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Detection of cocaine and cocaethylene in sweat by solid-phase microextraction and gas chromatography/mass spectrometry

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Abstract

In the present work, a semi-quantitative method was developed to detect simultaneously cocaine (COC) and cocaethylene (CE) (transesterification product of the coingestion of COC with ethanol) in sweat. Sweat samples were collected by means of a non-occlusive sweat patch device supplied by PharmChek™. The method was based on the dissolution of COC and CE incorporated into the patch, with 0.2 M sodium acetate buffer (pH 5.0) and the extraction of the analytes by solid-phase microextraction (SPME). Gas chromatography/mass spectrometry (GC–MS) was used to detect the analytes in selected ion monitoring mode (SIM). The method showed to be very simple, rapid and sensitive. The limits of detection were 5 ng/ml for COC and CE (12.5 ng/patch). Good inter and intra-assay precision was also observed (coefficient of variation <8%) with the use of deuterated internal standards.
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1. Introduction

Toxicological analyses of biological samples provide the most objective method for detecting drug abuse. Currently, the most common biological specimen used for drug testing is urine. Urine samples have been widely used in doping control, workplace drug testing, criminal justice (forensic) and drug abuse treatment testing programs [1–4]. Although urine is easily tested with standardised methods and non-invasively collected, it does have some minor disadvantages: the window of detection is dependent on the half-life of the drug and is relatively short for most drugs of abuse (2–3 days after a simple exposure) [4]. Consequently, urinalysis should be performed with a minimum frequency of two or three times a week for reliable detection of drug use [3–5]. Additionally, urine samples can be easily adulterated.

Recently, an increased interest has been observed in the use of alternative biological matrices to detect drug misuse and abuse: hair, saliva, nails and sweat are some of them with their own advantages and disadvantages. In the case of sweat, samples can be easily collected by means of a sweat patch applied to a hairless and of intense perspiration region of the body for a period of some days (up to 10 days), resulting in accumulation of drug in the patch. Moreover, it is collected in a non-invasive way and the samples obtained is difficult to be adulterated.

Several mechanisms of incorporation of drugs into sweat have been suggested, including passive diffusion from blood into sweat glands and transdermal passage of drugs across the skin. Non-ionised basic drugs diffuse into sweat and become ionised as a result of the lower pH of it (pH 5.8) as compared to blood (pH 7.4) [3]. Generally, parent drugs are found in sweat, rather than their polar metabolites which usually predominate in urine [1]. Drug abuse treatment testing, doping control analyses in sports activities on which athletes spend energy

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with abundant sweating (soccer, basketball, volleyball, etc) and drug testing in the workplace are some possibilities of application of sweat.

For detecting cocaine and metabolites in sweat, some methods have been published in the scientific literature using immunoassays techniques for screening and gas chromatography–mass spectrometry for confirmation [3–9]. As far as we know, solid phase microextraction (SPME) has not been used in sweat samples analyses.

So, the objective of this work was to propose a method to detect cocaine and cocaethylene (transesterification product of the coingestion of COC with ethanol) in sweat samples using solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Reagents and standards of reference

Cocaine (COC), cocaethylene (CE) and their respective deuterated-labeled analogues, COC-d3 and CE-d3 (internal standards) were purchased from Radian International (Austin, TX, USA). Sodium acetate, anhydrous sodium bicarbonate and potassium carbonate were of the analytical grade purchased from Merck (Darmstadt, Germany).

2.2. Preparation of standard solutions

Working solutions of cocaine (COC), cocaine-d3 (COC-d3), cocaethylene (CE) and cocaethylene-d3 (CE-d3) at a concentration of 1 μg/ml were prepared with acetonitrile in volumetric glassware. Stock solutions were stored at −20 °C when not in use.

2.3. Instrumentation

Solid-phase microextraction (SPME) devices obtained from Supelco (Bellefonte, PA, USA) were equipped with 100 μm polydimethylsiloxane coating fiber.

Sweat patches (PharmChek[TM]) were obtained from Pharmchem Laboratories Inc. (Menlo Park, CA, USA). The patch is a non-invasive and non-occlusive skin patch which consists of a transparent, hypoallergenic, water-resistant adhesive backing securing a cellulose absorption pad to the surface of the skin.

GC–MS analyses for COC and CE were performed using a gas chromatograph model 6890 coupled with a mass selective detector (MSD) model 5972 (Hewlett-Packard, Little Falls, DF, USA). Chromatographic separation was achieved on a HP-5MS 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. MSD was operated by electronic impact (70 eV) in selected ion monitoring (SIM) mode. The injector port and interface temperature was 250 °C. The oven temperature was maintained at 150 °C for

1 min; programmed at 10 °C/min to 250 °C with a hold at 250 °C for 7 min. The following ions were chosen for SIM analyses: cocaine: 182, 272, 303; cocaine-d3: 185, 306; cocaethylene: 196, 272, 317; cocaethylene-d3: 199, 320.

