Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of DUID

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Abstract

In recent years, the interest in the use of oral fluid as biological matrix has increased significantly, particularly for detecting driving under the influence of drugs (DUID). In this study, the relationship between the oral fluid and blood concentrations of drugs of abuse in drivers suspected of DUID is discussed. Blood and oral fluid samples were collected from drivers suspected of DUID or stopped during random controls by the police in Belgium, Germany, Finland and Norway for the ROSITA-2 project. The blood samples were analysed by GC-MS or LC-MS, sometimes preceded by immunoassay screening of blood or urine samples. The oral fluid samples were analysed by GC-MS or LC-MS(/MS). Scatter plots and trend lines of the blood and oral fluid concentrations as well as the median, mean, range and standard deviation of the oral fluid/blood (OF/B) ratios were calculated for amphetamines, benzodiazepines, cocaine, opiates and THC. The ratios found in this study are comparable with those that were previously published, but the range is wider. The OF/B ratios of basic drugs such as amphetamines, cocaine and opiates are > 1 (amphetamine: median [range] 13 [0.5-182]; MDA: 4 [1-15]; MDMA: 6 [0.9-88]; methamphetamine: 5 [2-23]; cocaine: 22 [4-119]; benzoylecgonine: 1 [0.2-11]; morphine: 2 [0.8-6]; codeine: 10 [0.8-39]). The ratios for benzodiazepines were very low, as could be expected as they are highly protein bound and weakly acidic, leading to low oral fluid concentrations (diazepam: 0.02 [0.01-0.15]; nordiazepam: 0.04 [0.01-0.23]; oxazepam: 0.05 [0.03-

0.14]; temazepam: 0.1 [0.06-0.54]). For THC an OF/B ratio of 15 was found (range [0.01-569]). In this study the time of last administration, the dose and the route of administration were unknown. Nevertheless the data reflect the variability of the OF/B ratios in drivers under the influence of drugs. The wide range of the ratios, however, does not allow reliable calculation of the blood concentrations from oral fluid concentrations.

Keywords: oral fluid/blood ratio, drugs of abuse, driving under the influence

Introduction

Drugs of abuse can be analysed in different biological matrices, and they all have their specific advantages and disadvantages. The advantages of blood are that usually the unchanged drug is detectable and that the blood matrix is relatively homogeneous. In cases of driving under the influence, blood is considered to be the best matrix for confirmation analysis, because the presence of drugs in blood corresponds best with recent use and impairment[1]. Difficulties may arise when only aged or haemolysed blood is available. Other disadvantages are the invasive way of sampling[2] and the difficulties encountered in some countries from the legal point of view in obtaining abusers' blood samples[3].

In recent years, the interest in the use of oral fluid as biological matrix has increased significantly, as this matrix displays some particularly interesting properties. Oral fluid can be obtained easily by non-medical personnel in a non-invasive and observable way. Other advantages of oral fluid analysis are less interference caused by endogenous compounds as compared to blood or urine[5] and the

presence of the parent drug[4]. On the other hand, the oral cavity can be contaminated by intranasal and smoked drug use, leading to extremely high concentrations in oral fluid. It is also difficult to obtain sufficient sample volume for the analysis[6], and the concentrations of benzodiazepines in this matrix are low[4]. Some correlation between oral fluid drug concentrations and impairment has been described[4,7,8,9,10]. In addition, Toennes *et al.*[11] demonstrate that oral fluid is superior to urine in correlating with serum analytical data and impairment symptoms of drivers under the influence of drugs of abuse. However, there is still some discussion concerning the use of oral fluid to determine impairment. Drummer[12] recently states that analysis of blood still enables a better interpretation of degree of exposure and likely drug effects. Therefore, research concerning the correlation of blood and oral fluid drug concentrations is of interest.

There exist some similarities between oral fluid and blood/plasma concentrations of drugs of abuse. The oral fluid concentrations, however, depend on the pH of oral fluid and blood, the protein binding of the drug and its pKa[13]. Oral fluid pH in healthy persons is usually between 6.2 and 7.4. For acidic drugs the equilibrium thus favours blood, hence oral fluid concentrations are lower than blood concentrations. Other factors that can influence the oral fluid/plasma ratio are molecular weight, lipid solubility, flow rate of oral fluid, fluctuating arterial-venous differences and elimination kinetics[14]. For basic drugs the oral fluid concentrations are higher and, as the pH decreases, a greater portion of the drug will be ionized and trapped in the oral fluid and consequently the oral fluid concentration increases. The correlation between drug concentrations in oral fluid and blood is not only influenced by factors associated with natural variation, but also by methodological aspects such as contamination and collection of oral fluid. The choice of oral fluid collection device plays a role, as *in vitro* experiments have shown that variations exist regarding the mean collection volume, the percentage of collected volume that can be recovered from the device and the recovery of the different types of drugs for the different collection devices. It is also known that stimulation of oral fluid affects oral fluid composition and resulting bicarbonate concentration, which in turn leads to a reduction in the concentration of basic drugs[15]. During the absorption phase, the oral fluid

concentrations are mostly higher because of local adsorption to the mucous membranes of the buccal cavity, leading to contamination of the oral fluid. This absorption effect is highest for THC because of its lipophilicity and ease of penetration through membranes. Another aspect of the lipophilicity of THC is that there is very little partitioning of THC between plasma and oral fluid[16].

In this article, the relationship between the oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs (DUID) is calculated and discussed. This study, however, does not give information concerning the correlation between oral fluid drug concentrations and the degree of impairment, as observations of impairment symptoms by police and medical officers were not rated and evaluated.

