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Solid-phase micro-extraction–gas chromatography–mass spectrometry and headspace-gas chromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples

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Abstract

In the present work, a method was developed aiming at the serial detection of tetrahydrocannabinol (THC), amphetamine, methamphetamine, cocaine and ethanol in saliva. Saliva samples were submitted to an initial headspace procedure for ethanol determination by gas chromatography/flame ionization detector (GC–FID). After this step, two consecutive solid-phase micro-extractions (SPME) were carried out: THC was extracted by submersing a polydimethylsiloxane fiber (100 μm) in the vial for 20 min; amphetamine, methamphetamine and cocaine were subsequently extracted after alkalinization. Derivatization of the amphetamines was carried out directly in the solution by adding 2 μl of butylchloroformate. Gas chromatography–mass spectrometry (GC–MS) was used to identify the analytes in selected ion monitoring (SIM) mode. Confidence parameters of validation of the method were: recovery, linearity, intra- and inter-assay precision as well as limits of detection and quantification of the analytes. The limits of quantification (LOQ) obtained were: ethanol (0.010 g/l); amphetamine (5.0 ng/ml); methamphetamine (0.5 ng/ml); cocaine (5 ng/ml) and THC (5 ng/ml). The method proved to be highly precise (coefficient of variation<8%) for all detected substances.

Keywords: Tetrahydrocannabinol; Amphetamine; Methamphetamine; Cocaine; Ethanol

1. Introduction

In recent years, an increased interest has been observed in the use of saliva as an alternative testing matrix to detect drug abuse. There are many potential application for saliva testing for drugs such as monitoring of suspected drivers under the influence, forensic investigations, epidemiological studies and monitoring of patients under drug detoxication [1]. Saliva can be easily collected under direct supervision in a noninvasive way, allowing the sample to freely flow from the mouth into a container, aspirating it through a vacuum pump tube or absorbing it through a cotton swab placed in the mouth [2]. Alternatively, sour candy or citric acid can be used to stimulate saliva secretion.

Drugs are transferred from blood to saliva by passive diffusion, ultrafiltration and active transport. Substances with low molecular mass like ethanol

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pass across cell membranes by ultrafiltration. This kind of transport is precluded to substances with molecular mass greater than 500, ionized molecules and protein-bound drugs. For other drugs, passive diffusion represents the most important route of entry [2]. Since saliva is a blood filtrate, salivary drug concentration should reflect plasmatic concentration of unbound drugs [3].

However, contamination of the oral cavity by smoking, intranasal and oral administration of the drug, produces increased saliva/plasma (S/P) ratios. Salivary drug concentration can also be affected by changes in saliva pH and its flow-rate [2]. In spite of this, the presence of drugs in this biological sample can reasonably be interpreted as an indication of recent use and a high probability that the subject was experiencing pharmacological effects at the time of sampling [1].

Unlike urine, parent drugs rather than their metabolites are found in saliva. This fact limits the use of immunoassay tests for saliva analyses since antibodies present in most commercially available immunoassay kits are specifically designed for drug metabolites [3].

For detecting ethanol and drugs of abuse in saliva some methods have been published using gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) [4–9].

The objective of this work was to propose a method to detect tetrahydrocannabinol (THC), amphetamine, methamphetamine, cocaine and ethanol in a unique small-volume saliva sample.

2. Experimental

2.1. Chemicals

Cocaine, cocaine-D3, amphetamine, amphetamine-D5, methamphetamine, methamphetamine-D5, THC and THC-D3 solutions (1 mg/ml) were purchased from Radian International (Austin, TX, USA). Ethanol standard (10.3% v/v) was purchased from Sigma (St Louis, MO, USA). *N*-Propanol and butylchloroformate were purchased from Aldrich (Milwaukee, WI, USA).

2.2. Preparation of standard solutions

Working solutions of cocaine and cocaine-D3 at a concentration of 1 μg/ml were prepared in acetonitrile with volumetric glassware. Working solutions of amphetamine, amphetamine-D5, methamphetamine, methamphetamine-D5, THC and THC-D3 were prepared in methanol. The stock solutions were stored at −20 °C when not in use.

Ethanol and *n*-propanol working solutions at concentrations of 0.6 g/l were prepared in distilled water.

2.3. Instrumentation

Solid-phase micro extraction devices obtained from Supelco (Bellefonte, PA, USA) were equipped with 100-μm polydimethylsiloxane coated fiber.

Saliva collector devices (Salivette) were obtained from Sarstedt (Nümbrecht, Germany).

