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Simultaneous detection of some drugs of abuse in saliva samples by SPME technique

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Abstract

A simple method for the simultaneous determination of many drugs of abuse in saliva is referred [methadone, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), cocaine, cocaethylene, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethyl amphetamine (MDEA), *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN)].

Head space-solid phase microextraction (HS-SPME) and direct immersion-solid phase microextraction (DI-SPME) followed by gas chromatographic/mass spectrometric analyses (GC/MS) were employed, and results obtained with both techniques are discussed.

The method was validated testing reproducibility, sensitivity, linearity.

Keywords: Saliva; SPME; GC/MS

1. Introduction

Lately, many authors studied solid phase microextraction (SPME) technique, applied to some drugs of abuse [1,2]. Recently, remarkable advances in sensitive techniques have encouraged the analysis of drugs in unconventional biological samples particularly saliva [3–5], hair [6] and sweat [7]. In this study, saliva has received special attention. In fact, human saliva offers many advantages as a matrix for the detection of drugs of abuse: it can be easily obtained by non-invasive techniques [8] and contains many analytes of interest useful for different purposes (toxicological screening, clinical diagnosis, etc.).

Recent advances in sensitive analytical techniques, such as gas chromatography/mass spectrometry (GC/MS) coupled with SPME, allowed the analysis of drugs also in saliva samples. In fact SPME has proven to be an important sample preparation technique because of the many advantages offered when it is applied to forensic specimens [1,9].

The increase in poly-drug abuse requires the development of an easy and fast technique able to simultaneously detect a wide range of drugs using a low amount of specimen. In this regard, we already studied the possibility of SPME application on saliva samples [5,10]. In the present study, we improved the procedure using SPME and GC/MS technique to test simultaneously many drugs of abuse in saliva.

2. Materials and methods

2.1. Sample collection

All the tests performed for the validation of the method have been done on saliva drug-free samples.

Fifty-three saliva samples obtained by drug-abusers were tested with the proposed method.

Saliva collection was performed by Cozart Rapiscan System (Cozart Bioscience Ltd., Abingdon, Oxfords, UK) according to Cozart Bioscience instructions as described in literature [10,11].

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Table 1
Substances and related internal standards considered

Compound	Internal standard
Amphetamine	Amphetamine-D5
Methamphetamine	Methamphetamine-D5
3,4-Methylenedioxymethamphetamine (MDMA)	MDMA-D5
3,4-Methylenedioxyethylamphetamine (MDEA)	MDEA-D5
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)	MBDB-D5
2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP)	EDDP-D3
Methadone	Methadone-D3
Cocaine	Cocaine-D3
Cocaethylene	Cocaethylene-D3
Cannabidiol (CBD)	THC-D3
Δ^9-Tetrahydrocannabinol (THC)	THC-D3
Cannabinol (CBN)	THC-D3

2.2. Sample preparation

Pure standards of drugs employed in this study were obtained by Radian International, LLC Austin, Texas 78728, USA.

Two hundred microliters of saliva drug-free samples were first spiked with the target compounds listed in Table 1 at concentrations ranging from 10 to 1000 ng/ml.

Deuterated internal standards (Table 1) analogs of most of the substances (Radian International) were used at concentrations of 250 ng/ml each for all the samples examined.

Twenty microliters of sodium hydroxide 0.1 M were added to saliva samples just prior to performing SPME to reach an alkaline pH; finally about 30–50 mg of sodium chloride were added to improve the recovery [9].

The same procedure was applied for real saliva samples.

2.3. SPME analysis

SPME technique was carried out using SPME fiber 30 μm polydimethylsiloxane coating (Supelco Bellefonte, PA). The sampling was performed both with direct immersion or head space modalities [12].

2.3.1. Direct immersion SPME (DI-SPME)

DI-SPME technique was performed by placing the sample in a 300 μl insert combo microtube, and directly submersing the fiber in the micro vial at room temperature for 30 min.

2.3.2. Head space-SPME (HS-SPME)

HS-SPME procedure was executed by introducing the sample in the bottom of a 2 ml micro vial hermetically plugged, and then placed in a water bath at approximately 60 °C to enhance the volatility. The fiber was then exposed for 45 min to the vapour phase of the head space vial.

