II.2.5 Pentazocine

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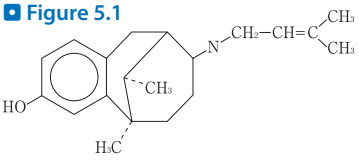
Introduction

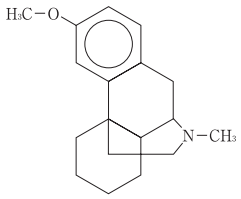
Pentazocine (*Fig. 5.1)* is a non-narcotic analgesic, but shows mild dependence; there are many cases of pentazocine abuse especially for medical and co-medical workers. The drug is being regulated as a subclass compound of narcotics (DEA class: IV).

Pentazocine in both antemortem and postmortem specimens is being analyzed by GC [1–4], HPLC [5–7] and GC/MS [8]. In this chapter, a simple method for GC/MS analysis of pentazocine in human whole blood and urine is presented.

Figure 5.1



pentazocine



dextromethorphan（IS）

Structures of pentazocine and dextromethorphan (IS).

Reagents and their preparation

i. Reagents

Ampoules for pentazocine injection were purchased from Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan. Each ampoule (1 mL) contains 15 mg of free pentazocine. The solution is transferred to a small glass centrifuge tube with a ground-in stopper, followed by addition of several mg of solid K_2CO_3 and 3 mL of chloroform. The tube is vortex-mixed well and centrifuged at 3,000 rpm for 5 min. The upper aqueous phase is carefully removed with a Pasteur pipette, and the lower organic phase is evaporated to dryness with a centrifugal freeze drier to obtain free pentazocine. However, at the present time, pure pentazocine is commercially available from Sigma (St. Louis, MO, USA). Dextromethorphan-HBr to be used as internal standard (IS) was purchased from Sigma; Sep-Pak C_{18} cartridges (classic type) from Waters

(Milford, MA, USA). Other common chemicals were of the highest purity commercially available.

ii. Preparation

- Pentazocine and dextromethorphan solutions: the drugs are separately dissolved in appropriate amount of methanol; a 10–20 μL aliquot was added to 1 mL of whole blood or urine.
- 1 M $NaHCO_3$: a 8.4-g aliquot of $NaHCO_3$ is dissolved in distilled water to prepare 100 mL solution.
- Chloroform/ethanol (9:1, v/v): a 100-mL volume of the mixture is prepared.

GC/MS conditions [8]

GC column[a]: a DB-17 fused silica capillary column (30 m × 0.32 mm i. d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA).

GC conditions: a GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan); column (oven) temperature: 150 °C (1 min) → 20 °C/min → 280 °C; injection temperature: 280 °C; carrier gas: He; its flow rate: 3 mL/min; injection: splitless mode for 1 min, followed by the split mode.

MS conditions; instrument: a Shimadzu QP-5050A quadrupole mass spectrometer[b] connected with the above GC; ionization: positive ion EI; electron energy: 70 eV; emission current: 60 μA; ion source temperature: 280 °C; accelerating voltage: 1.5 kV.

Procedure

i. To 1-mL of whole blood or urine, containing pentazocine, are added 50 ng dextromethorphan (IS, methanolic solution) and 6 mL distilled water, followed by mixing well. For a whole blood specimen, it is necessary to confirm the complete hemolysis. A 3-mL volume of 1 M $NaHCO_3$ solution is added to the above mixture to bring its pH to about 8.

ii. A 10-mL volume of ethanol and 10 mL distilled water are passed through a Sep-Pak C_{18} cartridge using a 10-mL volume glass syringe to activate the cartridge at a flow rate not faster than 5 mL/min.

iii. The cartridge is washed with 10 mL distilled water twice; pentazocine and IS are slowly eluted with 3 mL of chloroform/ethanol (9:1) into a glass vial.

iv. A small amount of upper aqueous layer is carefully removed by aspiration with a Pasteur pipette. The organic layer (chloroform) is evaporated to dryness under a stream of nitrogen. The residue is dissolved in 100 μL methanol; a 1-μL aliquot of it is injected into GC/MS being operated in the selected ion monitoring (SIM) mode.

v. Ions at *m/z* 271 plus 214 are analyzed for the IS from 3 to 7 min, and those at *m/z* 285 plus 217 for pentazocine from 7 to 8 min by SIM[c].

vi. A calibration curve is constructed by adding 50 ng IS and one of various concentrations of pentazocine to each vial containing 1 mL of blank whole blood or urine and 6 mL distilled water, followed by the above procedure. The number of different concentrations of pentazocine should be not smaller than 4 for the calibration curve, which is composed of pen-

tazocine concentration in the horizontal axis and peak area ratio of pentazocine to IS in the vertical axis. The peak area ratio of a test specimen is applied to the calibration curve to obtain its pentazocine concentration.

