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Short communication

Investigation of meperidine and its metabolites in urine of an addict by gas chromatography–flame ionization detection and gas chromatography–mass spectrometry

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Abstract

A gas chromatographic method with flame ionization detection (FID) for the quantitative analysis of meperidine and mass spectrometry (MS) for the qualitative analyses of meperidine and its metabolites in urine was established. Meperidine, normeperidine and acetyl normeperidine in urine were extracted with ether. Free and conjugated meperidinic and normeperidinic acids in urine, which are hydrophillic, were hydrolyzed by acid, esterified with methanol and derivatized with acetic anhydride. Meperidine and its four metabolites were identified by GC–MS. Meperidine was measured by GC–FID during 72 h after intramuscular administration of meperidine to an addict.

1. Introduction

Meperidine is an anaesthetic and analgesic drug. People are prone to become addicted to this drug when it is used for curing purposes. In earlier studies, radioisotopic [1], methyl orange dye [2], GC and GC–MS [3–8] methods were used to estimated meperidine and its metabolites. This paper presents a gas chromatographic method with flame ionization detection and mass spectrometry for the analysis of meperidine and its metabolites in urine in order to coordinate the clinical cure of a meperidine addict.

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2. Experimental

2.1. Chemicals

Meperidine was provided by the Chinese National Narcotic Lab. (Beijing, China). Eicosane was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used were of analytical reagent grade.

2.2. Extraction of meperidine and its metabolites from urine

Extraction of hydrophobic compounds

A 5-ml volume of urine was adjusted to pH 9–10 with 1 *M* NaOH and 1 ml of carbonate

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buffer (pH 9.3) was added. The solution was extracted with diethyl ether (2 × 5 ml). The ether extract was evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted with 45 μl of ethylene dichloride and 5 μl of internal standard (eicosane, 1 mg/ml). A 1-μl aliquot of the solution was injected onto the GC–MS system.

Extraction of hydrophillic compounds

Acid hydrolysis and esterification of conjugated metabolites: After extraction with diethyl ether, the aqueous phase was evaporated to 0.5 ml at 55°C under reduced pressure with an evaporator and then evaporated to dryness in a freeze drier. The residue was refluxed with 15 ml of methanol containing 10% sulphuric acid at 100°C for 5 h. This procedure hydrolyzed the conjugated metabolites to their free forms and changed the acids to their methyl esters [6]. The solution was evaporated to 2 ml at 55°C under reduced pressure, and then adjusted to pH 9–10 with concentrated ammonium hydroxide and extracted with diethyl ether (2 × 20 ml). The ether extract was evaporated to dryness under a stream of nitrogen at room temperature.

Acetylation of extracts: The residue was derivatized with 30 μl of acetic anhydride and 20 μl of pyridine at 60°C for 30 min. The solution was evaporated to dryness again under a stream of nitrogen and then reconstituted in 50 μl of methanol. A 1-μl aliquot of the solution was injected onto GC–MS system.

2.3. Instrumentation

GC–FID analysis was performed with a Perkin Elmer SIGMA 115 gas chromatograph. Chromatographic separation was accomplished with a 24 m × 0.24 mm I.D., 0.25 μm SE-54 capillary column. The oven temperature was programmed from 100°C (0 min) to 250°C (8 min hold) at 10°C/min. Injection port and detector temperature were 250°C and 260°C, respectively.

GC–MS analysis was performed with a VG TRIO-1000 gas chromatograph–mass spectrometer (VG Mass Lab, Manchester, UK). Chromatographic separation was accomplished with a 12 m × 0.32 mm, 0.25 μm DB-5 capillary column. Oven temperature was programmed from 80°C (0 min) to 250°C (10 min hold) at 10°C/min. Injection port and interface temperature were 250°C. Electron ionization energy and current were 70 eV and 180 μA, respectively. Source temperature was 200°C. The instrument was calibrated using perfluorotri-*n*-butylamine.

