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Sensitive determination of pethidine in body fluids by gas chromatography–tandem mass spectrometry

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Abstract

We have presented a simple and sensitive method for determining pethidine, a narcotic analgesic drug in body fluids by gas chromatography–tandem mass spectrometry (GC–MS/MS). Pethidine and 4′-piperidinoacetophenone (internal standard) were extracted from body fluids with Bond Elut C_{18} columns; the recoveries were above 85% for both compounds. The calibration curves for blood and urine showed good linearities in the range of 1.25–40 ng/ml. Its detection limits (signal-to-noise ratio=3) were estimated to be approximately 0.5 ng/ml of whole blood and urine.

Keywords: Pethidine

1. Introduction

Pethidine (meperidine) is a narcotic analgesic drug, which is widely prescribed in therapeutic practice; it is predominantly a μ-opioid receptor agonist, and it exerts its phamacological action on the central nervous system and the neural elements in the bowel [1]. Pethidine is readily absorbed after oral administration, and rapidly metabolized to norpethidine or pethidinic acid [2–4]. Pethidine was widely and most frequently abused by persons working in anaesthesia units in the US [5]. Thus it is necessary to detect and identify pethidine in human body fluids with high sensitivity. We have reported sensitive methods for determining pethidine in human body fluids by gas chromatography–surface ionization detector (GC–SID) [6] and GC/surface ionization organic mass spectrometer (SIOMS) [7].

GC–tandem mass spectrometry (MS/MS) has enabled sensitive and selective determination of target compounds from many impurities; it has been applied for some drugs-of-abuse [8–10]. In this

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paper, we have presented that pethidine in body fluids can be determined with very high sensitivity by GC–MS/MS.

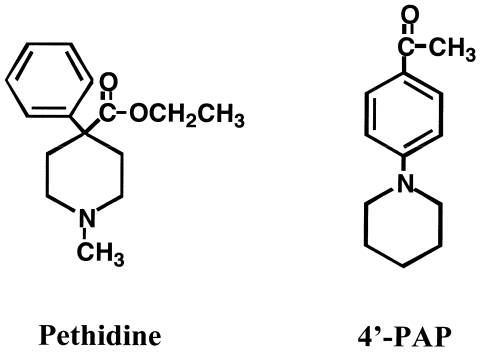
2. Experimental

2.1. Materials

Pethidine–hydrochloride and 4′-piperidino-acetophenone (internal standard, I.S.) was purchased from Tanabe Seiyaku Co. Ltd. (Osaka, Japan) and Aldrich Chemical Co. (Milwaukee, WI, USA), respectively; their chemical structures are shown in Fig. 1. Other chemicals used were of analytical grade. Bond Elut C_{18} columns and a CP-SIL 8CB-MS fused-silica capillary column (30 m×0.32 mm I.D., film thickness 0.25 μm) were obtained from Varian (Harbor City, CA, USA) and Chrompack (Middelburg, The Netherlands), respectively. A 1-μl plunger-in-needle syringe was obtained from Ito Seisakusho Co. Ltd. (Shizuoka, Japan). Human blood and urine samples were obtained from healthy volunteers.

2.2. Extraction of compounds with Bond Elut C_{18} columns

We extracted pethidine and I.S. from body fluids with Bond Elut C_{18} columns. The procedure followed Suzuki's method [11] with slight modifications. For pretreatment, the columns were washed with 10 ml of methanol and 15 ml of distilled water; this treatment was repeated twice. Eight milliliters of distilled water and 1 ml of 1 *M* $NaHCO_3$ solution were added to 1 ml of whole blood or urine samples with or without the compounds. In the case of whole blood samples, each mixture was spun down at 3500 rev./min for 15 min, and the supernatant was loaded onto a pretreated column. In the case of urine samples, the mixture was applied to a column without centrifugation. After washing the column with 15 ml of distilled water, the compounds were eluted with 3 ml of chloroform–methanol (9:1). The organic layer was evaporated to dryness under the flow of nitrogen. The residue was dissolved in 25 μl of methanol, and a 1-μl aliquot was subjected to the GC port using a 1-μl plunger-in-needle syringe.



