THE APPLICATION OF OPIATES DETERMINATION IN SALIVA FOR DIAGNOSTIC AND PROGNOSTIC PROPOSES DURING DETOXIFICATION OF ADDICTED PERSONS

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ABSTRACT: The abuse of "kompot" – home-made heroin – is very common in Poland. During detoxification and monitoring of abstinence, frequent blood sampling has to be done, which can cause some problems. Saliva can be an alternative body fluid for determination of morphine and other co-existing chemical substances in "kompot". The aim of the study was to compare serum and saliva concentrations of morphine and codeine in patients admitted to the Detoxification Unit, and during their stay at the Toxicology Clinic. The first step was to develop and validate the GC/MS methods of morphine and codeine determination. The study were carried out on 37 patients (29 male and 8 female) admitted to the Toxicology Clinic. In all patients the presence of opiates in urine was preliminary confirmed by enzymoimmunoassay. The concentration of morphine (measured by GC/MS) in serum ranged from 0 to 671 ng/ml (mean 179 ng/ml) and codeine from 0 to 553 ng/ml (mean 59 ng/ml), in saliva 0–142 ng/ml (mean 44 ng/ml) and 0–428 ng/ml (mean 63.4 ng/ml) respectively. In the studied group a correlation between concentration of morphine in serum and saliva was shown (r = 0.4854), but there was no correlation for the concentration of codeine in these body fluids. The results of this study indicate that the measuring of morphine in saliva could be used as a means of monitoring the taking of home-made heroin.

KEY WORDS: Alternative materials; Saliva analysis; Morphine; GC/MS.

Problems of Forensic Sciences, vol. XLII, 2000, 130–136
Received 9 September 1999; accepted 16 May 2000

INTRODUCTION

The testing of biological specimens for the presence of drug is the most objective means of determining drug exposure. The traditional media for the quantitative measurement of most pharmacologically active xenobiotics are blood, plasma or urine. Urine testing is an important element in most treatment programs for drug abusers; however, during last the decade, saliva has been increasingly used as an analytical tool in detection of illicit drugs [1, 5] and therapeutic drug monitoring [3, 4].

The concentration of drugs detected in saliva usually correlates with plasma concentration.

The required volume of saliva samples may be obtained through stimulating salivation by chewing paraffin wax, polystyrol tube with cotton wool elastic bands, or sour gum.

Particular interest has been expressed by law enforcement agencies in roadside testing of potentially intoxicated drivers [4, 6].

In the present study saliva was used for monitoring the detoxification process of home made heroin poisoned patients.

MATERIAL AND METHODS

Thirty seven patients (29 men and 8 women) at the Detoxification Unit with a history of intravenous opiates use participated in the study. The presence of opiates in urine was confirmed in all patients during admission to the Clinic by enzymoimmunoassay. Written informed consent was obtained from each subject in the study and blood samples were taken for diagnostic purpose.

Blood and saliva samples were taken immediately after admission to the Detoxification Unit and stored up till analysis (blood after serum separation) at –30°C. Morphine and codeine in studied specimens were determined by gas chromatography-ion trap mass spectrometry (GC/MS). Bond Elut Certify (Varian) columns were conditioned with methanol 3 ml and purified with 0.0115 M hydrochloric acid. 1 ml samples of serum or saliva with deutered internal standards (morphine-D_3 and codeine D_3) were hydrolysed (β-glucuronidase/arylsulphatase) and deproteinised (trichloroacetic acid). After extracting on SPE column and derivatisation (bis(trimethylsilil)trifluoracetamide), samples were analysed by GC/MS (Varian/Finnigan Mat Magnum System). A FSCC HP-5MS column and helium (1.2 ml/min) as a flow gas were used. The temperature program was 75°C (1 min) 250°C/min up to 275°C and 275°C for 7 min. Full scan (50–600 m/z) and selected ion monitoring were used. The parameters of the method are presented in Table I.

TABLE I. SELECTED PARAMETERS OF MORPHINE AND CODEINE DETERMINATION BY GC/MS

Substance	Limit of detection [ng/ml]	Limit of quantification [ng/ml]	Recovery [%]	Range of quantification [ng/ml]
Morphine	4	14	50–60	30–1000
Codeine	5	15	50–60	30–1000

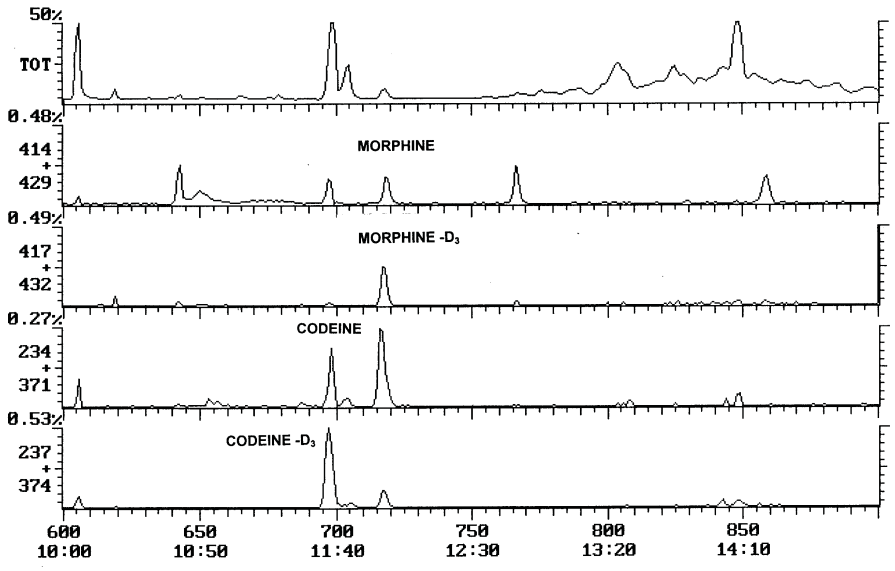

Fig. 1. GC/MS chromatogram of saliva specimen containing morphine and codeine (for details see "Material and methods").