2.4. Sample collection

Sweat samples were collected according to patch manufacturer's instructions. Sweat patches were applied to a hairless and of evident sweating part of the body after cleansing of skin with isopropanol 70% swab to avoid contamination and improve adherence. A period of 24 h is the minimum wear period recommended to verify drug use. Twenty-one samples were collected from 11 volunteers (cocaine users) of both sex, older than 21 years of age, in-patients of a drug abuse rehabilitation clinic. Patches were used for 3–7 days.

2.5. Sample preparation

Pads from sweat patches were immersed in a 2.5 ml of extraction medium (0.2 M acetate buffer pH 5.0) and maintained in this solution for 15 min. Afterwards, the pads were centrifuged 10 min at $300 \times g$ to obtain the eluent. Deuterated internal standards of COC-d3 and CE-d3 were added to the eluent (25 μl of a 1.0 μg/ml in acetonitrile). The analytes were extract using SPME, dipping a 100 μm polydimethylsiloxane fibre in the eluent for 20 min under magnetic stirring to obtain an optimal exchange surface. After the extraction time, the SPME device was introduced into the GC injector (glass liner) where the fibre is exposed to the heated chamber. The analytes were thermally desorbed at 250 °C (according to the manufacturer's instructions) for 20 min.

2.6. Validation of the method

Validation of the method was performed by establishing recovery values, intra and inter-assay precision, limits of detection (LOD) and stability as follows.

2.6.1. Recovery

The recovery studies for COC and CE were performed taking into consideration the possible loss of the analytes retained in the pads. Pads were spiked with 25 μl and 125 μl of a 1.0 μg/ml of both COC and CE in acetonitrile to obtain a final concentration in the eluent of 10 ng/ml and 25 ng/ml (25 ng and 125 ng/patch). This set of samples (Set A) was analysed in six replicates for each concentration according to the method described in Section 2.5. In the second one (Set B), also consisted of six replicates for each concentration (10 ng/ml and 25 ng/ml), the standards solutions (COC and CE) were spiked to 2.5 ml acetate buffer immediately before the SPME procedure. The absolute recovery was evaluated by comparison of the mean response obtained for the set A (processed) and the response of set B (unprocessed). The unprocessed response represented 100% recovery.

2.6.2. Intra and inter-assay precision

Precision, defined as the relative standard deviation or coefficient of variation (CV), was determined by intra and inter-assay. They were performed by analysing negative human sweat samples (quality controls) spiked with cocaine and cocaethylene in the concentration of 25 ng and 125 ng/patch (relative to the concentration of 10 ng/ml and 50 ng/ml in the final solution) in three different days. The analyses were performed in six replicates for each day.

2.6.3. Limit of detection (LOD)

LOD was determined by an empirical method that consists of analysing a series of sweat samples containing decreasing amounts of the analyte [10]. LOD was the lowest concentration that presented a CV that did not exceed 20%. The LOD should still satisfy the predetermined acceptance criteria of qualification (retention time within 1% of standards and ion ratios within 20%).

2.6.4. Stability

Analyte stability in sweat samples patches containing 10 ng/ml of COC and CE (25 ng/patch) was evaluated at room temperature during 7 days and at 4 °C during 14 days. Stability tests were performed in three replicates.

3. Results

Fig. 1 shows a chromatogram obtained with the practical use of this method to the analyses of sweat samples (blank sample, a sample spiked with 125 ng/patch of analytes and a positive sample deriving from a cocaine user). The analysis revealed the presence of cocaine and cocaethylene.

The confidence parameters of the validated method (LOD, intra- and inter-assay precision and recoveries) for the detection of cocaine and cocaethylene are shown in Table 1.

A loss of <2% of cocaine was observed after storage of sweat samples at room temperature (7 days) and at 4 °C (14

Table 1
Confidence parameters of the validated method for the detection of cocaine and cocaethylene in sweat samples

	Cocaine	Cocaethylene
Recovery (%)		
C1	103	97
C2	58	50
LOD (ng/patch)	12.5	12.5
Intra-assay precision (CV, %)		
C1	6.9	7.0
C2	5.9	5.5
Inter-assay precision (CV, %)		
C1	3.6	2.0
C2	7.6	8.6

