

Analysis of drugs of abuse in hair by automated solid-phase extraction, GC/EI/MS and GC ion trap/CI/MS

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Abstract

In our laboratory, analysis of human hair for the detection of drugs of abuse was first performed in 1995. Initially, requests for hair analysis were few, and it is only since 1997 that these analyses have become routine. As demand grew, we developed an automatic solid-phase extraction method; the use of a robot ASPEC allowed us to drop certain fastidious manipulations, and to treat a large number of samples at a time. This method is described, along with analysis by gas-chromatography-mass spectrometry (GC/MS) in selected ion monitoring mode (SIM), for the following drugs: codeine, 6-monoacetylmorphine (6-MAM), morphine, cocaine, methadone, ecstasy (MDMA) and Eve (MDE). This requires prior derivatization with propionic anhydride. The different validation parameters, linearity, repeatability, recovery and detection limits are described, as well as the application of this method to some real cases. Analysis of these cases is also performed by an ion trap GC/MS in chemical ionization mode (GC/IT/CI/MS) in order to demonstrate the usefulness of this technique as a complement to routine analysis. Analysis by GC/IT/CI/MS indeed avoids the risk of false-positive results by the identification of metabolites. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Drug testing is generally based on blood or urine analysis, sometimes on liver or kidney tissue in post-mortem cases. But blood and urine concentrations only reflect

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dosages of several hours and several days respectively. Hair analysis has become very important because it provides much information about consumption over a long period [1]. On the other hand, analysis of human hair can also provide evidence of the lack of use of drugs of abuse [2].

Different methodological approaches have been proposed for the pre-treatment of hair samples but after these pre-treatments, the extraction procedure remains an important step during the analysis. The most popular extraction technique is that of liquid–liquid (LLE) but the solid-phase extraction (SPE) is becoming more and more important, claiming a not inconsiderable place in the analysis of hair.

An automated solid-phase extraction method has previously been published for the analysis of opiates (codeine, morphine, 6-MAM) in hair [3]. Then this method was extended to extract other drugs such as cocaine, methadone, ecstasy (MDMA) and Eve (MDE).

This paper describes the validation and the utilization of this method in our laboratory for routine use.

2. Materials and methods

2.1. Chemical reagents and instrumentation

Solvents, acid buffer and pyridine were provided by Merck (Darmstadt, Germany). The propionic anhydride was provided by Aldrich (Gillingham, England). Drugs and nalorphine were provided by Sigma (Buchs, Switzerland). Hair samples were pulverised in a ball mill provided by Retsch (Schieritz, Hauenstein, Switzerland).

Automated extractions were performed on a robot ASPEC (Gilson Medical Electronics, Villiers-le-Bel, France). Isolute HCX cartridges, provided by IST (Hengoed, UK) were used for the extraction. Sample analyses were done using a Hewlett Packard (HP) 5890 gas chromatograph equipped with a mass spectrometer HP 5988 operating in electronic impact mode with an energy of 70 eV.

2.2. Materials for examination and decontamination

Soaked hair was prepared according to the procedure described by Edder et al. [4]. Real hair was obtained from deceased subjects and from subjects undergoing forensic examination.

Before analysis, the hair was washed successively by percolation with 5 ml of methylene chloride, 5 ml of water and finally 5 ml of methanol. This step is very important to eliminate possible external contamination. Then, the hair was dried at 60°C for 30 min and pulverised for 10 min at 70 s⁻¹ [4].

2.3. Digestion and extraction

Drugs are fixed inside the hair matrix, therefore a digestion procedure is necessary before the extraction. About 50 mg of powdered hair were placed in a tube and 1 ml of hydrochloric acid 0.01 M was added. After incubation at 60°C for 12 h, the solution was

neutralised with 1 ml of NaOH 0.01 M and buffered with 1 ml of phosphate buffer pH 7.0. After centrifugation at 4000 r.p.m. for 5 min, the supernatant was removed into a special tube for the extraction.

The ASPEC system was programmed to extract the samples with the following different steps:

1. Column conditioning with methanol
2. Column conditioning with water
3. Dispensing samples on the column
4. Rinsing with water
5. Rinsing column with acetate buffer pH 4
6. Rinsing column with methanol
7. Drying column with air
8. Elution with methylene chloride/isopropanol/ammoniac hydroxide
9. Addition of the standard Nalorphine.