Materials and methods

Sample collection

Blood and oral fluid samples were collected from drivers suspected of DUID or stopped during random controls by the police in Belgium, Germany, Finland and Norway for the ROSITA-2 project[17]. In Norway, samples were also obtained from drug addicts and in Belgium from volunteers, mostly passengers in the car admitting recent drug use. Sometimes screening of blood or urine samples by immunological methods preceded the collection of confirmative blood and oral fluid samples. Blood samples were collected based on the existing legislative systems in de different countries. Oral fluid samples were collected with Intercept® (OraSure Technologies, Inc. Bethlehem, PA, USA). The Intercept® collector was used according to the manufacture's guidelines: the device was kept in the mouth for 3 minutes after wiping a few times between the lower teeth and cheeks. The interval between the collection of oral fluid and whole blood samples was less than one hour in 90% of the

collections. More details concerning the collection protocols for each specific country can be found in several publications[11,18,19,20,21] .

Laboratory analysis

Blood samples were analysed by gas (GC) or liquid (LC) chromatography, coupled to mass spectrometry (MS). Oral fluid samples were analysed by GC-MS or LC-MS(/MS). The cut-offs used for the determination of a positive/negative in neat oral fluid or in blood are given in Table 1. The analytical methods are described in the Rosita-2 report[17] and in several scientific publications[19,20,22-33]. Inter-laboratory comparison of analytical results was achieved by analysis of 3 control samples (QC) consisting of an oral fluid/buffer mixture spiked with benzodiazepines, amphetamine and structural analogues, cocaine, THC and their metabolites, as well as morphine, methadone and codeine (Table 2).

Data analysis

Calculations were made in Microsoft Excel and MedCalc Software (Broekstraat 52, 9030 Mariakerke, Belgium). The Passing & Bablok method was used for the calculations of the trendlines, a linear regression procedure with no special assumptions regarding the distribution of the samples and the measurement errors. Linearity was evaluated by means of the Cusum test for linearity. When calculations were made for the sum of substances, the molar concentrations were used.

Outlier analysis was performed with WinSTAT®. All data for which either the distance of the mean is greater than four times the standard deviation of the variable or the probability of finding at least one value at this distance from the mean in a normally-distributed sample is less than 0.05 were considered as outliers.

For the calculation of the scatter plots and trend lines, only the data from individuals for whom the concentration of the particular drug in blood or in oral fluid or in both matrices was positive were used. For the calculation of the median, mean, range and standard deviation of the oral fluid/blood (OF/B) ratios only data from individuals for whom the concentration in blood and in oral fluid was positive for the particular drug were used.

Results

Relationship between blood and oral fluid concentrations

The scatter plots and trend lines of the relationship between the blood and oral fluid concentrations of the amphetamines, benzodiazepines, THC, cocaine (and benzoylecgonine) and opiates are given respectively in Figure 1 to 5. There were an insufficient number of data points for methamphetamine, MDEA, clonazepam, temazepam and lorazepam for these calculations. The Cusum test for linearity resulted in a p-value <0.05 for almost all substances. The test showed no significant deviation from linearity for the following substances: the sum of the benzodiazepines, nordiazepam, cocaine, BZE and the sum of cocaine and benzoylecgonine. In addition, a scatter plot showing the linear relationship between serum log[THC] and oral fluid log[THC] of the data in this study is given in Figure 6. In Table 3 the median, mean, range and standard deviation of the OF/B ratios and number of outliers of the different types of drugs of abuse are shown.

Time interval

The influence of the time interval between oral fluid and blood sampling on the OF/B ratio of THC is presented in Figure 7. These data show that the time interval between blood and oral fluid sampling has an influence on the ratio for THC. As could be expected, the ratio increases as the blood is sampled later and the oral fluid earlier. This trend was however not statistically significant ($p = 0.15$).

Discussion

According to the Cusum test, the presented data show a slight correlation between the serum and oral fluid drug concentrations for the sum of benzodiazepines, nordiazepam, cocaine, benzoylecgonine, as well as the sum of cocaine and its metabolite. In Fig. 6, the conclusion of Ramaekers *et al.*[34] that log[THC] levels in serum and oral fluid are correlated is confirmed. However, the correlation found in this study is less strong than the correlation found by Ramaekers *et al.* (R^2=0.21 compared to R^2=0.84)[34]. The difference is probably partially due to the fact that Ramaekers *et al.*[34] studied serum and oral fluid THC levels in subjects after controlled administration of the drug, while in this study there was a wide variation in dose, route of administration and time interval between use and sampling.

The OF/B ratios found in this article are comparable with those that were previously published (Table 4), but the range is wider. When comparing the results, a distinction should be made between OF/B and oral fluid/plasma ratios. Drug blood concentrations can differ from plasma concentrations due to binding onto red blood cell membranes or storage in red blood cell cytoplasm, depending on the drugs protein binding and its structural characteristics. The wider range could partially be due to analytical inter-laboratory variations as observed in Table 2. However, the variations observed during the ROSITA study are comparable to the ones described by Clarke and Wilson[35] evaluating a

proficiency testing of drugs in oral fluid. Moreover, the experimental set-up could also lead to variation, as not all laboratories determined the exact oral fluid sample volume obtained from the Intercept devices, leading to semi-quantitative results. Again, the more variable time of last administration, the differences in dose and route of administration will also result in a wider range as compared to experimental studies. These are limitations of the presented study; however, the presented results demonstrate the reality of roadside-testing.

The oral fluid concentrations of basic drugs such as amphetamines, cocaine and some opioids are higher than those in blood. The ratios for benzodiazepines were very low as result of their highly protein bound and weakly acidic characteristics, leading to low oral fluid concentrations. For THC, the median OF/B ratio was 15.4 in this study. When taken into consideration that the blood/plasma ratio of THC is 0.55[36], it can be estimated that the oral fluid/plasma ratio of the subjects in this article would be about 8.5. This ratio is higher than the ratios reported by Samyn and van Haeren[37] and by Huestis and Cone[38]. Samyn and van Haeren[37], however, mention that the stability of THC in their oral fluid samples stored at -18°C in a plastic tube and not centrifuged before storage was poor. Lower oral fluid concentrations in the study by Huestis and Cone[38] could partially be explained by the collection of oral fluid under stimulated conditions. The ratios found by Kauert *et al.*[39] were higher than the ratio in this article, while the oral fluid was also collected under non-stimulated conditions (Intercept®). However, in our study there was a large variation of OF/B THC ratios, which ranged from 0.01 to 568.91. In addition, differences in experimental setup between the controlled study performed by Kauert et al. and the ROSITA study, such as time interval between oral fluid and blood collection as demonstrated in Figure 7, can have an influence on the ratios. Although the trend between time interval of oral fluid and blood collection and the OF/B ratio was not statistically significant (Fig. 7), it should be studied more carefully in the future, especially concerning the influence of time of drug intake. Implementation of more standardised research protocols will hopefully lead to less variation of the results and yield more precise OF/B ratios.