Ethanol analyses were carried out on a gas chromatograph model 6890 (Hewlett-Packard, Little Falls, DE, USA) equipped with a Poraplot Q fused-silica capillary column (10 m×0.32 mm) (Varian, Midelburg, Netherlands). The oven temperature was isothermal at 130 °C and the injector and the flame ionization detector (FID) system were selected at 250 °C. Hydrogen was used as carrier gas at 2.6 ml/min.

GC–MS analyses for cocaine, amphetamine, methamphetamine and THC were carried out on a gas chromatograph model 6890 coupled with a mass selective detector model 5972 (Hewlett-Packard, Little Falls, DE, USA). Chromatographic separation was achieved on a HP5MS fused-silica capillary column (30 m×0.25 mm×0.25 μm film thickness) using helium as carrier gas at 0.6 ml/min in a constant flow-rate mode. The mass selective detector was operated by electronic impact (70 eV) in SIM (selected ion monitoring) mode.

For cocaine, amphetamine and methamphetamine analyses, the injector port and interface temperature was 250 °C. The oven temperature was maintained at 150 °C for 2 min; programmed at 15 °C/min to 220 °C; 20 °C/min to 250 °C with a hold at 250 °C for 5 min. The following ions were chosen for SIM analyses (quantification ions underlined): cocaine:

182, 272, 303; cocaine-D3: 185, 306; amphetamine: 144, 145, 162; amphetamine-D5: 148, 167; methamphetamine: 158, 159, 102 and methamphetamine-D5: 162, 106.

For THC analyses, the injector port and interface temperature was 280 °C. The oven temperature was maintained at 150 °C for 2 min; programmed at 15 °C/min to 250 °C with a hold at 250 °C for 8 min. The following ions were chosen for SIM analyses (quantification ions underlined): THC: 231, 299, 314 and THC-D3: 302, 317.

2.4. Sample extraction

Ethanol, THC, amphetamine, methamphetamine and cocaine were added to 2 ml of drug-free saliva absorbed on a cotton roll from a Salivette. The device was centrifuged at 700 *g* for 10 min to obtain the fluid sample. In a 10-ml glass vial, an aliquot of 1.0 ml of saliva was added to 1.0 ml of *n*-propanol 0.6 g/l (internal standard). The vial was sealed with a rubber cap and an aluminum crimp seal and incubated for 30 min at 70 °C. Afterwards, a homogenized 250-μl vapor aliquot was withdrawn through the rubber cap with a 500-μl gas-tight syringe (Hamilton, Reno, NV, USA) and injected directly into GC–FID. The remaining saliva solution was spiked with the deuterated internal standards and transferred to a 4-ml vial to have amphetamine, methamphetamine and cocaine extracted by SPME.

Amphetamine and methamphetamine derivatization was carried out directly in the solution by adding 2 μl of butylchloroformate and 200 mg of $NaHCO_3/K_2CO_3$ (2:1). The drugs were extracted by SPME submersing the polydimethylsiloxane fiber in the solution for 20 min under magnetic stirring. After the stated extraction time, the SPME device was transferred to the injector port of the GC–MS for a 15-min desorption time.

A procedure was developed to extract THC from the cotton roll. Two milliliters of 1 *M* NaOH solution was allowed to be absorbed by the cotton roll. After 10 min, the Salivette device was centrifuged at 700 *g* for 10 min and the liquid was transferred to a 4-ml vial and acidified with 0.5 ml of glacial acetic acid. THC-D3 (50 ng) was added to the solution. THC was extracted by submersing the fiber in the vial for 20 min under magnetic stirring. After the stated extraction time, the SPME device was transferred to the injector of the GC–MS for a 15-min desorption time.

2.5. Validation of the method

The validation of the method was carried out by establishing recovery values, linearity, intra- and inter-assay precision, limits of detection (LOD) and quantification (LOQ).

2.5.1. Recovery

The recovery study for cocaine, amphetamine and methamphetamine were carried out taking into consideration the possible loss of the analytes by thermal decomposition or volatilization after the headspace procedure or the retention of the drugs in the cotton roll of the saliva collector. The recovery study for THC was carried out taking into consideration the possible loss of the analyte retained in the cotton roll.

For both studies, two sets of samples of different concentrations were analyzed. One of them (set A), consisting of three concentrations for all drugs analyzed (10, 50 and 75 ng/ml) was analyzed according to the method described in Section 2.4 in six replicates for each concentration (processed). In the second one (set B), also consisting of six replicates for each concentration (10, 50 and 75 ng/ml), the standard solutions were spiked to the sample immediately before the SPME procedure (unprocessed). The absolute recovery was evaluated by comparison of the mean response of set A (processed) and the response of set B (unprocessed). The unprocessed response represented 100% recovery.