All the procedures have already been described in an earlier article [5].

2.4. Derivatization

GC/MS analysis required a procedure of derivatization by propionylation. 100 μ l of pyridine and 100 μ l of propionic anhydride (PA) were added to the extract and incubated for 30 min at 60°C. After evaporation under a stream of nitrogen, the extract was reconstituted in 50 μ l of ethyl acetate.

2.5. Gas chromatography-mass spectrometry method

Helium was used as carrier gas with the following capillary column (DB5MS, 15 m $\times$ 0.25 mm $\times$ 0.25 μ m, J&W Scientifics (Foldon, USA)). Temperatures: 85°C maintained for 1 min to 190°C at 15°C/min and maintained for 0.5 min, to 210°C at 2°C/min maintained for 1 min, to 270°C at 20°C/min for the final 8 min. Injector temperature was 270°C and injection was made in splitless mode.

Three μ l of the sample were injected into the GC/MS system operating in selected ion monitoring mode (SIM). The source and interface temperatures were 200 and 280°C respectively. The detection was performed with the following ions: codeine m/z =355 and 282, 6-MAM m/z =383 and 327, morphine m/z =397 and 341, cocaine m/z =303 and 182, methadone m/z =294 and 72, MDMA m/z =114 and 162, MDE m/z =162 and 72, nalorphine m/z =423 and 367.

3. Results and discussion

3.1. Validation process

Soaked hair was used for the validation process. In these conditions, all the compounds were well separated. Fig. 1 shows the chromatogram obtained.

