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Determination of dihydroetorphine in biological fluids by gas chromatography–mass spectrometry using selected-ion monitoring

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Abstract

A method for the determination of dihydroetorphine hydrochloride, a powerful anaesthetic and analgesic drug, in biological fluids by GC–MS with selected-ion monitoring using etorphine as internal standard was established. Dihydroetorphine was extracted from human blood and urine with dichloromethane and then derivatized with N-heptafluorobutyrylimidazole after concentration to dryness. A dihydroetorphine monoheptafluorobutyl derivative was formed which showed good behavior on GC–MS with electronic-impact ionization. The main fragment, *m/z* 522, which is the base peak, was selected as the ion for quantitation and the corresponding ion, *m/z* 520, was selected for monitoring the internal standard, etorphine. The recoveries and coefficients of variation of the whole procedure were determined with five controlled dihydroetorphine-free urine and plasma samples spiked with different concentrations of dihydroetorphine. The concentration of dihydroetorphine for quantitation was in the range 1–20 ng/ml for urine and 2.5–250 ng/ml for plasma. The correlation coefficients of the standard curves are sufficient to determine the dihydroetorphine. The accuracy for quantitation of dihydroetorphine in urine and plasma is less than 10.6%.

Keywords: Dihydroetorphine

1. Introduction

Dihydroetorphine [7α-(1-(*R*)-hydroxyl-1-methylbutyl)-6,14-*endo*-ethanotetrahydrooripavine], a 6,14-endoethenotetrahydrothebaine derivative (DHET), also a hydrogenated derivative of etorphine (ET), is an analgesic of unprecedented high potency, 12 000-times more potent than morphine [1,2]. As mentioned in the previous paper [3], it is characterized by its rapid onset and short duration of action. For 12 years now it has been widely used to relieve various pains in P.R. China, especially those who suffered from advanced-stage cancer. In recent years, it also has been successfully used as a substitute agent in opiate detoxification therapy, especially with heroin abusers. However, it has been noticed that misuse and abuse of dihydroetorphine can also result in addiction [4].

To prevent misuse and abuse, a GC–MS method with multiple selected-ion monitoring (SIM), which is both sensitive and specific for the detection of dihydroetorphine in biological fluids, has been established and successfully applied to identify dihydroetorphine intoxication in man [4]. In this

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paper, a quantitation method of GC–MS with SIM for urine and blood samples was developed to coordinating with a study on the detoxification therapy of opiates using dihydroetorphine.

2. Experimental

2.1. Chemicals

The standards of dihydroetorphine hydrochloride, etorphine hydrochloride, dihydroetorphine free base and etorphine free base (all >99% pure) were synthesized in our institute. N-Heptafluorobutyryl imidazole (HFBIM) was purchased from Lancaster synthesis (Windham, CI, USA). All solvents used were of analytical grade and were purchased from Beijing Chemical Company (Beijing, China).

2.2. Extraction of dihydroetorphine from urine

To 10 ml of urine were added 4 μg of etorphine hydrochloride (40 μl of 0.1 mg/ml of etorphine hydrochloride aqueous solution) and 0.5 ml of concentrated hydrochloric acid followed by hydrolysis for 1 h in a 80°C water-bath. The hydrolyzed urine was adjusted to pH 5–6 with 1 *M* NaOH and followed by adding 1 ml of 0.1 *M* phosphate buffer (pH 5.5) and extracted twice with 20-ml portions of dichloromethane. The organic fractions were combined and were then evaporated to dryness at 40°C under reduced pressure. The residue was then derivatized as described below and then determined by means of GC–SIM–MS. The recovery of dihydroetorphine spiked to control urine was studied at the 1 ng/ml, 10 ng/ml and 20 ng/ml levels, respectively.

2.3. Extraction of dihydroetorphine from blood

To 2 ml of plasma were added 4 μg of etorphine hydrochloride (40 μl of 0.1 mg/ml of etorphine hydrochloride aqueous solution) and 3 ml of 0.1 *M* phosphate buffer (pH 5.5). The solution was extracted twice with 5-ml portions of dichloromethane. The solvent fractions were combined and concentrated to dryness at 40°C under reduced pressure. The residue was then derivatized by the following procedure and then determined with GC–SIM–MS. The recovery of dihydroetorphine spiked to control plasma was studied at the 2.5, 50 and 250 ng/ml levels, respectively.

