Journal of Chromatography, 378 (1986) 467—472
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3111

Note

Quantitation of a new hypoglycaemic drug, 2-[2-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate (DG-5128), in plasma by gas chromatography—mass spectrometry

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(First received December 4th, 1985; revised manuscript received February 6th, 1986)

2-[2-(4,5-Dihydro-1H-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate, (I, DG-5128, Fig. 1) is a new oral hypoglycaemic drug [1] currently undergoing clinical trials. A sensitive and specific method for the analysis of I was needed to conduct clinical pharmacokinetic study.

This paper describes a quantitative method by gas chromatography—mass spectrometry (GC—MS) for the determination of I in plasma. This method was applied to determine I in plasma samples taken from healthy human subjects after oral administration of I.

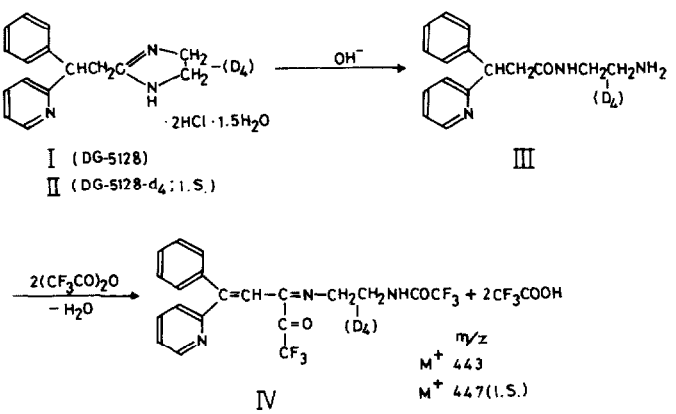


Fig. 1. Hydrolysis of I and II under alkaline conditions, and subsequent derivatization with TFAA.

0378-4347/86/$03.50

EXPERIMENTAL

Reagents

Compound I and ethyl 2-phenyl-2-(2-pyridyl)propionate were synthesized in our institute. Ethylenediamine-d_4 ($NH_2C^2H_2C^2H_2NH_2$) was obtained from Merck Sharp & Dohme (Montreal, Canada). Trifluoroacetic anhydride (TFAA), heptafluorobutyric anhydride (HFBA) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All other reagents were of analytical grade and were used without further purification.

Gas chromatography—mass spectrometry

A JEOL Model D-300 gas chromatograph—mass spectrometer (JEOL, Tokyo, Japan) was used. The system consisted of a JGC-20K gas chromatograph, a JMS D-300 mass spectrometer, and a JMA-2000 mass data analysis system. A glass column (1 m × 2 mm I.D.) packed with 2% OV-17 on 80—100 mesh Gas-Chrom Q was used. The electron energy was 70 eV. The injection port and the column temperatures were set at 280 and 250°C, respectively. The flow-rate of helium carrier gas was 90 ml/min. Quantitative analysis of samples was performed on the system described above by selected-ion monitoring at *m/z* 443 for I and *m/z* 447 for its deuterium-labelled compound (II, Fig. 1) employed as an internal standard (I.S.).

Extraction and derivatization

Internal standard dissolved in 2.0 ml of ethanol (II, 1 μg) was added to 1.0 ml of plasma in a test tube, which was capped and shaken for 10 min. After centrifugation at 1200 *g* for 10 min, the supernatant was alkalized with 2.0 ml of 0.1 *M* sodium hydroxide. Then the mixture was hydrolysed at 80°C for 1 h. The sample was extracted with 4.0 ml of chloroform by shaking for 10 min. After centrifugation, the lower organic fraction was transferred to a new test tube. The organic solution was evaporated in vacuo. The residue was dissolved in 50 μl of ethyl acetate–TFAA mixture (3:2). The solution was shaken with a Vortex mixer and allowed to stand for 5 min at room temperature. The sample was evaporated in a stream of nitrogen. The residue was dissolved in 50 μl of ethyl acetate, and 3—5 μl of this solution were injected into the gas chromatograph–mass spectrometer.

