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# Surface-activated chemical ionization ion trap mass spectrometry for the analysis of cocaine and benzoylecgonine in hair after extraction and sample dilution

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**Surface-activated chemical ionization (SACI) was employed for the analysis of cocaine and its metabolite, benzoylecgonine, extracted from hair. Following decontamination and acid hydrolysis procedures on the hair sample, the sample solution was diluted (1:10) and directly analyzed by liquid chromatography/surface-activated chemical ionization multiple collisional stage single reaction monitoring mass spectrometry (LC/SACI-MS$^3$-SRM) without solid-phase extraction (SPE) pre-purification and concentration procedures. To increase the selectivity of the method, MS$^3$ was chosen instead of the less selective MS/MS. This data was compared with that achieved using gas chromatography/mass spectrometry (GC/MS), the reference method used by the Italian Government Institute of Health protocol. The limits of detection (LODs) were 0.003 ng/(mg hair) for cocaine and 0.02 ng/(mg hair) for benzoylecgonine and the limits of quantitation (LOQs) were 0.01 ng/(mg hair) for cocaine and 0.04 ng/(mg hair) for benzoylecgonine. The squared correlation coefficient ($R^2$) of the calibration curve was 0.9887–0.9980 for cocaine and 0.9987–0.9997 for benzoylecgonine. The percent accuracy error was 2–5% for both cocaine and benzoylecgonine using the LC/SACI-MS$^3$-SRM approach, whereas it was higher for benzoylecgonine (20–25%) using the LC/SACI-MS/MS-SRM approach compared with the GC/MS data due to hair matrix contamination. In both cases, high precision was achieved (1–3% precision error), which confirmed the stability of the developed methods. Copyright © 2007 John Wiley & Sons, Ltd.**

Addicted drug abuse is an increasing problem in our modern society.[1–4] These chemical compounds give rise to serious toxicological and social problems. An increase in drug abuse has been confirmed by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[5] For this reason, robust analytical approaches are necessary to control known and suspected drug addicted subjects.

A variety of approaches have been employed to identify and quantify cocaine and their metabolites.[6–8] In particular, mass spectrometry is a powerful technique in terms of sensitivity and specificity for the detection of these drugs. Gas chromatography/mass spectrometry (GC/MS) has been widely employed for the characterization of cocaine and its primary metabolite, benzoylecgonine, using derivatization procedures.[9,10] In most cases, a derivatization step is essential for the achievement of valid results, despite the fact that it is time-consuming and sometimes results in sample loss. Other methods employed for the analysis of these drugs are based on the use of capillary electrophoresis (CE)[11] and liquid chromatography/mass spectrometry (LC/MS) coupled with various ionization sources.[12–14]

Cocaine (Scheme 1(a)) and its metabolite, benzoylecgonine (Scheme 1(b)), can be detected in various biological matrices (e.g. saliva, plasma and hair).[3,4,14] There is a growing interest in the analysis of drugs in hair samples[4,10–12] because of the easy sampling procedure and the ability to monitor the drug addicts more rigorously; for instance, in the cases of documented drug addicts who have cut their hair to hide their drug use. Hair sample analysis is also advantageous due to its precision in determining when the drug was consumed by the subject. In fact, the time of consumption is related to the location of the drug along the hair shaft: the

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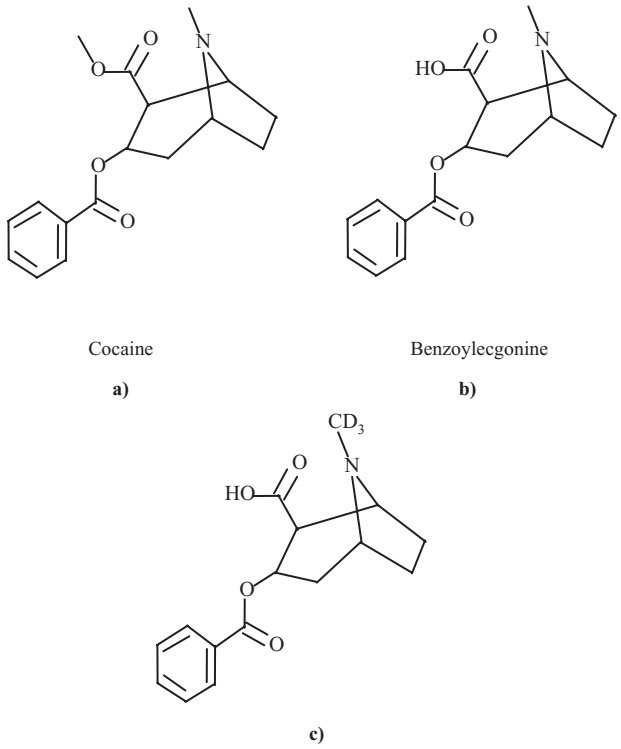


**Scheme 1.** Chemical structures of (a) cocaine, (b) benzoylecgonine, and (c) benzoylecgonineD3.

distance of the hair segment from the head is directly proportional to the drug consumption time.