Pethidine **4'-PAP**

Fig. 1. Chemical structures of pethidine and 4′-piperidino-acetophenone (I.S.).

2.3. GC conditions

GC analyses were performed on a Varian CP-3800 gas chromatograph with a split-splitless injector (Walnut Creek, CA, USA) coupled with a tandem mass spectrometer. The chromatograph was fitted with a CP-SIL 8CB-MS fused-silica column. The column temperature was maintained at 100 °C for 1 min and then programmed at 20 °C/min to 290 °C; the injection temperature was 250 °C. Helium (99.9999%) was used as a carrier gas at a flow-rate of 1.6 ml/min. The samples were injected in the splitless mode, and the splitter was opened 1 min after the completion of the injection.

2.4. MS conditions

The instrument used was a Varian Saturn 2000 ion-trap tandem mass spectrometer. Mass spectrometric measurements were performed in the positive ion chemical mode; methane was used as CI gas. MS conditions were as follows: interface temperature, 260 °C; manifold temperature, 45 °C; trap temperature, 210 °C; ionization current, 10 μA; electron energy, 70 eV; and detector voltage, 1.6 kV. The scan speed was 1000 amu/s, and scan was performed in the segments 2 and 3. The MS/MS method parameters for pethidine and I.S. are summarized in Table 1.

Table 1
MS/MS method parameters in the present method

	Acquisition segment	
	2	3
Compound	Pethidine	4′-PAP
Run time (min)	4.0–7.5	7.5–12
Mass range (m/z)	150–255	100–210
Mode	Resonant	Non-resonant
Precursor ion mass (m/z)	248	204
Isolation window (m/z)	4.0	3.0
Excitation rf (m/z)	109.3	89.8
Excitation amplitude (V)	0.48	30.0
Monitored product ions (m/z)	174 + 202	162

3. Results and discussion

3.1. Product ions and mass chromatograms of pethidine and I.S. extracted from human body fluids

The product ion profiles for pethidine and 4′-PAP are shown in Fig. 2. For pethidine, the peaks of major fragment ions appeared at m/z 174, 202 and 220. For 4′-PAP, the base peak was m/z 162; small peaks of fragment ions appeared at m/z 167, 114 and 70.

Fig. 3 shows the total ion chromatogram (TIC) and mass chromatograms of pethidine (20 ng/ml) and I.S. (40 ng/ml), extracted from spiked human whole blood. Background noises were very small, and both peaks were clearly found in mass chromatograms. The compounds were also extracted from spiked putrefied whole blood; pethidine was identified in 2.5 ng/ml whole blood. In the MS mode, many impurity peaks were found in TIC, the peak of I.S. was hardly discernible (data not shown).

3.2. Reliability of the present method

We quantitated pethidine in spiked human whole blood or urine by mass chromatography; the peak area ratio of both product ions, m/z 174 plus 202 to 162 was used for quantitation. The calibration curves for whole blood and urine were drawn by plotting six different concentrations of pethidine, using 40 ng/ml

