Selected Ion Monitoring for the Determination of Bromovalerylurea in Human Plasma

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A gas chromatography-selected ion monitoring procedure with chemical ionization is described for the determination of bromovalerylurea (BVU) in human plasma. BVU was extracted with ether after addition of 2-bromo-2-methylpropylurea as an internal standard. The lower limit of BVU quantification by this method was 2 ng/0.1 ml plasma volume. This procedure was used to determine the sequential plasma levels of BVU in a human volunteer following a single oral dose of a commercial analgesic.

Keywords bromovalerylurea; solvent extraction; gas chromatography-selected ion monitoring; chemical ionization; human plasma; oral administration

Bromovalerylurea, (2-bromo-3-methylbutyryl)urea (BVU, Fig. 1-I) has been used for many years as a sedative and hypnotic. Many analytical methods[1–6] have been reported for the identification of BVU and its metabolites in animals or humans after an overdose. To quantify therapeutic doses or misuse of BVU in humans, other analytical methods have been used, such as high-performance liquid chromatography (HPLC)[7] and electron imapact gas chromatography-mass spectrometry-selected ion monitoring (EI-GC/MS-SIM).[8] However, these techniques are often of limited value for detailed pharmacokinetic studies when a therapeutic dosage has been given, either because of inadequate sensitivity or poor specificity, especially when a dose has contained several other ingredients. This paper describes a sensitive and specific GC/MS method for the determination of BVU in human plasma, which consists of a single solvent extraction and isobutane chemical ionization (CI)-SIM, using BVU analogue as an internal standard (Fig. 1-II).

The specificity was checked by simultaneous monitoring of quasi-molecular ion intensity along with the isotope peak ion intensity derived from the ^{81}Br. This method was used to study the pharmacokinetics of BVU in a healthy subject following a single oral administration of a commercial analgesic which contained 200 mg of BVU.

Experimental

Material and Reagents BVU was purchased from Seitetsu Kagaku (Tokyo, Japan). The internal standard, 2-bromo-2-methylpropylurea was prepared according to a published procedure[9] from urea and 2-bromobutyryl bromide purchased from Tokyo Kasei Kogyo (Tokyo). This compound was characterized by mass spectrometry. All other solvents and reagents used were of reagent grade.

Heparinized blood samples were taken from a male adult volunteer before, then 10, 20, 40, 60, 120, 180, 240 and 480 min after administration of a drug containing 200 mg of BVU. The plasma samples were stored at −40 °C until analysis.

Instruments and Conditions A Hitachi M80-A GC/MS was equipped with a M-003 data processing system. The wide bore fused-silica column coated with DB-1 (15 m × 0.53 mm i.d.; coating thickness 1.5 μm, J&W Scientific, Rancho Cordova, CA, U.S.A.) was used for the analysis. The flow rate of carrier gas (helium) was 40 ml/min. The column temperature was maintained at 100 °C for 1 min, programmed up to 140 °C at 20 °C/min, and then raised to a final temperature of 250 °C at 25 °C/min to elute the decomposition products of unstable compounds or high molecular weight compounds that were not chromatographed. The mass spectrometer was operated at an ionization energy of 70 eV with an emission current of 100 μA. The temperatures of the injection port, interface between GC and Mass Spectrometer and the ion source were 170 °C, 200 °C and 200 °C, respectively. Mass spectrometry was performed by CI with isobutane as the reagent gas at a pressure of 2×10^{-6} Torr. For SIM quantification, the respective isotope ions of quasi-molecular ions derived from the ^{81}Br at *m/z* 225 for BVU and at *m/z* 211 for the internal standard were monitored. To confirm the selectivity of the GC peaks, the quasi-molecular ions at *m/z* 223 and 209 were also monitored simultaneously.

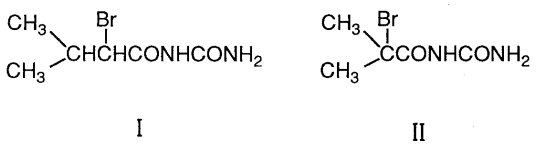

Fig. 1. Structures of BVU (I) and Internal Standard (II)

Extraction Procedure To 0.1 ml of plasma in a round bottom test tube were added 0.4 ml of distilled water, 0.1005 μg of internal standard in 25 μl of ethanolic solution and 3 ml of ether. The mixture was then shaken for 15 min at ambient temperature. After centrifugation at 4000 rpm for 20 min at 18 °C, the upper layer was transferred to another identical tube for evaporation under nitrogen. The dried residue was reconstituted in 10 μl of ethylacetate and an aliquot was analyzed by GC/MS.

