Driving Under the Influence of Drugs—Amphetamine Concentrations in Oral Fluid and Whole Blood Samples

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Abstract
This study investigated amphetamine concentrations in both oral fluid and whole blood samples of persons suspected of driving under the influence of drugs. The data for the study were obtained from 153 cases. The mean volume of oral fluid collected with the Intercept oral fluid collection device was 224 μL. Because of the small sample volume of oral fluid, the results of the amphetamine concentrations in oral fluid were not used in the calculations for 39 cases. The total number of cases positive for amphetamine in oral fluid was 100 out of 114. In seven cases the oral fluid sample was positive (cutoff 25 pg/L), even though the whole blood sample was negative (cutoff 20 pg/L). All of the cases found positive in whole blood (n = 93) were also positive in oral fluid. Oral fluid would therefore be well suited as a testing matrix for amphetamine when driving under the influence is suspected. The results nevertheless indicated that the cutoff used for amphetamine in oral fluid (i.e., 25 pg/L) could be higher to correspond to the window of detection given by the level of 20 pg/L in whole blood.

Introduction
The possibility of using oral fluid (OF) as a biological matrix for detecting drugs in forensic cases has been of great research interest during the past 10 years. Numerous articles have been published on the detection of drugs in OF. Some aspects that have been focused on recently are OF collection (1,2), impairment and drug concentration in OF (3), detection time with OF (4), and the correlation of drug concentration in OF and other matrices (5-7).

A full correlation between matrices may not be possible, as the relation for drugs between different sample matrices is dependent on the time after intake (8). Further factors that influence the diffusion of drugs from plasma to OF are the pKₐ, physical size and configuration, charge, dose, degree of protein binding and lipid solubility of the drug, and individual factors such as the pH of blood and saliva, salivary flow rate and salivary enzymes, metabolizing rate, and clearance (1,9).

The amphetamine (AM) levels in OF have been shown to be higher than in plasma (10), and they lack the pH dependence that causes variability in the AM concentrations in urine. AM abusers are prone to hyposalivation because of the adrenergic effect of the drug (9), a fact that must be taken into account when evaluating drug concentrations in OF and sample volume. Correlation studies have shown that when AM is detectable in serum samples, it is also detectable in OF samples (7).

Previous studies comparing AM levels in OF to blood/plasma have focused on pharmacokinetics, but only few are based on data from real cases of abuse (3,6,7). The aim of this study is to demonstrate the relationship between concentrations of AM in OF and those in whole blood for cases of driving under the influence. This study presents 153 cases of driving under the influence, in which the AM levels in both OF and whole blood (WB) have been quantified.

Materials and Methods
Sampling
The samples were collected as a part of the Roadside Testing Assessment 2 project (ROSITA2). The test subjects were persons suspected by the police for drug driving. All persons participating signed a consent form and gave an OF sample voluntarily. The blood samples used to confirm drug use were collected by routine procedures. The samples were collected between May 1, 2004, and June 30, 2005. The samples included in this study were the cases for which both WB and OF samples were available. The samples were collected during the clinical evaluation that was mandatory for all driving under the influence of drugs cases. The police collected the OF samples and were instructed to collect it at the same time as the WB sample.

OF samples
The samples of OF were collected with the Intercept device (OraSure Technologies, Bethlehem, PA). The use of the collector includes swabbing the inside of the lower cheek with a pad and keeping the pad in the oral cavity for 3 min. The pad...
was then placed in a cap containing buffer solution, sealed, and sent to the laboratory. The samples were weighed and stored at 
−20°C in the laboratory from a few days to two months until analyzed.

The maximum OF volume obtainable with this device was stated by the manufacturer to be 1 mL. The volume of buffer in the Intercept devices was calculated using six replicates, and the mean was used for the calculations. The sample volume was assessed by weighing sets of empty test tubes. The Intercept devices were placed in the empty test tubes and the contents of the Intercept device transferred to the test tubes by centrifugation at 2000 × g for 15 min. After centrifugation, the Intercept devices were removed from the test tubes, and the test tubes containing the sample and buffer were weighed. The sample volumes were calculated using the mean weight of the buffer and the weights of the test tubes. The masses were converted to volumes, assuming the density of OF to be 1 kg/L. The density was determined from the composition of OF (1) and previous studies using this assumption (2).