One problem in cocaine and benzoylecgonine hair analysis is external contamination from environmental drugs.[15] It is known that these compounds are present on many everyday objects, such as money.[16] Thus, it is possible that contamination occurs simply by manipulating these objects and then touching one's hair. In order to prevent cross-contamination, accurate hair-washing procedures are employed in drug sample analysis.[17] An acidic or basic hydrolysis[18] reaction is generally used to extract the compounds from hair samples. The matrix compounds, which can give rise to quantitation errors due to the matrix effect phenomenon,[18,19] are removed from the hair extracts using additional pre-purification steps and a pre-concentration step using solid phase extraction (SPE).[10,12] This approach strongly increases the sensitivity but these steps are time-consuming and not cost-effective. Unfortunately, they are usually necessary to increase the method sensitivity due to the low concentration of the drugs in the hair samples.[20]

The surface-activated chemical ionization source[21] coupled with liquid chromatography and ion trap mass spectrometry (LC-SACI-ITMS) has been used to analyze cocaine and benzoylecgonine in diluted urine samples,[22] thanks to its sensitivity, which is higher than that achieved by the usually employed electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) methods.[23] SACI is obtained by upgrading a commercially available APCI chamber, in the absence of corona discharge (no-discharge APCI), with the insertion of a gold surface, leading to a significant improvement in the ionization efficiency. Ionization of the sample occurs both by gas-phase and by surface-activated processes. This new ionization source improves sensitivity due to a strong chemical noise reduction in the spectrum. The SPE pre-purification and concentration steps are unnecessary using this approach due to the substantial benefits achieved by increasing the sensitivity. Thus, this approach has the significant advantages of lower cost and decreased analysis time.

We assessed the ability of the highly sensitive liquid chromatography/surface-activated chemical ionization tandem mass spectrometry single reaction monitoring (LC/SACI-MS/MS-SRM) and liquid chromatography/surface-activated chemical ionization multiple collisional stage mass spectrometry single reaction monitoring (LC/SACI-$MS^3$-SRM) techniques to analyze for the presence of diluted (1:10) cocaine and benzoylecgonine in hair sample extracts. The chromatographic methods were optimized to avoid the matrix effect phenomenon[24–27] that can lead to quantitation errors. It must be emphasized that it is not possible to recover drugs from externally spiked hair due to the differences in binding of exogenously versus endogenously applied drugs; they bind to hair tissue molecules in a different manner from internally consumed drugs. Thus, GC/MS was used as a reference method to validate the quantitation data and to help verify the stability and reliability of the technique.

## EXPERIMENTAL

### Chemicals and reagents

Standard cocaine (Scheme 1(a)), benzoylecgonine (Scheme 1(b)) and benzoylecgonine$D_3$ (Scheme 1(c)) standards were purchased from SALARS (Como, Italy), Medical Isotopes (Pelham, NH, USA) and Cerilliant (Austin, TX, USA), respectively. Acetonitrile, methanol, hydrochloric acid, dichloromethane and isopropanol were purchased from J.T. Baker (Deventer, The Netherlands). Formic acid, potassium hydroxide, potassium phosphate, ammonium hydroxide and *N,O*- bis ( trimethylsilyl ) trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS) were purchased from Sigma Aldrich (Milan, Italy). SPE $C_{18}$ pre-purification and concentration columns were purchased from Waters (Milan, Italy).

### Sample collection and preparation

Hair samples (20–50 mg each) were provided from drug-addicted subjects. Clean scissors were employed to cut the hair. The scissors were thoroughly washed using water/methanol 1:1 solution after removal of each sample to avoid cross-contamination. These samples were washed twice using 2 mL of dichloromethane in a 10 mL neutral glass tube. After each washing cycle, the samples were vortexed for 1 min, centrifuged at 3500 rpm for 5 min, and the supernatant was discarded. After this step, hair segments of 1–2 mm in length were obtained. The hair samples were spiked with 10 μL of a 2000 ng/mL solution of deuterated internal standard (benzoylecgonine$D_3$) and subjected to acid hydrolysis using 0.1 M hydrochloric acid (1 mL). The hydrolysis was performed at 45°C for 18 h. These solutions were diluted (1:10) with distilled water and directly analyzed by LC/SACI-MS/MS-SRM and LC/SACI-$MS^3$-SRM.

Seven different negative control hair extract samples (900 μL) were mixed with either cocaine or benzoylecgonine





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(100 μL each) for a final concentration of 0.1, 1.0, 5.0, 10, 50, 100 or 250 ng/mL to obtain calibration curves. These solutions were diluted 10 times to obtain the following final concentration values for each drug: 0.01, 0.1, 0.5, 1.0, 5.0, 10 and 25 ng/mL. The concentration of the internal standard (benzoylecgonine$D_3$) in the final standard solution was 20 ng/mL. These samples were directly analyzed by LC/SACI-MS/MS-SRM and LC/SACI-MS$^3$-SRM.