2.4. Gas chromatographic conditions

After the sampling, thermal desorption of analytes from the fiber was performed directly in the GC injection port for 1 min. A Hewlett-Packard model 5890 gas chromatograph fitted with split–splitless injector was equipped with a 12 m × 0.2 mm i.d. capillary column; 0.33 μm film thickness methylsilicone. The following temperature program was used: initial temperature set at 40 °C held for 1 min in isotherm, then 20 °C/min increments to 280 °C, and finally 5 min in isothermal mode. The injector temperature was set at 250 °C; helium was used as carrier gas at 1 ml/min flow rate. The capillary column was connected to a mass analyzer (HP5971A) operating by electronic impact (70 eV) in selected ion monitoring (SIM) mode.

Chromatographic characteristics regarding retention times and ions chosen for SIM analyses are reported in Table 2.

2.5. Quantitation

Standard calibration curves were obtained in double runs with the described method using drug-free control saliva spiked with standard solutions to obtain the concentrations of 10, 25, 50, 100, 250, 500, 1000 ng/ml for each compound as explained earlier.

Table 2
Chromatographic characteristics for SIM analysis

Compound	Retention time (min)	Target ion	Qualifiers	
Amphetamine	3.30	44	91	135
Amphetamine-D5	3.30	60	92	134
Methamphetamine	3.55	58	91	134
Methamphetamine-D5	3.55	62	92	139
MDMA	4.79	58	135	193
MDMA-D5	4.79	62	136	198
MDEA	4.96	72	207	44
MDEA-D5	4.96	77	212	45
MBDB	5.10	72	135	178
MBDB-D5	5.10	76	136	183
EDDP	6.95	277	276	262
EDDP-D3	6.95	280	279	265
Methadone	7.60	294	223	309
Methadone-D3	7.60	297	226	312
Cocaine	7.90	182	303	198
Cocaine-D3	7.90	185	306	201
Cocaethylene	8.20	196	317	272
Cocaethylene-D3	8.20	199	320	275
CBD	9.10	231	246	314
THC	9.80	299	231	314
THC-D3	9.80	302	234	317
CBN	10.30	295	296	310