Synthesis of II

For an internal standard, II was prepared by a modification of the procedure described for the preparation of I [2]. A mixture of 1.4 g (5.5 mmol) of ethyl 3-phenyl-3-(2-pyridyl)propionate and 0.5 g (7.8 mmol) of ethylenediamine-d_4 in 15 ml of ethanol was refluxed for 3 h to afford crude II. The crude product was purified by preparative high-performance liquid chromatography (HPLC) (column, Nucleosil 10 CN; detection, 254 nm). Elution with methanol—0.2% ammonium biphosphate, pH 7.0 (1:1) gave pure II (0.14 g, 0.40 mmol) in a yield of 5.1%/mmol ethylenediamine-d_4.

RESULTS AND DISCUSSION

Derivatization

In preliminary studies, compound I showed a strong tailing peak on the gas chromatogram owing to its column-adsorbing effect. In addition, attempts to derivatize I with TFAA, HFBA or BSTFA for a gas chromatographic analysis were unsuccessful.

On the other hand, it was found that alkali-catalysed hydrolysis of I afforded III quantitatively, and that the derivatization of III with TFAA gave IV in good yield. The GC—MS analysis of derivative IV showed a sharp peak and high sensitivity. Therefore, these chemical degradation and derivatization processes were applied for the quantitative analysis of I.

The structure of the hydrolysis product (III) was determined by the following spectroscopic data. The mass spectrum of III showed M^+ at *m/z* 268, 18 mass units greater than that of I. The IR spectrum of III showed an absorption band at 1640 cm^{-1} assignable to the carbonyl group. In the NMR spectrum of III, triplet signals at 2.6 ppm (2H) and 3.2 ppm (2H) assignable to the ethylene protons were observed. These results suggested that the ring opening had occurred in the imidazoline moiety of I, and thus the structure of the hydrolysis product was assumed to be 2-phenyl-3-(2-pyridyl)propionylamino-ethylamine (III, Fig. 1). The structure of III was confirmed by direct comparison with an authentic sample.

In order to obtain the derivative of III, some derivatizing reagents, such as acetic anhydride, TFAA, HFBA and BSTFA, were tested. The TFAA derivative of III had the highest sensitivity in the GC—MS analysis, and so TFAA was chosen as the derivatizing reagent for the analysis of III.

Structure of derivative IV

The structure of derivative IV was confirmed by elemental analysis, UV, IR, NMR and mass spectra.

The mass spectrum and elemental analysis of IV showed that its molecular formula was $C_{20}H_{15}N_3O_2F_6$ and its molecular weight was 443.35. These results suggested that the derivative was formed from III by two reaction processes, i.e. bis-trifluoroacetylation and dehydration.

The introduction of one trifluoroacetyl group at the primary amine moiety was demonstrated by characteristic fragment peaks at *m/z* 140 ($CF_3CONHCH_2$; calculated 140.0323, found 140.0324) and *m/z* 303 ($M^+ - CF_3CONHCH_2CH_2$; calculated 303.0745, found 303.0763).

The UV spectrum of IV (λ_{max} 303 nm, ϵ 12 700) showed a bathochromic shift compared with that of III (λ_{max} 263 nm, ϵ 3650), suggesting the presence of conjugated double bonds in IV. In comparison with the NMR spectrum of III, that of IV showed the appearance of a singlet (1H) at 7.3 ppm and the disappearance of multiplets at 3.2 ppm (2H) and 4.7 ppm (1H) owing to the $CHCH_2CO$ moiety. These results indicated that another trifluoroacetyl group was introduced at the $CHCH_2CO$ moiety of III and that dehydration took place at the same moiety.