2.4. Formation of derivatives

To the residues from urine and plasma samples were added 70 μl of toluene–acetonitrile (95:5, v/v) and 30 μl of HFBIM. The mixture was set for 15 min at room temperature and 1 ml of 0.1 *M* phosphate buffer (pH 7.0) was added to hydrolyze the excess reagent. A 1-μl aliquot of the supernatant was taken carefully and then injected onto the gas chromatograph–mass spectrometer.

2.5. Instrumentation

All GC–MS quantitative analyses utilized a VG-TRIO-2 system (VG Analytical, Manchester, UK). GC was achieved on a Model 5890-II (Hewlett-Packard) equipped with a 25 m×0.2 mm×0.5 μm HP-1 (crosslinked methyl silicone gum) capillary column and with helium as carrier at a column head pressure of 100 kPa at 280°C. Splitless injection was performed at 280°C. Oven temperature was programmed from 180°C (2 min) to 280°C (16 min) at 20°C/min. GC–MS interface temperature was 280°C. The GC–MS was operated in electron-impact mode with SIM at *m/z* 522 for dihydroetorphine and *m/z* 520 for etorphine, respectively. The multiplier was operated at 650 V and the source parameters were selected as follows: ion energy, 70 eV; ionization current, 180 μA; ion source temperature, 200°C. The instrument was calibrated daily using heptacosa (perfluortri-*n*-butylamine).

3. Results and discussion

3.1. Choice of selected ion and the internal standard

As mentioned in a previous paper [3], a dihydroetorphine monoheptafluorobutyl derivative was formed after derivatization (Fig. 1). As usual the base peak, *m/z* 522, which is a structure characteristic for dihydroetorphine, was selected as monitoring

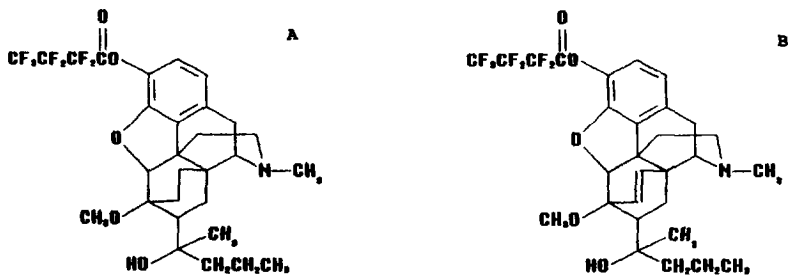

Fig. 1. Chemical structures of dihydroetorphine (A) and etorphine (B), monoheptafluorobutyl derivatives.

ion. Because no isotope dihydroetorphine was purchased or synthesized for the experiment, the etorphine whose structure is differentiated from dihydroetorphine by a double bond, was selected as internal standard. As the chemical property of etorphine is very similar to that of dihydroetorphine, it is a good internal standard for quantitation of dihydroetorphine. The precision for quantitation using etorphine as internal standard is not as good as that using isotope dihydroetorphine as internal standard due to the difference of mass behavior between etorphine and dihydroetorphine and the instability of mass behavior of compounds. However, etorphine as internal standard does not show the isotope effect which is very important at low concentration, compared to the isotope dihydroetorphine, as long as it can be separated completely by GC from dihydroetorphine. Furthermore, we needed much more etorphine than dihydroetorphine for the determination, since reducing dihydroetorphine was lost at ultra trace level due to adsorption to the surface of the capillary column. In the experiment, the amount of etorphine added was 4 μg which did not influence the area integration of dihydroetorphine.

3.2. Choice of GC–MS condition

It was very important to separate etorphine from dihydroetorphine completely as etorphine would have an isotope effect at m/z 522 if there was an overlap of peaks to some extent. Usually, the initial temperature is set low for splitless injection in order to reduce the solvent effect on the separation. However, it was found in the experiment that the initial temperature could not be set too low since the peak is too wide to obtain ultra-sensitivity even though they could be separated completely. By optimizing experiments, the GC–MS conditions listed above were selected which could gain both complete separation for etorphine and dihydroetorphine and good peak shapes for ultra-sensitivity. A thick-film capillary column was also selected in order to increase the capacity of separation, especially for biosamples. The GC–SIM–MS chromatogram of dihydroetorphine and etorphine in plasma under the selected conditions is shown in Fig. 2.

