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Short communication

Determination of cocaine, benzoylecgonine and cocaethylene in human hair by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

The present work describes a highly precise and sensitive method developed to detect cocaine (COC), benzoylecgonine (BE, its main metabolite) and cocaethylene (CE, transesterification product of the coingestion of COC with ethanol) in human head hair samples. The method was based on an alkylchloroformate derivatization of benzoylecgonine and the extraction of the analytes by solid-phase microextraction (SPME). Gas chromatography–mass spectrometry (GC–MS) was used to identify and quantify the analytes in selected ion monitoring mode (SIM). The limits of quantification and detection (LOQ and LOD) were: 0.1 ng/mg for COC and CE, and 0.5 ng/mg for BE. Good inter- and intra-assay precision was observed. The dynamic range of the assay was 0.1–50 ng/mg. The method is not time consuming and was shown to be easy to perform.

Keywords: Cocaine; Benzoylecgonine; Cocaethylene

1. Introduction

Recently, many studies are being developed with the aim of improving drug detection in specimens other than the conventional ones such as urine and blood. So, a large number of drug abuse studies, forensic toxicology analyses, drugs in the workplace and even in doping control in sports activities related to drug detection in human hair have been described in the literature [1–3]. This is specially true for cocaine and metabolites (Fig. 1) [4–11]. The interest in the development and optimisation of analytical techniques to detect them in hair is explained by the several advantages hair has over other biological matrices. Hair is a sample obtainable in a non-invasive way, it is not easily adulterated and it can be stored and transported without special conditions thanks to its stability. Moreover, hair analysis permits the assessment of early cocaine exposure and at the same time allowing a chronological profile of the consumption depending on the length (segments) of hair to be obtained.

There are some gas chromatography–mass spectrometry (GC–MS) methods in the literature for the determination of cocaine (COC), benzoylecgonine (BE) and cocaethylene (CE) in hair samples. Sample preparation for cocaine and metabolites in hair generally involves a decontamination step, sample incubation in aqueous solution or solvents, derivatization of its polar metabolites and extraction of the analytes. Liquid–liquid extraction (LLE) or more commonly solid-phase extraction (SPE) are utilised for this purpose [4–9].

Besides preventing chemical instability, one of the requirements for an appropriate liberation of the drugs from hair structures is that the solvent used can reach the various places in which the compounds are located in the matrix. The complete digestion of hair using alkaline solutions and temperature is the most applied technique to liberate stable drugs such as cannabinoids and amphetamines [12]. However, for ester compounds like cocaine and metabolites, such strong chemical conditions will invariably hydrolyse the analytes.

Eser et al. found good results for drug recovery (opiates, cocaine and benzoylecgonine) when hair samples were treated with methanol or aqueous solution extractions. In

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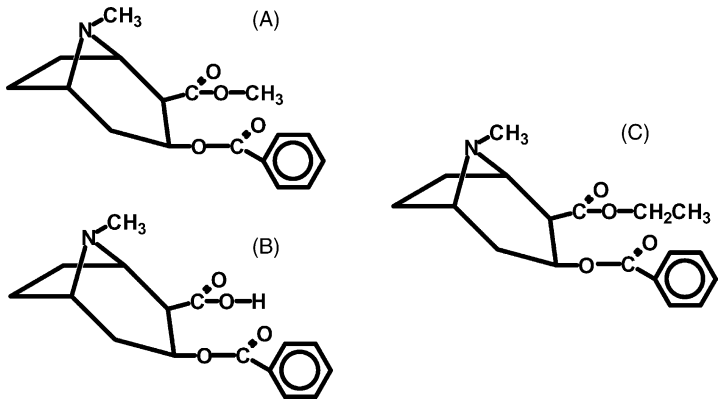

Fig. 1. Chemical structures of: (A) cocaine; (B) benzoylecgonine; (C) cocaethylene.

general, drug liberation from hair with aqueous solution showed to be similar to methanol, but better than other organic solvents [13]. In fact, both are the most employed system in the literature to liberate cocaine and metabolites from hair [4–11].

Recently, solid-phase microextraction (SPME), a relatively new technique, gained considerable of interest in a broad field of analysis including drug abuse area. Simplicity, rapidity, less sample manipulation and solvent-free extraction are some advantages of its use. It consist of a fibre coated with stationary phase which is dipped for some minutes in an aqueous solution in which the drugs to be analysed are present. The fibre is then placed in the injection port of the chromatograph in order to strip the adsorbed substances. So, substances dissolved in an aqueous solution at part per billion levels can be detected [14–16].