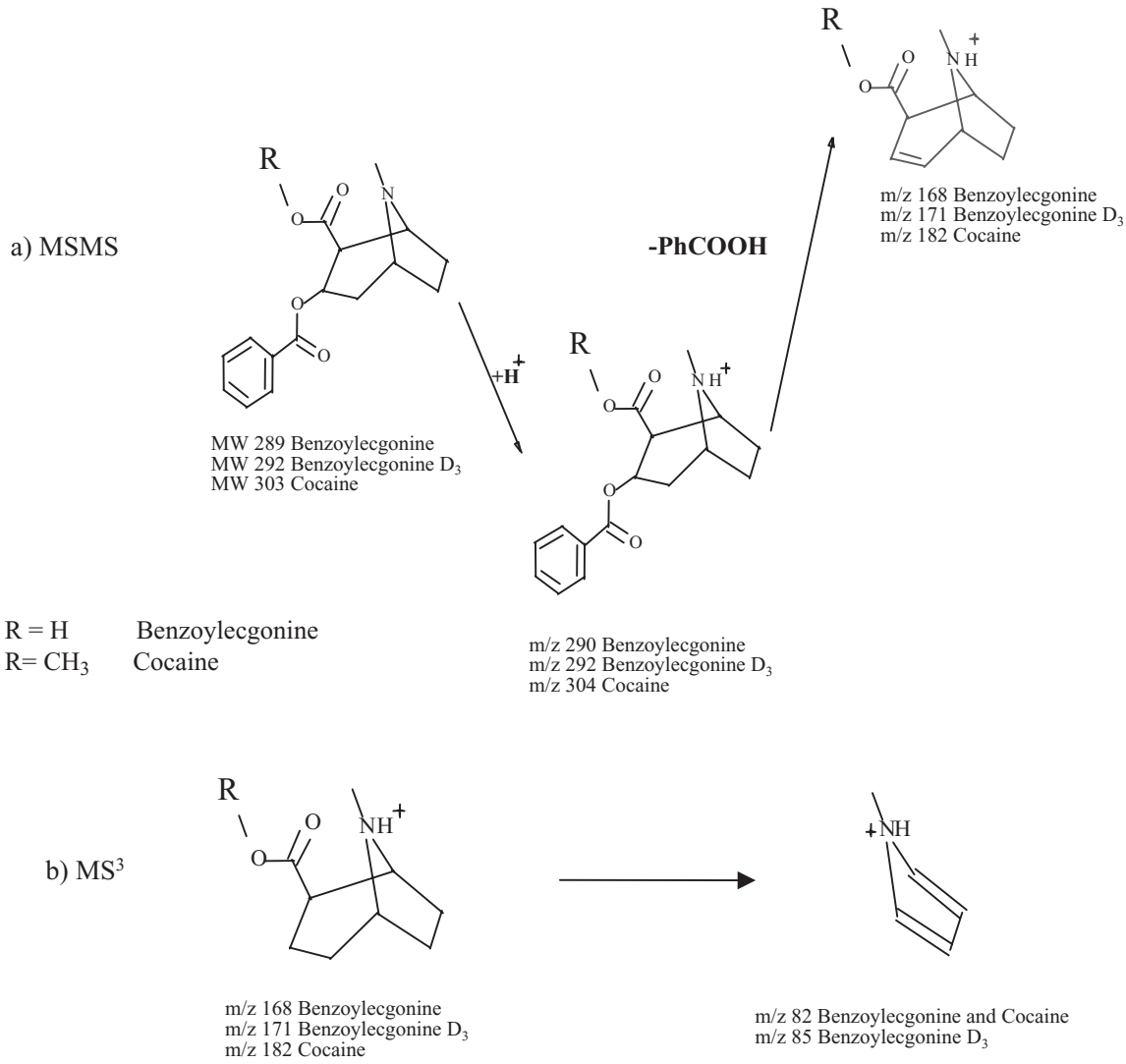
For GC/MS analysis of hair samples, a derivatization reaction and SPE pre-concentration and purification steps were necessary. After acid hydrolysis, a final sample solution (5 mL) was obtained with the drug extraction solution (900 μL), 2000 ng/mL benzoylecgonine$D_3$ (100 μL), and the negative control sample matrix (4 mL). A solution of pH 6.0 was achieved by adding 0.1 M potassium phosphate buffer (1 mL) and then adjusting with potassium hydroxide. SPE $C_{18}$ columns were conditioned using methanol (2 mL) and 0.1 M potassium phosphate buffer (pH 6.0) (2 mL). The drug matrix solution was loaded onto the SPE $C_{18}$ stationary column. The SPE cartridge was washed with distilled water (2 mL), followed by 0.1 N hydrochloric acid solution (3 mL). The final washing step was performed using methanol (5 mL). The $C_{18}$ cartridge was left to dry for 5 min and the sample was then eluted with dichloromethane/isopropanol (80/20 v/v) (2 mL) + 2% (v/v) ammonium hydroxide solution. The eluted solution was evaporated under a flow of nitrogen. The dried sample was derivatized by addition of 50 μL of BSTFA + 1% TMCS solution at 75°C for 15 min. The obtained solution (2 μL) was injected into the GC/MS instrument.

## Gas chromatography

A HP6890N GC/MS system (Agilent Technologies, Santa Clara, CA, USA) operated in electron ionization (EI) mode was used to analyze hair samples. A 12 m × 0.20 mm i.d. 0.33 μm, 5% methyl-silica fused capillary column was employed at an injector temperature of 250°C. The column temperature was increased from 100 to 180°C at a rate of 40°C/min, then at 10°C/min until reaching 290°C.

## Liquid chromatography

A HP1100 instrument (Agilent Technologies) was used for liquid chromatography (LC). The chromatographic column was a reversed-phase $C_{18}$ 50 × 4.6 mm, 1.8 μm, 300 Å. The HPLC gradient used two eluents at a flow rate of 500 μL/min:




**Scheme 2.** (a) MS/MS fragmentation pathway of $[M+H]^+$ ion of cocaine, benzoylecgonine and benzoylecgonineD3 and (b) $MS^3$ fragmentation pathway of the most abundant product ion of the same compounds.