Blood samples

WB samples were collected for laboratory analysis from test subjects suspected of drugged driving. All samples were collected by health care professionals, and the test subjects were referred to them by the police. WB samples were collected into vacuum tubes containing potassium oxalate and sodium fluoride (Venoject® Terumo Europe, Leuven, Belgium). The samples were stored at 4°C in the laboratory for a few days until analyzed.

AM assay

The AM assay for OF was performed as described previously (11). The method described (12) was used for WB samples. The AM cutoff values used were 20 and 25 µg/L for WB and OF matrices, respectively. The results were normalized using the weighed volume of OF sample to achieve concentrations corresponding to the standard sample volume of 250 µL. Those samples, where less than 50 µL was recovered, were analyzed but omitted from the statistical evaluation of the data. The reason for choosing 50 µL as a limit was that it was regarded that the quantitative result could not be considered reliable because of the increase in variation.

Data handling

The gas chromatography–mass spectrometry (GC–MS) data system used was a Hewlett-Packard (now Agilent) Chem-Station A.03.00. Further processing was done with Microsoft Excel 2003 and Statistical Package for Social Sciences (SPSS) 14.0 for Windows.

Results and Discussion

Case descriptions

GC–MS confirmation of AM in OF and WB samples was done in 153 cases. The oldest test subject was 59 years old, and the youngest was 19 years old. The mean age of the subjects was 32.3 years [standard deviation (SD) = 8.82 years], and the percentage of women was 20.9%.

Polydrug use, especially the use of benzodiazepines together with drugs of abuse, is quite common in the Nordic countries (13). Many other illegal drugs and medicinal drugs besides AM, especially benzodiazepines, were found in the blood samples. Some kind of illegal drug or medicinal drug were found in all samples and of the WB samples 83.6% were positive for AM. Of the 25 blood samples negative for AM, benzodiazepines and other medicines were found in 10, other drugs of abuse were found in 8, and combinations of an illegal drug and benzodiazepines were found in 6 samples. Of all the samples positive for AM, 42 were also positive for benzodiazepines, and five samples were positive for other medicines. Some other drug than AM was found in 26 samples, and in 17 of these benzodiazepines were also found. Two blood samples were positive for AM, another drug of abuse, benzodiazepine, and some other medicinal drug.

The reason for this high prevalence of AM and other substances is that the police, who apprehended the persons suspected for driving under the influence, were directed to further investigation in only the cases where they had a clear and well-stated reason for presuming the use of drugs. The police aids used for determining drug use were a mini-DRE test and point-of-collection rapid tests such as Securetec Drugwipe.

OF sample volume

The volume of the buffer in Intercept was weighed into six replicates. The mean volume of the buffer was 753 µL. The range of the six samples varied from 730 to 776 µL. The SD was 15.5.

The weighted mean sample volume was 224 µL, the SD was 231 µL, and the median was 140 µL. This included samples with volumes less than 50 µL. The maximum sample volume collected was 795 µL. The weighted volumes are shown as a histogram in Figure 1.

In the majority of samples, the weighted volume was below the volume of the standard samples (Figure 1). The maximum volume collectable with Intercept was stated by the manufacturer to be 1 mL. The sampling capacity of Intercept had been studied previously (2) with the aid of an actual situation simulating the weighing method. The mean volume recovered in that study (n = 10) was 640 µL. The mean sample volume collected from these real measurements with the described calculating system was considerably lower.

AM concentration in OF

The distribution of the AM concentrations confirmed to be positive in OF was randomly spread: the median was 7,440 µg/L and the SD was 17,600 µg/L. High concentrations as well as low concentrations were found (min = 27.8 µg/L, max = 131 000 µg/L) (Figure 2). Fourteen cases were negative for AM, rendering a percentage of 87.7% (100 out of 114) positive for OF when OF sample volume was sufficient.

Concentration level and volume of OF

A large proportion of the samples in which the volume of OF was insufficient were confirmed positive for AM in blood. AM
is known to cause hyposalivation, but conclusions of this cannot be drawn directly from this study. The majority of the subjects were under the influence of other substances in addition to AM as stated in the Case Description section.

The relationship between OF volume and AM in OF is shown in Figure 3 for the individual cases. All negatives are included to show the range of sample volume among the negatives with a sufficient sample volume for quantification (n = 14, median = 116 μL, and SD = 256 μL). As the concentration and the limits of quantification are dependent on OF volume, Figure 3 must be interpreted with caution.

