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Forensic Science International
74 (1995) 205–211

Forensic Science International

Sensitive and selective determination of bromisovalum by high-performance liquid chromatography/particle beam mass spectrometry

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Received 4 January 1995; accepted 4 April 1995

Abstract

A specific procedure using high-performance liquid chromatography/particle beam mass spectrometry (HPLC/PBMS) has facilitated determination of bromisovalum in human plasma and whole blood. Bromisovalum, a sedative and hypnotic, was effectively extracted with Sep-Pak C_{18} cartridges and selectively determined by HPLC/PBMS of EI mode. An originally synthesized 2-bromohexanoylurea served as the internal standard (IS). The calibration curve was linear over the concentration range 0.5–5.0 μg/g and the lower limit of detection was 0.1 μg/g (10 ng at the time of direct injection). This method could be practically applied to detect bromisovalum in the whole blood of an autopsied individual.

Keywords: Toxicology; Bromisovalum; High-performance liquid chromatography/particle beam mass spectrometry

1. Introduction

Bromisovalum (Fig. 1), a widely used sedative and hypnotic, is one of the drugs most frequently requiring toxicological analyses in forensic practice in Japan. Determinations of bromisovalum in tissue samples have been made using gas chromatography (GC) [1,2] and gas chromatography/mass spectrometry (GC/MS) [3,4]. These methods have drawbacks such as thermal instability and decomposition of the

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0379-0738/95/$09.50
SSDI 0379-0738(95)01762-8

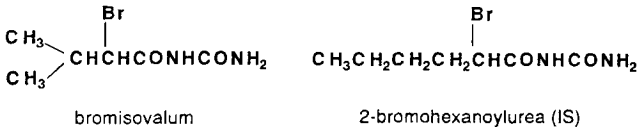

Fig. 1. Molecular structure of bromisovalum and 2-bromohexanoylurea.

drug during GC analysis, especially when a narrow-bore capillary column is used [1,5]. Although high-performance liquid chromatography (HPLC) with UV detection can provide a stable analysis of bromisovalum [6–10], the specificity was unsatisfactory compared with that of GC/MS, in case of mixing or contamination with any other components. We have now made use of HPLC/particle beam mass spectrometry (PBMS).

This advanced system is equipped with a nebulizer, which generates an aerosol from the effluent of HPLC with helium. As the aerosol passes through a slightly heated desolvation chamber, the mobile phase evaporates to a mixture of vapor, so that particles enter a two-stage momentum separator. Thus, the low momentum vapor molecules are pumped away and the high momentum particles are carried into the mass spectrometer source to be ionized and analyzed [11,12]. Both EI and CI mass spectrum can be obtained with this method, and complicated adjustments for other types of LC/MS interface are unnecessary, such as the mobile phase, buffer or matrix.

We devised an even more specific method for determining bromisovalum in the human plasma and whole blood, using the HPLC/PBMS technique. A case of practical application is briefly described.

2. Experimental

2.1. Materials and reagents

Bromisovalum was obtained from Nippon Shinyaku Co. Ltd. (Osaka, Japan). 2-Bromohexanoyl bromide was purchased from Aldrich Chemical Company (Milwaukee, WI). Dichloromethane, acetonitrile and methanol were of analytical grade and were purified by distillation. Sep-Pak C_{18} cartridges were purchased from Waters Millipore Corporation (Milford, MA) and Hypersil ODS column (5 μm, 100 × 2.1 mm I.D) from Hewlett Packard Co. (Palo Alto, CA).

Human whole blood was obtained at the time of autopsy and was kept at −20°C until analysis. Outdated plasma was obtained from a blood bank.

2.2. Preparation of internal standard (IS)

2-Bromohexanoylurea was synthesized in our laboratory from urea and 2-bromohexanoyl bromide, referring to a published procedure for the synthesis of bromisovalum [13]. This compound presented a single peak on HPLC, and was clearly characterized by mass spectrometry.

2.3. Extraction procedure

The method of extracting bromisovalum reported by Kumazawa et al. [1] was used, as follows: Sep-Pak C_{18} cartridge was preactivated by passing through 10 ml dichloromethane/methanol (9:1 v/v), 10 ml acetonitrile and 20 ml distilled water and this procedure was repeated once. Next, 1 g of plasma or whole blood containing 2-bromohexanoylurea (IS, 1 μg) was mixed with distilled water (4 ml for plasma and 9 ml for whole blood) and poured into the preactivated cartridge. The cartridge was washed with 20 ml distilled water, and the bromisovalum and IS were eluted with 3 ml dichloromethane/methanol (9:1 v/v). After discarding the aqueous layer (upper phase) with a Pasteur pipette, the organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl methanol/distilled water (1:1 v/v), and a 10-μl aliquot of this solution was used for LC/MS analysis.

