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RECENT ADVANCES
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Gas Chromatographic - Mass Spectrometric Identification of Codeine,
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Gas Chromatographic - Mass Spectrometric Identification of Codeine, Morphine and 6-Monoacetyl Morphine in Human Urine as Trimethylsilyl Derivatives.

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1. INTRODUCTION

The sensitive and selective methods of different legal and illegal opiates are necessary to discriminate legal use of prescribed drugs from illegal use of banned drugs due to the increasing use of illegal drugs.

The immunoassay methods are widely used for initial screening of opiates in urine. This method generally cannot differentiate legal from illegal opiates. Therefore we have to confirm the positive urine by GC-MS.

The most common opiate substances detected in biological fluids are codeine, morphine and 6-monoacetyl morphine (6-MAM, a metabolite of heroin which is classified as an illegal drug). Heroin, morphine and codeine are narcotic analgesic which have been widely abused and are addictive.

Codeine (legally used) as an antitussive agent is metabolized in the liver to glucuronide and sulphate conjugates of codeine and morphine. Heroin (illegal drug) is rapidly hydrolyzed in the body to 2 products, 6-MAM and morphine. Therefore morphine can be either from codeine or heroin and the detection of morphine alone does not provide complete information about the source of the compound.

A sensitive and selective method has been developed in our laboratory to identify codeine, morphine and 6-monoacetyl morphine (6-MAM) in human urine using GC-MS and derivatization with bis - (trimethylsilyl) trifluoroacetamide (BSTFA). This method has been demonstrated to be useful to identify and discriminate among several drugs describe above.

Key words: Opiate, codeine, morphine, 6-MAM

2. EXPERIMENTAL

Sample Preparation

The urines from the abuser were collected in the clean bottle and tested both in screening and confirmation. The screening was performed by EMIT (immunoassay). The confirmation was performed by the following procedure: to 2.0 mL of urine (urine blank, spiked standard mix of codeine, morphine, 6-MAM and samples) were added 100 μ l of Nalorphine solution (10 μ g/mL) as internal standard, 100 μ l of 2 M acetate buffer pH 5, 25 μ l of β -glucuronidase (*H. Pomatia*). The mixture was mixed and hydrolyzed at 55⁰ C for 2 hours. After cooling 80 μ l of 1 M KOH were added, and columns (C₁₈ Sep- Pak[®]) were loaded with this mixture. The columns were washed with 2 mL of water and 1 mL of 0.1 M acetate buffer. Elute the columns with 4X 1 mL of ethyl acetate. The eluents were dried under a stream of nitrogen at 35⁰ C. The residues were derivatized with 50 μ l of BSTFA: TMCS (99: 1) for 20 minutes at 60⁰ C before finally transferred to the autosampler vial. The samples were injected in GCMS.

Instrument Condition

GC-MS: HP 6890-HP 5973

Column: HP Ultra 2, 17 m, 0.2 mm i.d., 0.11 μ m film thickness.

Carrier gas: Helium, pressure 17.7 psi, split ratio 6:1,

Temperature program: initial temp. 140⁰C, hold 2 minutes, 15⁰C/min to 260⁰C, 20⁰C/min to 310⁰C hold 1 minute

Injection volume: 2.0 μ l

Total run time: 13,5 minutes

3. RESULTS AND DISCUSSION

The retention times (RT), relative retention times (RRT), and characteristic ions of codeine, morphine, 6-MAM and ISTD as TMS derivatives for the identification are listed in Table I.

The intra and inter day precision at the concentrations 0.25 – 2.0 μ g/ml were between 0.8 – 8.82% for codeine, 4.39 – 7.84% for morphine, and 1.09 – 8.55% for 6-MAM (n=6). The reproducibility data are summarized in Table II.

There was a good resolution of each drug and the total ion chromatogram of TMS derivatives of codeine, morphine, 6-MAM and ISTD is shown in Figure 1. The typical positive sample contained morphine and 6-MAM is shown in Figure 2.