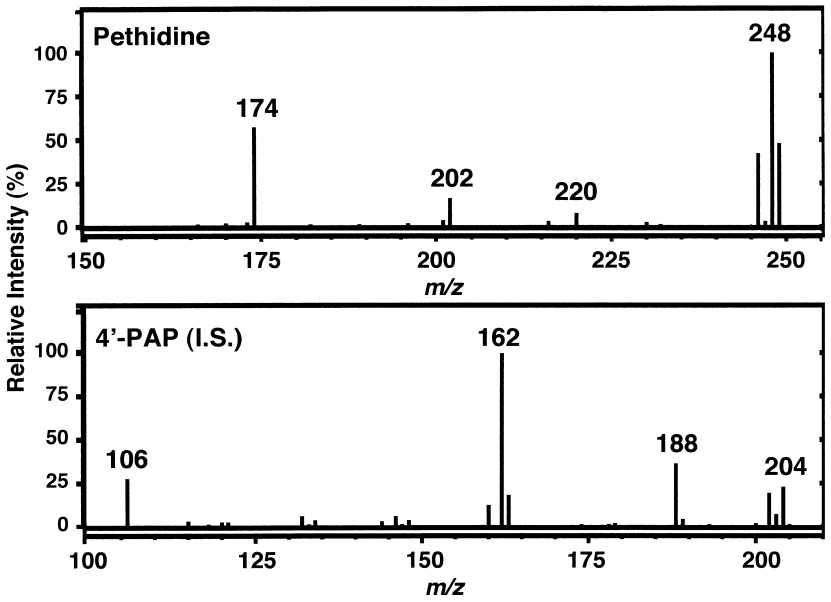


Fig. 2. Product ion profiles of pethidine (upper panel) and I.S. (lower panel). The amounts of injected pethidine and I.S. were 0.8 and 1.6 ng on-column, respectively.

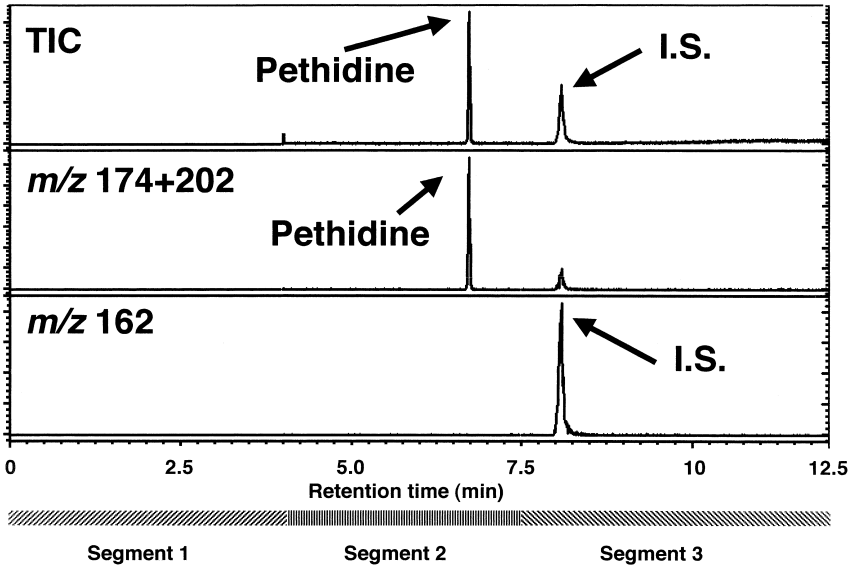


Fig. 3. Total-ion chromatogram (TIC) (upper panel) and the mass chromatograms of product ions of pethidine (m/z 174+202, middle panel) and I.S. (m/z 162, lower panel), extracted from 1 ml of spiked human whole blood (20 ng of pethidine and 40 ng of I.S.).

of I.S.; they gave good linearities in the range of 1.25–40 ng/ml pethidine in whole blood and urine. The equations and r values were $y=0.0299x-0.0623$ and $r=0.986$ for whole blood, and $y=0.0393x-0.0884$ and $r=0.989$ for urine. The detection limit (signal-to-noise ratio=3) for pethidine was estimated to be 0.5 ng/ml for both whole blood or urine.

Several researchers quantitated pethidine by SIM of conventional EI-MS [12–14]. Their detection limits were 25 ng/ml (plasma) [12], 0.17 ng/ml (serum) [13], and 1 ng/ml (breast milk) [14]. The sensitivity of our present method is almost comparable to the previous methods.