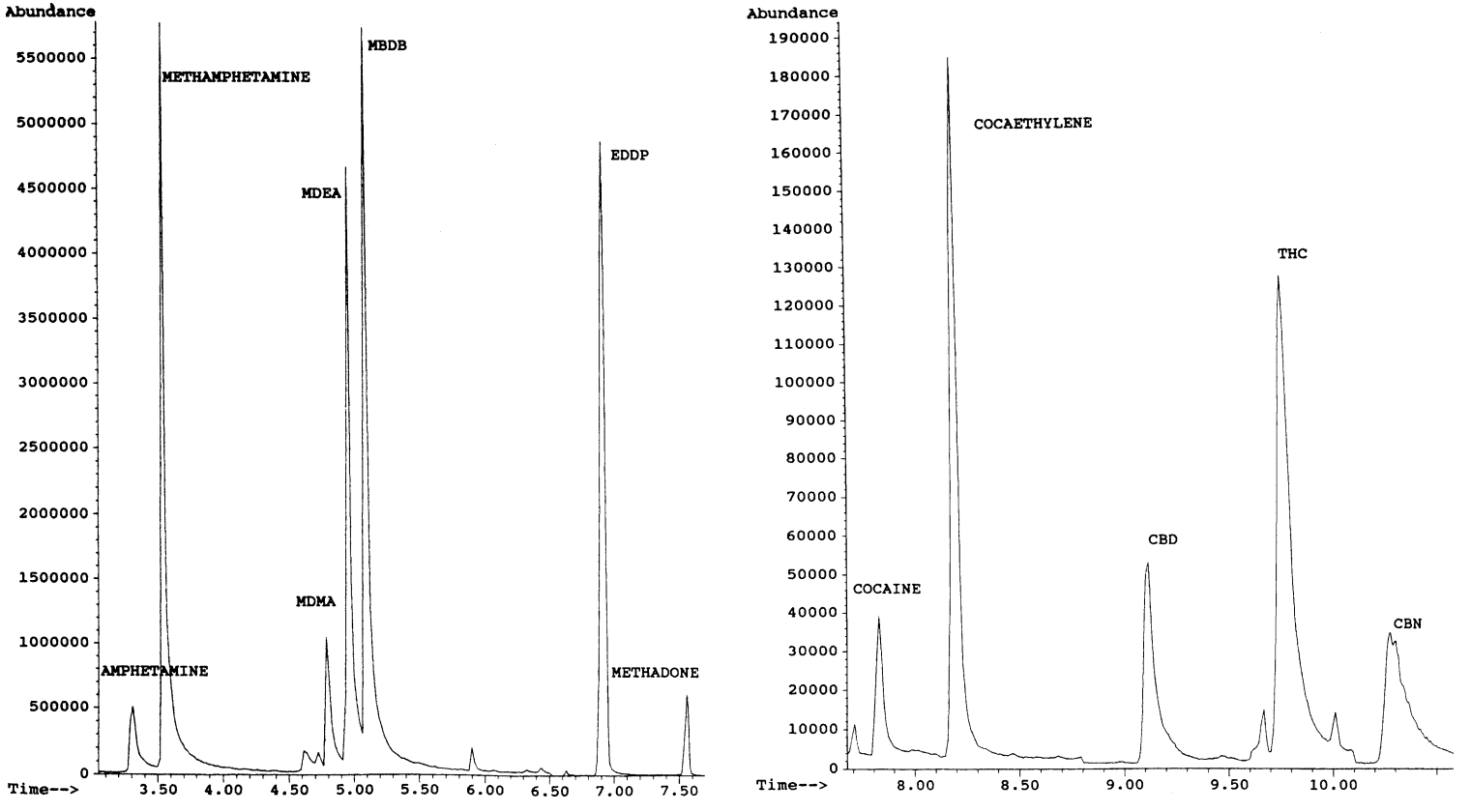

Fig. 1. Chromatographic pattern of a drug-free saliva sample spiked with all the substances considered, submitted to DI-SPME and GC/MS analysis.

Quantitation was based on target peak area ratios of various compounds with their respective internal standards.

3. Results

The choice of analytical conditions, regarding the coating of the fiber, the temperature and the time of adsorption, the alkalinization rate, both for DI- or HS-SPME, was performed according to our previous experience [10].

Since the SPME fiber coatings are neutral, the pH should be adjusted such that the analyte of interest is also neutral. Recoveries of basic analytes are enhanced by basic pH [12].

In order to increase the concentration of the analytes in the gas phase of HS-SPME, the sample is usually heated ranging from 45 to 100 °C [2,12]; our experience showed that 60 °C was the most efficient temperature for the substances considered.

The gas chromatographic method used was effective in separating all the substances studied (Fig. 1) in a short time (about 11 min). Chromatographic pattern of a saliva drug-free sample showed no interferences. No carry-over with the desorption time of 1 min at 250 °C was observed when the fiber was re-tested after sampling-desorption.

Cannabinoids were detected only for qualitative purposes, hence no reproducibility, nor linearity were studied. It seems that Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) do not pass into the saliva from the blood but rather are sequestered in the buccal cavity during smoking [13]. For this reason cannabinoids may sometimes be detected in saliva for a longer time than in plasma [5], hence the quantitative in saliva detection is not useful.

However, qualitative determination of cannabinoids in saliva could be very interesting to detect the recent drug intake.

3.1. Reproducibility

The precision of the method for the compounds in saliva was studied through the within-batch precision for five replicate analysis at two concentration levels (100 and 1000 ng/ml) prepared with drug-free saliva spiked with standard solutions and submitted to SPME procedure. The results for both the sampling procedures are reported in Table 3. As reported in the table, the reproducibility is acceptable for all the substances. Moreover, we did not observe significant differences between the two sampling modalities used.

3.2. Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for DI-SPME and HS-SPME are reported in Table 4.

While DI-SPME shows high sensitivity for all the substances, ensuring the detection of little amounts, HS-SPME seems to be less sensitive for some compounds. DI-SPME is more suitable for cocaine and cocaethylene because of their lower volatility.

Table 3

Compound	Reproducibility			
	1000 ng/ml		100 ng/ml	
	DI-SPME (%)	HS-SPME (%)	DI-SPME (%)	HS-SPME (%)
Amphetamine	14.57	14.70	25.85	27.05
Methamphetamine	13.80	15.93	10.21	12.45
MDMA	6.67	10.89	9.69	11.33
MDEA	11.82	10.95	22.59	23.56
MBDB	11.45	14.35	10.40	12.58
EDDP	2.46	0.60	3.94	2.95
Methadone	3.81	0.47	6.35	3.68
Cocaine	4.55	ND	10.05	ND
Cocaethylene	6.00	ND	28.20	ND

ND, not detected; DI-SPME, direct immersion solid phase microextraction; HS-SPME, head space solid phase microextraction; ng/ml, nanograms/milliliter.