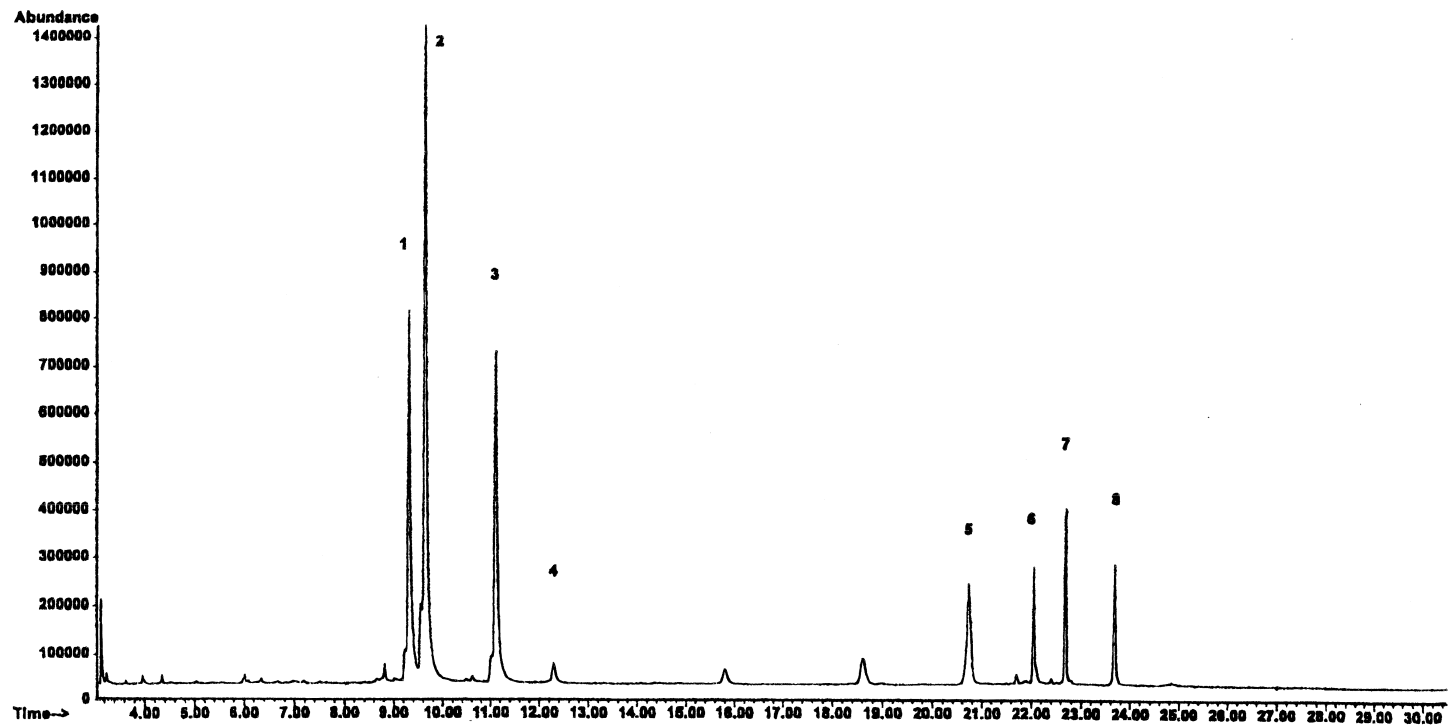


Fig. 1. Chromatogram of soaked hair (GC/EI/MS): (1) MDMA (25 ng/mg); (2) MDE (20 ng/mg); (3) Methadone (15 ng/mg); (4) Cocaine (15 ng/mg); (5) Codeine (18 ng/mg); (6) 6-MAM (20 ng/mg); (7) Morphine (23 ng/mg); (8) Nalorphine (SI).

Table 1
Calibration curves and correlation coefficients

Compound	Target ion	Equation	R
Codeine	355	$y = 0.00286x - 0.03355$	0.9987
6-MAM	383	$y = 0.00146x - 0.02924$	0.9979
Morphine	397	$y = 0.00127x - 0.00468$	0.9999
Cocaine	303	$y = 0.00055x + 0.00071$	0.9984
Methadone	294	$y = 0.00067x - 0.00487$	0.9963
MDMA	114	$y = 0.00510x + 0.16091$	0.9969
MDE	162	$y = 0.03426x + 1.52329$	0.9958

3.1.1. Linearity

Standard calibration was done in hydrochloric acid (0.01 M) in a domain of linearity between 50 and 1000 ng corresponding to a domain between 1 and 20 ng/mg of hair. These standard were analysed by the complete procedure. Table 1 shows the linearity and the correlation coefficients obtained for all the compounds.

3.1.2. Limits of detection

The limit of detection (LOD) was evaluated as the lowest concentration giving a chromatographic peak with the signal to noise ratio $S/N=3$. The limit of detection obtained for the opiates and MDE is 0.05 ng/mg. 0.10, 0.15 and 0.20 ng/mg were obtained for MDMA, cocaine and methadone respectively (Table 2).

3.1.3. Extraction recovery

Extraction recovery was determined by comparing the peak areas of an extracted hydrochloric acid solution with the peak areas of a methanolic solution both at the same concentration. These solutions were prepared six times each. Extraction recovery obtained was between 79 and 103% (Table 2). The recovery of 103% obtained for the morphine was probably due to a slight hydrolysis of the 6-MAM.

Table 2
Limits of detection, extraction recovery and repeatability evaluated with soaked hair ($n=6$)

Compound	LOD (ng/mg)	Recovery (%)	Cv (%)	High conc. (ng/mg)	Cv (%)	Low conc. (ng/mg)	Cv (%)
Codeine	0.05	95	6.0	31.9	7.0	4.2	7.5
6-MAM	0.05	80	3.1	26.6	2.4	3.5	7.9
Morphine	0.05	103	5.4	32.9	3.8	3.5	6.4
Cocaine	0.15	79	7.3	41.1	6.2	5.5	11.6
Methadone	0.20	94	7.9	31.9	6.8	3.5	9.7
MDMA	0.10	81	5.6	27.7	5.0	7.9	6.1
MDE	0.05	71	6.6	37.1	5.2	10.9	8.0

3.1.4. Repeatability

Six replicates of soaked hair of low concentration and high concentration were analysed through the complete procedure. The relative standard deviation (RSD) obtained is generally inferior to 10% for the two concentrations studied (Table 2).

3.2. Forensic applications

Three different hair samples were chosen to show the different applications of this method. Sample A was obtained from a driver being tested. The hair sample was divided into four segments of 3 cm each, from the roots to the tips. The drug concentrations found in each segment are shown in Table 3. The driver in question was a heroin and cocaine user who had been in methadone treatment for several months. There are no apparent ambiguities in the confirmation and interpretation of the results. The next two samples came from deceased subjects. In sample B, a significant concentration of methadone was found in the hair sample (methadone 6 ng/mg); in sample C, cocaine alone was identified (cocaine 246 ng/mg). In both these cases, the consumption of drugs of abuse was proved. This method can thus reveal past or recent use of a certain category of substances. There remain, however, a large number of compounds that still escape identification.

