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Determination of morphine and codeine in urine by gas chromatography–mass spectrometry*

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Abstract: GC–MS is one of the recommended analytical techniques for the identification and confirmation of opiates in urine. A method for the qualitative detection and quantitation of codeine and morphine in urine samples by this technique has been developed. This method is also suitable for the detection of their main metabolites in urine: norcodeine and normorphine. It also allows the identification of 6-monoacetylmorphine in urine, which can be used as a confirmatory marker of heroine abuse.

The derivatized compounds are separated by capillary gas chromatography (GC) and identified by mass spectrometry (MS) in the selective ion monitoring acquisition mode (SIM).

The recoveries from urine at concentrations of 1000 ng ml^{-1} are 72% for codeine and 80% for morphine. The method is linear in the range studied (0–1000 ng ml^{-1}) for codeine and morphine.

Keywords: *Codeine, morphine and metabolites; 6-monoacetylmorphine; solid-phase extraction; capillary GC–MS; quantitation.*

Introduction

There is a need in our society for the development of analytical methods for the detection and confirmation of opiates abuse. The interpretation of results is not easy principally due to the fact that some compounds like morphine and codeine are biotransformation compounds common to heroine, morphine, codeine [1–3] and some natural products (i.e. poppy seeds) [4].

Some authors have designed analytical techniques for the detection of 6-monoacetylmorphine (6-MAM) and morphine as markers of heroine ingestion [5, 6]. Nevertheless, the small amount excreted in urine and the short half life of 6-MAM [7] renders the detection of this compound useful only when there has been very recent consumption of heroine. Other analytical techniques are focused in the quantitation of morphine and codeine and in the study of some ratios between such compounds in order to distinguish between the ingestion of different opiates [1, 8–10]. A complementary approach, could include the detection of other metabolites of morphine and codeine such as normorphine and norcodeine to add more information to the metabolic urinary profile.

An analytical method has been developed to take account of these different approaches, including the agreement with some new regulatory cut-off [11] concentrations for morphine and codeine and the ability to detect other opiates that can crossreact with some common immunoassays used as screening techniques.

Materials and Methods

Chemicals and reagents

Morphine hydrochloride, codeine phosphate, 6-monoacetylmorphine base and norcodeine base were a gift of the Research Triangle Institute (RTI, NC, USA). Internal standard levallorphan tartrate was kindly donated by Roche (Basel). Urine samples from patients were obtained at the Hospital del Mar (Barcelona).

N-methyl-*N*-trymethylsilyl-trifluoroacetamide (MSTFA) and *N*-methyl-bis-trifluoroacetamide (MBTFA) were obtained from Macherey-Nagel (Düren, FRG). β-Glucuronidase from *Helix pomatia* was supplied by Sigma Chemicals (St Louis, USA). Methanol HPLC grade, chloroform and glacial acetic acid analytical grade were purchased from Merck (Darmstadt, FRG). Isopropyl alcohol and ammonium hydroxide 25% reagent grade were supplied by Scharlau (Barcelona, Spain).

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Deionized water was obtained by MILLI-Q system (Waters). Bond Elut Certify columns were provided by Analytichem International (Harbor City, USA).

Sodium acetate buffer (1.1 M) was prepared by dissolving 145.9 g of sodium acetate in deionized water, adding 22 ml glacial acetic acid, and further diluting to a total volume of 1 l with deionized water for a pH of 5.2.

Acetate buffer (0.1 M pH 4) was prepared by mixing 100 ml of deionized water with 570 μl of glacial acetic acid and 1.6 ml of 1.0 M potassium hydroxide, and adjusting pH to 4.0 if it was necessary.

1.0 M Potassium hydroxide was prepared by dissolving 56 g of potassium hydroxide in deionized water and diluting to a total volume of 1 l.

Stock solutions of all narcotics (1 mg ml^{-1}, free base) were prepared in methanol. Working solutions of 0.1, 0.01 and 0.001 mg ml^{-1} were prepared by dilution of stock solutions. Solutions were checked by UV spectrophotometry and stored at −20°C.

