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Note

Quantitative analysis of budralazine in human plasma and urine by gas chromatography–mass spectrometry

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4-Methyl-3-penten-2-one (1-phthalazinyl)hydrazone (I, budralazine, Fig. 1) is a new vasodilator antihypertensive drug [1,2]. Determination of plasma concentrations and urinary excretion of the drug in humans is important for clinical pharmacokinetic studies. It was assumed, from the results of investigation into the metabolism of budralazine in animals [3], that the plasma concentration of unchanged budralazine in humans would be extremely low. Thus a sensitive and specific analytical method for budralazine in plasma and urine was required. This paper reports development of such a method.

EXPERIMENTAL

Reagents

Budralazine (I) and deuterium-labelled budralazine as an internal standard (I.S., [$^2H_4$]budralazine, II) were synthesized in the Research Institute of Daiichi Seiyaku, and [^{14}C]budralazine (III) was obtained from Daiichi Pure Chemicals (Fig. 1).

All solvents and reagents employed were of analytical grade and were used without further purification.

Gas chromatography–mass spectrometry (GC–MS)

A Hitachi Model RMU-6MG mass spectrometer equipped with a gas chromatograph was used. The GC conditions were as follows. A glass column (1 m×3 mm I.D.) packed with 3% silicone OV-225 on 80–100 mesh Chromosorb W HP (Gasukuro Kogyo, Japan) was used. The temperatures of the oven and the injec-

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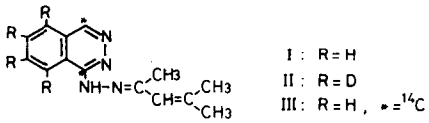

Fig. 1. Chemical structures of budralazine (I), deuterium-labelled budralazine (II) and ^{14}C-labelled budralazine (III).

tion port were set at 230 and 250 °C, respectively. The flow-rate of helium carrier gas was 50 ml/min. The MS conditions were as follows. The ionization voltage was 30 eV. The target current was 100 μA. The ion-source temperature was 200 °C. Quantitative analysis was performed by selected-ion monitoring at m/z 225 for I and m/z 229 for I.S.

Analytical procedure

To 1 ml of plasma, 100 ng of I.S. and 4 ml of 0.03 *M* borate buffer (pH 10) were added. The solution was extracted with 3 ml of chloroform–2-propanol (3:1) by shaking for 10 min. After centrifugation at 1800 *g* for 10 min, the organic solution was separated and evaporated in vacuo. The residue was dissolved in 0.5 ml of *n*-hexane and applied to a column (4.6 mm I.D.) of aluminium oxide (1 g, activity 2–3, Merck, Darmstadt, F.R.G.). The column was washed with 3 ml of *n*-hexane, and the fraction containing I and I.S. was eluted with 2 ml of benzene. The eluate was evaporated and the residue was redissolved in 100 μl of methanol, and 3–5 μl aliquots were injected into the gas chromatograph–mass spectrometer.

To 5–10 ml of urine, 500 ng of I.S. and 10 ml of 0.03 *M* borate buffer (pH 10) were added and extracted with 10 ml of chloroform–2-propanol (3:1). The extract was separated and evaporated in vacuo. The residue was dissolved in 200 μl of methanol, and 3–5 μl aliquots were injected into the gas chromatograph–mass spectrometer.

Recovery test of I from the aluminium oxide column

The test sample was prepared by adding 100 ng of I (containing [^{14}C]budralazine, ca. 10 000 dpm) to 1 ml of human plasma. The sample was extracted as described above, and the extract was evaporated. The residue was dissolved in 0.5 ml of *n*-hexane and subjected to clean-up with the aluminium oxide column. After eluting successively with three 1-ml aliquots of *n*-hexane, five 1-ml aliquots of benzene and two 2.5-ml aliquots of chloroform, the radioactivity of each fraction was measured with a liquid scintillation spectrometer (Aloka, LSC-652).