2.4. Preparation of calibration curve

Plasma and whole blood samples were prepared to contain bromisovalum at concentrations of 0.5, 1, 3 and 5 μg/g, each containing 1 μg/g IS. These samples were extracted in the same manner as described above. The standard curve was obtained by plotting the peak area ratio of bromisovalum to IS versus the amount of bromisovalum.

2.5. LC/MS conditions

The PBMS analysis was performed on a Hewlett Packard 5988A particle beam interface (Palo Alto, CA). The EI mass spectra were acquired at 70 eV, with a source temperature of 250°C by a Hewlett Packard 5989A MS Engine. The desolvation chamber temperature was held at 55°C. The helium nebulizer pressure was set at 40 psi. Analyses were performed on a Hewlett Packard Hypersil ODS column (100 mm × 2.1 mm) at 40°C. The mobile phase used was methanol/distilled water (1:1 v/v) delivered at a flow rate of 0.2 ml/min from a Hewlett Packard 1090M HPLC system employing a 20-μl injector loop.

3. Results and discussion

3.1. Synthesis of new IS, 2-bromohexanoylurea

At first, 2-bromo-2-methylpropylurea, reported to be an appropriate IS for GC/CI-MS analysis [4] was used for particle beam LC/MS analysis. This compound, however, was inadequate for our purpose because of an extremely low intensity of total ions compared with that of bromisovalum. On the other hand, the 2-bromohexanoylurea we synthesized from 2-bromohexanoyl bromide and urea was completely separated from bromisovalum by LC and the intensity was sufficient for total ions in order to analyze bromisovalum by particle beam LC/MS.

3.2. Determination of bromisovalum by particle beam LC/MS

The mass spectra of bromisovalum and IS obtained by LC/MS are shown in Fig. 2. Major ions in each compound were checked and *m/z* 182 was selected as an appropriate ion for selected ion monitoring (SIM). A SIM chromatogram of the extract