3.3. Choice of solvent for extraction of dihydroetorphine from urine and plasma

Several solvents or mixed solvents were chosen to compare the effectiveness of extraction. Using the largest peak area of dihydroetorphine (4 μg in 10 ml urine) or etorphine (4 μg in 10 ml urine) as 100%, the results of the comparison of extraction effectiveness of different solvents are shown in Table 1. It is obvious in Table 1 that dichloromethane is the most effective extraction solvent for dihydroetorphine and etorphine. The amount of dichloromethane used for extraction was 20 ml each time which could reduce the emulsification of the solution.

3.4. pH for extraction of dihydroetorphine from urine and plasma

In the last paper [3], dihydroetorphine was extracted at pH 8.0 with dichloromethane. However, it

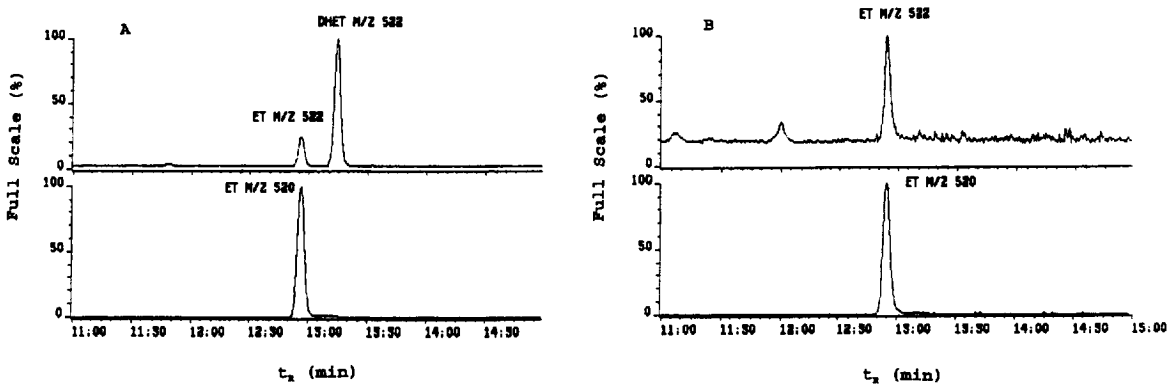

Fig. 2. GC–SIM–MS chromatogram of (A) a plasma sample spiked with 50 ng/ml dihydroetorphine and 4 μg/ml etorphine (the internal standard) and (B) a blank plasma spiked with the internal standard only.

Table 1
Results of different solvents for extraction of dihydroetorphine and etorphine spiked in urine, $n=5$

Solvent	Peak area of dihydroetorphine		Peak area of etorphine	
	Mean±S.D.	RRc (%)[a]	Mean±S.D.	RRc (%)
Ethyl acetate	1560±275	45.1	1972±331	47.3
Chloroform	1494±244	43.2	1853±256	44.4
Dichloromethane	3459±95	100.0	4173±504	100.0
Diethyl ether	174±213	5.0	105±137	2.5
Dichloromethane–propyl alcohol (7:3)[b]	1296	37.5	576	37.8
Chloroform–propyl alcohol (7:3)	1017±80	29.4	1308±131	31.4

[a]RRc is the abbrevation of relative recovery.
[b]Because the mixed solvent dichloromethane–propyl alcohol emulsified the solution, only two experimental data could be obtained.

was found that a greater recovery could be obtained at pH 5.5 than at pH 8.0 by a more detailed experiment, the result of which is listed in Table 2. This could be explained by the existence of the acidic phenolic group and the tertiary amine in the structure of dihydroetorphine. Therefore, pH 5.5 was chosen for the extraction in this experiment.

Table 2
Results for extraction of dihydroetorphine (DHET) and etorpnine (ET) spiked in urine at different pH[a], $n=3$

pH	Peak area		DHET/ET ratio (mean±S.D.)
	DHET	ET	
3.5	109.5	192.6	0.5699±0.0199
4.5	285.7	447.3	0.6366±0.0263
5.5	5290.1	4569.9	1.1583±0.0194
6.0	2263.0	2522.3	0.8899±0.0288
7.0	2780.9	4527.5	0.6142±0.0216
8.0	2759.0	4688.7	0.5884±0.0022
9.0	2816.4	4151.0	0.6807±0.0031
10.0	2546.4	3000.2	0.8482±0.0063

[a]4 μg of DHET and ET in 10 ml urine extracted with dichloromethane was used.