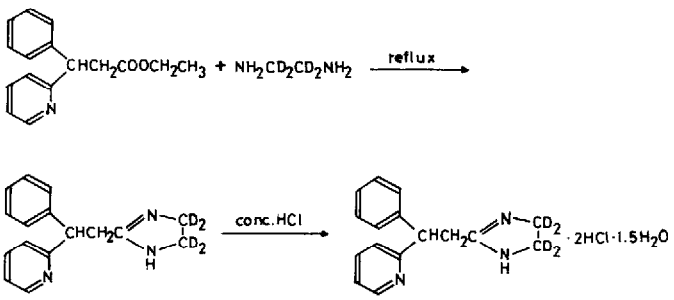
Accordingly, the structure of the derivative was determined to be IV, as shown in Fig. 1.

Recovery

A plasma specimen was deproteinized with ethanol. The ethanolic supernatant was alkalized with 0.1 *M* sodium hydroxide and then the sample was hydrolysed for 1 h at 80°C. The hydrolysed sample was extracted with chloroform. In these procedures, the recovery of the drug was examined using the ^{14}C-labelled compound of I. In the chloroform extract, 67% of the radioactivity added to the plasma was recovered.

Internal standard (II)

As shown in Fig. 2, the deuterium-labelled compound (II) was prepared as an internal standard from ethyl 3-phenyl-3-(2-pyridyl)propionate and ethylenediamine-d_4.



II

Fig. 2. Reaction scheme for the synthesis of II.

After II was hydrolysed under alkaline conditions, its hydrolysis product was derivatized with TFAA in a similar way as in the analytical procedure for I. The TFAA derivative of the deuterated compound showed M^+ at *m/z* 447, 4 mass units greater than that of the corresponding undeuterated compound. This finding revealed that four deuterium atoms of II were not removed from the molecule during hydrolysis in alkaline medium and subsequent derivatization with TFAA.

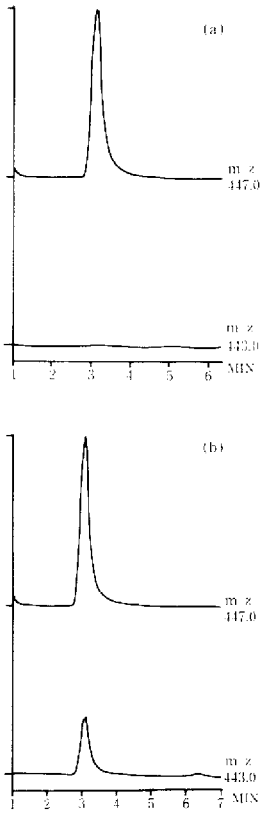
The isotopic purity of II was determined by comparison of the peak heights at *m/z* 443 (from d_0) and *m/z* 447 (from d_4) of the derivative. The ratio d_0/d_4 was found to be 0.007, indicating an isotopic purity of 99.3%.

Precision and limits of detection

Fig. 3 shows typical chromatograms obtained by monitoring molecular ion peaks at *m/z* 443 and *m/z* 447 (I.S.). No interference peak was observed. Calibration curves were obtained by plotting the peak-height ratio (I/I.S.) against concentration of I. The relationship was linear over the concentration range 25–2000 ng/ml (r = 0.997). The coefficients of variation at concentrations of 50 and 500 ng/ml were 3.04 and 0.585% (n = 5), respectively. The lower limit of detection was 20 ng/ml.

Selectivity

If the alkali hydrolysis product (III) and its conjugates exist as the metabolites of I, they would interfere with the quantitation of I, because the



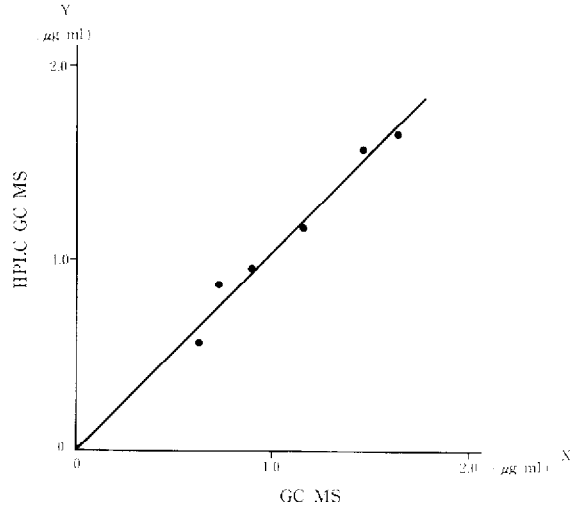


Fig. 3. Chromatograms of plasma samples. (a) Blank plasma sample; (b) plasma sample containing I (166 ng/ml).