In hair analysis of drugs of abuse, SPME has been used mainly for the determination of amphetamines [17–20]. The detection of cannabinoids and methadone in hair were also reported [21–23]. Strano-Rossi and Chiarotti proposed a method to detect cannabinoids in hair by SPME and suggested its possible application to other drugs, including cocaine. According to the authors, a screening test to detect cocaine could be performed using an enzymatic digestion with subsequent SPME extraction. To confirm positive results, the authors suggested the use of the remaining solution for the determination of cocaine metabolites [23]. However, as far as we know, no analytical method in the scientific literature describes the use of SPME for simultaneous analysis of cocaine and metabolites present in hair.

So, the aim of this study was to develop a derivatization/solid-phase microextraction method for the identification and quantification of cocaine, cocaethylene and benzoylecgonine in human head hair using GC–MS. Butylchloroformate was used as derivatization reagent to convert benzoylecgonine in butylbenzoylecgonine, a less polar substance, prior to the extraction by SPME using a 100 μm polydimethylsiloxane fibre.

2. Experimental

2.1. Reagents and standards of reference

Methanol and acetonitrile (Merck, Darmstadt, Germany) were reagent grade solvents. Cocaine, benzoylecgonine and cocaethylene used as calibrators and their respective deuterated-labelled analogues, COC-d3, BE-d3 and CE-d3 (internal standards) solutions (1 mg/ml) were purchased from Radian International (Austin, TX, USA). According to the certificate of analysis supplied by the manufacturer, the purity of all standards was 99%. Butylchloroformate was purchased from Aldrich (Milwaukee, WI, USA). Pyridine, anhydrous sodium bicarbonate and potassium carbonate were of the analytical grade purchased from Merck.

2.2. Preparation of standard solutions

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

2.3. Instrumentation

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

GC–MS analyses for COC, BE 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 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. 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 9 min. The following ions were chosen for SIM analyses (quantification ions underlined): cocaine: 182, 272, 303; cocaine-d3: 185, 306; benzoylecgonine: 224, 272, 345; benzoylecgonine-d3: 227, 348; cocaethylene: 196, 272, 317; cocaethylene-d3: 199, 320.

2.4. Preparation of spiked hair

Negative human head hair samples were submitted to an initial procedure of decontamination by washing them with dichloromethane (2 ml/50 mg of sample) for 15 min at 37 °C to remove external contamination. Then they were dried, cut into small pieces and 50 mg aliquots were used for analyses.

2.5. Preparation of the sample

2.5.1. Sample digestion

In a stoppered glass tube, 50 ng of each deuterated internal standard (cocaine-d3, benzoylecgonine-d3 and cocaethylene-d3) were added to the hair sample. Afterwards, 2 ml of methanol was added to the sample. The system was maintained at 50 °C for 18 h for drug liberation from the matrix. The methanol was transferred to a 4 ml silanised vial and evaporated at 50 °C under a stream of nitrogen.

2.5.2. Derivatization

The residue obtained was treated with 100 μl of acetonitrile, 2 μl of pyridine and 2 μl of butylchloroformate under ultrasonification at room temperature for 6 min to derivatise BE to a less polar compound. Afterwards, 2 ml of deionised water and 30 mg of a solid buffer ($NaHCO_3$:K_2CO_3 ratio of 2:1) were added directly to the derivatization solution in order to reach a pH value of approximately 9–10.

2.5.3. Solid-phase microextraction

SPME was performed by directly dipping a 100 μm polydimethylsiloxane fibre in the derivatization solution for 20 min under magnetic stirring to obtain an optimal exchange surface. After the extraction time, the SPME device was directly inserted in the GC injector port at 250 °C for a 20 min desorption.