3.5. Calibration curves and detection limits

The ion-current ratios of m/z 522 to m/z 520 generated by monitoring for dihydroetorphine and etorphine were determined from their peak areas at appropriate GC retention times (Fig. 2). The concentrations of dihydroetorphine in unknown or quality control samples were then calculated from their ion-current ratios using the calibration curves listed in Table 3. The calibration curves for both plasma and urine, in which dihydroetorphine and etorphine were spiked with different concentration, were calculated with SAS (Statistics Analysis System) software. Reliable detection limits for dihydroetorphine

Table 3
Calibration curves for dihydroetorphine in urine and plasma and the limits of quantitation

Sample	n	Calibration curve (ng/ml)	r^a	C.R.[b] (ng/ml)	LOD[c] (ng/ml)	LOQ[d] (ng/ml)
Plasma	7	$y=0.000270x+0.000415$	0.9995	2.5–250[e]	0.5	2.5
Urine	5	$y=0.000359x+0.000298$	0.9995	1.0–20[e]	0.2	1.0

[a] $P<0.0001$ for the calibration curves of dihydroetorphine in both plasma and urine.
[b] Concentration range.
[c] Limit of detection.
[d] Limit of quantitation.
[e] Calibration curves for dihydroetorphine in plasma and urine were calculated by unweighted linear regression.

from spiked plasma and urine were obtained with a signal-to-noise ratio greater than 5.

3.6. Recovery, precision and accuracy

Recoveries, coefficients of variation and accuracy of the whole procedure were determined with five controlled dihydroetorphine-free urine and plasma samples spiked with different concentrations of dihydroetorphine (Table 4 and Table 5). The standard values for calculating the recoveries of dihydroetorphine were determined from the samples in which etorphine was spiked before extraction of the samples and dihydroetorphine was spiked just before the derivation. All data was calculated by SAS software.

Table 4
Recoveries of dihydroetorphine from plasma and urine, $n=5$

Sample	Concentration (ng/ml)	Recovery (%)	C.V.[a] (%)
Plasma	250.0	67.7	9.6
	50.0	67.6	4.9
	2.5	63.3	10.9
Urine	20.0	59.9	5.4
	10.0	49.9	9.3
	1.0	43.3	9.0

[a] Coefficient of variation.

Table 5 shows that the developed method is reliable and can meet the requirements for the determination of dihyroetorphine in biological fluids.

The stability of the instrument was determined by repeatedly injecting the same concentration sample (1 ng/ml dihydroetorphine and 400 ng/ml of etorphine standard solution) on five separate days. The coefficient of variation for inter-day was 5.7% ($n=5$), indicating satisfactory instrumental reproducibility.

4. Conclusion

The purpose to develop a quantitative method for dihydroetorphine is to study the pharmacokinetics in the detoxification therapy of opiates using dihydroetorphine. Since dihydroetorphine is a very

Table 5
Accuracy and precision of the method, $n=5$

Sample	DHET added (ng/ml)	DHET detected (ng/ml)	C.V.[a] (%)	Rel.diff.[b] (%)	P^c
Plasma	2000.00	2027.00±50.03	2.5	1.4	>0.05
	50.00	47.24± 2.39	5.1	5.5	>0.05
	2.50	2.23± 0.41	18.4	10.6	>0.05
Urine	800.00	781.72± 7.23	0.9	2.3	>0.05
	100.00	93.74± 2.51	2.7	6.3	>0.05
	10.00	10.28± 1.03	10.0	2.8	>0.05
	1.00	1.07± 0.20	18.7	7.0	>0.05

[a] Coefficient of variation.
[b] Rel.diff=(DHET added−DHET detected)/DHET added.
[c] $P>0.05$ indicates that there was no significant difference between DHET added and DHET detected.

potent drug which acts on human beings at dose levels of 20 μg per person in a single sublingual dose or intravenous injection, the sensitivity of this method is still not sufficient enough to detect the concentrations of dihydroetorphine in humans at different times after administration of dihydroetorphine at therapeutic level for obtaining the pharmacokinetics data. It is proposed to get the data for pharmacokinetics of dihydroetorphine in humans using a dihydroetorphine addict, who could be administrated several doses of dihydroetorphine at set time-points, in a clinical study. This is very important as these data could help us to improve the medication of dihydroetorphine and to lengthen the short duration of action on humans of dihydroetorphine.

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