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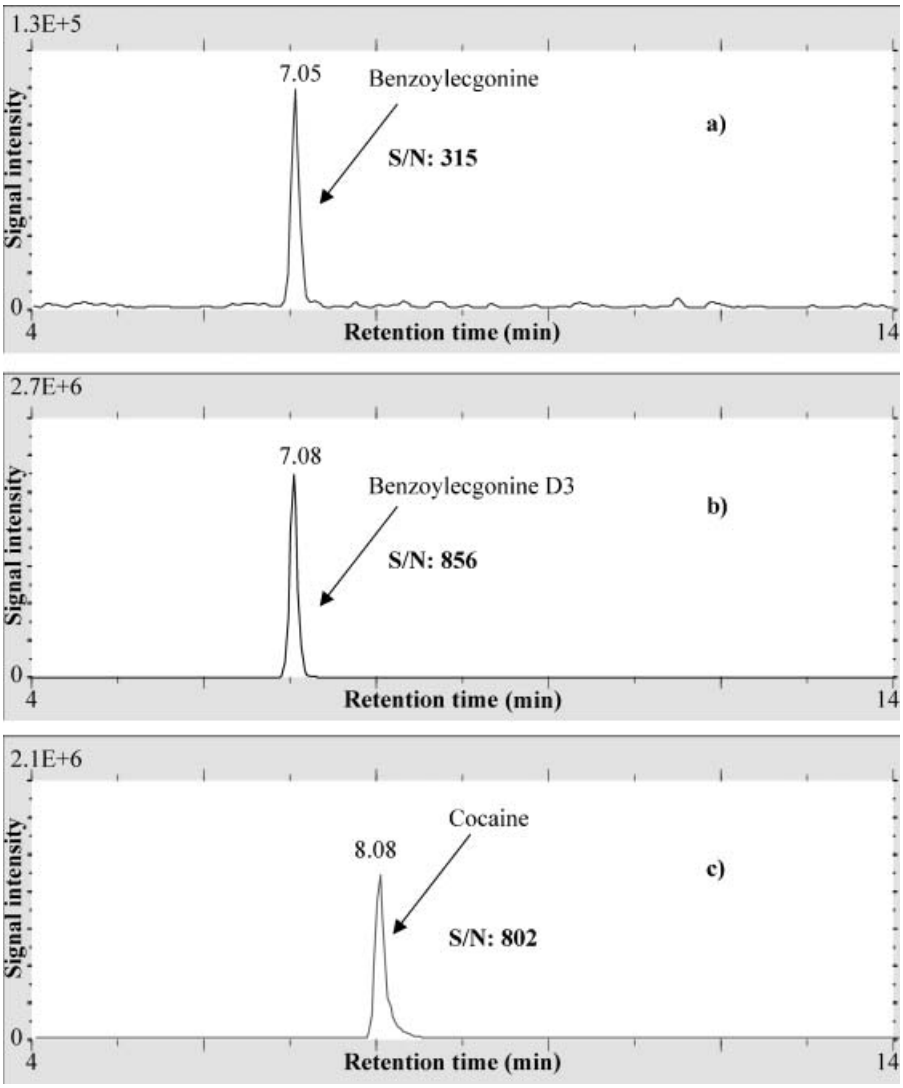


**Figure 1.** LC/SACI-MS/MS-SRM extracted mass chromatograms of (a) benzoylecgonine (*m/z* 168), (b) benzoylecgonine$D_3$ (*m/z* 171), and (c) cocaine (*m/z* 182). The concentration of benzoylecgonine and cocaine was 1 ng/mL each and that of benzoylecgonine$D_3$ was 20 ng/mL. The injection volume was 20 μL and the eluent flow rate was 500 μL/min.

**Table 1.** Analysis of 15 drug-spiked negative hair matrix solutions prepared using 15 different subject matrices at the same sample concentrations (10 ng/mL of benzoylecgonine and cocaine). The LC/SACI-MS/MS-SRM approach was employed. The extracted samples were diluted (1:10). The mother solution concentration is reported in ng/mL. The accuracy and precision percentage errors, with respect to GC/MS data, are also reported

| Sample ID | LC/SACI-MS/MS-SRM (cocaine/benzoylecgonine ng/mL) | GC/MS (cocaine/benzoylecgonine ng/mL) | % accuracy error (cocaine/benzoylecgonine) | % precision error (cocaine/benzoylecgonine) |
|---|---|---|---|---|
| 1 | 10.4/12.1 | 10.2/10.1 | 2/20 | 1/2 |
| 2 | 9.8/12.6 | 10.1/10.3 | 3/22 | 3/2 |
| 3 | 10.4/12.2 | 10.2/10.2 | 2/20 | 1/1 |
| 4 | 9.7/12.7 | 10.1/10.3 | 4/23 | 2/2 |
| 5 | 10.7/12.7 | 10.4/10.3 | 3/23 | 2/3 |
| 6 | 10.4/12.8 | 10.1/10.3 | 3/24 | 3/2 |
| 7 | 9.8/12.2 | 10.2/10.1 | 4/21 | 3/2 |
| 8 | 10.7/12.6 | 10.3/10.1 | 4/25 | 1/2 |
| 9 | 10.4/12.6 | 10.2/10.2 | 2/24 | 1/3 |
| 10 | 10.5/12.6 | 10.3/10.2 | 2/24 | 1/1 |
| 11 | 10.5/12.6 | 10.1/10.4 | 4/21 | 2/2 |
| 12 | 9.7/12.6 | 10.1/10.1 | 4/25 | 3/1 |
| 13 | 10.4/12.9 | 10.2/10.3 | 2/25 | 3/2 |
| 14 | 10.5/12.9 | 10.3/10.5 | 2/23 | 3/2 |
| 15 | 10.5/12.8 | 10.2/10.6 | 3/21 | 3/1 |





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(A) $H_2O + 0.05\%$ formic acid and (B) $CH_3CN + 0.05\%$ formic acid. Solution B (5%) was maintained for 2 min, then a linear gradient was used passing from 5 to 40% of B in 13 min. Solution B was maintained for 3 min at 40%, and then re-equilibrated back to the starting conditions after 2 min. Thus, the chromatographic analysis was performed in 20 min, but the acquisition time was set to 25 min to allow the chromatographic column to re-equilibrate.

## Mass spectrometry

The SACI operating source was employed to obtain direct infusion spectra and LC mass chromatograms. The SACI mass spectra were obtained using an HCT plus ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) and a steel surface. The steel surface was chosen for two reasons: (1) the low cost and (2) the ionization efficiency is essentially the same as that obtained using the gold surface.[21] The vaporizer temperature was 400°C and the entrance capillary temperature was 150°C. The surface voltage was 50 V. The surface temperature was monitored using an optical pyrometer at a temperature of 110°C. The flow rate of nebulizing sheath gas (nitrogen) was 9.0 L/min. The dry gas flow rate was 2.0 L/min. The spray needle voltage was set to 0 V. The maximum number of ions per scan was 100 000, with an average of three microscans per spectrum. The ion charge control (IGC) was on.