**AM concentrations in WB**

The distribution of the results over the concentrations in blood is shown in Figure 4. The median for the concentrations of the positive cases was 455 μg/L, the SD was 503 μg/L, and the range was 45–2750 μg/L. The number of negative cases was 25, and 83.6% (128 out of 153) were positive for AM in WB.

These concentrations are well in agreement with previous reports on AM in WB for offenders driving under the influence in Scandinavia. In a group of driving under the influence offenders in Norway who were found positive for AM and/or methamphetamine (878 cases), the highest concentration of total AM and methamphetamine in blood was 3740 μg/L and the median was 520 μg/L (14). Blood AM concentrations for driving under the influence cases have been found to be as high as 17,000 μg/L without fatal outcome (15), the median AM concentration was 700 μg/L in a population of 6613
Swedish driving under the influence of drug offenders. Such high concentrations (> 5000 µg/L) were not detected in the blood samples of this study.

Correlation

The numbers of negative and positive samples for both matrices are shown in Table I. All of the OF samples confirmed negative were also confirmed negative in the WB sample. However, seven samples confirmed negative in the WB matrix were found positive in the OF matrix. The numerical data for these are shown in Table II, along with other substances confirmed positive for these cases.

The correlations (r = 0.433 Pearson’s bivariate two-tailed, P = 0.000) between AM concentrations for both matrices are shown in Figure 5. The time interval between administration of the drug and taking the sample is not known and vary from case-to-case. A full correlation between the quantitative results in different body fluids could not be obtained due to the different time profile that the excretion and metabolism has in the different matrices (4,8). Our study is in line with others (6,10), indicating that the AM concentrations are higher in OF than in blood. The median ratio of OF to WB AM concentration is 15.3, but as the data is obtained in different unrestrained factors affecting the ratio, no assumptions can be drawn to a general conclusion. The range (2.57-210) and SD of 32.1 also clearly indicated that the ratio heavily depends on the time profile and individual variations. The dose consumed was not known, and this might affect the numerical correlation.

For methamphetamine, the dose seemed to affect the disposition in OF (16). Chronic drug use may also be a factor causing higher concentrations in OF than in blood (17).

The few extremely high AM concentrations in OF that were not equivalent to the concentrations in WB could be explained as contamination of the oral cavity. A large proportion of the AM collected would in these contamination cases originate from residuals of ingested AM and not from excretion via the salivary glands.

Conclusions

The present study demonstrates in a large number of drugged driving cases the correlation between AM in OF and in WB. A statistically significant correlation of the AM concentrations of both matrices could not be shown in this material. The interpretation of the results is, however, congruent in that the cases positive for AM in WB were also deemed positive for OF. The AM concentrations in OF were significantly higher than those in WB. The detection time window for which AM was found was also longer for OF than for WB using these cutoff points. The present results therefore indicate that the limits of detection for AM in OF should be higher than for WB in order for the window of detection to be comparable.

Table I. Descriptive Statistics for the Samples

<table>
<thead>
<tr>
<th>Oral Fluid</th>
<th>Whole Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Positive</td>
</tr>
<tr>
<td>Oral Fluid</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>93</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Insufficient amount of oral fluid</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
</tr>
</tbody>
</table>

Table II. Cases Positive for Amphetamine in Oral Fluid but Not in Whole Blood

<table>
<thead>
<tr>
<th>Oral Fluid Sample Amount (µL)</th>
<th>Amphetamine in Oral Fluid (µg/L)</th>
<th>Other Substances Found in Whole Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>1230 negative</td>
<td>THC and THCC</td>
</tr>
<tr>
<td>86</td>
<td>160 negative</td>
<td>ethanol, temazepam, and nor Diazepam</td>
</tr>
<tr>
<td>795</td>
<td>150 negative</td>
<td>THCC, buprenorphine, diazepam, nordiazepam, oxazepam, and temazepam</td>
</tr>
<tr>
<td>536</td>
<td>144 negative</td>
<td>THCC, diazepam, nordiazepam, clonazepam, midazolam, and norbuprenorphine</td>
</tr>
<tr>
<td>412</td>
<td>86.6 negative</td>
<td>ethanol, phenazepam, and alprazolam</td>
</tr>
<tr>
<td>170</td>
<td>70.1 negative</td>
<td>MDMA and MDEA</td>
</tr>
<tr>
<td>413</td>
<td>27.8 negative</td>
<td>diazepam and nordiazepam</td>
</tr>
</tbody>
</table>
Acknowledgments

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References