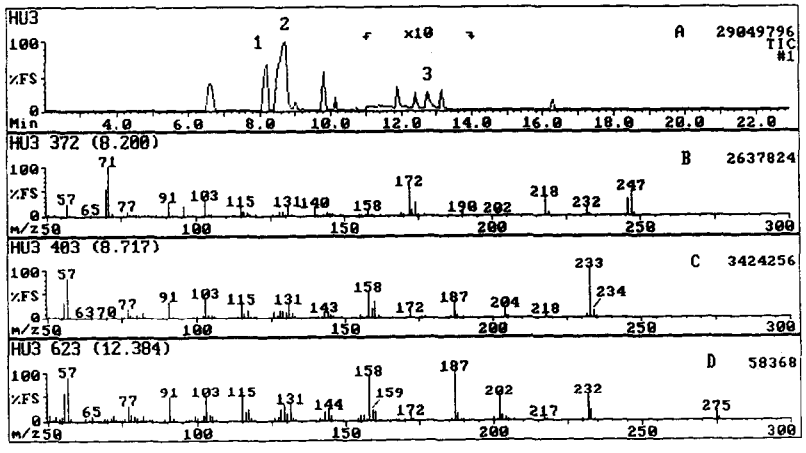

Fig. 1. (A) GC–MS total ion chromatogram of diethyl ether extract of urine of an addict 14 h after intramuscular injection of 100 mg of meperidine; (B) spectrum of meperidine (peak 1); (C) spectrum of normeperidine (peak 2); (D) spectrum of acetyl meperidine (peak 3).

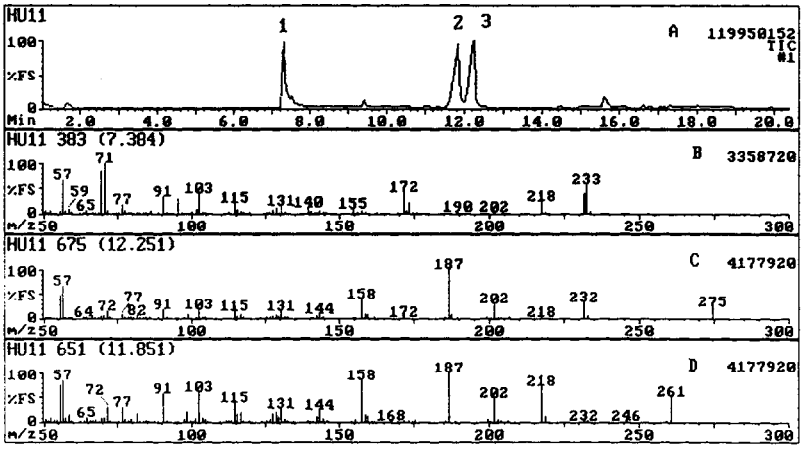

Fig. 2. (A) GC–MS total ion chromatogram of hydrolyzed, methyl esterified and acetylated extract from urine of an addict 14 h after intramuscular injection of 100 mg of meperidine; (B) spectrum of methyl-esterified meperidinic acid (free and conjugated, peak 1); (C) spectrum of acetyl meperidine (peak 3); (D) spectrum of acetylated methyl-esterified normeperidinic acid (free and conjugated, peak 2).

3. Results and discussion

3.1. Identification of meperidine and its metabolites

Figs. 1 and 2 show chromatograms of extracts from urine and the corresponding mass spectra. These spectra were identical with the spectra of the authentic compounds [8]. The structures of meperidine and its detected and identified metabolites are summarized in Fig. 3. Acetylated normeperidine was found in urine without