Instrumentation

A model 5890/5970 gas chromatograph–mass spectrometer (Hewlett–Packard) was employed. Data acquisition and manipulation were performed using HP Chemstation 59940 (HP–UX series). GC was done in a 25 m × 0.2 mm 5% phenyl-methylsilicone (0.33-μm film thickness) capillary column (Hewlett–Packard, Palo Alto, USA). Injector (split mode, ratio 1:10) and detector temperatures were 280°C. Oven temperature was programmed from 100°C (initial time, 3 min) to 290°C at 20°C min^{-1} (final time, 5 min) and solvent delay was 10 min. Helium flow was 0.8 ml min^{-1} and the sample injection volume was 2 μl.

The mass spectrometer was used in the single ion monitoring acquisition mode (SIM), the ions (*m/z*) acquired being listed in Table 1.

Vacuum manifold for the semi-automatic processing of the columns was from Analytichem International.

Extraction procedure

Urine samples prior to extraction were submitted to an enzymatic hydrolysis. To 2.5 ml of urine, 2.5 μg levallorphan (Int.Stand.), 0.5 ml of sodium acetate buffer (1.1 M) pH 5.2 and 50 μl β-glucuronidase were added in a 15-ml glass tube. The urine was vortexed and the tube incubated in a dry block at 55°C for 3 h. After cooling at room temperature, sample pH was adjusted to 6–7 with 1 M potassium hydroxide and the tube was centrifuged for 10 min at 3000 rpm.

Bond Elut Certify columns were inserted into a vacuum manifold and conditioned by washing once with 2 ml of methanol and 2 ml of deionized water. The columns were prevented from drying out before applying specimens. Samples were poured into each column reservoir and drawn slowly through the column. The columns were washed with 2 ml of deionized water, 1 ml of 0.1 M acetate buffer pH 4 and 2 ml of methanol.

Elution of opiates was performed with 2 ml of a mixture of chloroform–isopropyl alcohol (80:20, v/v) with 2% of ammonium hydroxide. The extracts were evaporated to dryness under a stream of nitrogen in a 40°C water bath.

Sequential derivatization [12]

TMS-derivatives of hydroxyl and phenolic groups. 50 μl of MSTFA were added to the dried residue, vortexed for 20 s and kept at 60°C for 10 min.

TFA-derivatives of primary and secondary amines. After cooling at room temperature, 20 μl of MBTFA were added and the mixture was vortexed for 20 s and incubated at 60°C for

Table 1

Compound	Derivative	Tr (min)	*m/z*
Levallorphan	Levallorphan-O-TMS	12.72	355, 272, 176
Dihydrocodeine	Dihydrocodeine-O-TMS	13.13	373, 236, 176
Codeine	Codeine-O-TMS	13.45	371, 234, 178
Morphine	Morphine-bis-O-TMS	13.73	429, 401, 236
Oxycodone	Oxycodone-O-TMS	13.76	315, 255, 225
Norcodeine	Norcodeine-O-TMS-N-TFA	13.92	453, 282, 313
Normorphine	Normorphine-bis-O-TMS-N-TFA	14.18	511, 281, 354
6-MAM	6-Monoacetylmorphine-O-TMS	14.30	399, 340, 287

5 min. 2 μl of this mixture were injected into the gas chromatograph.

Results and Discussion

In the described analytical method some modifications have been introduced to those proposed by Analytichem [13]. The acidic hydrolysis (0.5 ml conc. HCl, 120°C, 20 min) has been substituted by an enzymatic hydrolysis. With the latter procedure, the hydrolysis of morphine-3-glucuronide (1000 ng ml^{-1}) was almost complete. Using the procedure proposed by Analytichem the rate of hydrolysis achieved was 70% for the same concentration of glucuronide. It was also verified that in such conditions 6-monoacetylmorphine (6-MAM) was hydrolysed to morphine. Using the enzymatic hydrolysis, 6-MAM was unaffected.