LC/SACI mass chromatograms were obtained using tandem mass spectrometry (MS/MS) and multiple collision dissociation ($MS^3$) approaches. The isolation width of the ions was 3 $m/z$ units. The collision energy was 100% of its maximum value (2 V peak-to-peak) operating in both MS/MS and $MS^3$ conditions. The mass spectra were acquired in the positive ion mode. The direct infusion mass spectra were obtained using a sample flow rate of 30 μL/min.

GC/EI-MS spectra were obtained using an HP5973 (Agilent Technologies) single quadrupole mass spectrometer. The electron energy was 70 eV. The mass chromatogram was acquired in single ion monitoring (SIM) mode.

## Data analysis

The signal-to-noise (S/N) ratio was calculated using the root mean square (RMS) algorithm.[28] The LC/MS/MS and $MS^3$ data were converted into the mzXML universal format using the mzBruker converter. The mzXML format is an XML (eXtensible Markup Language)-based common file format for mass spectrometric data. Most mass spectrometers do not directly produce mzXML data, but there are several tools available that generate mzXML files from native acquisition files.[29] Currently, there are converters available at Sashimi for ThermoFinnigan (Xcalibur), MassWolf (Micromass MassLynx), mzStar (SCIEX/ABI Analyst) and Bruker (CompassXport) that will easily generate mzXML files for many of their native file formats. The calibration curves and quantitation data were obtained using Excel software.

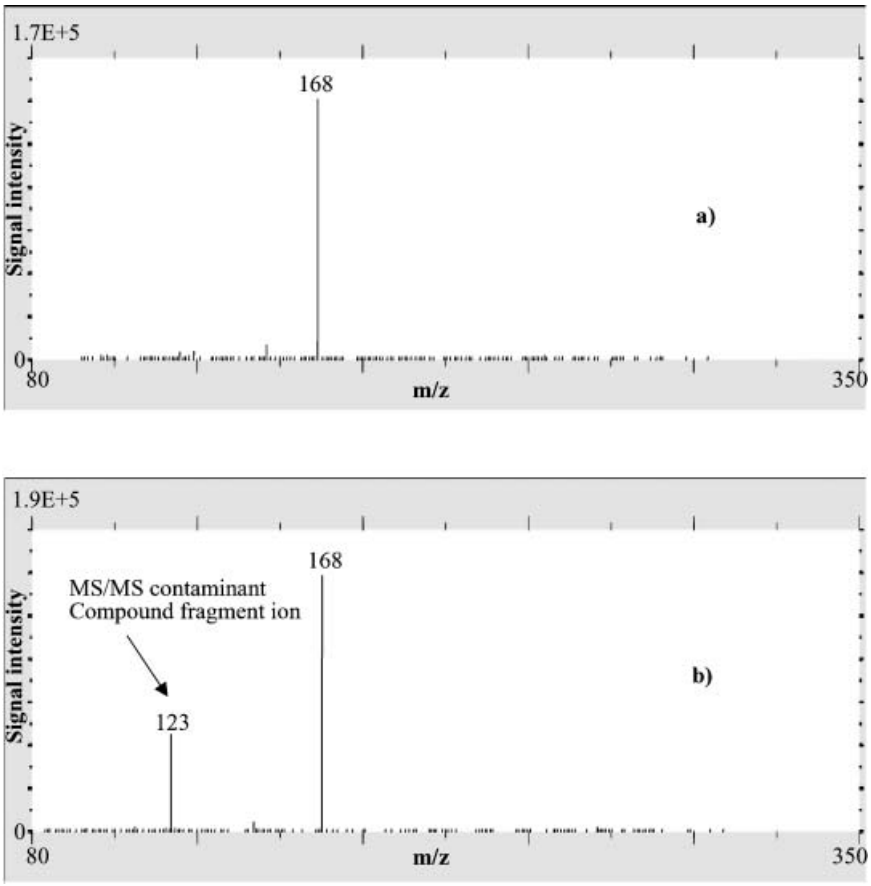


**Figure 2.** Benzoylecgonine LC/SACI-MS/MS spectra obtained analyzing (a) an aqueous standard solution (1 ng/mL) and (b) extracted hair sample of an addicted subject. The injection volume was 20 μL and the eluent flow rate was 500 μL/min.





