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Liquid chromatography–fast atom bombardment mass spectrometry for detection and determination of pentazocine in human tissues

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Abstract

A reliable and sensitive method was developed for the detection and determination of pentazocine in human solid tissues using liquid chromatography–dynamic fast atom bombardment (FAB) mass spectrometry, combined with a three-step liquid–liquid extraction procedure. Levallorphan tartrate served as an internal standard. The extract was evaporated to dryness and dissolved in the mobile phase, acetonitrile–10 m*M* ammonium acetate solution (20:80, pH 4.0) containing 0.5% glycerol as FAB matrix. The eluent was pumped at a flow rate of 25 μl/min and split before introduction to FAB mass spectrometer. Quantitative analysis was carried out by means of monitoring quasi-molecular ions with *m*/*z* 286 for pentazocine and *m*/*z* 284 for levallorphan. The lower limit of detection of pentazocine in each tissue tested was 1 ng/g with scan mode and 0.1 ng/g with SIM mode. Using this method, the concentrations of pentazocine were determined in the tissues of an autopsied individual to perform toxicological evaluation.

Keywords: Pentazocine

1. Introduction

Pentazocine, a synthetic benzomorphan derivative, is widely prescribed as a potent analgesic drug, and has received attention in forensic toxicology in relation to addiction or fatal intoxication [1–3], where the reproducibility of the analytical method was given little attention. On the other hand, reports on the analysis of pentazocine by high-performance liquid chromatography (HPLC) [4–6], gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) have been published [7–10], but most focused on the determination of the drug in biological fluids such as plasma, cerebrospinal fluid, urine etc. Using fast atom bombardment (FAB) mass spectrometry for pentazocine-related substances, qualitative analysis of pentazocine hydrate in human urine has been carried out in relation to forensic toxicology [11], no quantitative results were, however, discussed in the paper.

In forensic toxicological examinations, fresh body fluids are not always available because putrefaction, degradation and contamination have often occurred. Even whole blood cannot always be obtained owing to hemorrhage. In this paper, we report a sensitive, selective and reliable method, applicable to both detection and determination of pentazocine in human-derived solid tissues, using a liquid chromatograph–FAB–mass spectrometer (LC–

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FAB–MS), the objective being to procure a precise toxicological evaluation.

2. Experimental

2.1. Reagents

Pentazocine was provided by Yamanouchi Pharmaceutical (Tokyo, Japan) and levallorphan, an internal standard (I.S.), was done as a tartrate salt by Takeda Chemical Industries (Osaka, Japan). A buffer solution of pH 9 was prepared by mixing 21.3 ml of 0.1 *M* sodium hydroxide solution with 50 ml of each of 0.1 *M* boric acid and 0.1 *M* potassium chloride solution and diluting to 100 ml with distilled water. Methanol and *tert*.-butyl methyl ether were of analytical-reagent grade and were purified by distillation. Bromothymol blue indicator solution was purchased from Ishizu Seiyaku (Osaka, Japan). Other chemicals used were of analytical grade.

2.2. Biological samples

Human tissues including whole blood were obtained at the time of forensic autopsy to provide control samples. These samples were kept at −20°C until analysis.

2.3. Standard solutions of pentazocine and I.S.

Pentazocine (1 mg) was dissolved in methanol and the volume was adjusted to 10 ml with methanol to give a concentration of 100 ng/μl. This solution was further diluted to give concentrations of 10, 1 and 0.1 ng/μl. Standard solution of the I.S. salt was dissolved in water and the volume was adjusted to 10 ml to give a concentration of 100 ng/μl.

2.4. Extraction procedure

A method we developed for extraction of diazepam was used [12]. Approximately 1.0 g of whole blood or tissues was weighed and homogenized with a tissue homogenizer in a mixture of 5 ml of borate buffer (pH 9.0) and 1 μl of I.S. salt solution (100 ng of levallorphan tartrate) in a 30-ml centrifuge tube. A 10-ml volume of *tert*.-butyl methyl ether was added and the preparation was shaken for 10 min and centrifuged at 850 *g* for 20 min. The organic layer was transferred into a 30-ml centrifuge tube containing 2.0 ml of 2 *M* hydrochloric acid. The mixture was then shaken for 10 min and centrifuged at 850 *g* for 20 min. The aqueous layer was transferred into a 10-ml centrifuge tube containing two drops of bromothymol blue solution (0.04%) as an indicator and the mixture was made alkaline by adding 2 *M* sodium hydroxide solution until the indicator turned blue (about pH 9). A 2-ml aliquot of *tert*.-butyl methyl ether was added to the solution and the preparation was shaken for 10 min. After centrifugation, the solvent layer was dried with sodium sulphate and evaporated. The residue was dissolved in 50 μl of eluent and an aliquot of the solution was injected onto the liquid chromatograph–FAB–mass spectrometer.

