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Short communication

Identification of dihydroetorphine in biological fluids by gas chromatography–mass spectrometry

Yi Luo*, Jian-lin Feng, Feng Liu, Xu-ying Hu

Institute of Pharmacology and Toxicology, Beijing 100850, China

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Abstract

A method for the monitoring of dihydroetorphine hydrochloride, a powerful anaesthetic and analgesic drug, in biological fluids was developed, involving GC–MS with multiple selected-ion monitoring. Dihydroetorphine was extracted from human blood and urine with dichloromethane and then derivatized with N-heptafluorobutyrylimidazole after having been concentrated to dryness. A dihydroetorphine monoheptafluorobutyl derivative was formed, which showed good behaviour in GC–MS with electron impact ionization. Its molecular ion, m/z 609, and its main fragments, m/z 576, 534, 522 and 508, were selected as the ions for identification owing to their relative peak intensities and characteristics. The target drug was identified based on its retention time, its selected multiple ions and their relative intensities. This method was successfully used for the detection of dihydroetorphine in blood and urine from a dihydroetorphine addict and a poisoned patient, respectively.

1. Introduction

Dihydroetorphine {7α - [1 - (*R*) - hydroxy - 1 - methylbutyl] - 6,14 - *endo* - ethanotetrahydrooripavine}, a 6,14 - *endo* - ethenotetrahydrothebaine derivative, and also a hydrogenated derivative of etorphine, is an analgesic of unprecedented high potency, being 12 000 times more potent than morphine [1,2]. It is characterized by its rapid onset and short duration of action. Therefore, it has been widely used to relieve various types of pain in patients, especially those suffering from advanced-stage cancer, for about 12 years in China. In recent years, it also has been successfully used as a substitute agent in the detoxification therapy of opiates, especially heroin abusers. However, its misuse can also result in dihydroetorphine addiction [3].

Only a few papers have been published on GC–MS confirmatory methods for etorphine [4,5], and for the determination of dihydroetorphine only phosphorimetric methods have been established [6,7]. However, they were not suitable for the identification of dihydroetorphine owing to their non-specificity and low sensitivity. The low dose level of dihydroetorphine (20 μg in a sublingual tablet) demands a more sensitive measurement technique for its detection in biological fluids. As its misuse and abuse have become increasingly serious and no analytical methods have been established that can be used for the identification of dihydroetorphine in biological fluids, we have developed a GC–MS method with multiple selected-ion monitoring,

* Corresponding author.

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which is both sensitive and specific for the detection of the target compound in biological fluids. The developed method was applied to identify human intoxication by dihydroetorphine.

2. Experimental

2.1. Chemicals

Standards of dihydroetorphine hydrochloride and dihydroetorphine free base were synthesized in our Institute. Their purities were >99%. N-Heptafluorobutyrylimidazole (HFBIM) was purchased from Lancaster Synthesis (Windham, USA). All solvents were of analytical-reagent grade and were purchased from Beijing Chemical (Beijing China).

2.2. Extraction of dihydroetorphine from urine

To 10 ml of urine were added 5 ml of concentrated hydrochloric acid, followed by hydrolysis for 1 h at 80°C on a water-bath. The hydrolysed urine was adjusted to pH 8.5 with 1 *M* NaOH, 1 ml of 0.1 *M* phosphate buffer (pH 8.5) was added and the mixture was extracted twice with 20-ml portions of dichloromethane. The organic fractions were combined and evaporated to dryness at 40°C under reduced pressure. The recovery of dihydroetorphine added to control urine was studied at the 6 ng/ml level.

2.3. Extraction of dihydroetorphine from blood

A 2-ml volume of whole blood was adjusted to pH 8.5 with 1 ml of 0.1 *M* phosphate buffer (pH 8.5) and extracted twice with 5-ml portions of dichloromethane. The solvent fractions were combined and evaporated to dryness at 40°C under reduced pressure. The recovery of dihydroetorphine added to control blood was studied at the 25 ng/ml level.

2.4. Formation of derivatives

Dihydroetorphine has two hydroxyl groups and either both or one of these must be selectively and quantitatively derivatized in order to have good chromatographic behaviour. Dihydroetorphine (100 ng) was placed in a Reacti-Vial and 170 μl of toluene–acetonitrile (95:5, v/v) were added, followed by 30 μl of HFBIM. The mixture was allowed to stand for 15 min at room temperature, 1 ml of 0.1 *M* phosphate buffer (pH 7.0) was added to hydrolyse excess of reagent. A 1-μl aliquot of the supernatant was injected into the gas chromatograph–mass spectrometer. The mass spectrum of derivatized dihydroetorphine (see Fig. 2) showed that one of its hydroxyl groups had been replaced.

