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## Short Communication

# Determination of an irreversible inhibitor of monoamine oxidase B (MDL 72974A) in human plasma and urine by gas chromatography–positive-ion chemical ionization mass spectrometry

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ABSTRACT

A sensitive and specific assay has been developed for the quantitative measurement in human plasma and urine of the irreversible inhibitor of monoamine oxidase B [(*E*)-4-fluoro-β-fluoromethylenebenzenebutanamine HCl salt] (MDL 72974A) (I). This assay is based on gas chromatography–mass spectrometry with ammonia as the chemical ionization reagent gas. After addition of 1-fluoro-2-(4-chlorobenzene)-ethanamine HCl salt (MDL 71946A) as the internal standard, plasma (1 ml) and urine (100 μl) samples were extracted using an automated solid–liquid extraction procedure on CN columns. The eluent was dried with a stream of nitrogen, and the residue was derivatized with pentafluoropropionic anhydride. Selected-ion monitoring of the $[MNH_4]^+$ ions *m/z* 361 (I) and 351 (internal standard) was used for quantification. The method yielded a linear response over the concentration range 0.25–100 pmol/ml in plasma with a limit of quantitation of 0.25 pmol/ml. The within-day reproducibility at a concentration of 5 pmol/ml was 4.6% and at a concentration of 50 pmol/ml was 1.3%. The day-to-day reproducibility was 5.2 and 7.0% at concentrations of 10 and 30 pmol/ml, respectively. The method was applied to the quantification of I in plasma and urine after the administration of 12-mg doses of I to a healthy male volunteer.

## INTRODUCTION

Recent interest has focused on the possibility that inhibition of the B form of monoamine oxidase (MAO) may delay or prevent the progression of Parkinson's disease [1–5]. This suggestion is based on two hypotheses of the pathogenesis of the disease. The first holds that the destruction of dopaminergic neurons is initiated by a reactive oxygen species formed during the MAO-catalysed metabolism of dopamine [6]. The second is based on the possibility that the cause of Parkinson's disease is related to the formation of a toxic metabolite [7–9].

*In vivo* and *in vitro* studies in animals have shown that I [(*E*)-4-fluoro-β-fluoro-

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methylenebenzenebutanamine] is a potent and selective enzyme-activated, irreversible inhibitor of the B form of MAO. The *in vitro* $IC_{50}$ (concentration of inhibitor to produce a 50% inhibition of enzyme activity) values for the inhibition of rat brain mitochondrial MAO-A *versus* MAO-B activity show a selectivity ratio of 190 in favour of MAO-B inhibition for I, compared with only 17 for L-deprenyl. At MAO-B-selective doses *in vivo*, this drug was orally active, potent, and did not potentiate the cardiovascular effects of intraduodenally administered tyramine [10].

To obtain sufficient sensitivity and specificity, a capillary gas chromatographic–mass spectrometric (GC–MS) method was developed for the quantification of I in samples of human plasma and urine.

The developed method is based on positive-ion chemical ionization (PICI) GC–MS with ammonia as reagent gas, to quantify with precision and accuracy the low plasma concentrations of I. The assay is performed with 1 ml of plasma or 100 μl of urine. The biological extracts were treated with pentafluoropropionic anhydride (PFPA), and the N-PFP derivatives gave intense and characteristic ions at *m/z* 361 (I) and *m/z* 351 (internal standard, I.S.) which were recorded by selected-ion monitoring (SIM). The applicability of the method was demonstrated by analysing I in the plasma and urine of a healthy volunteer given I orally.

## EXPERIMENTAL

### *Reagents*

(*E*)-4-Fluoro-β-fluoromethylenebenzenebutanamine HCl salt (MDL 72974A) (I) and 1-fluoro-2-(4-chlorobenzene)ethanamine HCl salt (MDL 71946A) (I.S.) were supplied by Merion Merrell Dow (Strasbourg, France).

Methanol and dichloromethane were reagent grade (Merck, Darmstadt, Germany). Acetonitrile was purchased from S.D.S. (Peypin, France). PFPA was purchased from Pierce (Oudbeijerland, Netherlands). Disposable CN extraction columns (3 ml) and *n*-hexane came from Baker (Deventer, Netherlands).

### *Standard curves*

Stock solution of I and the I.S. were prepared by dissolving each pure reference compound in methanol at a concentration of 1 μmol/ml and stored at −20°C until used. Working solutions obtained from appropriate dilutions in methanol were prepared daily. Aliquots of 1 ml of drug-free human plasma were spiked with 50 pmol of the I.S. and various amounts of I over the concentration range 0.25–100 pmol/ml. For urine samples (100 μl) the amount of I.S. added was the same and the standard curve was prepared in the same range as for plasma samples (0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 25, 50, 75 and 100 pmol). The blank plasma or urine samples were prepared in a similar way by spiking control plasma or urine with the I.S. only.