RESULTS AND DISCUSSION

Mass spectra of I and II

Figs. 2 and 3 show the mass spectra of I and II (I.S.), respectively. Since the molecular ions of I and II (m/z 240 and m/z 244, respectively) were of low abundance under the instrumental conditions used, the fragment ions at m/z 225 for I and m/z 229 for II, observed as the most abundant peaks, were chosen for selected-ion monitoring.

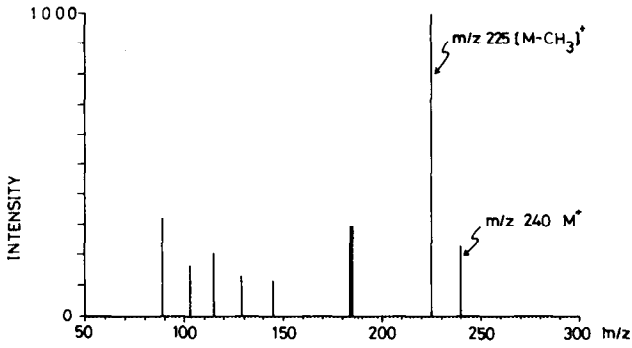

Fig. 2. Mass spectrum of I.

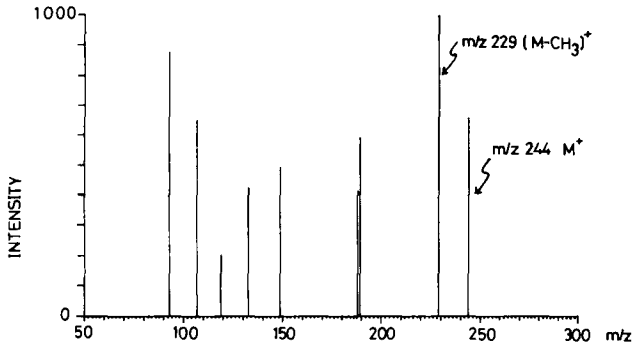

Fig. 3. Mass spectrum of II.

Extraction

Chloroform–2-propanol (3:1) was found to be suitable for the extraction of I from plasma and urine. The efficiency of extraction, examined using [^{14}C]budralazine, was 99–100%.

Clean-up procedure

In the case of plasma samples, a clean-up procedure was required to remove substances that interfered with the detection of I on the mass chromatogram. Purification of the samples was attempted with the aluminium oxide column and a Sep-Pak silica cartridge (Waters Assoc., Milford, MA, U.S.A.). Fig. 4 shows the recoveries of I from the aluminium oxide column and Sep-Pak cartridge. The recovery of added drug from the aluminium oxide column was 60% in the initial 2 ml of benzene eluate, in which there was no interference peak on the mass chromatogram. On the other hand, the recovery of I from Sep-Pak cartridge, eluted successively with *n*-hexane, benzene and chloroform, was extremely low. Moreover, the interfering substances on the mass chromatogram were eluted together. Consequently, the aluminium oxide column was found to be more efficient for the purification of I in plasma than Sep-Pak silica cartridge.

In urine samples, no clean-up procedure was needed for the detection of I.