Assessment of the method

Table 5.1 shows EI mass spectra of pentazocine and IS. The base peaks unfortunately appeared in a low mass range at *m/z* 70 and 59, respectively. Therefore, the author et al. [8] used the combined SIM mode as shown in Figure 5.2. Ions at *m/z* 271 (M^+) plus 214 and at *m/z* 285 (M^+) plus 217 were used for detection of IS and pentazocine, respectively. When SIM with a single ion is made, ions at *m/z* 217 and 271 (M^+) should be used for pentazocine and IS, respectively.

The detection limits[d] (S/N=3) in the SIM mode were 20–30 ng/mL for pentazocine and 5–10 ng/mL for IS. The therapeutic concentrations of pentazocine in blood was reported to be 50–200 ng/mL; the acute toxic blood level more than 1 μg/mL [9]. Therefore, the present method can be used for detection and quantitation of both therapeutic and toxic levels of pentazocine in blood.

The recovery of pentazocine using the Sep-Pak C_{18} cartridge was close to 100 % for both whole blood and urine [8].

In this method, dextromethorphan is used as IS; the drug is being widely used as an antitussive and a fatal case, suggesting dextromethorphan poisoning, was reported [10]. To measure dextromethorphan, the same method can be used with pentazocine as IS conversely.

Table 5.1

EI mass spectra of pentazocine and dextromethorphan (IS)

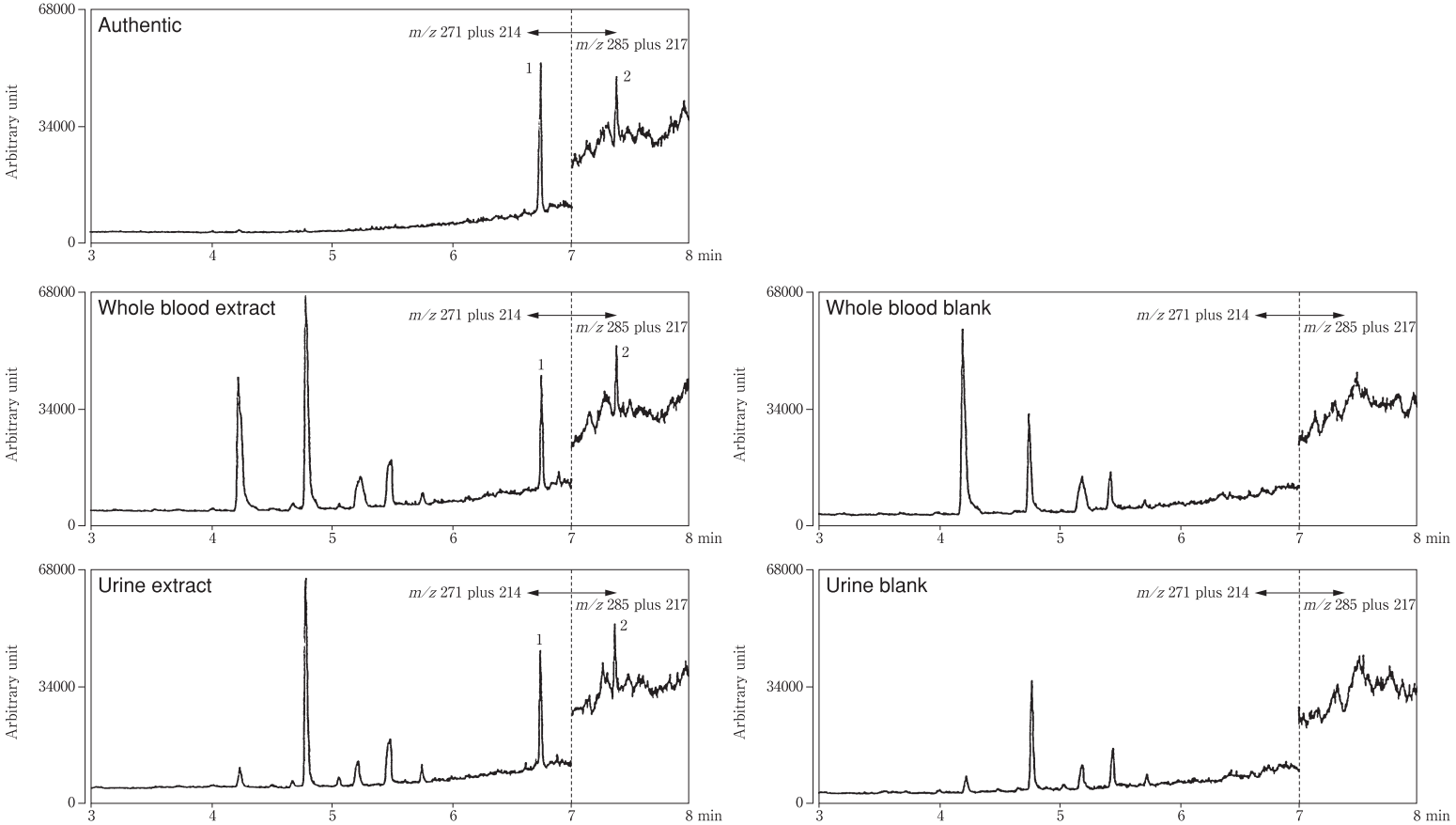
Compound	*m/z* (% peak intensity)				
Pentazocine	70 (100)	110 (39)	217 (45)	270 (9)	285 (9)
IS	59 (100)	150 (17)	214 (9)	271 (17)	