After modifying the hydrolysis conditions of the sample, the recoveries observed for codeine and morphine (1000 ng ml^{-1} of each) using the proposed standard procedure were 68 and 65%, respectively. Since these recoveries were quite low, the influence of the enzyme on the extraction procedure was studied. Different reagents such as acetonitrile, methanol, sulphosalicylic acid in 50% of methanol were evaluated with negative results. The reduction of the volume of sample to one-half (from 5 to

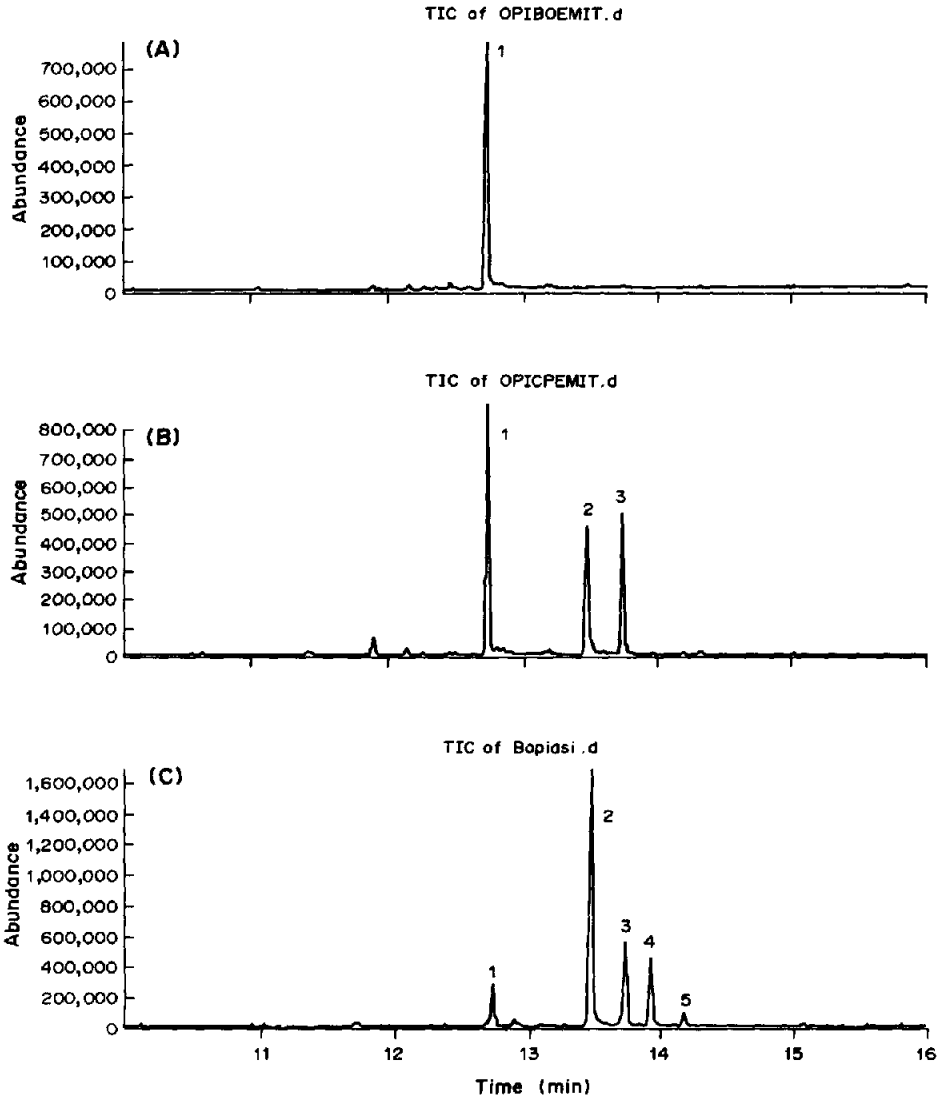

Figure 1
Total ion chromatograms (SIM acquisition mode) obtained from urines processed through this procedure. (A) drug-free urine spiked with levallorphan (ISTD); (B) control urine containing codeine and morphine; (C) urine specimen from a codeine excretion study (0–8 h). (1) Levallorphan, (2) codeine, (3) morphine, (4) norcodeine, (5) normorphine.