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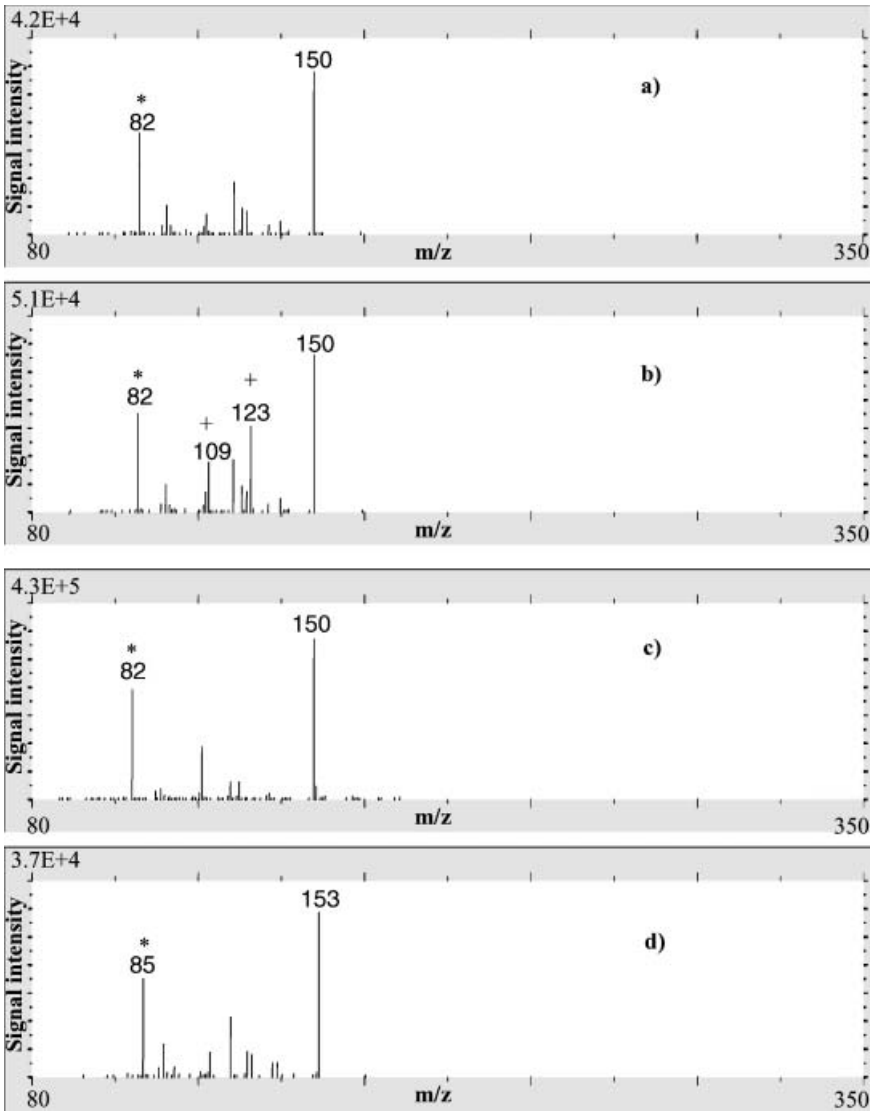


**Figure 3.** LC/SACI-MS$^3$ spectra of (a) benzoylecgonine product ion at *m/z* 168 (1 ng/mL in water), (b) benzoylecgonine product ion at *m/z* 168 (1 ng/mL in hair matrix), (c) cocaine product ion at *m/z* 182 (1 ng/mL in water), and (d) cocaine product ion at *m/z* 182 (1 ng/mL in hair matrix). The higher abundant MS$^3$ benzoylecgonine product ions (*) and the co-eluted contaminant MS$^3$ product ions (+) are indicated. The injection volume was 20 μL and the eluent flow rate was 500 μL/min.

## RESULTS AND DISCUSSION

A method for the analysis of cocaine and benzoylecgonine in urine, by means of LC/SACI-MS/MS, was previously described.[22] In particular, the MS/MS spectra of the $[M+H]^+$ ions of the two compounds led to the same neutral loss (PhCOOH; Scheme 2(a)). The fragmentation of $[M+H]^+$ ions of cocaine at *m/z* 304, benzoylecgonine at *m/z* 290 and benzoylecgonine$D_3$ at *m/z* 293 led to the formation of the ions at *m/z* 182 (cocaine), *m/z* 168 (benzoylecgonine) and *m/z* 171 (benzoylecgonine$D_3$) (Scheme 2(a)). The LC/SACI-MS/MS approach was combined with a column that had a lower particle size (50 × 4.6 mm, 1.8 μm) to analyze the above compounds extracted from hair using acid hydrolysis. With this column, the surface area exposed to the active stationary phase increased while the empty column volume decreased. Therefore, the column could be washed with higher efficiency with a lower flow rate ratio than a column of the same length and internal diameter dimensions, but with a higher particle size. A previous report on the analysis of the pharmaceutical drug tacrolimus in a diluted plasma matrix sample showed that washing a classical LC column (100 × 4.6mm, particle size 5 μm) at a flow rate of 1300 μL/min strongly reduced the matrix effect.[30] Therefore, reducing the particle size from 5.0 to 1.8 μm at a flow rate of 500 μL/min is sufficient to obtain the same washing results during the same washing time. This approach is also known as high flow gradient chromatography.[31,32] An aqueous solution of 1 ng/mL of each of the selected addicted drugs (cocaine and benzoylecgonine) and 20 ng/mL of the internal standard (benzoylecgonine$D_3$) was prepared and analyzed. The LC/SACI-MS/MS-SRM mass chromatograms obtained by monitoring the addicted drug product ions at *m/z* 168 for benzoylecgonine, 171 for benzoylecgonine$D_3$ and 182 for