*Sample work-up*

Sample preparation was developed using an ASPEC (automatic sample preparation with extraction column) system (Gilson Medical Electronics, Villier-le-Bel, France). Plasma samples (1 ml), spiked with 50 pmol of the I.S., were placed in a 4-ml vial. Disposable extraction columns (CN, 3 ml) were conditioned with 3 ml of methanol and washed with 2 ml of distilled water. The plasma was added to the top of the solid phase, and pushed through the column with 1 ml of air, then washed with 2 ml of distilled water and 2 ml of methanol–water (60:40, v/v). The column was dried with air (10 ml), and the compounds were eluted with 1.5 ml of acetonitrile containing 10% 1 *M* HCl. The column was dried with 10 ml of air, and the sample was evaporated under a stream of nitrogen at room temperature.

*Derivatization*

To the extracted sample were added 100 μl of dichloromethane and 50 μl of PFPA, and the mixture was allowed to react for 30 min at room temperature. This was followed by evaporation of the solvent; then the sample was reconstituted in 50 μl of *n*-hexane. A 1-μl aliquot was injected into the GC column for GC–MS analysis.

*GC–MS analysis*

MS analyses were carried out with a Carlo Erba QMD 1000 GC–MS system or a Finnigan TSQ46 GC–MS system coupled on-line to an Incos data system Nova 4X. The chromatographic column was a 15 m × 0.32 mm I.D. 5% phenylmethylsilicone fused-silica capillary column (Hewlett Packard, Les Ulis, France). The film thickness of the column was 0.52 μm. The end of the column was directly introduced into the ion source via a stainless-steel transfer line kept at 270°C. Samples were injected into a splitless injector with helium used as carrier gas (inlet pressure of 60 p.s.i.). The temperature of the injector was maintained at 270°C. The oven temperature was raised from 60 to 120°C at 30°C/min and from 120 to 200°C at 6°C/min, and the final temperature was held for 1 min.

The GC–MS system was operated in the PICI mode with an electron energy of 70 eV, an emission current of 250 μA and a source temperature of 160°C for the QMD 1000 and 100°C for the TSQ46. The PICI mass spectra of the N-PFP derivatives of MDL 72974 and the I.S. were recorded during a GC run by scanning from 90 to 500 in 0.5 s. Quantification of I was performed by focusing the mass spectrometer in the ion monitoring selected in order to measure *m/z* 361 and *m/z* 351, the $[MNH_4]^+$ ions of the compounds.

*Precision and accuracy*

To assess the precision and the accuracy of the method, reproducibility assays were carried out at different concentrations (5, 10 and 50 pmol/ml). The spiked plasma samples were analysed the same day or the day after. For each concentration level, the coefficient of variation (C.V.) was calculated.