Conclusion

The wide range of the ratios for the different drugs of abuse in this study does not allow reliable calculation of the blood concentrations from oral fluid concentrations. Limitations of this study are the unknown time of last administration, dose and route of administration, as well as the unknown exact oral fluid sample volume obtained from the Intercept devices. These limitations together with the inter-laboratory analytical variation result in a wider range of OF/B ratios compared to published experimental studies. Nevertheless these data reflect the realistic variability of the OF/B ratios in drivers under the influence of drugs.

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Table 1: Cut-offs used in oral fluid or in blood

Substance	**Cut-off blood (ng/mL)**	**Cut-off oral fluid (ng/mL)***
Amphetamine	20	25
Benzoylecgonine (BE)	20	8
Clonazepam	5	5
Cocaine	20	8
Codeine	10	20
Diazepam	50	5
Lorazepam	10	5
MDA	20	25
MDEA	20	25
MDMA	20	25
Methamphetamine	20	25
Morphine	10	20
Nordiazepam	50	5
Oxazepam	50	5
Temazepam	50	5
THC	1	2

*: in neat oral fluid: the Intercept® device contains about 800 µL of buffer, leading to a dilution of the collected oral fluid of about 1:3. All measured concentrations were thus multiplied by 3.

Table 2: Analyte concentrations spiked to oral fluid QC samples for inter-laboratory comparisons and the range of the coefficients of variation (CV)

Analyte	Concentrations (µg/L)	CV% (range)
Amphetamine	21, 41, 62	6-15
Methamphetamine	21, 63	19-20
MDMA	20, 40, 60	16-66
MDA	23, 68	16-17
MDEA	21, 62	18-24
Cocaine	7, 20	20-21
Benzoylecgonine	6,13,19	33-69
6-monoacetylmorphine	2, 5	35-45
Codeine	19, 56	24-25
Methadone	19, 37, 56	14-25
THC	2, 5	50-67
11-OH-THC	1, 3	43-44
THC-acid	5, 15	37-48
Diazepam	5, 13	34-63
N-desmethyldiazepam	5, 13	10
Temazepam	5, 14	14-27
7-aminoflunitrazepam	2, 7	28-62
Bromazepam	14, 42	35
Lorazepam	4, 13	26-32
Clonazepam	4, 9, 13	29-35
Zopiclone	12, 37	42
Zolpidem	10, 29	29-35

Table 3: Median, mean, range and standard deviation of the oral fluid/blood ratios and the acid dissociation constant (pKa) and the number of outliers of the different types of drugs of abuse

Substance	**pKa [36,40]**	**Median**	**Mean**	**Range**	**N**	**Outliers**	**Standard deviation**
Amphetamines (µmol/L)		12.07	18.20	0.27 – 182.13	177	10	22.43
Amphetamine	9.9	13.43	19.01	0.47 – 182.13	148	9	22.85
MDA	9.7	4.38	5.14	1.28 – 14.61	22	7	3.40
MDMA	9.4;8.7;8.8	5.57	10.37	0.88 – 88.19	41	11	15.20
Methamphetamine	9.9	5.19	8.05	2.20 – 23.00	6	2	7.69
Benzodiazepines (µmol/L)		0.04	0.59	0.002 – 19.02	48	4	2.77
Diazepam	3.4	0.02	0.04	0.01 – 0.15	21	7	0.10
Nordiazepam	3.5;12.0	0.04	0.05	0.01 – 0.23	22	6	0.04
Oxazepam	1.7;11.6	0.05	0.07	0.03 – 0.14	6	6	0.04
Temazepam	1.3	0.10	0.18	0.06 – 0.54	5	4	0.20
THC	10.6	15.37	34.08	0.01 – 568.91	277	10	63.41
Cocaine + benzoylecgonine (µmol/L)		1.80	4.57	0.19 – 78.89	40	8	12.33
Cocaine	8.6	21.84	30.24	3.76 – 119.35	18	10	28.62
Benzoylecgonine		0.91	1.47	0.19 – 10.62	40	8	1.80
Morphine + codeine (µmol/L)		7.17	7.16	0.91 – 13.36	14	5	4.34
Morphine	8.0;9.9	2.25	2.80	0.77 – 5.70	6	5	1.81
Codeine	8.2	9.61	10.19	0.79 – 39.0	13	3	9.30

Table 4: Oral fluid/blood and oral fluid/plasma ratios for several drugs of abuse found in literature and median and range of oral fluid/blood ratios found during ROSITA 2

Type of drug	Literature: Oral fluid/plasma	ROSITA 2: oral fluid/blood	
		Median	Range
Alcohol (ethanol)	1.08 (1.06-1.09)[41]*		
Amphetamine	2.8[42] 6.6 – 20.2[37] 15.3 (2.6 – 210)[43]	13.4	0.5 – 182.1
Barbiturates	0.3[44-47]		
Benzoylecgonine	0.4 (0.3-0.5)[48] 0.6 – 1.3[37]	0.9	0.2 – 10.6
Buprenorphine	IM: 0.1 – 0.4; SL: >1[49]		
Codeine	3.7 (±0.3)[50] 4.0 (±0.5)[51] 7.5 – 43.7[37]	9.6	0.8 – 39.0
Cocaine	0.5[52] 3[53] 8.7 (3.8-13.2)[48] 15 – 36[37]	21.8	3.8 – 119.4
Diazepam	0.01-0.02[54,55]	0.02	0.01 – 0.15
GHB	0.2 – 0.5[56] <1[57]*		
Heroin	IV: 0 – 1.9[58]* smoking: 0 – 784[58]*		
MDMA	6.4-18.1[59] 0.8 – 22.4[60]	5.6	0.9 – 88.2