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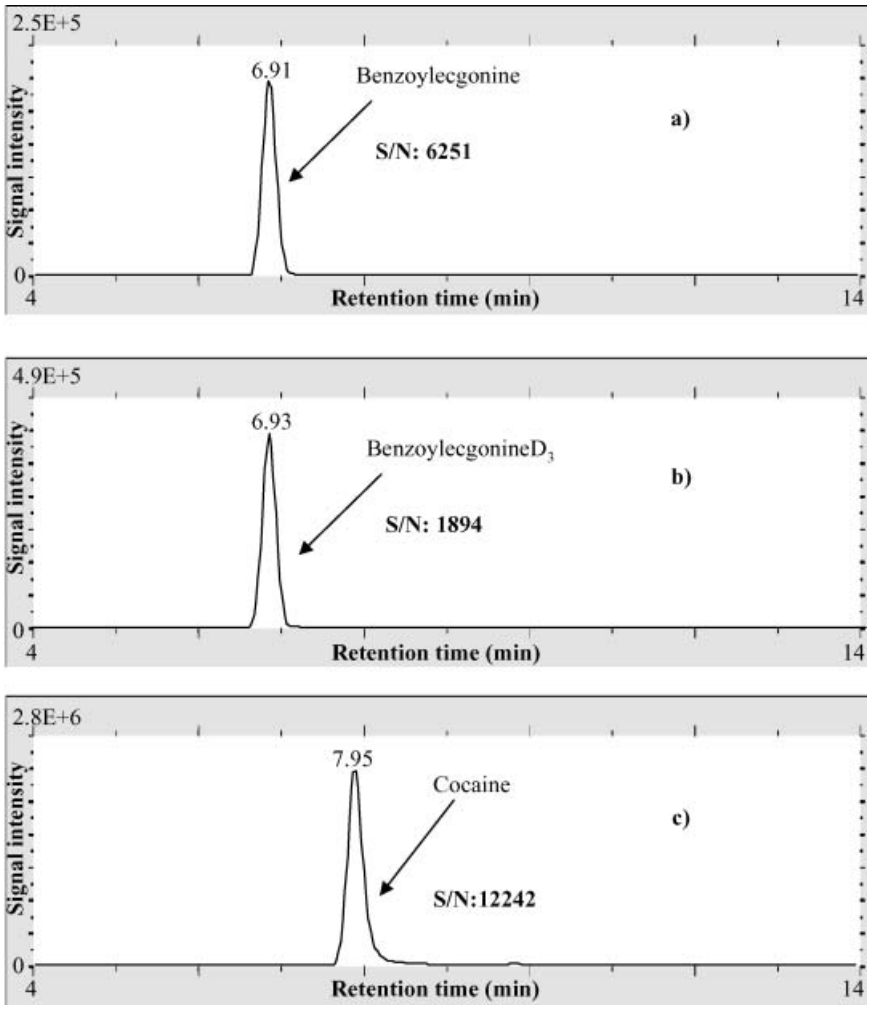


**Figure 4.** LC/SACI-MS$^3$-SRM mass chromatograms obtained analyzing (a) benzoylecgonine (10 ng/mL), (b) benzoylecgonine$D_3$ (10 ng/mL), and (c) cocaine (20 ng/mL) in hair matrix. The injection volume was 20 μL and the eluent flow rate was 500 μL/min.

**Table 2.** Analysis of 15 drug-spiked negative hair matrix solutions prepared using different matrix extracts and the same sample concentrations (10 ng/mL of benzoylecgonine and cocaine). The LC/SACI-MS$^3$-SRM approach was employed. The extracted samples were diluted 1:10. The mother solution concentration is reported in ng/mL. The accuracy and precision percentage errors, with respect to GC/MS data are also reported

| Sample ID | LC/SACI-MS$^3$-SRM (cocaine/benzoylecgonine ng/mL) | GC/MS (cocaine/benzoylecgonine ng/mL) | % accuracy error (cocaine/benzoylecgonine) | % precision error (cocaine/benzoylecgonine) |
|---|---|---|---|---|
| 1 | 10.5/10.4 | 10.2/10.1 | 3/3 | 2/3 |
| 2 | 9.9/10.6 | 10.1/10.3 | 2/3 | 1/3 |
| 3 | 10.5/9.8 | 10.2/10.2 | 3/4 | 1/3 |
| 4 | 9.7/10.5 | 10.1/10.3 | 4/2 | 3/1 |
| 5 | 10.2/10.6 | 10.4/10.3 | 2/3 | 2/1 |
| 6 | 10.4/10.7 | 10.1/10.3 | 3/4 | 2/2 |
| 7 | 10.0/10.4 | 10.2/10.1 | 2/3 | 2/3 |
| 8 | 10.7/9.6 | 10.3/10.1 | 4/5 | 3/1 |
| 9 | 10.7/10.4 | 10.2/10.2 | 5/2 | 3/2 |
| 10 | 10.6/10.7 | 10.3/10.2 | 3/5 | 3/2 |
| 11 | 10.5/10.6 | 10.1/10.4 | 4/2 | 1/3 |
| 12 | 9.6/9.7 | 10.1/10.1 | 5/4 | 2/3 |
| 13 | 10.5/10.1 | 10.2/10.3 | 3/2 | 3/3 |
| 14 | 10.6/10.8 | 10.3/10.5 | 3/3 | 3/3 |
| 15 | 10.6/10.9 | 10.2/10.6 | 4/3 | 3/2 |





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cocaine are shown in Figs. 1(a), 1(b) and 1(c), respectively. The chromatographic peaks of these three compounds were clearly detected with high S/N ratios (315 for benzoylecgonine, 856 for benzoylecgonine$D_3$, and 802 for cocaine). To evaluate the absence of the matrix effect, 15 drug-spiked negative hair extracts (10 ng/mL of benzoylecgonine and cocaine for each extract) were analyzed. The calibration curves for cocaine and benzoylecgonine were obtained using benzoylecgonine$D_3$ as an internal standard. The benzoylecgonine$D_3$ chromatographic peak does not co-elute with the cocaine peak (see Figs. 1(b) and 1(c)). We determined whether matrix compounds led to quantitation errors under the described chromatographic conditions. The $R^2$ of the calibration curve was in the range 0.9887–0.9980 for cocaine and 0.9987–0.9997 for benzoylecgonine. The limits of detection (LODs) were 0.003 ng/(mg hair) for cocaine and 0.02 ng/(mg hair) for benzoylecgonine. The limits of quantitation (LOQs) were 0.01 ng/(mg hair) for cocaine and 0.04 ng/(mg hair) for benzoylecgonine.