Fig. 4. Correlation of plasma concentration of I assayed by the GC–MS method (X) and the HPLC–GC–MS method (Y). Plasma samples were obtained from healthy human subjects given I orally. Regression line is $Y = 1.028X + 0.027$, $r = 0.98549$.

analytical procedure of this GC–MS method involved the alkali hydrolysis process.

However, this possibility was excluded by the following evidence. First, when the hydrolysis step was omitted in the clean-up procedure, no peak corresponding to IV was observed in the GC–MS analysis of plasma sample from a human given I. Second, the fraction containing I in the plasma sample was selectively collected by preparative HPLC and then quantified by the GC–MS method. This modified GC–MS method (HPLC–GC–MS; Y), which was specific but had low sensitivity, was compared with the present GC–MS method (GC–MS; X). Fig. 4 shows a good agreement between the two methods in the analysis of the same plasma samples obtained from subjects given I ($Y = 1.028X + 0.027$, $r = 0.985$).

These results indicated that III and its conjugates did not exist as the metabolites in human plasma.

In rat and dog studies, several metabolites of I were known [3]. Authentic samples of these metabolites were synthesized [3] and found to have no influence on the quantitation of I.

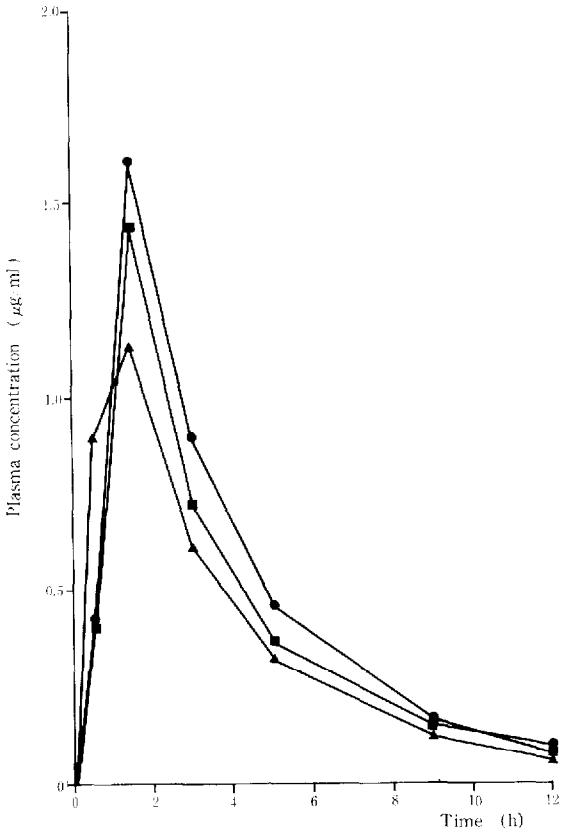


Fig. 5. Plasma concentration of I in healthy human subjects after oral administration of 250 mg of I: subject A (●); subject B (■); subject C (▲).

In conclusion, it was demonstrated that the metabolites did not disturb the determination of I and this analytical method had a high selectivity.

Application of the method

Fig. 5 shows the plasma concentration of the unchanged drug after oral administration of 250 mg of I to three healthy human subjects. In each subject, I was rapidly absorbed, with higher plasma levels being achieved at shorter intervals after dosing. The maximum plasma concentration was 1.61, 1.44 and 1.14 μg/ml for subjects A, B and C, respectively. Thereafter, the plasma concentration declined exponentially with a mean apparent half-life of 2.6 h.

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