3.3. Ion-trap/CI/MS

It is sometimes necessary to confirm a result by another analytic technique. This is especially relevant for drug concentrations that are close to cut-off. Also to avoid a false-positive interpretation due to external contaminants, testing is then carried out for the presence of drug metabolites. Furthermore, in some cases, testing for other substances such as benzodiazepines and antidepressants can be useful to prove long-term excessive use.

3.3.1. Apparatus

Positive ion chemical ionization mass spectrometric analysis of hair extracts was performed on a Varian Saturn 2000 (Walnut Creek, CA) ion-trap mass spectrometer, coupled to a Varian 3400 GC equipped with a capillary column (DB5MS, 15 m×0.25 mm, 0.25 µm film thickness, J&W Scientific, Folsom, CA). Ultra-high purity helium was used as a carrier gas with a head pressure of 7 psi; 1 µl of reconstituted extract was injected in splitless mode. The column temperature was programmed for an initial

Table 3
Analysis of sample A by GC/EI/MS and GC/IT/CI/MS (concentration in ng/mg)

	Codeine	Morphine	6-MAM	Methadone	Cocaine
Segment 1	1.4/1.7	2.1/1.9	1.7/2.2	4.9/4.9	11/9
Segment 2	1.1/1.2	1.7/1.9	3.2/4	0/0.5	14/9
Segment 3	0/0.2	0.4/0.2	2.5/2.9	0/0.2	21/14
Segment 4	0/0.1	0.5/0.3	7.2/7.2	0/0	56/22

temperature of 85°C held for 1 min, increased to 190°C at 15°C/min and held for 0.5 min, then to 210°C at 2°C/min, held for 0.5 min, and then to 290°C at 20°C/min for the final 6 min. The injector temperature was 270°C.

The ion trap was operated with a transfer line and a source temperature of 280 and 200°C respectively. The filament emission current was 10 μ A. Acetonitrile was used as the reagent gas with the following ionization parameters: maximum ionization time, 200 μ s; maximum reaction time, 40 ms. A full-scan GC/MS analysis of mass range 100–450 amu was performed at 1 s/scan, on all extracts in routine qualitative assays.

3.3.2. Forensic applications

Chemical ionization (CI) has the advantage of being a fairly soft ionization process. Compounds comprising ions with a relatively low mass-to-charge ratio, but high intensity in electronic impact, receive ions with a higher m/z ratio, thus more compound-specific, in chemical ionization. This is the case with methadone, with an m/z ratio of 72 in EI, and two ions with m/z ratios of 310 and 265 in CI. Chemical ionization thus demonstrates higher specificity for this compound. Furthermore, acquisition being performed in full-scan, a wider range of compounds can be detected. Sample A, previously analysed by EI, was injected in CI (Fig. 2), with the aim of proving the reproducibility of the results (Table 3). The results obtained were within the same range, with slightly lower concentrations for cocaine. The quantification limit is however lower for all of these compounds. Sample B showed the presence of methadone, and a high concentration of venlafaxine, an antidepressant. Both these compounds were identified in CI (Fig. 3). Cocaine was revealed in the hair of sample C, and 0.4% of alcohol was present in blood. Metabolites were tested for to confirm the consumption of cocaine; cocaethylene (CET) was identified from the propionylated extract and a supplementary derivatization using pentafluoropropionic anhydride (PFPA) was necessary to show the presence of benzoylecgonine (BZE) (Fig. 4). The detection of metabolites in hair is not easy. Kintz et al. [6] observed that benzoylecgonine could only be detected when the cocaine concentration was higher than 2 ng/mg. Cocaine was present in concentrations approximately 3–6 times higher than those of its main metabolites. This was also observed for opiates where the ratio of 6-MAM to morphine was in the range 1.3–10 [7].

Moeller et al. [8] showed that the methadone to EDDP ratio ranged from 1.5 to 7.2.