The recoveries of pethidine and I.S. were determined by mass chromatography; the peak areas in whole blood spiked with known amounts (4 or 20 ng/ml for pethidine and 40 ng/ml for I.S.) of the compounds were compared with the peak areas of authentic samples. The recoveries of pethidine from whole blood were 109±17.1% (mean±SD, $n=4$) at 4 ng/ml and 109±10.9% at 20 ng/ml; that for I.S. was 89.4±12.4% ($n=8$) at 40 ng/ml.

To check reproducibility of our present method, we quantitated 4 and 20 ng/ml pethidine, spiked to whole blood using each calibration curve by mass chromatography. The coefficients of intra-day variations were 12.9% at 4 ng/ml and 14.1% at 20 ng/ml, respectively ($n=5$); those of day-to-day variations were 12.7% at 4 ng/ml and 14.4% at 20 ng/ml, respectively ($n=5$). The C.V. values of the present method were above 10%; the difference of the ionization efficiencies among separate experiments would be one of the causes of such high C.V. values. This may be due to the difference of coexisting impurities in the extracted samples. The use of deuterium-labeled pethidine as I.S. could improve the reproducibility, as in the quantitation of midazolam in human body fluids by high-performance liquid chromatography–fast atom bombardement–mass spectrometry (HPLC–FAB–MS) [15].

4. Conclusion

This is the first report describing the determination of pethidine in human body fluids by GC–MS/MS. The present method is highly selective for detecting and identifying pethidine in human body fluids. GC–MS/MS may be applicable for the identification of pethidine in putrefied body fluids or samples containing many impurities.

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References

[1] H.B. Gutstein, H. Akil, in: A. Goodman Gilman, J.G. Hardman, L.E. Limbird (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition, McGraw-Hill, New York, 2001, p. 569.
[2] A.C. Moffat, J.V. Jackson, M.S. Moss, B Widdop (Eds.), Clarke's Isolation and Identification of Drugs, 2nd edition, The Pharmaceutical Press, London, 1986, p. 867.
[3] L.E. Mather, P.J. Meffin, Clin. Pharmacokinet. 3 (1978) 352.
[4] D.J. Edwards, C.K. Svensson, J.P. Visco, D. Lalka, Clin. Pharmacokinet. 7 (1982) 421.
[5] C.F. Ward, G.C. Ward, L.J. Saidman, J. Am. Med. Assoc. 250 (1983) 922.
[6] H. Seno, H. Hattori, T. Iizumi, T. Kumazawa, O. Suzuki, Jpn. J. Forensic Toxicol. 10 (1992) 241.
[7] A. Ishii, R. Kurihara, K. Watanabe-Suzuki, T. Kumazawa, H. Seno, H. Matsushima, O. Suzuki, Y. Katsumata, J. Chromatogr. B 758 (2001) 117.
[8] A. Polettini, A. Groppi, M. Montagna, Forensic Sci. Int. 63 (1993) 217.
[9] T. Mieczkowski, Forensic Sci. Int. 70 (1995) 83.
[10] U. Hofmann, S. Seefried, E. Schweizer, T. Ebner, G. Mikus, M. Eichelbaum, J. Chromatogr. B 727 (1999) 81.
[11] O. Suzuki, T. Kumazawa, H. Seno, H. Hattori, Med. Sci. Law 29 (1989) 242.
[12] C. Lindberg, M. Berg, L.O. Boréus, P. Hartvig, K.-E. Karlsson, L. Palmér, A.-M. Thörnblad, Biomed. Mass Spectrom. 5 (1978) 540.
[13] E.L. Todd, D.T. Stafford, J.C. Morrison, J. Anal. Toxicol. 3 (1979) 256.
[14] P.G. Quinn, B.R. Kuhnert, C.J. Kaine, C.D. Syracuse, Biomed. Environ. Mass Spectrom. 13 (1986) 133.
[15] T. Sano, K. Sato, K. Kurihara, Y. Mizuno, T. Kojima, Y. Yamakawa, T. Yamada, A. Ishii, Y. Katsumata, Legal Med. 3 (2001) 149.