2.6. Validation of the method

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

2.6.1. Recovery

The recovery studies for COC, BE and CE were performed taking into consideration the possible loss of the analytes by decomposition (hydrolysis) after the digestion procedure of the matrix. Samples spiked with different concentrations of calibrators were analysed. One set of samples (set A) consisting of three concentrations for all drugs (1, 15 and 30 ng/mg) was analysed in six replicates for each concentration according to the method described in Section 2.5. The other one (set B), also consisted of samples analysed in six replicates for each concentration (1, 15 and 30 ng/mg). However, for this set, the calibrators were spiked to the sample 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. Linearity

The study of linearity was estimated by the analysis of extracts obtained from aliquots of hair spiked, in triplicate, with 50 ng of internal standard of each analyte and standards of the respective non-deuterated ones (calibrators) at the following concentrations: 0.1, 5.0, 10, 20, 40 and 50 ng/mg.

2.6.3. 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 human hair samples in the concentration of 1, 15 and 30 ng/mg for all analytes in three different days. The analyses were performed in six replicates for each concentration.

2.6.4. Limit of detection and limit of quantification

LOD and LOQ were determined by an empirical method that consists of analysing a series of hair samples containing decreasing amounts of the analyte [24]. 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%. 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. 2 shows chromatograms obtained with the practical use of this method to the analyses of head hair samples (blank sample, a sample spiked with 1 ng/mg of analytes and a positive sample deriving from an individual victim of violent death). The analysis revealed the presence of cocaine at a concentration of 47.4 ng/mg, butylbenzoylecgonine (4.1 ng/mg) and cocaethylene (1.3 ng/mg).

The calibration curves were linear over the specified range (0.1–50 ng/mg). The linear regression equations and coefficients of correlation were: $Y = 1.503X - 2.914$, $r^2 = 0.989$ (cocaine), $Y = 0.945X + 0.638$, $r^2 = 0.991$ (benzoylecgonine), and $Y = 1.153X - 1.848$, $r^2 = 0.988$ (cocaethylene), where Y and X represent the relationship between the peak area ratio (compound/internal standard) and the corresponding calibration concentrations, respectively.