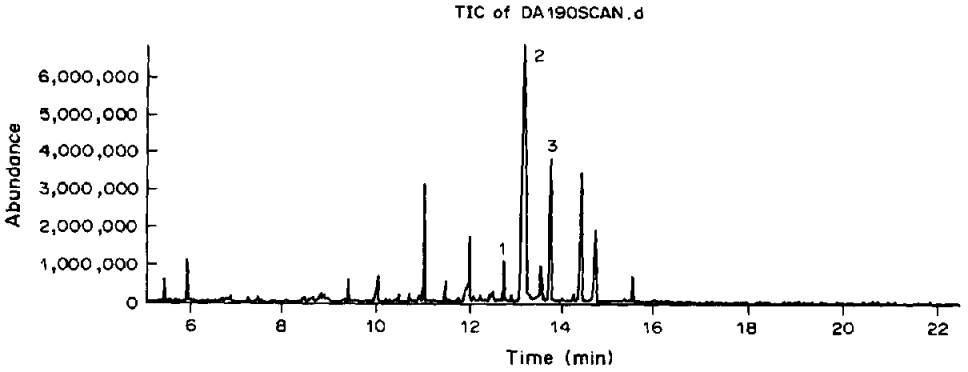

Figure 2
Total ion chromatogram obtained in SCAN acquisition mode from a urine specimen containing dihydrocodeine (2) and oxycodone (3). (1) Levallorphan.

2.5 ml) to allow a greater interaction between the sample and the column improved the recoveries. For the same concentration of codeine and morphine recoveries of 72 and 80%, respectively, were found.

Under the described analytical conditions, the linearity of the method was checked in the range 0–1000 ng ml^{-1} by adding to a blank urine the following concentrations: 20, 50, 100, 200, 500 and 1000 ng ml^{-1} of morphine and codeine (expressed as free base). Quantitation has been done using the area ratios between codeine-O-TMS and morphine-bis-O-TMS and the internal standard levallorphan-O-TMS. A linear regression model has been applied.

The linearity for morphine and codeine in the range studied was $y = 2.036x + 0.037$ (intercept 0.037 ± 0.005, $r = 0.998$) and $y = 2.375x + 0.065$ (intercept 0.065 ± 0.002, $r = 0.996$), respectively. Referring to the limit of detection, this was lower than 20 ng ml^{-1}. The same detection limit was observed for norcodeine and 6-MAM. The coefficients of variation intraday ($n = 5$) were 6.2 and 8.2% for codeine and 11.1 and 6.8% for morphine at concentrations of 100 and 1000 ng ml^{-1}, respectively.

In Fig. 1 are presented some chromatograms (total ion chromatogram), corresponding to a blank urine (spiked with 1000 ng ml^{-1} of ISTD), a control urine (CON-DOA™, Euro DPC Ltd, Oxford, UK) and an excretion study of codeine (0–8 h collection period). The versatility of the analytical technique is exemplified in Fig. 2 where a chromatogram of a positive urine for opiates by an immunological technique is presented. Neither morphine nor codeine were detected but in the sample were present hydrocodeine, hydrocodone and oxycodone.

Conclusions

The present analytical technique, introduces some modifications to the method proposed by Analytichem, increasing its specificity (6-MAM unaltered) and sensitivity (higher recovery of morphine after hydrolysis). The clean extract obtained after the extraction and derivatization procedures and the possibilities of full automatization of the technique renders it very useful for the confirmation of drug testing for opiates.

The sensitivity achieved (more than 20 ng ml^{-1}) is well above the confirmatory cut-off of the NIDA guidelines [11] for drug testing (300 ng ml^{-1} for morphine and codeine) and other concentrations (200 ng ml^{-1} for morphine) proposed by some expert committees [14] when evaluating ratios between morphine and codeine. The linearity and the recoveries found allows a good quantitation of morphine and codeine.

Additionally the method is able to detect other metabolites of opiates as 6-MAM, normorphine, norcodeine and some related compounds (i.e. hydrocodeine, hydrocodone, oxycodone . . .).

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