RESULTS AND DISCUSSION

Fluid known as "saliva" or "oral fluid" is mainly excreted by the major saliva glands. The matrix of this fluid is less complex than whole blood or serum and it can be collected by a non-invasive method. This means that saliva can be very useful as a medium for drug monitoring; however, the concentration of xenobiotics in it is lower than in other body fluids. Determination of such a low level of drugs is possible only by very sensitive methods (GC/MS, LC/MS).

The developed GC/MS method allowed determination of morphine and codeine in serum and saliva (Table I). A GC/MS chromatogram of saliva containing morphine and codeine is shown in Figure 1 and the mass spectrum of morphine in Figure 2.

The results of a preliminary study of morphine and codeine in the serum and saliva of 37 addicts taking home made heroin – "kompot" – are presented in Table II.

TABLE II. DESCRIPTIVE STATISTICS OF MORPHINE AND CODEINE CONCENTRATION [ng/ml] IN SERUM AND SALIVA

Substance/material	Mean	SEM	Median	Range

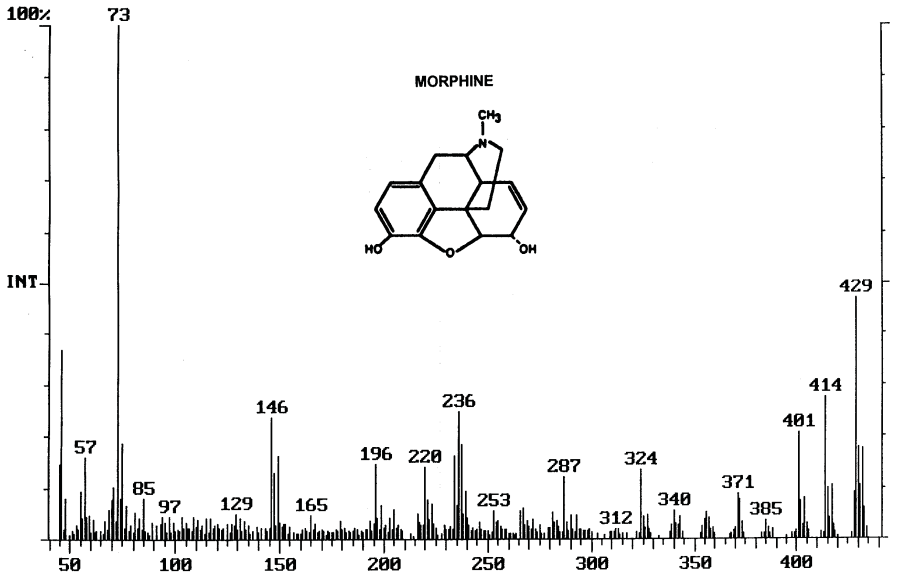

Fig. 2. Mass spectrum of morphine (for details see "Material and methods").

Morphine in serum	179.0	30.94	96.5	0–671
Morphine in saliva	44.1	7.23	29.8	0–142
Codeine in serum	58.8	16.29	31.3	0.553
Codeine in saliva	63.4	16.02	38.2	0–428

The distribution of concentration of studied drugs in patients' serum and saliva is presented in the Figure 3.

In all the serum and saliva samples in which the morphine was present, codeine was also determined, but a correlation between these two drugs, neither in serum nor saliva was not found.

The lack of correlation may be caused by contamination of home-made heroin by codeine. The amount of codeine present in body fluids is a result not only the biotransformation of morphine but also of the injection of codeine itself. Furthermore, a correlation between the concentration of codeine in serum and saliva was not found, which is in consistent with previous studies [2] in which the saliva/serum ratio ranged from 2 to 6.6.

Conversely, a correlation between morphine concentration in serum and saliva was proved; however, the concentrations in saliva were much lower (Figure 5). Other authors also observed 4 to 6 times lower level of morphine in saliva than in serum [2].

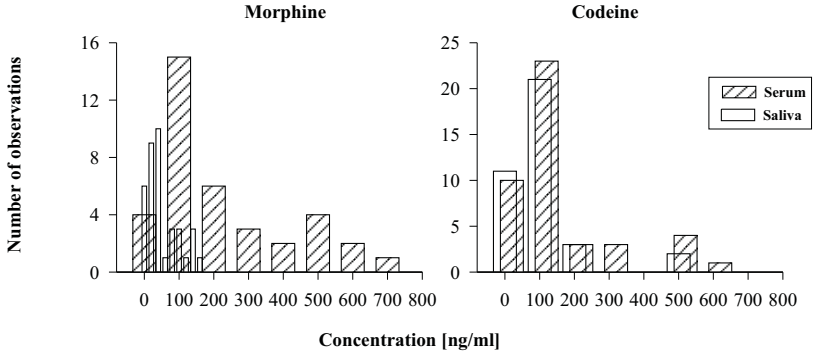

Fig. 3. Histogram of morphine and codeine in serum and saliva.

Fig. 4. Relationship between concentration of morphine and codeine in serum and saliva.

After administration of codeine which is metabolised to morphine, the correlation between the concentration of morphine in serum and saliva was also described.

The acceptable correlation between concentration of morphine in serum and saliva in the "kompot" users allowed application the measurement of morphine in saliva in the monitoring the abstinence during therapy of opiate addicts.

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Fig. 5. Relationship between serum and saliva concentration of morphine and codeine.