4. Conclusion and perspectives

This paper describes a method (GC/MS/EI) used in our laboratory for three years. The number of forensic hair cases has grown so that an automated SPE method had to be developed. We obtained a good repeatability and avoided long and fastidious manipulations. The use of gas chromatography/mass spectrometry in selected ion monitoring mode allowed for the identification of drugs of abuse with a high reliability and we showed the important complementarity of the use of an ion trap/mass spectrometer in chemical ionization mode. This one is important to confirm drugs consumption by the detection of metabolites. For the analysis of low concentrations in

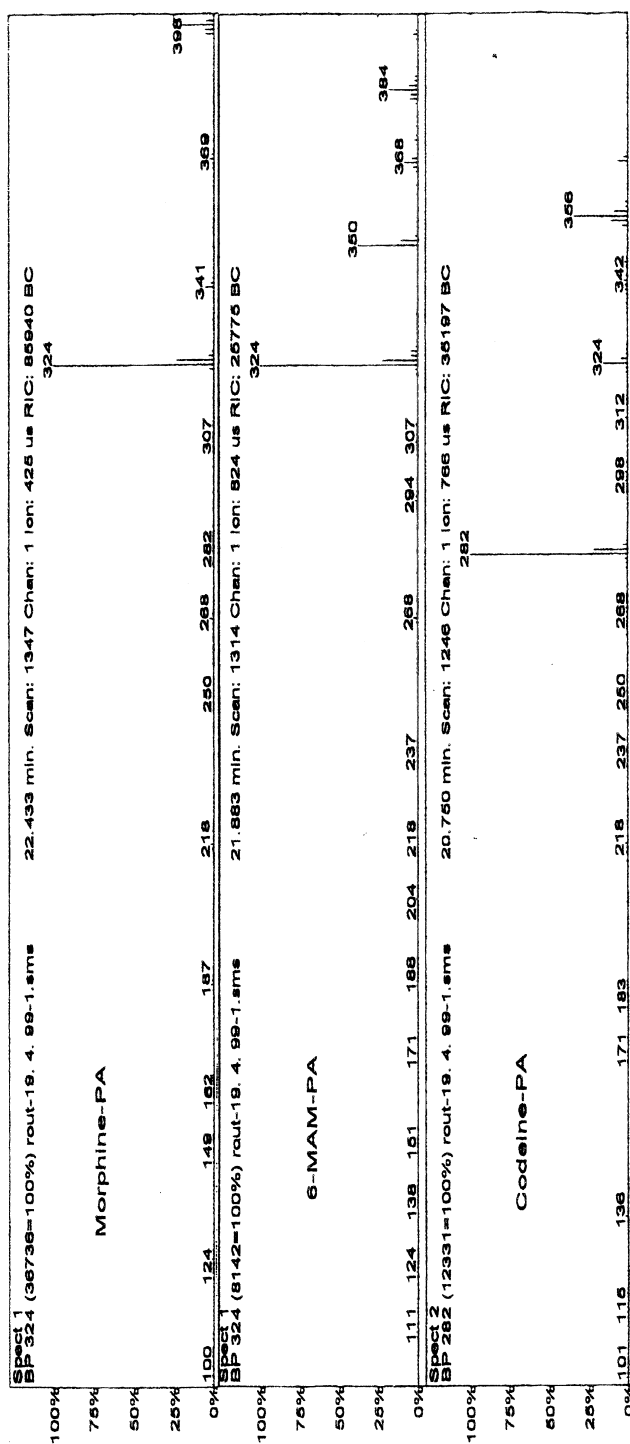


Fig. 2. Spectra of opiates in CI mode.