4. CONCLUSION

The procedures described here are sensitive, selective, and specific to identify and discriminate codeine, morphine and 6-MAM. The GCMS method and derivatization technique using BSTFA can discriminate between codeine, morphine and 6-MAM, a metabolite of heroin. The presence of 6-MAM indicates that the sample is from a heroin user. All of the 10 samples, which came from the Drug Abuse Hospital in Jakarta and screened at the Jakarta Doping Control Laboratory by immunoassay were positive for opiates. The samples were confirmed by GCMS: 8 samples contained codeine, all samples contained morphine and 7 samples contained 6-MAM.

5. REFERENCES

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TABLE I

Relative Retention Times (RRT) of Codeine, Morphine and 6-MAM to Nalorphine (ISTD), and characteristic ions of TMS derivatives

Compound	RT (min)	RRT	Ions
Codeine	8.67	0.90	371, 234, 178
Morphine	8.98	0.93	429, 236, 196
6-MAM	9.28	0.97	399, 340, 287
Nalorphine (ISTD)	9.64	1.0	414

TABLE II

The Intra and Inter Day Precision (% CV) of Codeine, Morphine and 6-MAM with Concentration between 0.25 – 2 µg/ml (n = 6)

Compound	Intra day				Inter day		
	0.25	0.50	1.0	2.0	0.25	0.50	1.0
Codeine	1.41	2.92	0.96	1.58	8.82	8.25	7.29
Morphine	4.39	6.94	4.72	0.8	4.9	7.84	5.17
6-MAM	2.33	4.37	3.66	1.09	8.55	6.23	4.71