2.5. Instrumentation

All GC–MS analyses were carried out with a VG-TRIO-1000 GC–MS system (VG Analytical, Manchester, UK). Gas chromatography was carried out on a Model 5890 instrument (Hewlett-Packard) equipped with a 0.5-μm HP-5 (cross-linked 5% phenyl–methylsilicone) capillary column (12 m × 0.2 mm I.D.), with helium as the carrier gas at a column head pressure of 50 kPa at 280°C. Splitless injection was performed at 280°C. The oven temperature was programmed from 100°C (0 min) to 280°C (20 min) at 20°C/min. The GC–MS interface temperature was 250°C. The GC–MS system was operated in the electron impact mode with full scan at 50–650 u/s or multiple selected-ion monitoring (SIM) tuned on *m/z* 508, 522, 534, 576 and 609. The multiplier was operated at 650 V and the source parameters were selected as ion energy 70 eV, ionization current 180 μA and ion source temperature 200°C. The instrument was calibrated daily using heptacosa(perfluorotri-*n*-butylamine).

3. Results and discussion

3.1. GC–MS of derivatized dihydroetorphine

Fig. 1 shows the structure of dihydroetorphine (DHE). Its two hydroxyl groups imply poor chromatographic behaviour. For this reason, it is

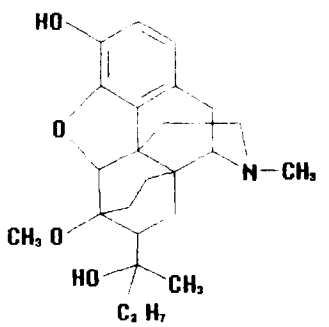

Fig. 1. Structure of dihydroetorphine.

necessary to improve its behaviour by derivatization.

Fig. 2 shows the mass spectrum of the HFB derivative of dihydroetorphine. It shows a molecular ion peak at m/z 609, a base peak at m/z 522 and other major peaks at m/z 576, 534 and 508. The molecular ion at m/z 609 results from the derivatization of the hydroxy groups at the C-3 position in the structure of dihydroetorphine, whereas the tertiary hydroxy group cannot be derivatized owing to steric hindrance. The base peak at m/z 522 is formed by the loss of a stable tertiary radical $[CH_3C(OH)C_3H_7]$. The ions at m/z 609, 576, 534, 522 and 508 are all characteristic and have considerable relative intensities so that they can be selected as ions for multiple ion monitoring. The retention time and intensity ratios of these ions are also used for the purpose of identification of dihydroetorphine.

The recovery of the whole procedure was determined using five controlled dihydroetorphine-free urine samples spiked with 6 ng/ml of the drug. A yield of $75.0 \pm 2.7\%$ was obtained. Another set of five controlled blood samples spiked with 25 ng/ml of dihydroetorphine were processed as above. The recovery was estimated to be $72.4 \pm 10.0\%$. Control blood and urine samples subjected to the above-described procedure for dihydroetorphine determination indicated no significant background interference at m/z 508, 522, 534, 576 and 609.

3.2. Human data

A blood sample from an addict, who was administered sublingually 20 μg of dihydroetorphine per hour, was analysed by the proposed method. The results are shown in Fig. 3 and demonstrate the presence of dihydroetorphine in the blood sample.

Other samples were collected from a patient in a coma who had misadministered an overdose of an unknown drug. Blood and urine samples were analysed as described above. Dihydroetorphine was detected in both blood and urine samples (Figs. 4 and 5). These results indicated that dihydroetorphine could have been the cause of the intoxication.

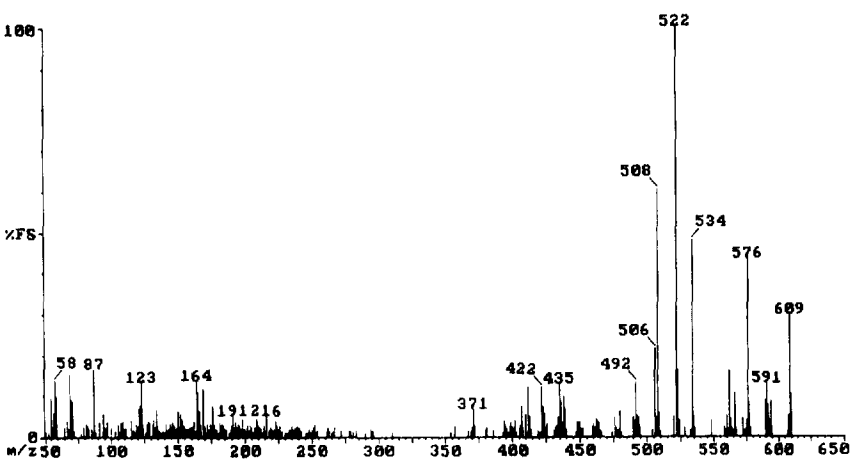

Fig. 2. Positive-ion electron impact mass spectrum of dihydroetorphine heptafluorobutyl derivative.