	1.0 – 16.5[37]		
Methadone	0.5 (±0.1)[61] 1.3[62] 1.5 - 1.7 (oral fluid/serum)[63] 0.6 – 7.2[64]		
Methamphetamine	Oral: 2.0 (0.0-23.0)[65] 7.8 (±0.5)[66] IV: 6[67] smoking: 5.1[67]	5.2	2.2 – 23
Morphine	4.0 – 154.2[37] IV: 0 – 1.8[58]* smoking: 0 – 29[58]*	2.3	0.8 – 5.7
THC	0.2 – 3.1[37] 1.2 (±0.6)[38] 46.2 (±27.0) (low dose: 18.2 ± 2.8 mg); 35.8 (±20.3) (high dose: 36.5 ± 5.6 mg)[39]	15.4	0.01 – 568.9

IV: intravenous / IM: intramuscular / SL: sublingual

*: oral fluid/blood ratio

Figure 1: Scatter plots and trend lines of the blood and oral fluid concentrations (in ng/mL) of amphetamine, MDA, MDMA, methamphetamine, and the sum of the concentrations of these substances (µmol/L)

Figure 2: Scatter plots and trend lines of the blood and oral fluid concentrations (in ng/mL) of diazepam, nordiazepam, oxazepam, temazepam, and their sum (µmol/L)

Fig 3: Scatter plot and trend line of the blood and oral fluid concentrations (in ng/mL) of THC

Figure 4: Scatter plots and trend lines of the blood and oral fluid concentrations (in ng/mL) of cocaine, benzoylecgonine (BZE) and the sum of the concentrations (µmol/L) of these substances

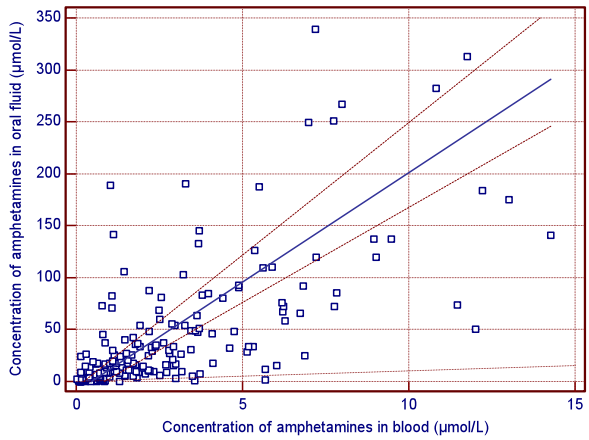
Figure 5: Scatter plots and trend lines of the blood and oral fluid concentrations (in ng/mL) of morphine, codeine and the sum of the concentrations (in µmol/L) of these substances

Figure 6: Scatter plot showing the linear (95% CI) relationship between serum log[THC] and oral fluid log[THC] (Regression equation: $Y=0.9248+0.8445X$; $R^2=0.21$)

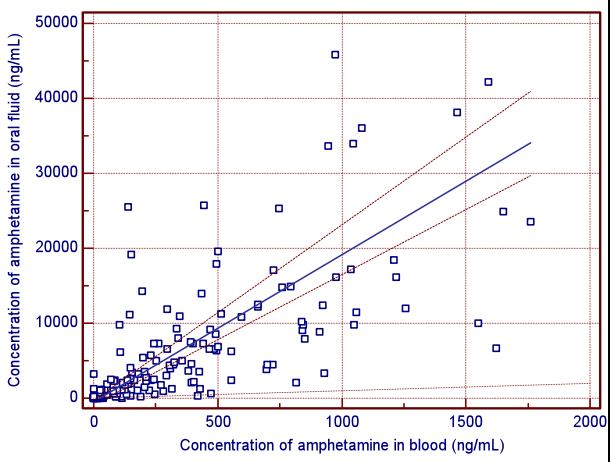
Figure 7: Box-and-whisker plots of the oral fluid/blood ratio of THC in function of the time interval (in minutes) between oral fluid and blood sampling. In interval A blood was sampled between 70 and 20 minutes before oral fluid. In interval B blood was sampled between 19 minutes before and 29

minutes after oral fluid and in interval C blood was sampled between 30 and 95 minutes after oral fluid

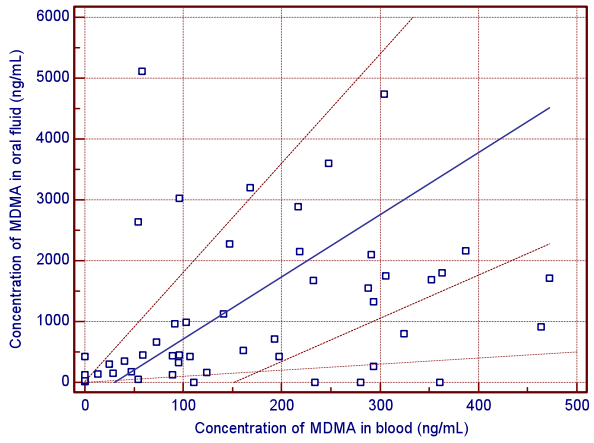
Amphetamines (sum) (y = -9.58+21.07x)



Amphetamine (y = -493.18+19.66x)



MDMA (y = -311.16+10.22x)



MDA (y = -114.29+13.44x)