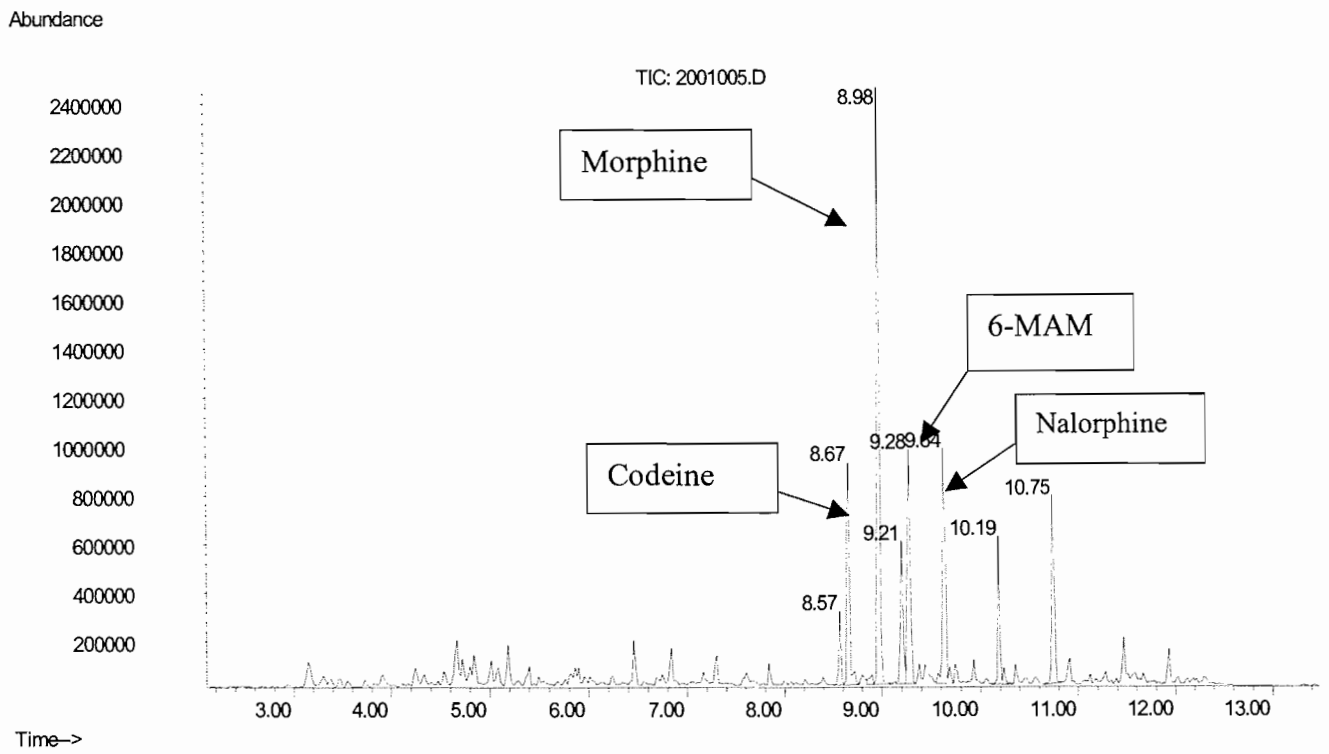


Figure 1: Chromatogram of a BSTFA-derivatized standard spiked with 0.5 µg/mL Codeine, Morphine and 6-MAM

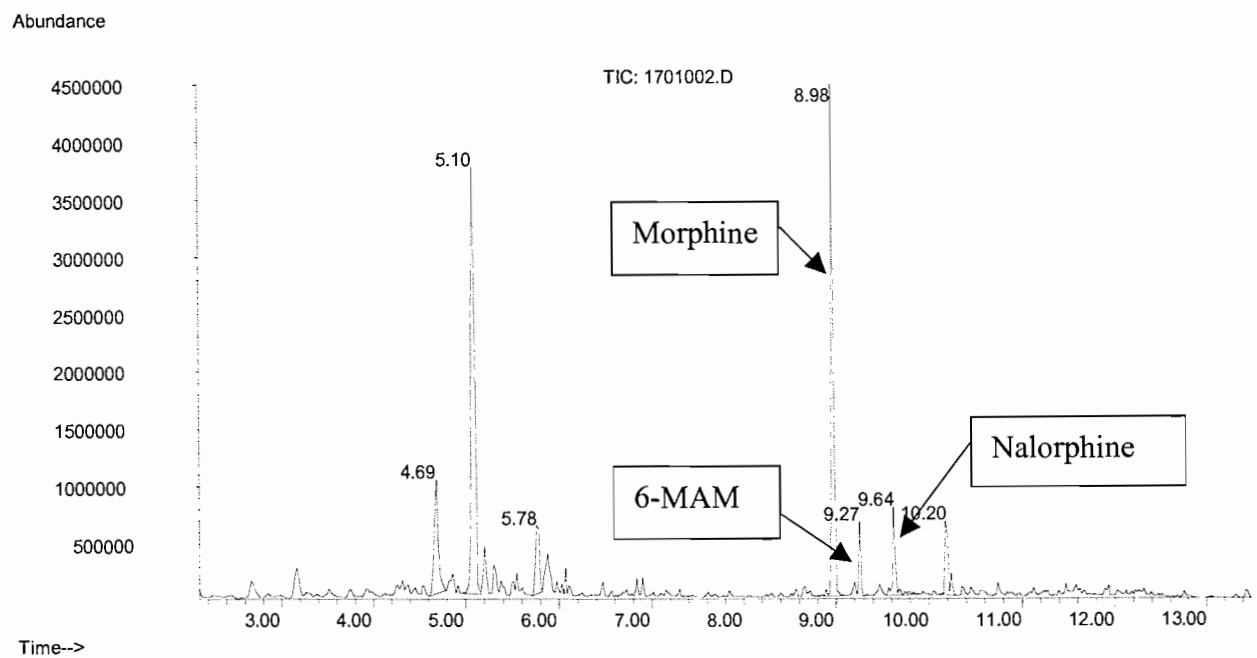


Figure 2: Chromatogram of a BSTFA-derivatized positive sample