2.5. Preparation of calibration graph

Whole blood samples were prepared to contain pentazocine at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 1000 ng/g and 10.0 μg/g, each containing 100 ng/g levallorphan tartrate. These samples were extracted in the same manner as described above. Calibration graphs were obtained by plotting the peak-area ratio of pentazocine to levallorphan versus the amount of pentazocine, measured by a computer connected with the FAB–mass spectrometer.

2.6. Conditions of LC–FAB–MS

The liquid-chromatograph pump used was a Shimadzu LC-10 AD (Kyoto, Japan), and the FAB mass spectrometer utilized was JEOL LX-1000 (Akishima, Japan). The analytical column was Inertsil ODS-2 (5 μm, 150 mm×0.7 mm I.D.) commercially pre-packed by GL Sciences (Tokyo, Japan). The mobile phase, acetonitrile–10 m*M* ammonium acetate solution (20:80, adjusted to pH 4.0 with acetic acid), was pumped at a flow rate of 25 μl/min. At the FAB matrix, glycerol was mixed to the mobile phase to give a concentration of 0.5% (v/v). The effluent was split with a built-in splitter, about 5 μl/ml of which was introduced to the

FAB–mass spectrometer. Xenon was used for the bombardment atom. The scan was performed from m/z 80 to m/z 400 by means of altering the magnetic field, and the scan speed was set at 5 s/scan. The other analytical parameters of the mass spectrometer were held as follows: temperature of ion source= 45°C, accelerating voltage=3 kV and mass resolution=500 (10% valley). This chromatographic–mass spectrometric analysis was carried out at room temperature.

3. Results and discussion

3.1. Monitoring ions

At the time of the preliminary experiments, we compared both the modes of monitoring, the positive ion monitoring and the negative ion mode. As a result, positive-ion monitoring gave more abundance of produced ions and a better signal-to-noise ratio, thus we decided to monitor positive ions.

3.2. Detection of pentazocine and I.S.

The FAB–mass spectra of pentazocine and levallorphan are shown in Fig. 1. Pentazocine showed a quasi-molecular ion at m/z 286 and levallorphan at m/z 284, respectively. Since these ions were the most abundant ions and glycerol-added ions were found in each mass spectrum, pentazocine and the I.S. could be detected without difficulty. A reconstructed ion chromatogram between m/z 280 and m/z 290 of the extract both from whole blood containing 100 ng each of pentazocine and I.S. are shown in Fig. 2. The retention times of pentazocine and the I.S. were 15.7 and 11.0 min, respectively. The peaks were clearly separated and no interfering peaks appeared in the chromatograms of the pentazocine-free human tissue and blood. We were able to detect pentazocine and I.S. from the chromatogram