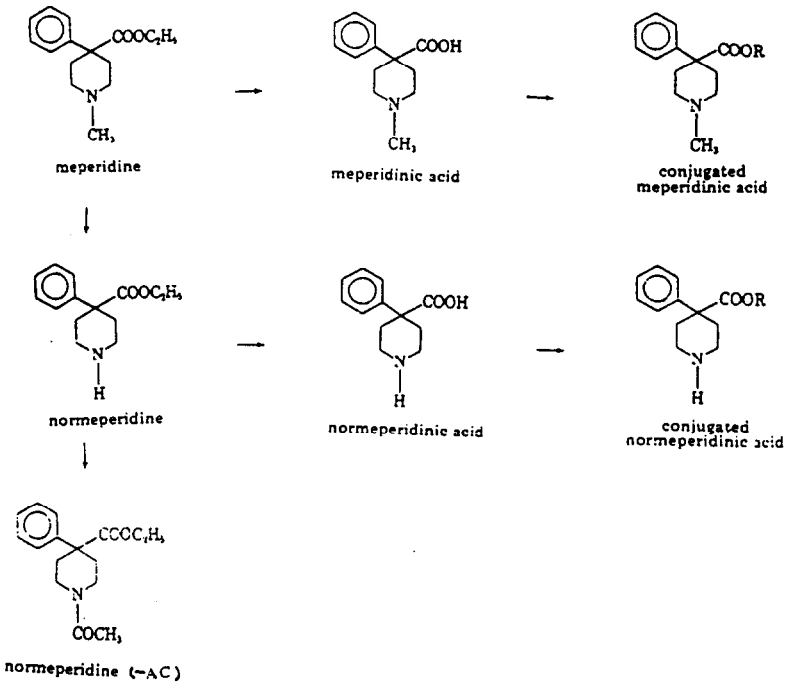

Fig. 3. The structures of meperidine and its main metabolites.

Table 1
Recovery and coefficient of variation

Amount added (μg/ml)	Recovery (%)	C.V. (%)
0.50	71.9	17.6
2.00	81.1	10.7

acetylation with acetic anhydride; this has not been reported in the literature before. Because in the experiments all solvents used were free from acetic acid, this compound possibly occurs as a metabolite in human. In the esterification, methanol was used for methylation of the acids, instead of ethanol for ethylation of the acids [6], in order to distinct the acetylated meperidine already present in the aqueous phase in free or conjugated form (Fig. 2).

3.2. Quantitation of meperidine

Extraction recovery, standard curve and detection limit: quantitation of meperidine was accomplished by GC–FID. The linear equation was $y = 0.466x + 0.120$ with correlation coefficient 0.9996 ($n = 6$) in the range of 0.1–8.0 μg/ml. The detection limit was 20 ng/ml of urine. Table 1 lists the coefficient of variation (C.V.) and the recovery of extraction by this method.

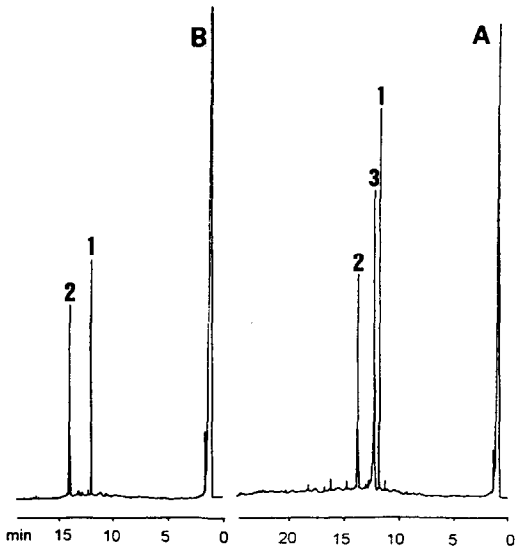

Fig. 4. Chromatogram of extracts from urine of an addict (A) and control urine spiked with meperidine (B). Peak 1: meperidine; peak 2: internal standard; peak 3: normeperidine.

Table 2
Amount of meperidine in urine of an addict at different times after injection

Time (h)	Amount of urine (ml)	Meperidine (μg/ml)
75	200	5.90
14	200	11.75
24	800	4.23
38	600	5.98
48	600	2.78
62	600	0.63
72	600	0.05

3.3. Human data

For 72 h after intramuscular injection of 100 mg of meperidine to an addict, the clinical samples were quantitatively analyzed by GC–FID. Fig. 4 shows chromatograms of extracts from a clinical sample and a blank urine spiked with meperidine and internal standard. Table 2 lists the amount of meperidine over time.

4. Conclusions

It was clearly demonstrated that the new established method is suitable for the analysis of meperidine and its metabolites in urine. This method may provide valuable data for clinical cure of meperidine addicts.

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