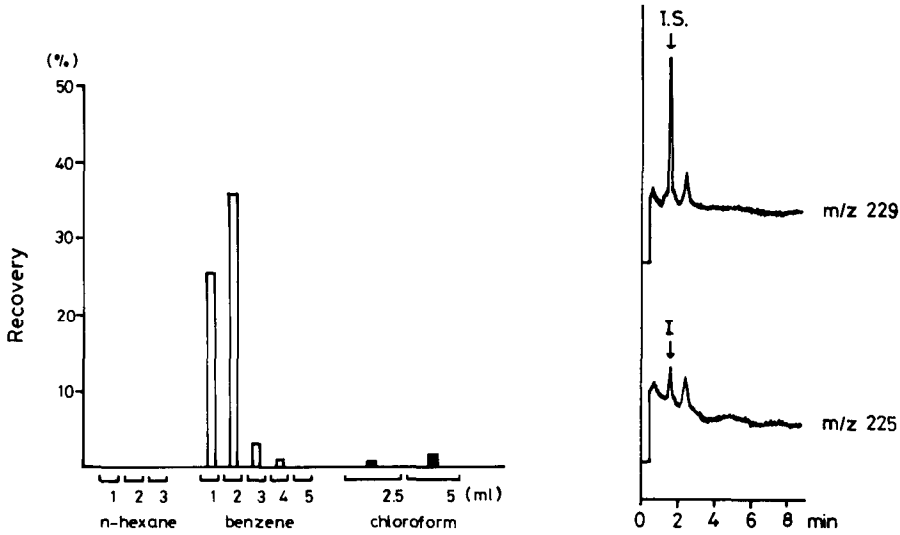

Fig. 4. Recovery of I from the aluminium oxide column (□) or the Sep-Pak silica cartridge (■). Budralazine (I) was added to human plasma at a concentration of 100 ng/ml; the column was eluted successively with 3 ml of *n*-hexane, 5 ml of benzene and 5 ml of chloroform.

Fig. 5. Mass fragmentogram of human plasma spiked with I (25 ng/ml) and II (100 ng/ml).

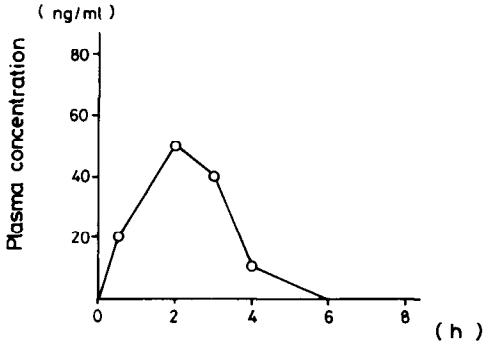

Fig. 6. Plasma concentration of the unchanged drug after a single oral administration of I at a dose of 60 mg.

Precision and limit of detection

Fig. 5 shows a typical chromatogram obtained from plasma spiked with I (25 ng/ml) and I.S. (100 ng/ml).

Calibration curves were prepared by plotting the peak-height ratio (I/I.S.) against the concentration of I. The relationship was linear over the concentration range 12.5–100 ng/ml in plasma ($r=0.9915$) and 50–980 ng/ml in urine ($r=0.9997$). The coefficients of variation at 25 and 50 ng/ml were 2.4 and 2.6% ($n=3$), respectively. The lower limit of detection was 10 ng/ml.

Application of the method

The plasma concentration and the cumulative urinary excretion of the unchanged drug after a single oral dose of 60 mg of I to a healthy human subject

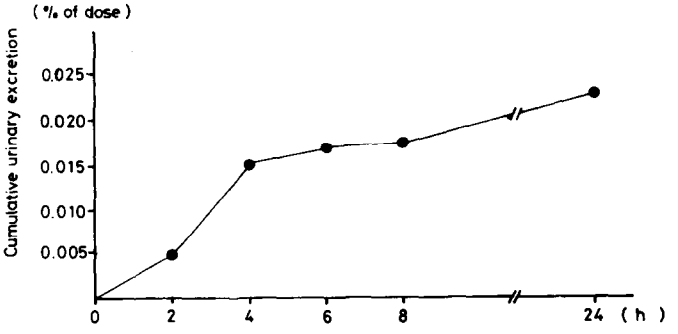

Fig. 7. Cumulative urinary excretion of the unchanged drug after a single oral administration of I at a dose of 60 mg.

are shown in Figs. 6 and 7, respectively. The maximum plasma concentration of 50 ng/ml occurred 2 h after dosing, and the cumulative urinary excretion during 24 h was 0.025% of the dose.

Our results confirm that the present method is sensitive and very effective for the quantitative analysis of I in human plasma and urine.

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