2.5.2. Linearity

The study of linearity was carried out by the analysis of saliva samples in triplicate submitted to the method with the following concentrations: ethanol (0.01, 0.06, 0.15, 0.6, 1.5 and 3.0 g/l); cocaine, amphetamine, methamphetamine and THC (5, 20, 40, 60, 80 and 100 ng/ml).

2.5.3. Intra- and inter-assay precision

Precision, defined as the relative standard deviation or coefficient of variation (CV), was determined intra- and inter-assay. They were carried out by analyzing saliva samples (quality controls) on three different days at the following concentrations: ethanol: 0.03, 0.30, 1.20 g/l; cocaine, amphetamine, methamphetamine and THC: 10, 50 and 75 ng/ml. The analyses were carried out in six replicates for each concentration.

2.5.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined by an empirical method that consisted of analyzing a series of saliva samples containing decreasing amounts of the analyte [10]. The LOD was the lowest concentration that presented a CV that did not exceed 20% and the LOQ the lowest concentration that presented a CV that did not exceed 10%. For GC–MS analyses, the LOD and LOQ should still satisfy the predetermined acceptance criteria of qualification (retention time within 1% of calibrator and ion ratios within 20%).

3. Results

Fig. 1 shows the chromatogram obtained with the GC–FID analysis of a saliva sample spiked with ethanol (0.6 g/l). Fig. 2 shows the chromatogram obtained with the GC–MS analysis of a saliva sample spiked with cocaine, amphetamine and methamphetamine at the concentration of 50 ng/ml each. Fig. 3 shows the chromatogram obtained with the GC–MS analysis of a saliva sample containing 50 ng/ml of THC.

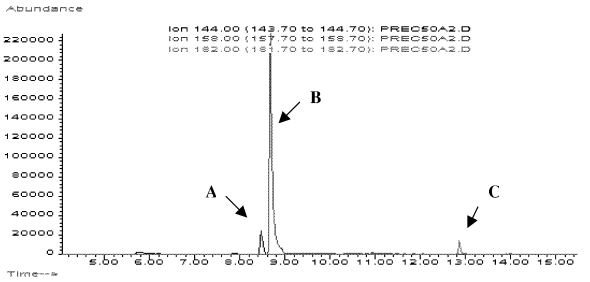

Fig. 2. Chromatogram obtained with the GC–MS analysis of a saliva sample spiked with amphetamine (A), methamphetamine (B) and cocaine (C) at concentrations of 50 ng/ml each. Time in minutes.

Calibration curves were obtained from 0.01 to 3.0 g/l for ethanol analyses and from 5 to 100 ng/ml for the other drugs. The linear regression equations and coefficients of correlation were: ethanol: $y = 0.7724x + 0.0037$, $r = 0.999$; cocaine: $y = 0.0167x + 0.0095$, $r = 0.998$; amphetamine: $y = 0.0172x + 0.0227$, $r = 0.998$; methamphetamine: $y = 0.016x + 0.0287$; $r = 0.998$; THC: $y = 0.0692x + 0.0823$, $r = 0.996$.

The confidence parameters of the validated method (LOD, LOQ, intra- and inter-assay precision and recoveries) for the determination of ethanol, cocaine, amphetamine, methamphetamine and THC are shown in Table 1.

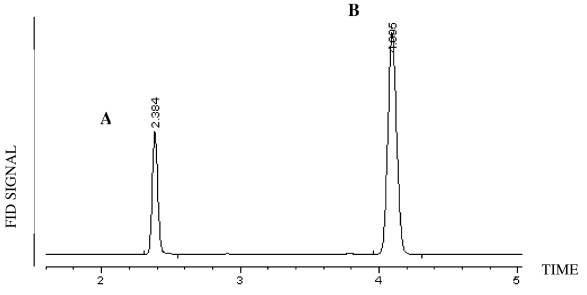

Fig. 1. Chromatogram obtained with the GC–FID analysis of a saliva sample spiked with ethanol at a concentration of 0.6 g/l (A) and *n*-propanol (B) (internal standard). Time in minutes.

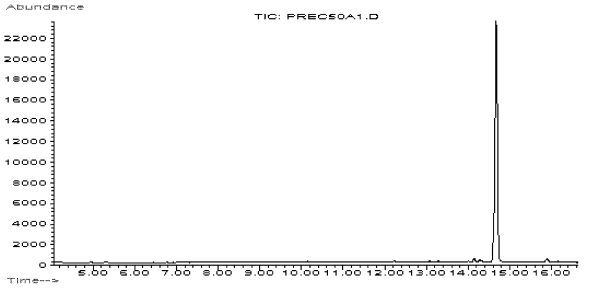

Fig. 3. Chromatogram obtained with the GC–MS analysis of a saliva sample spiked with THC at a concentration of 50 ng/ml. Time in minutes.

