Methods of Saliva Analysis and the Relationship between Saliva and Blood Concentration

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ABSTRACT

Immunologic analysis, originally developed for urine, is also effective for saliva. Using fluorescence polarization immunoassay (FPIA), we analyzed saliva directly (without former extraction) for barbiturates, opiates, cannabinoids, amphetamines, and cocaine. By using calibration standards, cut-off limits were established. The presence of benzodiazepines was tested using radioimmunoassay. Substance concentration in saliva, given some conditions, is equivalent to the nonprotein-linked serum concentration. In a series of analyses with codeine and cyclobarbital, we showed that an essential prerequisite is a certain ph-value in saliva. Thus, we determined the following saliva/plasma ratios: amphetamine, 2.4; barbiturates, up to 0.5; cannabinoids, 1 to 2; cocaine, 1 to 2; morphine, 0.2; codeine, 1 to 3; benzodiazepines, 0.03. In addition, saliva was tested for aliphatic alcohols by head space gas chromatography. Studies with subjects showed that the dynamics of elimination, and thereby the concentration ratio between saliva and blood, are strongly interdependent. Thus, the saliva method proved to be an efficient tool for epidemiologic studies dealing with psychoactive substances, including alcohol and licit and illicit drugs.

INTRODUCTION

The aim of forensic-toxicological analysis is to detect and quantify exogenous substances, poisonous agents or drugs in different body fluids. Of those, urine samples are better apt to identify active substances whereas blood samples should be used to measure the concentration of effective compounds. Knowledge about the distribution of drugs in plasma and in saliva is important because saliva is much easier to collect for drug analysis (Muelenbruch, 1982). The advantage of analysing drugs in saliva is to receive an equivalent of the non-protein bound part of a substance without necessarily taking blood samples. This method can be easily applied in but there are some inconveniences:

1. The pH-value of saliva is not constant and therefore influences the proportion between the distribution ratio of plasma and saliva.
2. Portions of drugs orally taken can cause contaminations producing high concentration of the drug in saliva.
3. The saliva-plasma concentration quotient is increasing in the resorption phase.
Despite these shortcomings saliva analysis is a promising tool. This was shown in a series of experiments with alcohol, different drugs and different measuring methods done by the Institut of Forensic Medizine in Wuerzburg. In the following paper, typical results for ethanol, its congeners, and codeine are shown.

MATERIAL AND METHODS

Saliva was sampled with the so-called “salivette” (SARSTED). This cotton swab is centrifuged afterwards. However, using the “salivette” frequently yields insufficient amounts of saliva. Whereas 0.2 mL of saliva is sufficient for the identification of ethanol, at least 0.5 to 1.0 mL is required for simultaneously identifying other aliphatic alcohols.

The individual alcohols were identified by Headspace-gaschromatography (model 8500 PERKING-ELMER). The Fluorescence-polarisations-immunoassay (FPIA) System from ABBOTT was used to analyse the saliva samples for licit and illicit drugs. The double antibody radioimmunoassay from BIERMANN Company was used to measure benzodiazepines and benzodiazepine metabolites.

RESULTS

Ethanol

Approximately 2 hours after alcohol consumption, there is a close relationship between saliva and blood ethanol concentrations. The largest difference between these two concentrations was about 0.30 mg/g.
Figure 1 shows a typical example for the course of ethanol concentration in blood and saliva. The test subject was a 24 years old female who consumed wine. The dosage was 0.7 g ethanol/kg body weight; drinking time was 60 minutes (Schulz et al. 1986).

**Methanol**

Encouraged by these results, we analysed saliva for congeners, too, which are present in alcoholic beverages and are found in blood after consuming these beverages (Figure 2).

