Possibility of Use a Saliva for Determination Ethanol and Opiates

Wojciech Piekoszewski¹,², Wojciech Guba³¹, Ewa Janowska¹, Janusz Pach³, Dariusz Zuba¹

¹Institute of Forensic Research, Westerplatte 9, 31-033 Kraków, Poland
²Department of Clinical and Industrial Toxicology, Jagiellonian University, Zlotej Jesieni 1, 31-826 Krakow, Poland
³Toxicology Clinic, Jagiellonian University, Zlotej Jesieni 1, 31-826 Krakow, Poland

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Abstract

The research was aimed at verification of possibility to use saliva for ethanol and main opiates (morphine and codeine) determination as exact equivalent of blood samples.

For the measurement of alcohol the headspace gas chromatography was used and for opiate alkaloids the gas chromatography/mass spectrometry was applied. The headspace GC method applied for ethanol determination in saliva was specific (resolution>1), shows good accuracy (recovery = 100.7%) and precision (SD = 0.0155 g/l).

Saliva and blood samples were collected from forty-nine volunteers and over 700 values of ethanol concentration were obtained. The correlation between blood and saliva concentrations of ethanol, estimated by the linear regression analysis, was very strong. Pearson’ correlation coefficient amounted to 0.94 and it was statistically significant (p<0.0001). The concentrations of ethanol in saliva and blood were on a similar level. The mean value of saliva/blood ratio of ethanol amounted to 1.06 ± 0.21.

The morphine and codeine concentrations were measured in serum and saliva collected from thirty-seven patients with a history of intravenous opiates use. The concentration of morphine in saliva during admission to hospital was four times lower than in serum (saliva/serum ratio 0.28±0.32) but the concentration of codeine was on the similar level in both mediums (saliva/serum ratio 0.89±1.36). The concentrations of morphine and codeine in serum and saliva have shown a good correlation.

Introduction

Alcohol consumption is a long habit in Poland but during the last twenty years there has been a substantial increase in illicit drug intoxication. The most frequently used drugs are cannabis and amphetamines, however its low acute toxicity cause that the major numbers of hospitalise patients are opiates poisoned. One of the most commonly abused forms of opiates in Poland is a liquid domestic product, produced from poppy straw or poppy head juice (1). For treatment of acute poisoned patients and in some forensic cases (alcohol drug and driving) the quantitative measurement of pharmacologically active xenobiotics are needed. The traditional media for these types of analyse are blood, plasma or urine. In the last ten years advances in analytical methods have enabled the determination of xenobiotics in alternative material such as sweat, saliva, and hair. The sampling of this material is not invasive and easy for collecting even from unconscious patients (2).
The aim of the present study was to develop the methods for determination of main opiates and ethanol in saliva and prove its validity by comparison with determination of these xenobiotics in blood or serum.

**Materials and methods**

For the alcohol determination saliva and blood samples were collected from forty-nine volunteers (37 men and 12 women), ageing 23–60 years before and in 15-minute intervals after consumption of 0.7g (men) and 0.6g (women) of ethanol per kg of body weight in the form of undiluted 40 % v/v vodka. The research performed on the studied group yielded over 500 values of ethanol concentration in saliva and blood.

The alcohol concentration in studied media was measured by headspace gas chromatography (GC) using Perkin Elmer AutoSystem XL with HS 40 autosampler and flame ionisation detector (FID). Separation was achieved on a 0.2% Carbowax 1500/Graphpack-GC column under isothermal conditions (at 100°C). A 0.2 ml of saliva or blood sample was mixed with 1.8 ml of 0.02 g/l 2-methyl-2-propanol (internal standard-IS). The samples were incubated in the autosampler for 22 min at 60°C.

For the opiates studies blood and saliva samples from thirty-seven patients (29 men and 8 women) with a history of intravenous opiate use, were collected at the Toxicology Clinic in Krakow. Written informed consent was obtained from each subject in the study and blood samples were taken for diagnostic purposes.

Blood (serum) and saliva samples were taken immediately after admission to the Clinic and were stored till analysis at –30°C. 1 ml samples of serum or saliva with deuterred internal standards (morphine-D3 and codeine D-3) were hydrolysed (β-glucuronidase/arylsulphatase) and deproteinised (trichloroacetic acid). For extraction, Bond Elute Certify (Varian) columns were used. Columns were conditioned with 3 ml methanol and purified with 0.0115 M hydrochloric acid. After extracting, studied compounds were derivatised with bis (trimethylsilyl) trifluoracetamide.