Calibration Curve and Extraction Recovery The calibration curve for the BVU determination by GC/MS-SIM was obtained by plotting the ratio of the peak area of BVU to that of internal standard (0.1005 μg/ml of plasma) against the concentration. Standard solutions of BVU at 0.0199, 0.0497, 0.1988, 0.7952 and 3.1808 μg/ml of plasma and a blank were analyzed in triplicate to generate a calibration curve for BVU. The extraction recovery percentage was determined by comparing peak areas of selected ion recordings (SIRs) obtained from extracted plasma samples to those of the standard stock solutions for each concentration in the calibration curve.

Results and Discussion

GC/MS Conditions In the EI mass spectra of BVU, the most abundant ion was the fragment at *m/z* 137 together with a very weak molecular ion peak at *m/z* 222 (data not shown). Monitoring the base peak at *m/z* 137, human plasma BVU levels have been reported following oral administration using EI-SIM.[8] Although the sensitivity of the reported EI-SIM method is higher than that of HPLC,[7] it was not sensitive and specific enough to measure BVU at levels below 0.2 μg/ml plasma, owing to interfering peaks. Therefore, it is not suitable for routine clinical monitoring of BVU levels at therapeutic doses. The CI mass spectra of the compound, however, showed a very dominant quasi-molecular ion at *m/z* 223 followed by the isotope peak at *m/z* 225 as previously reported.[10] (Fig. 2)

This quasi-molecular ion current was approximately 2 times greater than that of the *m/z* 132 for the EI mode, when ion source pressure of the isobutane reagent gas was 2×10^{-6} Torr and adjusted to maximize the intensity of the BVU quasi-molecular ion. This situation was the same for the synthesized internal standard, which showed a

quasi-molecular ion peak at m/z 209. The maximum intensity of respective quasi-molecular ions was found when the temperature of the ion source was 200 °C.

Since bromoacetylurea derivatives and alkyl substituted ureas are thermally decomposed during GC analysis,[11] it is necessary to optimize the temperature of the GC injection port and the column oven. Therefore, these temperatures were adjusted and optimized to give symmetrical peaks without derivatization reactions such as trifluoroacetylation.[12]

Sample Preparations Although the extraction recovery of BVU was approximately 50% ($n=3$) in 0.01 μg/ml of plasma, diethyl ether was found to be a preferable solvent to others such as ethyl acetate and dichloromethane, the use of which resulted in the appearance of interfering peaks on SIRs. Only a single ether extraction with 0.1 ml of the sample plasma was simple enough to be analyzed directly by GC/MS, allowing analysis of a large number of samples per day. The recovery was almost quantitative by the use of Bond-Elut C_{18} solid phase extraction. The SIRs of the extract residues after extraction with ether indicated that less endogenous material was extracted from the plasma specimens at neutral pH than at acidic pH. Typical SIRs are shown in Fig. 3. The blank was prepared from the plasma of BVU-free volunteers, and no interfering peaks were observed at the retention times of the compounds of interest. The retention times for BVU and the internal standard were approximately 2.8 and 1.9 min, respectively, and each peak was sharp and symmetric.

A linear response curve for the determination of BVU was obtained in the concentration range from 0.02 to 3.18 μg/ml plasma with a correlation coefficient of 0.9998, and passed through the origin (data not shown). As shown in Table I, the coefficient of variation ($n=3$) was less than 5%. The detection limit of BVU was below 0.001 μg/0.1 ml with a signal-to-noise ratio of 7 by the present method.

Determination of Plasma BVU Although BVU metabolites such as glutathione related conjugates[1] and debrominated compound[2] have been investigated, these metabolites were not converted back into the parent drug during the GC/MS analysis. Ingredients such as caffeine, ethenzamide and ibuprofen in a single commercial tablet, also do not have fragment ions of the same m/z value as those of the SIM monitoring ions for BVU. However, another possibility for contaminants is endogenous co-extracted material or unknown metabolites derived from each constituent drug. Therefore, the use of the quasi-molecular ion is more reliable than that of the fragment ion in EI mode to reduce the possibility of interference and enhance the selectivity. In the present

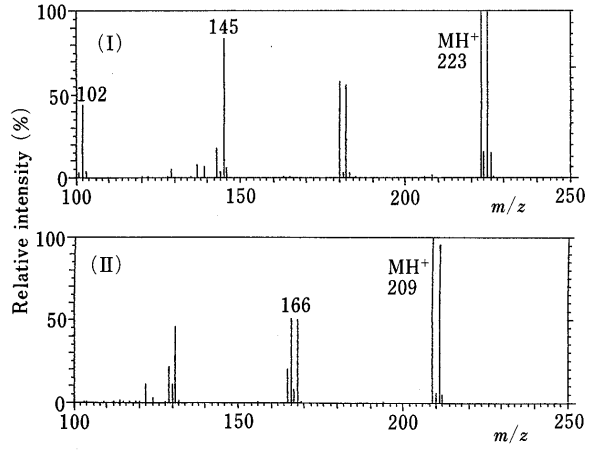

Fig. 2. Isobutane CI Mass Spectra of BVU (I) and Internal Standard (II)