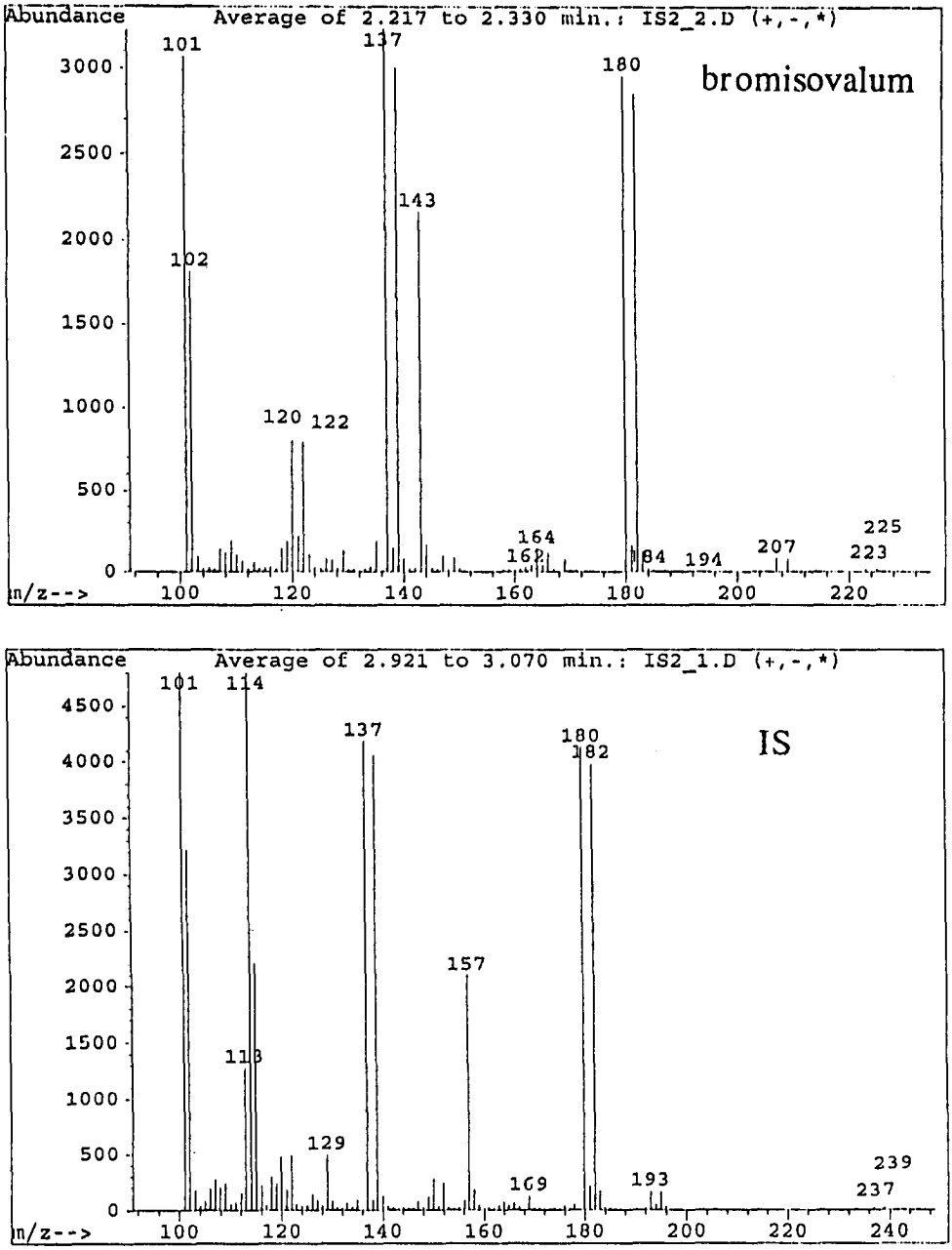

Fig. 2. EI/MS of bromisovalum and IS obtained by particle beam LC/MS.

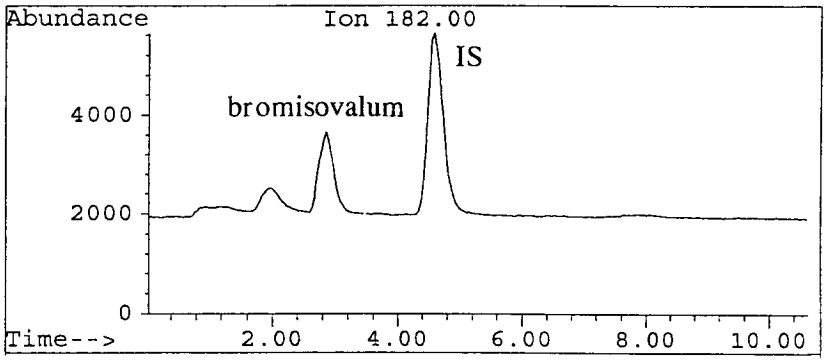

Fig. 3. SIM chromatogram of the extract from a plasma containing 1 μg/g each of bromisovalum and IS.

from a plasma containing 1 μg/g each of bromisovalum and IS, is shown in Fig. 3. Each peak was clearly separated on the SIM chromatogram. No interfering peaks were observed in the drug-free human plasma and whole blood (Fig. 4).

The calibration curve for bromisovalum was linear in the concentration range 0.5–5 μg/g. Although the calibration range with linearity is not always wide, it is considered adequate to cover the concentrations for a practical forensic analysis. The limit of detection was approximately 0.1 μg/g (10 ng per injected volume).

3.3. Case study

A 21-year-old woman was found dead in her room, her whole body surface covered with bruises. Although cause of death was at first considered to be due to