LOD: limit of detection; C1 = 25 ng/patch; C2 = 125 ng/patch; CV: coefficient of variation.
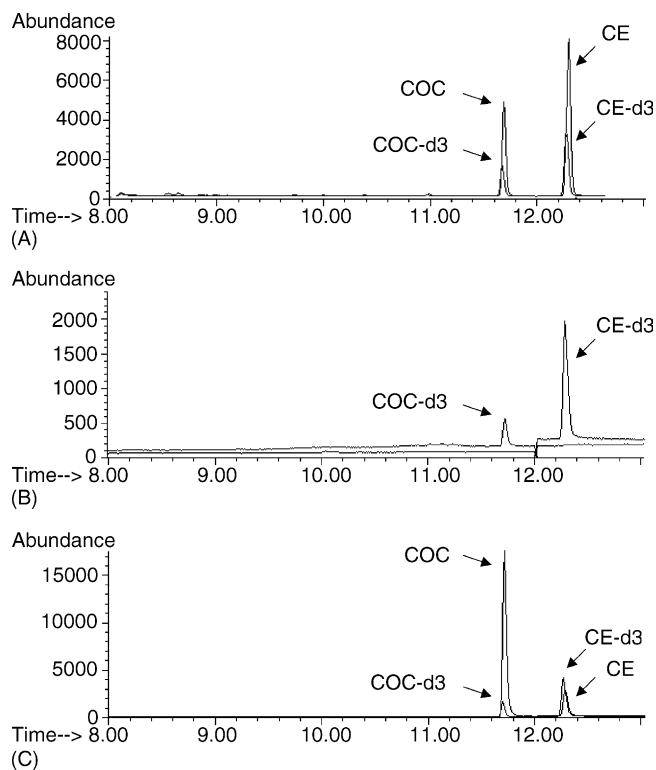

Fig. 1. (A) Chromatographic profile obtained with the SPME/GC–MS analysis of a spiked sweat sample containing 125 ng/patch of both cocaine (COC) and cocaethylene (CE). (B) Blank sample. (C) Authentic sweat sample deriving from a cocaine user.

days). For cocaethylene a loss of approximately 17% was observed in the fifth day at room temperature and in the 14 day at 4 °C.

4. Discussion

In the methods for determining COC, CE and metabolites in sweat samples that have been reported in the scientific literature [3–7], acetate buffer 0.2 M (pH = 5) with methanol (25:75), 0.1% Triton X-100 in 0.2 M acetate buffer or only acetate buffer 0.5M (pH 4) are the solutions used to elute sweat samples from the cellulose pads. The time of immersion of the pad in the eluent ranged from 30 min to 2 h. Moreover, some authors also recommend to shake the system (pads and elution buffer). After this step, a solid phase extraction (SPE) is normally used to extract the analytes from the obtained solution.

In the present work, the pads were immersed in 2.5 ml of acetate buffer 0.2 M for 15 min without shaking. Using this method, average recoveries of 80% and 74% for cocaine and cocethylene were obtained, respectively. Solid-phase microextraction (SPME), a relatively new technique, was used to extract COC and CE from the eluate. Simplicity, rapidity, less sample manipulation and solvent-free extraction were some advantages obtained with the use of this technique compared to SPE [11].

Despite parent drugs are predominant in sweat samples, the detection of metabolites could increase the reliability of positive results. This fact could distinguish environmental contamination from consumption of drugs. However, studies have shown that the quantity of benzoylecgonine, the main metabolite of cocaine, excreted in sweat is very low (<10%) [8]. Besides, benzoylecgonine is present to a small extent in street-grade cocaine and appears to be produced by cocaine degradation on the skin [12]. Hence, benzoylecgonine was not considered in this study. Its extraction and detection would present a great analytical difficulty due to its polar nature that would demand an additional step of derivatization.

The parameters of validation study (recovery, precision and stability) were obtained taking into consideration the reference value (cut-off) proposed by Substance Abuse and Mental Health Services Administration (SAMHSA) for COC in sweat (10 ng/ml or 25 ng/patch) [13,14]. The use of a cut off criteria is a common practice for reporting positive results in drug abuse area. In the case of sweat samples collected with a non-oclusive patch device, quantification of analytes does not have practical meaning. Concentrations of analytes cannot be determined due to the impossibility to measure the volume of secreted sweat. Moreover, the concentration of drugs in sweat is highly dependent upon the individual perspiration, the site of collection and the time of wearing of the patch. Patch technology is more suitable to detect drug use and should not be used to determine the amount of drug consumed or specific time of use. Nine out 21 sweat samples presented positive results for cocaine by the proposed SPME/GC–MS method. In one of these positive samples, cocaethylene were also detected.

In short, although the proposed method is a semi-quantitative one, it may find useful application in the treatment and monitoring of drug abusers. The method showed to be highly precise with the use of deuterated internal standards for the analyses of cocaine and cocaethylene. Good sensitivity was also obtained for both analytes.

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