal drugs). In cases of dry mouth, the sample volume of oral fluid may be significantly lower than average sampling volumes, even if a volume indicator suggests that sufficient volume has been collected. The collected volume of oral fluid therefore should be determined if the aim is to estimate the drug concentration in undiluted oral fluid. The easiest way would be to weigh the samples before analytical testing; the drug concentrations in undiluted oral fluid may then easily be calculated. This was not done in our study. Instead, we determined the dilution by determining the concentration of the blue coloring agent, which is present in the buffer mixture of the Intercept collection kit. The primary advantage with our procedure is that variations in the buffer content in the oral fluid sampling kit do not affect the calculation of the dilution factor for oral fluid.

Conclusions

We observed large variations in drug concentration ratios between oral fluid and blood for a number of drugs. These results may better reflect the real-life variation in drug concentration ratios than those observed in some well-controlled pharmacokinetic studies. Our findings indicate that drug concentrations in oral fluid may not be used to accurately estimate drug concentrations in blood. A drug finding in oral fluid confirms recent drug use and may provide a semiquantitative suggestion of the blood drug concentration for some drugs. For psychiatric patients, oral fluid testing may be used as a non-invasive instrument in assessing substance use. For suspected drugged drivers, oral fluid testing may be used as initial on-site screening before it is decided whether a blood sample should be taken for forensic drug analysis.

Acknowledgments

The collection and analysis of samples from patients was financially supported by the Eastern Norway Health Authorities (grant no. 206/2005), and the analysis of samples from suspected drugged drivers was supported by the European Commission through the Rosita-2 project.

Thanks to the staff at Lovisenberg Deaconal Hospital and Sørlandet Hospital for collecting samples, and thanks to the staff at the Department of Analytical Method Development at the Norwegian Institute of Public Health for analyzing drug concentrations in samples of oral fluid and blood. Thanks to Åse Ripel for the administration of Rosita-2 samples and Inger Hasvold for the determination of the dilution factor for samples of oral fluid. The recruitment of participants and the collection of samples for this study were approved by the Norwegian Regional Ethical Committee.

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Manuscript received August 4, 2009;
revision received October 28, 2009.