Poisoning case, and toxic and fatal concentrations

A 17-year-old female [11] ingested about 100 tablets of pentazocine (total amount estimated, 2.5 g) and fell into unconsciousness with convulsion like grand mal. She was sent to an emergency room, but still showed general cyanosis, convulsions and severe tachycardia. Because of her irregular spontaneous respiration, endotracheal intubation was accomplished and oxygen supply started. Since she showed severe acidosis, 100 mg of sodium bicarbonate, 1.6 mg of naloxone and diazepam (anticonvulsant) were administered to her. Her recovery was smooth, and she got free of the artificial respiration device after 3 h; her consciousness became clear.

A 45-mg aliquot each of pentazocine was administered to 8 subjects intramuscularly. The maximum blood concentrations were obtained 1 h after the administration; they were 0.11–0.24 μg/mL (average 0.14 μg/mL). A 75 mg aliquot each of pentazocine was orally administered to 5 subjects; the maximum blood concentrations (0.11–0.30 μg/mL; average 0.16 μg/mL) were obtained 1–3 h after the administration [12].

Figure 5.2



SIM chromatograms for pentazocine (peak 2) and dextromethorphan (IS, peak 1) extracted from whole blood and urine. In this system, ions at *m/z* 271 plus 214 (IS) and at *m/z* 285 plus 217 (pentazocine) were used at the retention times of 3–7 min and 7–8 min, respectively. The amount of pentazocine and IS spiked into 1 mL of blank whole blood or urine was equally 50 ng.

In two autopsy cases of pentazocine poisoning, its concentrations were 9.2 and 3.3 μg/mL in blood, 43 and 34 μg/g in the liver, and not detectable and 4.5 μg/mL in urine, respectively [12].

The blood concentrations of dextromethorphan, which has been used as IS in this chapter, were reported to be 100–950 ng/mL (average 380 ng/mL) in its poisoning fatal cases of infants [10].

Notes

a) Any capillary column of a 50 % phenylsilicone/50 % dimethylsilicone stationary phase (intermediately polar) can be used, regardless of manufacturers.
b) Any type of GC/MS instruments, to which a capillary column can be attached, can be used.
c) With a computer system unable to perform such combined SIM detection, the usual SIM using a single ion can be made by the method described below.
d) In view of sensitivity only, GC-NPD was reported to give similar sensitivity to that of the present GC/MS for pentazocine [1–3].

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