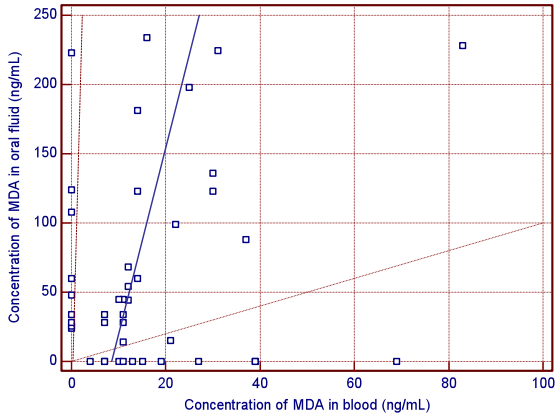


Figure 1

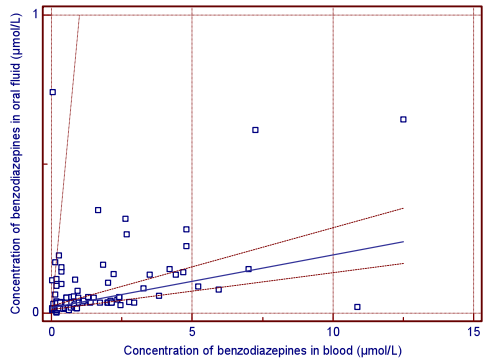
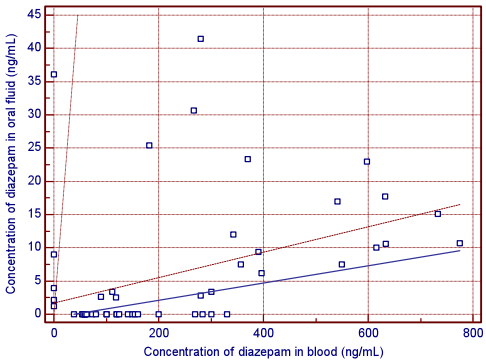
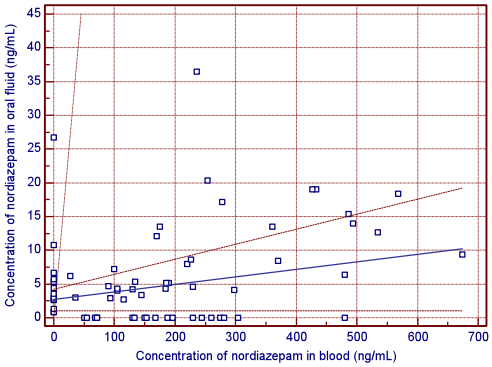
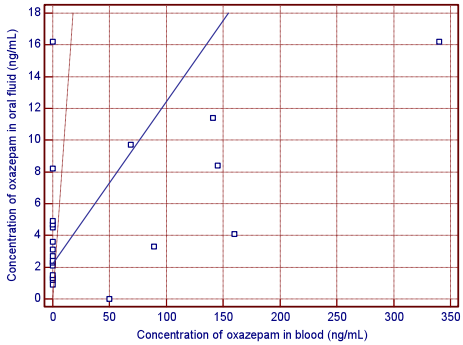
Benzodiazepines (sum) (y = 0.018+0.018x)	**Diazepam** (y = -0.49+0.01x)
	
Nordiazepam (y = 2.70+0.01x)	**Oxazepam** (y = 2.20+0.10x)
	