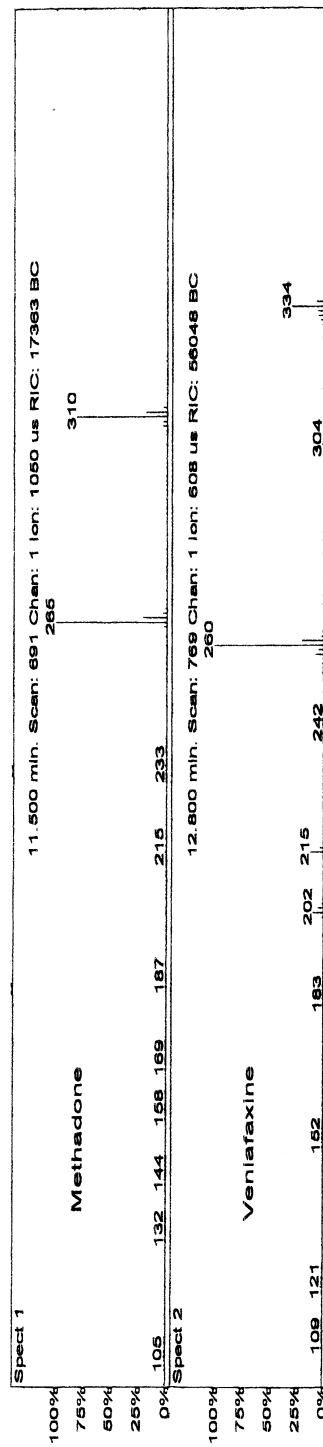


Fig. 3. Spectra of methadone and venlafaxine in CI mode.

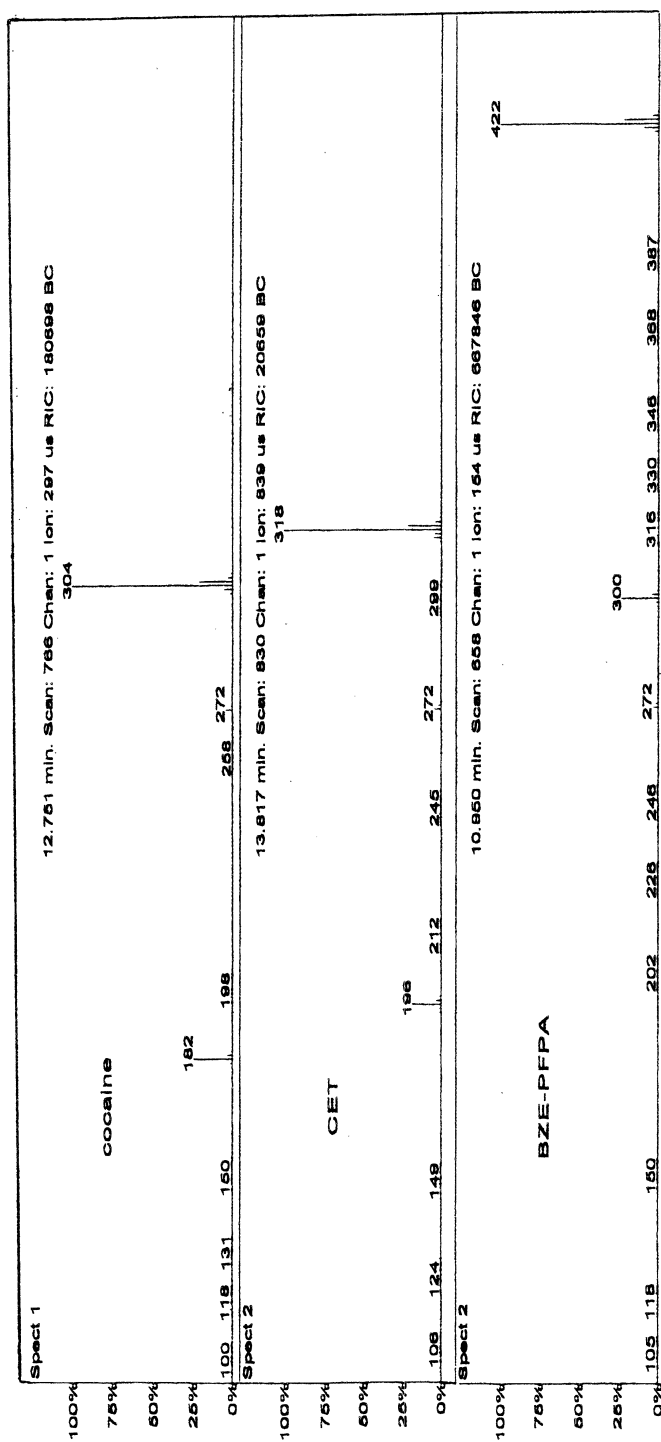


Fig. 4. Spectra of cocaine and metabolites in CI mode.

hair, the use of an advanced technique like CI/MS/MS should be required in order to reach the necessary sensitivity and specificity.

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