Table 1
Confidence parameters of the validated method for the determination of ethanol, cocaine, amphetamine, methamphetamine and THC in saliva samples

	ETOH	AMP	MET	COC	THC
Recovery (%)					
QC1	–	104.4	90.5	93.5	28.7
QC2	–	82.5	86.3	83.2	51.7
QC3	–	68.2	75.4	73.4	57.7
LOD	0.006 g/l	5.0 ng/ml	0.5 ng/ml	5.0 ng/ml	3.0 ng/ml
LOQ	0.010 g/l	5.0 ng/ml	0.5 ng/ml	5.0 ng/ml	5.0 ng/ml
Intra-assay precision (CV%)					
QC1	7.7	4.0	4.0	5.2	5.1
QC2	2.8	2.4	4.6	6.3	3.5
QC3	1.7	5.1	1.6	3.9	2.4
Inter-assay precision (CV%)					
QC1	3.2	1.0	4.8	2.7	2.0
QC2	4.0	2.7	1.5	6.3	5.4
QC3	3.1	5.2	3.3	2.6	5.0

ETOH, ethanol; AMP, amphetamine; MET, methamphetamine; COC, cocaine; THC, tetrahydrocannabinol; LOQ, limit of quantification; LOD, limit of detection; QC, quality control, for ethanol analyses: QC1 = 0.03 g/l; QC2 = 0.3 g/l; QC3 = 1.2 g/l; for other drugs: QC1 = 10 ng/ml; QC2 = 50 ng/ml; QC3 = 75 ng/ml.

4. Discussion

A number of gas chromatographic methods proposed for determination of ethanol and drugs of abuse in saliva have been reported [4–9]; however, none of them proposes the detection of volatile drugs simultaneously to other fixed compounds in a unique sample.

The headspace method for ethanol described in this work presented good linearity and precision, using 1 ml of saliva sample. The commonly used saturated NaCl to promote a ''salting-out'' effect was not considered for this procedure in order to make possible the consecutive SPME in the clean remaining solution. However, good limits of detection and quantification of ethanol were obtained (0.01 g/l).

The study of recovery of amphetamine, methamphetamine and cocaine showed that the previous headspace procedure for ethanol did not cause significant loss of analytes. Percentage of recovery for the three analytes was higher for lower concentrations in the samples. In our study, the value higher than 100% recovery for amphetamine was obviously due to the imprecision of the method. For the analyses of amphetamines, butylchloroformate was chosen as derivatizing agent to convert the substances in less polar compounds. Most derivatization reactions are not compatible with aqueous media, whereas chloroformates convert amines rapidly into carbamates in buffered aqueous media at room temperature [11].

This proposed method based on the simultaneous butylchloroformate derivatization of amphetamines and extraction by SPME was rapid and practical. Over 20 saliva samples could be extracted, injected and analyzed by one analyst in approximately 8 h. A similar method was proposed [12] for determination of amphetamines in urine with good results.

In our study, it was observed that cocaine could be determined under the same conditions as those suitable for amphetamines. However, to avoid cocaine hydrolysis in alkaline solution, the solid buffer ($NaHCO_3/K_2CO_3$) and the derivatizing agent (butylchloroformate) were added to the sample only a few minutes before the SPME procedure.

As observed in another study [7], THC was absorbed on the Salivette roll and the classic centrifugation was insufficient to release the drug. These authors used 5 ml of hexane/ethyl acetate (90:10, v/v) to remove THC from the cotton roll, obtaining a recovery of approximately 82%. In our proposed method, 2 ml of NaOH solution (1 *M*) was used to extract the THC from the Salivette roll obtaining an average recovery of 46%. In spite of the lower

recovery obtained, the new method has the advantage of not using solvents to extract the analyte.

The analytical technique proposed for the determination of the drugs (ethanol, amphetamine, methamphetamine, cocaine and THC) in saliva is not too time consuming and is easy to manipulate. Furthermore, the use of non-destructive techniques (headspace and SPME) is of great benefit when only small volumes of sample are available.

The method proved to be highly precise with the use of deuterated internal standards for the analysis of amphetamine, methamphetamine, cocaine and THC. Good sensitivity and linearity were also obtained for all drugs analyzed.

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