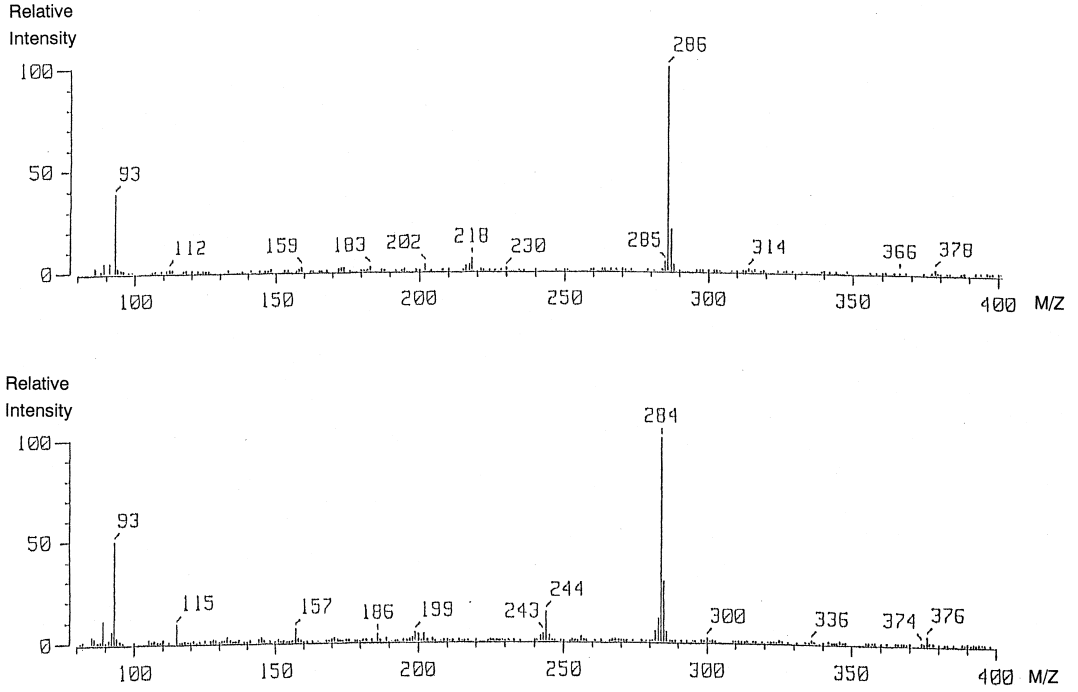

Fig. 1. Positive FAB–mass spectra of pentazocine (upper) and levallorphan (lower).

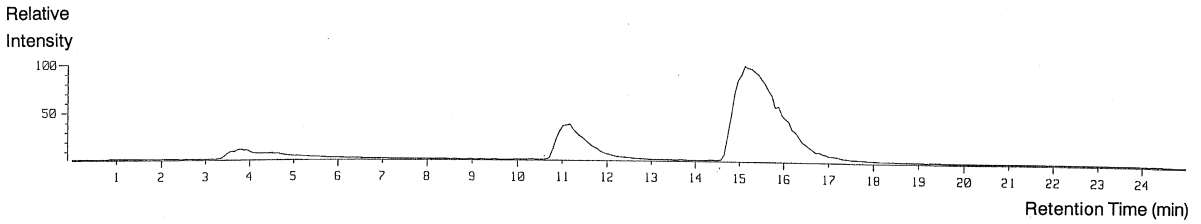

Fig. 2. Reconstructed ion chromatogram of an extract from whole blood containing 100 ng/g each of pentazocine and the I.S. salt.

by means of proper selection of reconstructed ions for glycerol-derived ions to be eliminated.

3.3. Determination of pentazocine

The respective quasi-molecular ions of *m/z* 286 and 284 were chosen to measure pentazocine and the I.S. with mass chromatography or selected ion monitoring (SIM), as the appropriate ions for determination from the aspect of signal-to-noise ratio. The limit of detection for pentazocine was about 100 pg/g with selected ion monitoring and 1.0 ng/g with scan mode in each tissue examined.

The calibration graph drawn for quantitation of pentazocine in each tissue tested was nearly equal to the value of the slope and was linear in the concentration range from about 1.0 ng/g to 1 μg/g when a 25-μl aliquot of a dissolved extract was injected. In addition, we confirmed that the graph was linear from 10 ng/g to at least 10 μg/g with correlation coefficients of 0.999, when the injected volume was reduced to 5 μl. This dynamic range is considered feasible to determine pentazocine concentration from trace to toxic/fatal level.

Within-day and between-day precisions were obtained using two concentrations (100 ng/g and 5.0 μg/g) by adding pentazocine to the blank blood. The first concentration is considered a therapeutic level and the second a toxic level. Spiked blood for between-day precisions were analyzed after one week of storage in a freezer. The coefficients of variation (C.V.) for the compound was between 7.4 and 8.4% for the within-day and between 7.7 and 9.1% for the between-day precision as seen in Table 1.

4. Practical application

A toxicological examination was required on a 67-year-old female who fainted suddenly after an intramuscular injection of 15-mg pentazocine to remove pain in her lower extremities. She died about 30 min later. Concerning this case, a toxicological examination was then ordered to determine whether or not the clinical treatment had been appropriate.