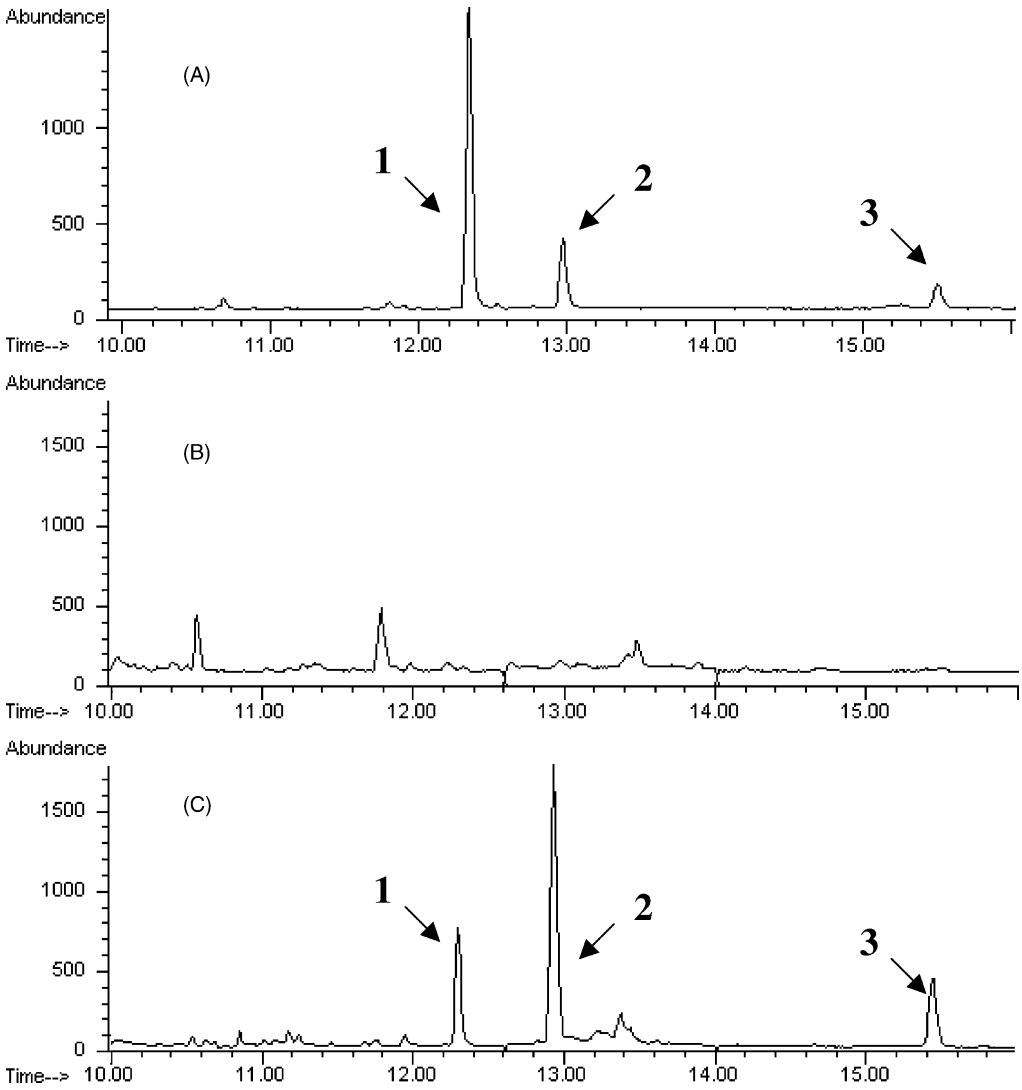

Fig. 2. (A) Chromatographic profile obtained with the GC–MS analysis of an authentic hair sample containing: (1) cocaine at a concentration of 47.4 ng/mg; (2) cocaethylene at a concentration of 1.3 ng/mg; (3) benzoylecgonine at a concentration of 4.1 ng/mg. (B) Blank sample. (C) Spiked hair sample with 1 ng/mg of analytes.

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

4. Discussion

The use of a lengthy incubation time with aqueous solution or methanol (overnight or 18 h) and mild temperature seems to be now a consensus in the scientific literature to liberate cocaine and metabolites from hair [4–11]. So, hair analyses of cocaine and metabolites is time consuming. In our point of view, everything that can be done to minimise the time of analyses is of great value in analytical toxicology. Before the development of the present method, solid-phase extraction was used in our laboratory to extract cocaine and metabolites from hair samples, similarly to the method described by Montagna et al. [7]. However, SPE has some important limitations: multi-step process and relatively high solvent volumes are necessary to clean up and elute the analytes. With the use of SPME, less solvents are spent and consequently less residues are generated. Moreover, the use of SPME represented savings of time. Whilst the new method allows the analysis of approximately 20 hair samples in 8 h after the incubation time, the method using SPE allows only the analysis of no more than 12 hair samples.

In the present study, methanol (2 ml) was used as solvent due to its efficiency in liberating the analytes from the biological matrix [13] and its great facility to evaporate prior to benzoylecgonine derivatization. In spite of the long incubation time (18 h) and high temperature (50 °C), studies of recovery showed that using methanol the losses of analytes

Table 1
Confidence parameters of the validated method for the determination of cocaine, butylbenzoylecgonine and cocaethylene in hair samples

	Cocaine	Butylbenzoylecgonine	Cocaethylene
Recovery (%)			
*C*1	74.6	90.8	88.1
*C*2	108.1	101.0	104.5
*C*3	98.4	94.4	98.0
LOD (ng/mg)	0.1	0.5	0.1
LOQ (ng/mg)	0.1	0.5	0.1
Intra-assay precision (CV, %)			
*C*1	5.9	2.1	7.0
*C*2	4.9	2.7	6.5
*C*3	5.1	3.8	4.9
Inter-assay precision (CV, %)			
*C*1	1.8	0.3	1.1
*C*2	9.3	3.8	9.7
*C*3	8.0	5.6	14.2

LOQ: limit of quantification; LOD: limit of detection; $C1 = 1$ ng/mg; $C2 = 15$ ng/mg; $C3 = 30$ ng/mg; CV: coefficient of variation.

by hydrolysis or decomposition were acceptable (more than 74% recoveries for all analytes).

A variety of derivatization reagents are used in the analysis of drugs of abuse. After examination of several of these reagents, butylchloroformate with the appropriate catalyst was chosen to convert benzoylecgonine to butylbenzoylecgonine. This substance is more stable in aqueous solution than other products such as pentafluoropropyl or silyl derivatives frequently described in the literature. This derivatization step in the analysis enables the compatibility of benzoylecgonine with SPME and provides good separation on the GC capillary column. However, to avoid the hydrolysis of cocaine, cocaethylene and the formed product butylbenzoylecgonine in alkaline solution, the solid buffer ($NaHCO_3/K_2CO_3$) was added to the sample only a few minutes before the SPME procedure.