**Figure 2**

*Methanol in Blood and Saliva (in µg/g) after the Consumption of Wine: Mean Values with Standard Deviation*

Compared to the blood-methanol concentration, the saliva-methanol concentration is lower by a factor of about 0.9. The course of both curves is parallel and ends in a plateau. This is due to the fact that methanol is not metabolized as long as there is ethanol in blood. The same constant relationship between blood and saliva was also found for the aliphatic alcohols 1-Propanol, 2-Propanol, 1-Butanol, 2-Butanol and Isobutanol (Hein et al., 1989). This is quite remarkable because these alcohols are known to be more lipophylic than methanol and ethanol.

**Codeine**

There is still the question whether saliva is suitable for the detection of typical forensic-relevant drugs like morphine derivates. For our tests we chose codeine. From the chemical
structure, codeine (as a typical morphine derivative) is very similar to the compound heroin which is often analysed in blood and urine. The first step was to develop validation curves for the use of FPIA-urine-test that was also applied to detect codeine in saliva. By a series of in-vitro experiments we found a linear relationship between codeine concentration in saliva (µg/ml) and values given by the FPIA test. Thereafter, saliva concentration was measured in 5 human subjects over 4 hours who got 1 mg Codeine per kg body weight (Figure 3).

![Figure 3](image)

Saliva-Codeine-Concentration in µg/ml, minutes after ingestion

Within the first two hours we found large differences between the subjects. Afterwards, the concentration curves of all test subjects became more and more alike. One possible explanation for the high variation in the initial phase may be the pH-value of the saliva, which was found to be more different at the beginning of the experiment than after 2 hours. It seems that the transport and the secretion of basic codeine in saliva are considerably influenced by the pH-value.

The course of saliva curves became alike after approximately 2 hours. This course is similar to the course of the secretion of ethanol in saliva. However, we can basically state that in all test subjects, the saliva-codeine concentration was about 3 times as high as the respective serum concentrations.

For practical purposes, the detection of codeine should better be done in saliva than in plasma because the concentrations are remarkably higher. However, there is no direct relationship between concentrations in blood and saliva, mostly caused by the varying pH-values. This variation also restricts the saliva analysis within one subject. On the other hand, for the detection of drugs in saliva, immunological tests can be directly used without
The quantitative course for codeine in saliva must be interpreted cautiously because we noticed high intra- and interindividual variations in the quantitative and qualitative course characteristics of individual saliva.

The drug concentration in saliva is (under certain prerequisites) equivalent to the non-binding protein portion in plasma. This only holds true for a few drug groups. In fact, this relationship is constant only with lipophylic substances with a dissociation constant pKa<5.5 (base) and >8.5 (acid), as well as in indifferent substances, e.g. alcohols where the constant is nearly 1 (Feller, Le Petit 1977). But this relationship does not hold true for the most of illicit drugs. Their concentration in saliva mostly depends on the saliva pH value and seldom exceeds the plasma concentration.

With the same procedure as used with codeine, we investigated in blood and saliva the concentrations of Cyclobarbital and Diazepam after oral ingestion. The saliva/blood ratio for the barbiturate was found to be <1, for Diazepam <0.1. These empirically determined constants are very similar to those published by Schramm et al. (1992):

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codein</td>
<td>3</td>
</tr>
<tr>
<td>Morphin</td>
<td>0.2</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1</td>
</tr>
<tr>
<td>Amphetamin</td>
<td>2.8</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>0.5</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>0.1</td>
</tr>
</tbody>
</table>

CONCLUSION

The analysis of saliva as compared to blood yielded the following results: For ethanol and for the other aliphatic alcohols both methods are equivalent. In case of drugs, the relationships are more complex. They depend strongly on the pH-value of saliva and the intensity of the plasma protein binding of the drug. Therefore, it is not possible to estimate blood concentration directly from saliva. On the other hand, saliva has clear advantages with regard to the sampling procedure and the handling in the analysis. For example, saliva can be used in difficult sampling situations (like in a roadside survey) and should also be discussed as a pretest for detecting drugs in suspected drivers.

REFERENCES