The percent accuracy error, in relation to the GC/MS data, was between 2 and 4% for cocaine and between 20 and 25% for benzoylecgonine (Table 1). These results clearly show that the matrix effect does not have a strong influence on the analysis of either compound. For benzoylecgonine studies, the quantitation values were systematically higher using LC/SACI-MS/MS-SRM than those obtained using GC/MS. Moreover, a higher precision was achieved (1–3% precision error) in the analysis of both compounds, leading to confirmation of the stability of the LC/SACI-MS/MS-SRM approach. The presence of a contaminant decreased the accuracy in the determination of benzoylecgonine when using the less selective LC/SACI-MS/MS-SRM technique. For GC/MS, the quantitation data was obtained using the highly specific benzoylecgonine fragment ion at *m/z* 240 ($CH_3NH_2$ and $H_2O$ neutral losses). However, this ion is not observed when using low-energy MS/MS collision-induced dissociation (CID). The only ion obtained and monitored using the CID-MS/MS approach was at *m/z* 168 (data not shown). Thus, the quantitation error can be attributed to the low specificity of the transition selected when operating in SACI conditions. In other words, other compounds with a similar *m/z* value to that of the $[M+H]^+$ ion could co-elute and give rise to the same product ion. Initial evidence of this can be obtained by considering the results for benzoylecgonine. The benzoylecgonine LC/SACI-MS/MS spectra of an aqueous standard solution (1 ng/mL) and a hair sample from an addicted subject support this hypothesis (Figs. 2(a) and 2(b)). For the aqueous standard spectrum, only the ion at *m/z* 168 was detected, whereas an additional ion was present in the MS/MS spectrum of the hair sample (*m/z* 123). This fact confirms the co-elution of another compound with a $[M+H]^+$ ion at the same *m/z* ratio as benzoylecgonine that gives rise to the product ion at *m/z* 123. In principle, this compound could also generate an ion at *m/z* 168. To better investigate this hypothesis, the LC/SACI-$MS^3$ spectrum of the product ion at *m/z* 168 for the benzoylecgonine standard (1 ng/mL) aqueous solution (Fig. 3(a)) was compared with that using the LC/SACI-$MS^3$ approach analyzing a standard spiked (1 ng/mL) hair sample (Fig. 3(b)). The LC/SACI-$MS^3$ spectrum of the hair sample (Fig. 3(b)) showed two abundant ions at *m/z* 123 and 109 that were not present in the $MS^3$ spectrum of the aqueous solutions (Fig. 3(a)). The cocaine $MS^3$ spectra of the product ion at *m/z* 182 (1 ng/mL aqueous solution) shown in Fig. 3(c) exhibited a fragmentation behavior similar to that of the benzoylecgonine product ion at *m/z* 168 (Fig. 3(a)). The contaminant ions at *m/z* 123 and 109 were not observed when analyzing these compounds in the hair matrix (Fig. 3(d)). Thus, these ions are probably related to a contaminant that elutes at the same retention time for benzoylecgonine and that produces the same *m/z* ratio. The formation of the specific $MS^3$ ion at *m/z* 82 for cocaine and benzoylecgonine (Figs. 3(a), 3(b), and 3(c)) and 85 for benzoylecgonine$D_3$ (Fig. 3(d)) was monitored to increase the selectivity of the analysis and to avoid quantitative overestimation of benzoylecgonine amounts due to matrix contamination. These ions are due to neutral losses of $C_4H_6O_2$ (benzoylecgonine) and $C_5H_8O_2$ (cocaine). The fragmentation pathway that leads to the formation of the ions at *m/z* 82 and 85 is shown in Scheme 2(b). Thus, highly specific and abundant $MS^3$ product ions were selected for quantitative analysis using the SRM approach. The LC/SACI-$MS^3$-SRM mass chromatograms were obtained using cocaine (10 ng/mL), benzoylecgonine (10 ng/mL) and benzoylecgonine$D_3$ (20 ng/mL) in hair matrices (Figs. 4(a), 4(b), and 4(c)). A greater sensitivity was achieved (by a factor of four for cocaine and two for benzoylecgonine) for the LC/SACI-$MS^3$-SRM approach than for the LC/SACI-MS/MS-SRM approach. As reported by Busch and Cooks,[33] the analyte S/N ratio usually increases in proportion to the number of fragmentation steps. The samples reported in Table 1 were also analyzed using the developed LC/SACI-$MS^3$-SRM method (Table 2). Using this method, the quantitation data is in agreement with that obtained using the GC/MS approach. In fact, the percent accuracy error compared with that obtained by GC/MS was between 2 and 5% for both compounds and maintained a high precision error (1–3%).

On the basis of these results, extracted hair samples from 20 addicted subjects diluted 1:10 were analyzed by LC/SACI-$MS^3$-SRM and the results were compared with those achieved by the well-established GC/MS approach (data not shown). After sample dilution, the LC/SACI-$MS^3$-SRM results were in strong agreement with the GC/MS results. The percent accuracy error of the quantitation values obtained by LC/SACI-$MS^3$-SRM compared with those obtained using GC/MS was between 2 and 5%.

## CONCLUSIONS

The LC/SACI-$MS^3$-SRM approach demonstrated here is capable of detecting and measuring the quantity of cocaine and its metabolite benzoylecgonine in diluted extracts of hydrolyzed hair samples. The LC/SACI-MS/MS-SRM approach had low selectivity due to poor product ion spectrum information (PhCOOH loss only) and the co-elution of a compound with the same *m/z* ratio as the benzoylecgonine $[M+H]^+$ ion and the same neutral loss.

Our approach gave high sensitivity and selectivity, and there was no matrix effect due to the optimization of the chromatographic conditions, thus avoiding the SPE pre-purification and concentration analytical steps. This





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approach offers several important advantages – mainly reduced cost and time per analysis. Further studies will focus on applying the same approach in the analysis of other addictive drugs of interest (e.g. amphetamines and morphines).

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