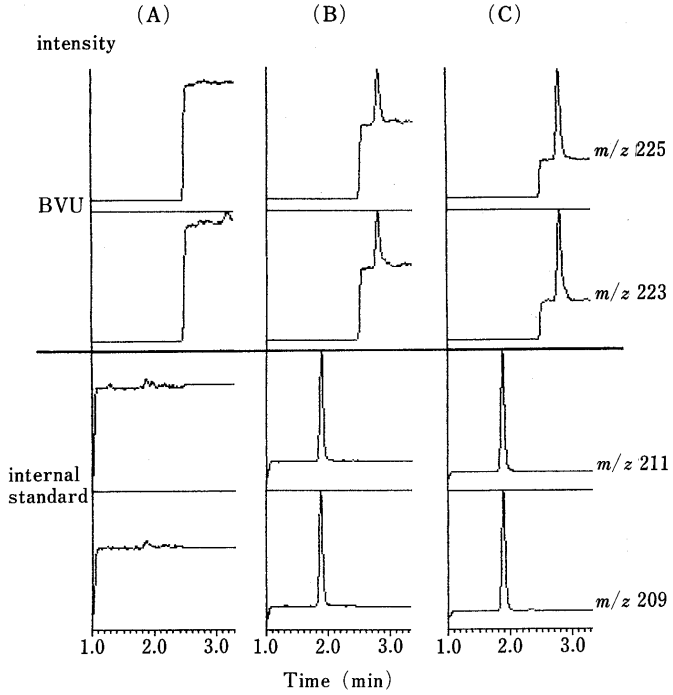

Fig. 3. Selected-Ion Recordings of (A) Blank Human Plasma, (B) Human Plasma Spiked with 0.020 μg/ml of BVU and 0.100 μg/ml of Internal Standard and (C) Plasma Collected 3 h Following Administration of a Tablet Containing 200 mg of BVU and Spiked with 0.100 μg/ml of Internal Standard (Equivalent to BVU of 0.13 μg/ml)

TABLE I. Precision and Accuracy of the BVU Determination in Spiked Plasma

Concentration added (μg/ml)	Measured concentration (mean ± S.D., $n=3$) (μg/ml)	Coefficient of variation (%)	Accuracy (%)
0.0199	0.0237 ± 0.0005	2.1	119
0.0497	0.0418 ± 0.0009	2.1	84
0.1988	0.1927 ± 0.0093	4.8	97
0.7952	0.7549 ± 0.0060	0.8	95
3.1808	3.1852 ± 0.0431	1.4	100

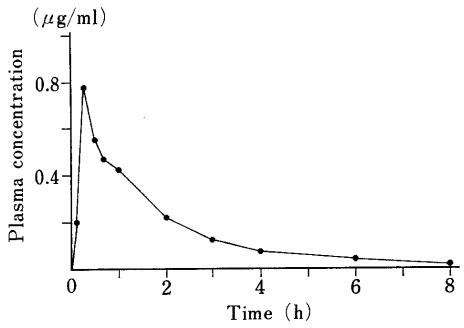

Fig. 4. Plasma Concentrations of BVU *versus* Time Following a Single Oral Administration of One Analgesic Tablet (200 mg of BVU) to a Healthy Volunteer

method, in order to confirm the GC purity, each characteristic doublet ion was monitored simultaneously to compare the ratios of m/z 225 to m/z 223 (BVU) and m/z 211 to m/z 209 (internal standard) with those of respective standard solutions during analysis.

This method was used to determine the BVU concentration in human plasma. Figure 4 shows the plasma levels of BVU after oral administration of a commercial mixture of analgesics containing 200 mg of BVU. At 20 min and 3 h after dosing, the observed ratios of BVU and internal standard were comparable (within $\pm 3\%$ variation) to those of the corresponding standard solutions. Therefore, there was no interference.

As shown in Fig. 4, the maximum BVU plasma concentration of 0.78 μg/ml was observed 20 min after dosing, and then it decreased with an elimination half-life of about 3 h in a human volunteer. With this method, the plasma BVU concentration was measured for up to 8 h after dosing, when levels dropped as low as 0.002 μg/0.1 ml.

Conclusion

The present CI-SIM method enables us not only to improve the sensitivity but also to enhance the selectivity without any interference. The result of the plasma concentration determined for up to 8 h following the oral administration of a commercial drug containing 200 mg of BVU confirmed the applicability of this method for human subjects.

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