Figure 2

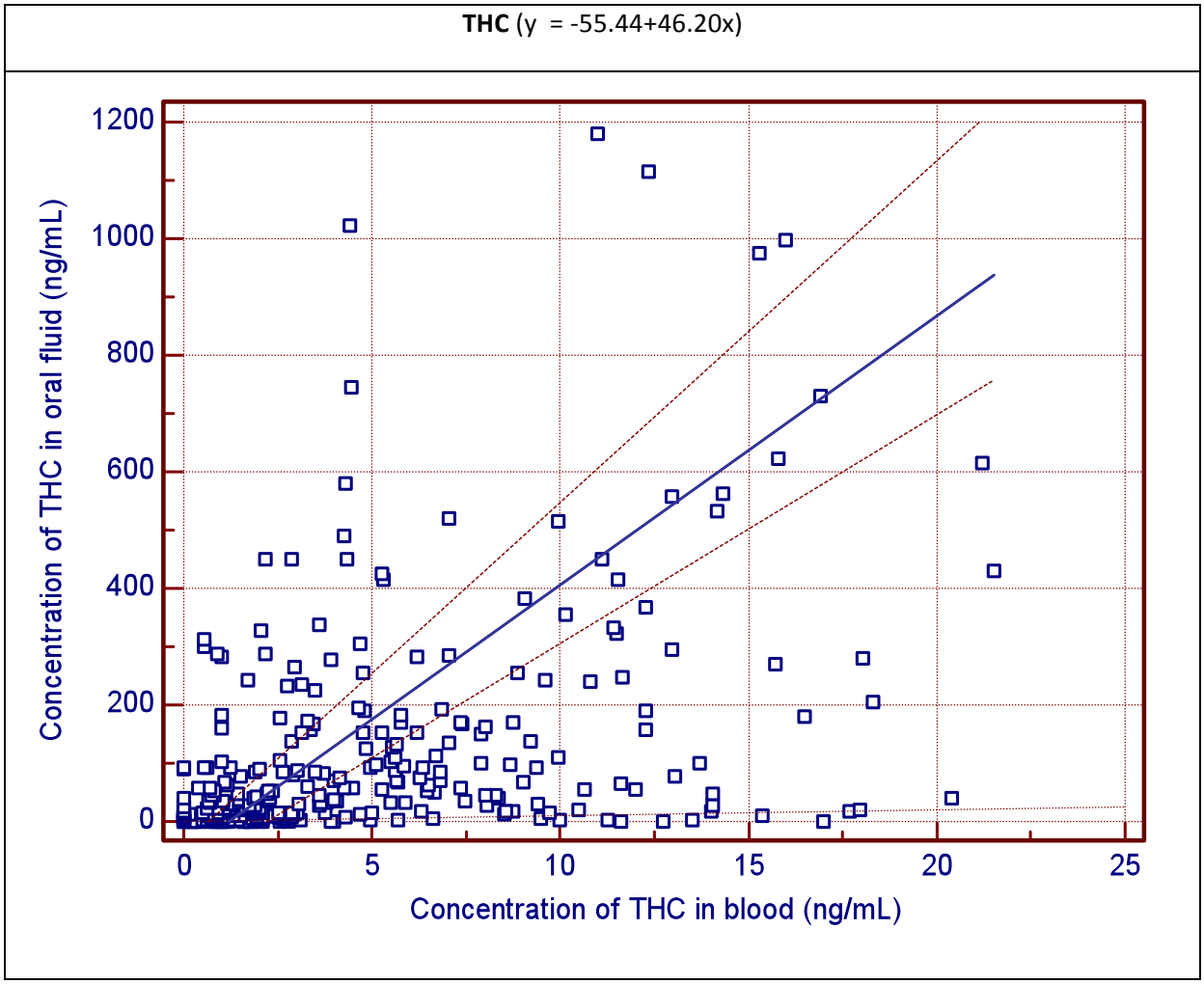
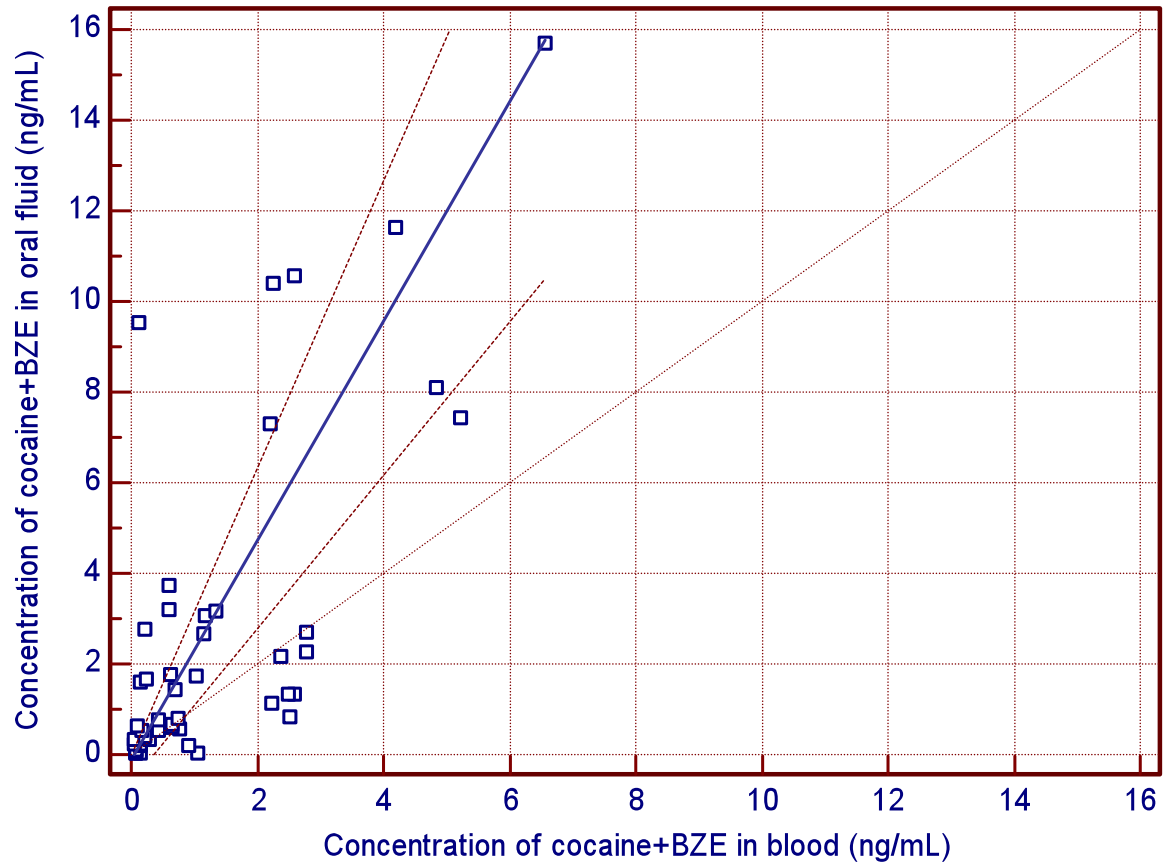
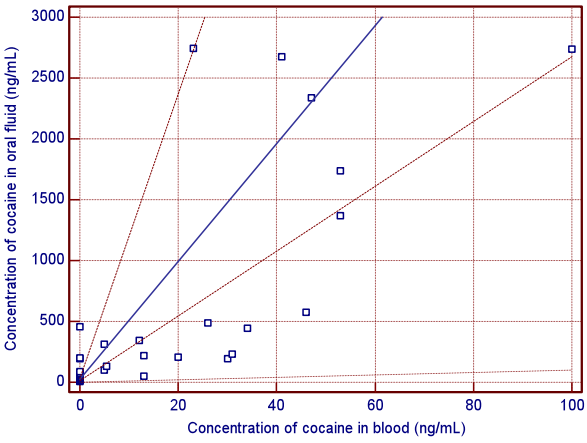

THC (y = -55.44+46.20x)
Concentration of THC in oral fluid (ng/mL)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Concentration of THC in blood (ng/mL)

Figure 3

Cocaine + Benzoylecgonine (y = -0.13+2.42x)



Cocaine (y = 22.20+48.49x)



BZE (y = 28.84+0.62 x)