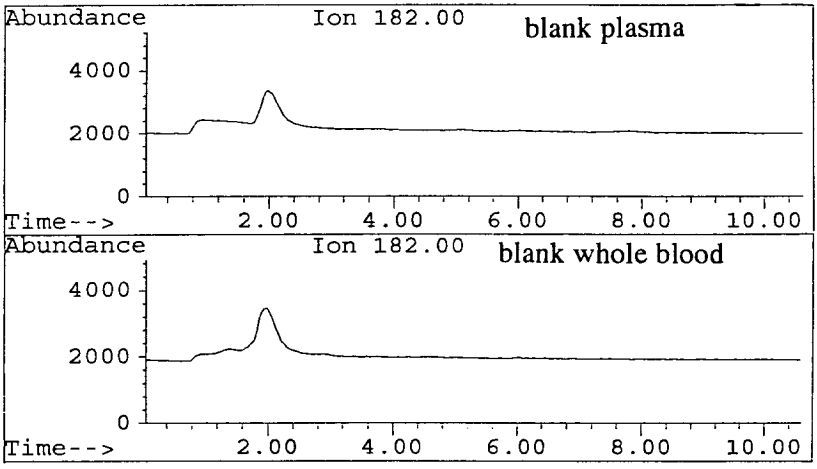

Fig. 4. SIM chromatograms of the extracts from blank plasma and blank whole blood.

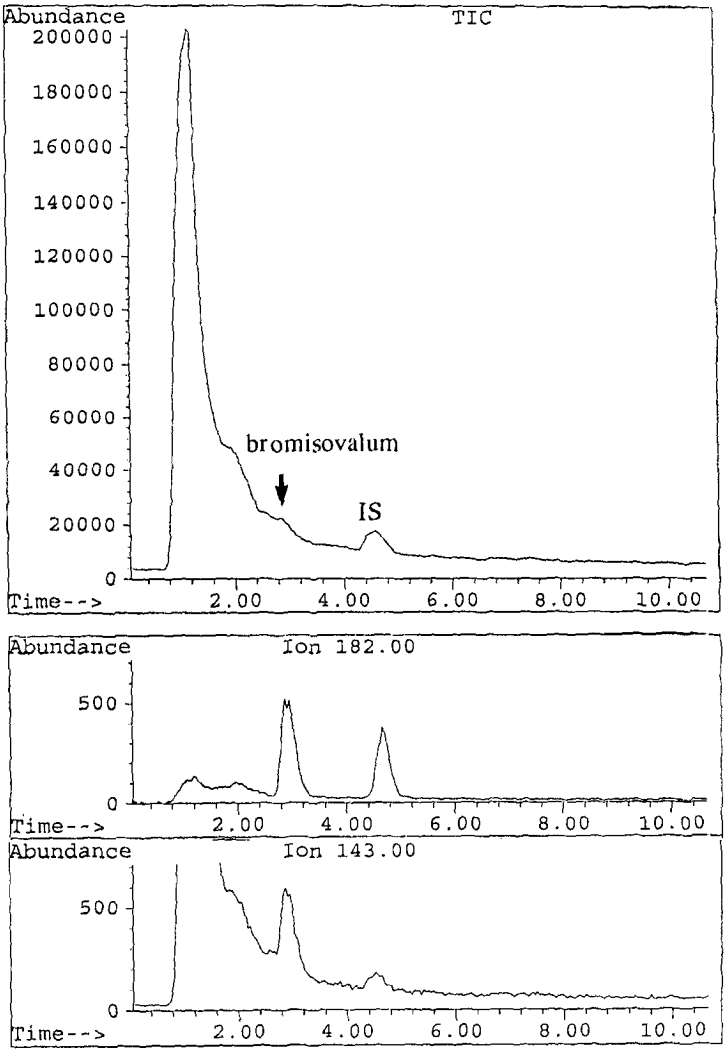

Fig. 5. Total ion chromatogram and mass chromatograms of the extract from the blood of an autopsied person.

an intensive beating by a male friend, the assailant strongly insisted that the victim must have died of poisoning as a hypnotic drug was found near her body.

Toxicological analysis was done to elucidate the cause of death, using the method described above. Although a large peak appeared at the solvent front with tailing and interfered with the detection of the small peak at the position of bromisovalum in total ions of scan mode, the specific ions in bromisovalum (*m/z* 182 and 143) were clearly detected (Fig. 5). The concentration of bromisovalum in the whole blood

determined by SIM mode was 2.31 μg/g, that is a therapeutic level. As ingestion of this drug was confirmed to be at the level of common use, the male friend was held under suspicion of beating the woman, which led to her death. Cause of death was determined to be a traumatic shock due to beating.

4. Conclusions

The analytical problems of bromisovalum in cases of GC, GC/MS and HPLC were overcome by the use of HPLC combined with particle beam mass spectrometry, using 2-bromohexanoylurea as IS. This method is sensitive, selective and can readily be used to test biological samples in forensic toxicological examinations.

Acknowledgement

We thank M. Ohara for helpful comments on the manuscript.

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