Hall et al. were the first group to describe the alkylchloroformate derivatization of benzoylecgonine in conjunction with SPME. In their method, benzoylecgonine was directly derivatised in urine, using hexylchloroformate and a mixture containing acetonitrile:water:hexanol:2-dimethylaminopyridine (5:2:2:1, v/v/v/v) to yield hexylbenzoylecgonine as the product [25]. In order to improve our method in terms of labour and time, we tried to adapt this procedure in hair analysis. However, our attempt to derivatise benzoylecgonine directly in the aqueous solution was unsuccessful.

The analytical technique proposed for the determination of cocaine, cocaethylene and benzoylecgonine showed to be highly precise with the use of the respective deuterated internal standards. Good sensitivity and linearity were also obtained for all analytes. Recoveries for COC, BE and CE were very similar in our method and varied between 74.6 and 108.1%. The coefficients of correlation (r^2) were higher than 0.98 for the dynamic range studied (up to 50 ng/mg).

References

[1] C.M. Selavka, F. Riders, Forensic Sci. Int. 70 (1995) 155.
[2] M.A. Huestis, Ther. Drug Monit. 18 (1996) 456.
[3] A.F. Midio, R.L.M. Moreau, O.A. Silva, Sports Med. 31 (2001) 321.
[4] M. Montagna, A. Polettini, C. Stramesi, A. Groppi, C. Vignali, Forensic Sci. Int. 128 (2002) 79.
[5] J.A. Bourland, E.F. Hayes, R.C. Kelly, S.A. Sweeney, M.M. Hatab, J. Anal. Toxicol. 24 (2000) 489.
[6] S. Paterson, N. McLachlan-Troup, R. Cordero, M. Dohnal, S. Carman, J. Anal. Toxicol. 25 (2001) 203.
[7] M. Montagna, C. Stramesi, C. Vignali, A. Groppi, A. Polettini, Forensic Sci. Int. 107 (2000) 157.
[8] L. Skender, V. Karacic, I. Brcic, A. Bagaric, Forensic Sci. Int. 125 (2002) 120.
[9] C. Jurado, M.P. Giménez, M. Menéndez, M. Repetto, Forensic Sci. Int. 70 (1995) 165.
[10] P. Kintz, Ther. Drug Monit. 18 (1996) 450.
[11] M. Uhl, Forensic Sci Int. 84 (1997) 281.
[12] Y. Nakahara, J. Chromatog. B 733 (1999) 161.
[13] H.P. Eser, L. Pötsch, G. Skopp, M.R. Moeller, Forensic Sci. Int. 84 (1997) 271.
[14] H. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[15] S. Ulrich, J. Chromatogr. A 902 (2000) 167.
[16] G. Theodoridis, E.H.M. Koster, G.J. de Jong, J. Chromatogr. B 745 (2000) 49.
[17] S. Gentili, A. Torresi, R. Marsili, M. Chiarotti, T. Macchia, J. Chromatogr. B 780 (2002) 183.
[18] F. Musshoff, H.P. Junker, D.W. Lachenmeier, L. Kroener, B. Madea, J. Chromatogr. Sci. 40 (2002) 359.
[19] J. Liu, K. Hara, S. Kashimura, M. Kashiwagi, M. Kageura, J. Chromatogr. B: Biomed. Sci. Appl. 758 (2001) 95.
[20] I. Koide, O. Noguchi, K. Okada, A. Yokoyama, H. Oda, S. Yamamoto, H. Kataoka, J. Chromatogr. B: Biomed. Sci. Appl. 707 (1998) 99.
[21] F. Sporkert, F. Pragst, J. Chromatogr. B: Biomed. Sci. Appl. 746 (2000) 255.
[22] A.C. Lucas, A.M. Bermejo, M.J. Tabernero, P. Fernandez, S. Strano-Rossi, Forensic Sci. Int. 107 (2000) 225.
[23] S. Strano-Rossi, M. Chiarotti, J. Anal. Toxicol. 23 (1999) 7.
[24] D.A. Ambruster, M.D. Tilman, L.M. Hubbs, Clin. Chem. 40 (1994) 1233.
[25] B.J. Hall, A.R. Parikh, J.S. Brodbelt, J. Forensic Sci. 44 (1999) 527.