Extracts (serum and saliva) were analysed by a GC/MS (Varian/Finnigan Mat Magnum) ion trap in full scan (50 – 600 m/e) and selected ion monitoring EI mode. A FSCC HP-5MS column (30 m x 0.25 mm x 0.25 μm) and helium (1.2 ml/min) as a flow gas were used. The temperature program was: 75°C (1 min), 25°C/min up to 275°C and 275°C for 7 min. Multiplier voltage was 1150 V and ionisation mode – electron impact 70 eV. Full scan (50 – 600 m/e) and selected ion monitoring were used. The retention times were 12.05 min for morphine 12.03 min for morphine D3 and 11.43 and 11.41 min for codeine and codeine D3 respectively.

**Results**

**Determination of ethanol in blood and saliva**

The developed gas chromatography headspace method allow for good separation of ethanol from its metabolite (acetaldehyde) and other possible co-exist in blood and saliva volatile compounds (methanol, acetone, n-propanol and iso-propanol). Linearity of the method ranged from 0.1 to 4.0 g/l of ethanol in blood and saliva. The limit of determination (LOD) was lower than 0.05 g/l. The recovery calculated for all calibration levels amounted to 100.7%. The precision of the method was checked using duplicate determinations of 100 randomly chosen routine samples used for ethanol level evaluation in saliva. The intra-assay precision expressed as standard deviation (SD) amounted to 0.016, while reproducibility (r) was 0.045,
and relative standard deviation (RSD) was 3.6 %. The data show the absence of systematic error when the aqueous solutions were used instead of saliva for calibration (3).

Determination of morphine and codeine in serum and saliva
However a lot of information concerning the determination of opiates in serum appeared in literature, information about quantification morphine and its analogies in saliva are seldom [6]. The applied chromatographic conditions allowed to separate the compound of interest from a biological background. Low limits of detection of this method were 4 ng ml\(^{-1}\) for morphine and 5 ng ml\(^{-1}\) for codeine and the limits of quantification were evaluated to be equal 14 and 15 ng ml\(^{-1}\) respectively. The range of quantification was from 20 to 1000 ng ml\(^{-1}\), what covers most often observed concentrations of morphine and codeine in clinical and forensic cases. The recovery of solid phase extraction was between 50 and 60 %. The coefficients of between day’s variation for measurement of morphine and codeine concentration (concentrations 40 and 900 ng ml\(^{-1}\)) in serum and saliva were below 15%.

Concentrations of ethanol, morphine and codeine in blood and saliva

![Fig. 1. Correlation between ethanol concentrations in blood and saliva.](image1)

![Fig. 2. Relationship between saliva/blood ratios and blood concentration of ethanol.](image2)

![Fig. 3. Correlation between morphine concentrations in blood and saliva.](image3)

![Fig. 4. Relationship between saliva/serum ratios and blood concentration of morphine.](image4)
Discussion

The correlation between blood and saliva concentrations of ethanol, morphine and codeine was estimated by the linear regression analysis, according to the least squares method. The results indicate strong correlation between ethanol concentration in blood and saliva. Pearson’s correlation coefficient, r, amounted to 0.94 and it was statistically significant (p<0.0001). The correlation between the concentration of morphine in serum and saliva was also observed. The acceptable correlation between concentration of morphine in saliva and serum in the home-made heroine users allowed application its determination in saliva in the monitoring the clinical and forensic cases.

The present of codeine in all samples containing morphine with lack of correlation between these two drugs could be caused by contamination of home-made heroine by codeine. On the other hand, a correlation between the concentration of morphine but not codeine in serum and saliva was observed. A similar relationship has been described by other authors in patients taking heroin intravenously.

The concentrations of ethanol and codeine in saliva and blood (serum) are on a similar level, whereas the concentrations of morphine in saliva are about four times lower than in serum. The mean value of saliva/blood ratio of ethanol amounted to 1.06 ± 0.21. The saliva/serum ratio of morphine concentration was pH independent and averaged 0.28 ± 0.32 and for codeine 0.89 ± 1.36. The obtained values of ratio were similar as in previous literature (1, 4). The lack of influence of pH on saliva/serum ratio of studied opiates could have been caused by the imprecise method of measurement of pH applied in the experiment.

References