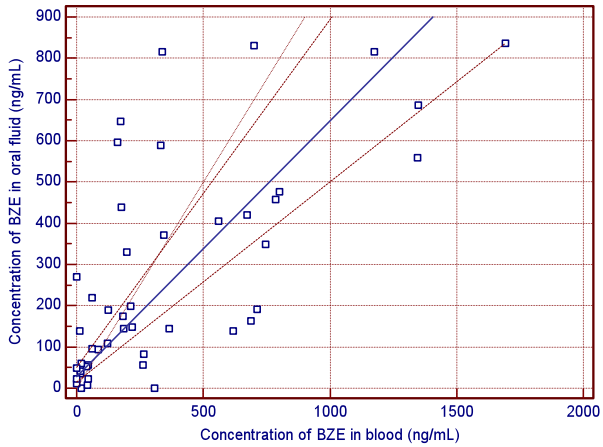
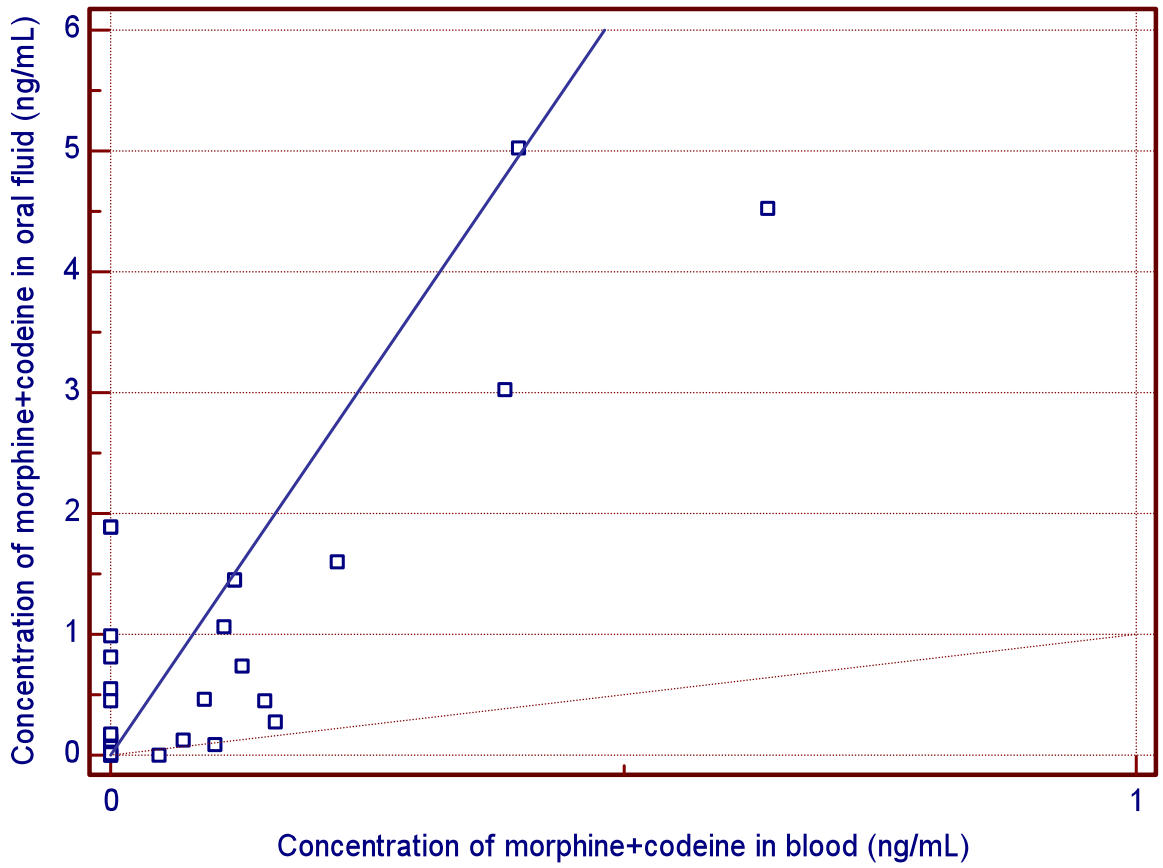
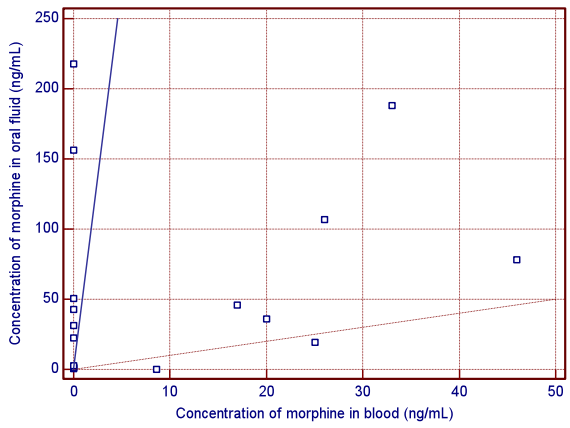


Figure 4

Morphine + Codeine (y = 0.004+12.46x)



Morphine (y = 1.14+54.39x)



Codeine (y = -12.62 +13.55x)