Table 4

Compound	LOD (ng/ml)		LOQ (ng/ml)	
	DI-SPME	HS-SPME	DI-SPME	HS-SPME
Amphetamine	10	5	25	25
Methamphetamine	1	1	10	10
MDMA	5	10	10	25
MDEA	1	1	10	10
MBDB	1	1	10	10
EDDP	1	1	10	10
Methadone	5	1	10	10
Cocaine	20	100	50	250
Cocaethylene	5	20	10	100
CBD	20	50	–	–
THC	20	50	–	–
CBN	20	20	–	–

LOD, limit of detection; LOQ, limit of quantitation; DI-SPME, direct immersion solid phase micro extraction; HS-SPME, head space solid phase micro extraction; ng/ml, nanograms/milliliter.

3.3. Linearity

The linear range of the method was studied by spiking saliva drug-free samples covering the range 10–1000 ng/ml.

Simple linear regression analyses were performed; calibration curve equations and correlation coefficients (r) are shown in Table 5.

Correlation coefficients are acceptable for all the considered compounds, and the linearity covers a wide range.

3.4. Real samples

We analyzed 53 samples coming from heroin addicts receiving methadone: the timing of collecting saliva samples was approximately 2 min. In 66% of the samples methadone was detected with the proposed method with concentration

Table 5

Compound	Linearity range (ng/ml)	Calibration curve	r
Direct immersion-solid phase micro extraction			
Amphetamine	25–1000	$y = 3.67\ x + 1.06$	0.970
Methamphetamine	10–1000	$y = 0.817\ x - 0.008$	0.991
MDMA	10–1000	$y = 0.737\ x - 0.012$	0.992
MDEA	10–1000	$y = 0.990\ x + 0.059$	0.955
MBDB	10–1000	$y = 0.752\ x + 0.022$	0.974
EDDP	10–1000	$y = 1.740\ x - 0.209$	0.989
Methadone	10–1000	$y = 0.519\ x - 0.076$	0.986
Cocaine	50–1000	$y = 0.297\ x + 0.184$	0.941
Cocaethylene	10–1000	$y = 0.561\ x + 0.101$	0.982
Head space-solid phase micro extraction			
Amphetamine	25–1000	$y = 1.270\ x + 0.375$	0.963
Methamphetamine	10–1000	$y = 0.702\ x + 0.004$	0.984
MDMA	25–1000	$y = 0.814\ x + 0.085$	0.981
MDEA	10–1000	$y = 0.748\ x + 0.019$	0.984
MBDB	10–1000	$y = 1.060\ x - 0.038$	0.998
EDDP	10–1000	$y = 1.330\ x - 0.075$	0.999
Methadone	10–1000	$y = 0.477\ x - 0.080$	0.995

ng/ml, nanograms/milliliter; r, correlation coefficient.

ranges from 10 to 3800 ng/ml; in 15% of cases we found methadone at concentrations lower than 10 ng/ml. In 41% of cases, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP) was found at lower concentration than methadone (10–991 ng/ml); only trace amounts of EDDP were seen in 28% of samples.

In many cases we observed the presence of cannabinoids (26%) but the quantitation was not performed as previously explained. Only few cases of cocaine (7%) and amphetamines (11%) (mainly methamphetamine) were noted at very low concentrations (near to the LOD value).

4. Discussion

Toxicological analyses of saliva specimens can be useful to investigate the ''actuality'' of the drug use, but because of the limited volume available, the use of a sensitive technique, such as SPME, is desirable. SPME coupled to GC/MS has been applied to extract some abused drugs from saliva samples. To compare with conventional techniques, SPME has various advantages as no use of solvents, easy handling, and fast method.

Moreover, the high sensitivity of this technique becomes more important in saliva analysis, because of the low concentrations of drugs of abuse [14].

The selection of a well-suited internal standard is crucial for the SPME in biological samples. Hence we chose internal standard calibration with isotopically labelled spikes for the chemical structure very similar to the analytes, therefore, reducing any error.

SPME can be accomplished by two kinds of extraction approaches: HS-SPME and DI-SPME. We compared both methods finding the outstanding advantage of HS-SPME, the prevention of direct contact of the fiber with the sample and therefore, prevention of contamination of the surface of the fiber with organic polymers.

On the other hand, HS-SPME is limited to special analytes because of the requirement of a high vapour pressure (Table 4).

The results obtained are satisfactory enough to propose the technique not only as a screening procedure, but also for the quantitation of drugs usually encountered in saliva samples. In fact sensitivity and reproducibility were good for all the substances and the correlation coefficients were acceptable for all the compounds studied.

However, despite the numerous advantages, the method reported should not be seen as panacea, a substitute or an opponent of the existing standard methods. It should instead be considered as a complementary technique, which offers an attractive alternative to more conventional systems.

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