The peak of pentazocine was detected in the cadaveric blood as seen in Fig. 3. Qualitative results are shown in Table 2, at measured concentrations of 64.2, 35.4, 61.3 and 1.5 ng/g in the whole blood, brain, lung and liver, respectively. The pentazocine concentration in blood of the cadaver was much

Table 1
Precision and accuracy for pentazocine analysis in whole blood

Spiked concentration (ng/g)	Within day (n=5)		Between day (n=5)	
	Determined concentration (Mean±SD) (ng/g)	C.V. (%)	Determined concentration (Mean±SD) (ng/g)	C.V. (%)
100.0	96.2±8.1	8.4	98.9±9.0	9.1
5000.0	5130.2±379.6	7.4	5065.0±390.0	7.7

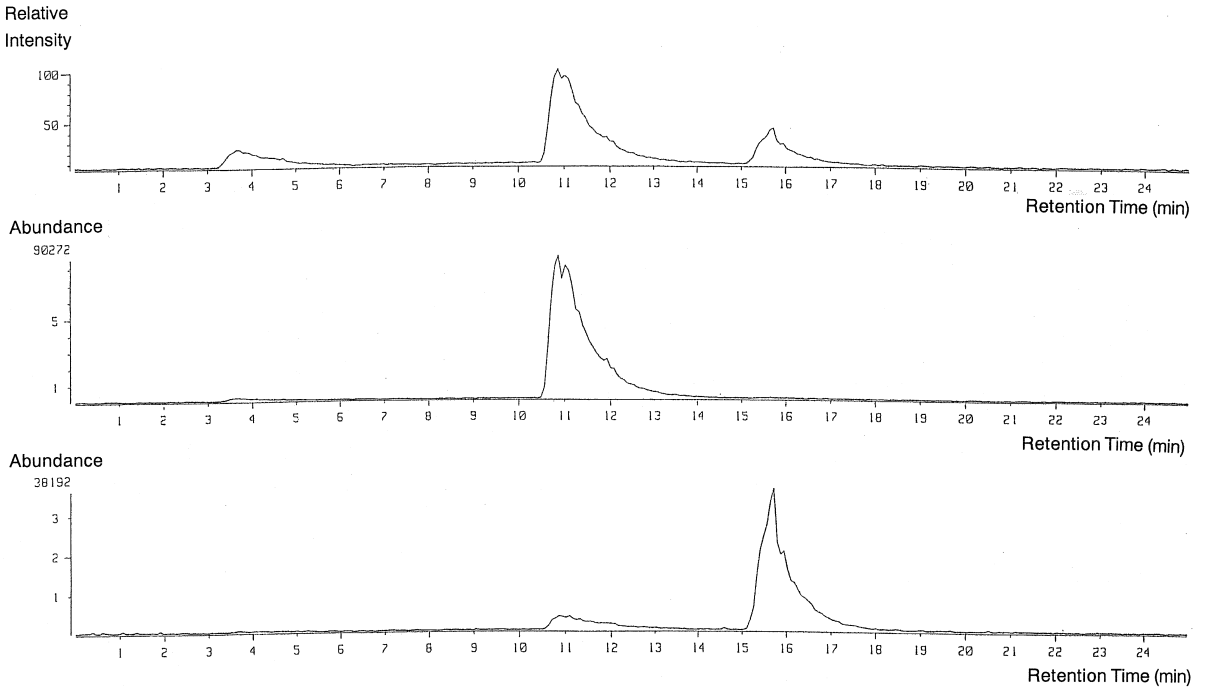

Fig. 3. Reconstructed ion chromatogram and mass chromatograms of the extract from cadaveric blood.

Table 2
Pentazocine concentrations in the tissues obtained from an autopsied individual

Tissue	Determined concentrations (ng/g)
Whole blood	64.2
Brain	35.4
Lung	61.3
Liver	1.5

lower than concentrations considered as toxic or lethal levels [2,3]. Based on these results, an overdose of the drug and misadministration were both denied.

5. Conclusion

A sensitive reliable and specific LC–FAB-MS assay was developed for the detection and determination of pentazocine. This method is applicable to solid tissues and fluids obtained at autopsy. Practical application of this method was also described.

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