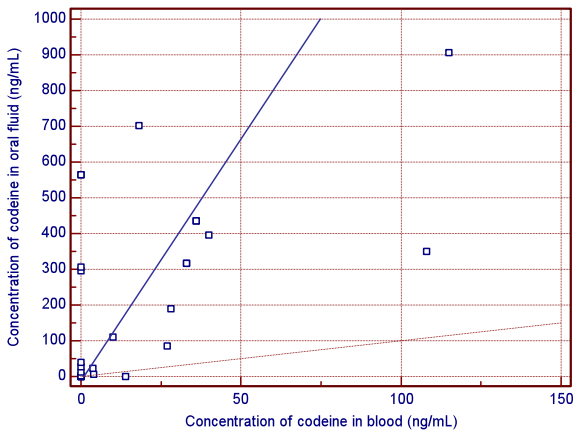


Figure 5

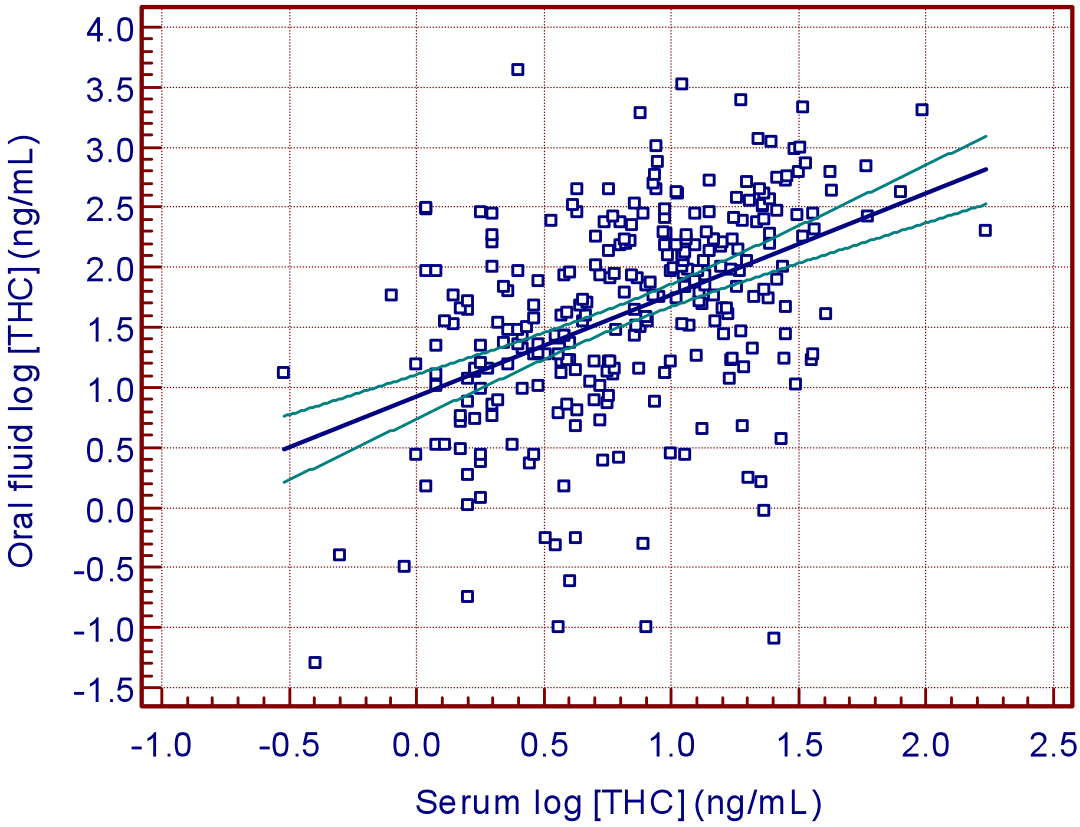

Oral fluid log [THC] (ng/mL)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
Serum log [THC] (ng/mL)

Figure 6

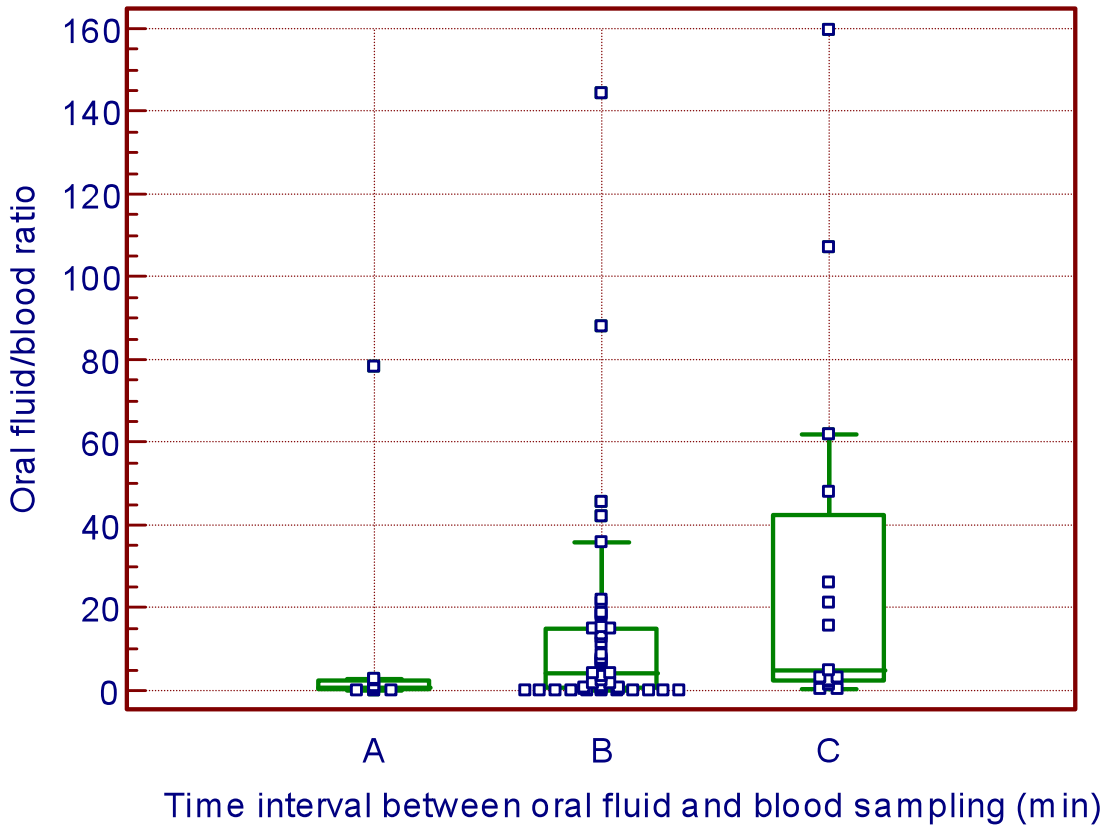

160
140
120
100
80
60
40
20
0
Oral fluid/blood ratio
A
B
C
Time interval between oral fluid and blood sampling (min)

Figure 7