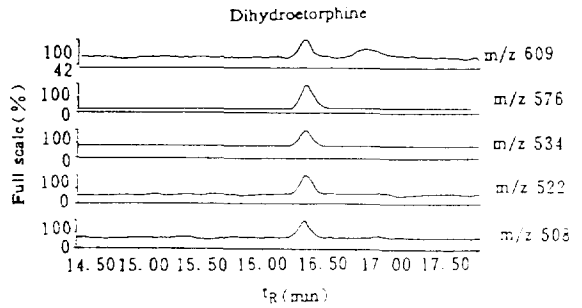

Fig. 3. GC–MS-SIM of dihydroetorphine extracted from 2 ml of blood from a dihydroetorphine addict 0.5 h after an intravenous injection of dihydroetorphine.

4. Conclusion

Dihydroetorphine is one of the best examples of very potent drugs which act on humans at dose levels of 20 μg in a single sublingual dose. It has been used to ease pain in cancer patients who have reached an advanced stage of the disease. However, dihydroetorphine addiction may occur after prolonged administration even though the drug is administrated at a very low dose level. The determination of this kind of substance is very difficult for several reasons other than the need for high sensitivity. First,

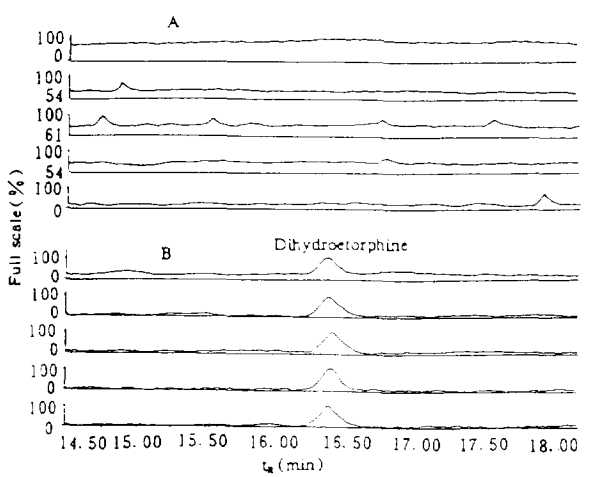

Fig. 4. GC–MS-SIM of dihydroetorphine extracted from 2 ml of blood 40 h after dihydroetorphine hydrochloride intoxication. (A) Blood control; (B) blood extract 40 h after dihydroetorphine hydrochloride intoxication.

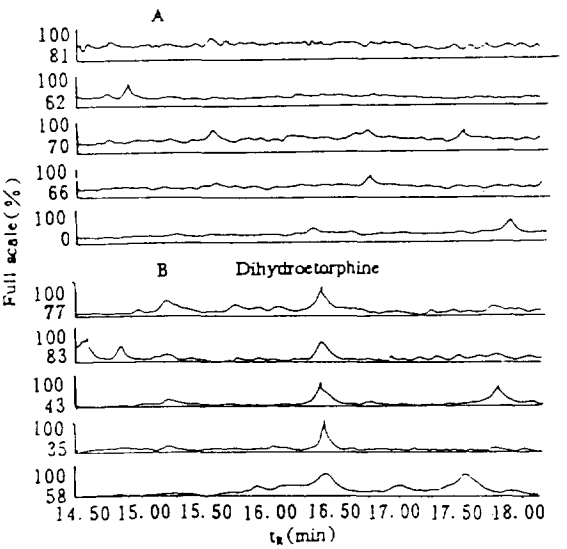

Fig. 5. GC–MS-SIM of dihydroetorphine extracted from 10 ml of urine 40 h after dihydroetorphine hydrochloride intoxication. (A) Urine control; (B) urine extract 40 h after dihydroetorphine hydrochloride intoxication.

hydrolysis of conjugates is necessary. Second, because of the acidic phenolic group and the tertiary amine, all the extraction steps have to be performed at precisely pH 8.5. Third, derivatization of the molecule has to be performed prior to GC–MS determination and HFBIM seems to be the most reliable reagent. From the point of view of sensitivity, the proposed procedure allows the detection of dihydroetorphine in urine and blood at low doses estimated to be about 20 μg per person with intravenous injection at 1-h intervals. However, it cannot be detected in both urine and blood at a single dose of 20 μg per person after sublingual administration. A more sensitive and quantitative method for the determination of dihydroetorphine is being developed in our laboratory and will be reported later.

In conclusion, the described GC–MS procedure is well suited for confirming the presence of dihydroetorphine in urine and blood from humans after intravenous administration of doses as low as 20 μg. It is in use for the routine control of dihydroetorphine abuse and intoxication.

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