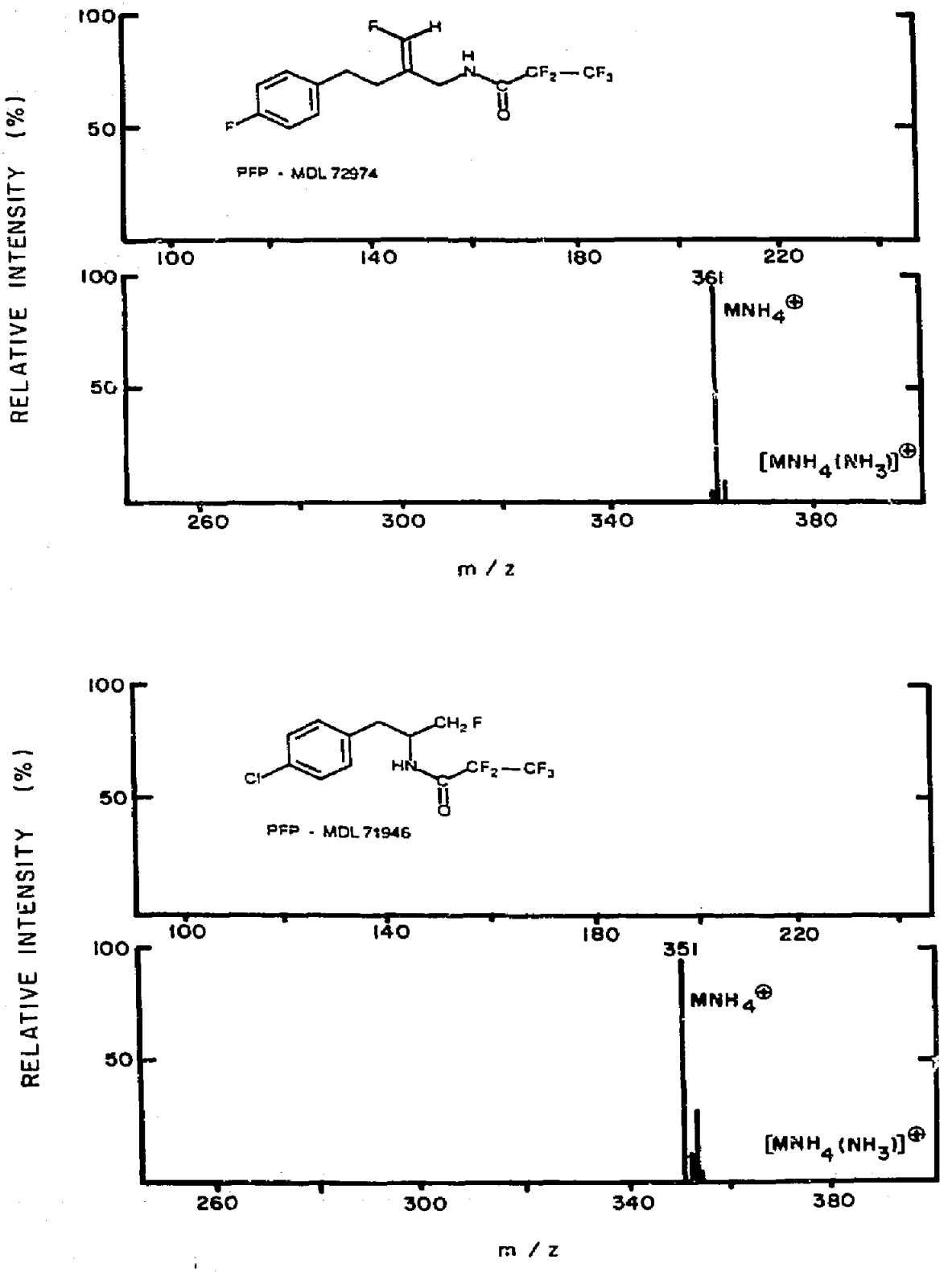


Fig. 1. Ammonia PICI mass spectra of the N-pentafluoroacetyl derivatives of I and the I.S.

*Drug administration*

A healthy volunteer was administered single daily oral doses of 12 mg of I over a ten-day period. Blood samples (10 ml) were collected into Sarstedt-Monovette tubes containing potasssium EDTA at 0, 15, 30 and 45 min and 1, 2, 3, 4, 6, 8 and 24 h following drug administration on the final day of treatment. A 24-h urine sample was collected to determine the extent of urinary excretion. The plasma concentrations were measured to establish the plasma concentration–time course.

## RESULTS AND DISCUSSION

*Derivatization procedure*

The PICI mass spectra of the N-PFP derivatives of I and the I.S. obtained with ammonia as reagent gas showed characteristic ions (Fig. 1). These compounds gave only a peak at $m/z$ 361 and $m/z$ 351 corresponding to the $[MNH_4]^+$ ions of I and the I.S., respectively.

*Standard curve*

The calibration curve obtained by plotting the peak-area ratio $m/z$ 361/351 *versus* the known plasma concentration of I produced a straight line over the concentration range 0.25–100 pmol/ml. Linear regression analysis gave the equation $y = 0.0092x - 0.002$ with a correlation coefficient ($r$) of 0.9996.

*Precision and accuracy*

The within-day precision was checked by analysing five plasma samples at three different concentrations of I (5, 10 and 50 pmol/ml). The C.V. were less than 4.6% at each of the concentrations (Table I).

The day-to-day precision was checked by measuring two concentrations (10.15 and 30.45 pmol/ml) between days 1 and 15 using the calibration curve obtained on day 1. The results obtained with this procedure are given in Table II.

A chromatogram obtained after GC–MS analysis of a blank plasma sample is shown in Fig. 2, and those obtained from a control spiked with 5 and 50 pmol/ml are shown in Fig. 3. There was no interference from endogenous compounds in

TABLE I

WITHIN-DAY PRECISION AND ACCURACY FOR I IN SPIKED PLASMA SAMPLES

| Concentration added (pmol/ml) | Concentration found (mean ± S.D., $n$ = 3) (pmol/ml) | C.V. (%) |
|---|---|---|
| 5 | 4.99 ± 0.23 | 4.6 |
| 10 | 10.09 ± 0.09 | 0.9 |
| 50 | 49.06 ± 0.66 | 1.3 |

TABLE II

DAY-TO-DAY PRECISION AND ACCURACY FOR I IN SPIKED PLASMA SAMPLES WITH A CALIBRATION CURVE OBTAINED ON DAY 1

| Day of analysis | Amount found (pmol/ml) | |
|---|---|---|
| | 10.15 pmol/ml added | 30.45 pmol/ml added |
| 1 | 11.22 | 34.11 |
| 2 | 10.55 | 32.28 |
| 5 | 10.77 | 29.24 |
| 9 | 10.05 | 29.69 |
| 11 | 9.79 | 34.50 |
| 12 | 10.30 | 29.84 |
| 15 | 9.75 | 30.10 |
| Mean | 10.35 | 31.39 |
| S.D. | 0.54 | 2.22 |
| C.V. (%) | 5.20 | 7.04 |

the SIM trace of the ions, enabling the quantitative measurement of I for pharmacokinetics. The limit of quantitation for I was 250 fmol/ml in plasma at a signal-to-noise ratio of 4. A chromatogram of a 24-h urine sample is shown in Fig. 4.

*Application*

The application of the GC–MS method was shown by quantification of I in plasma and urine samples of a healthy male volunteer who had recived daily single oral doses of I over a ten-day period.

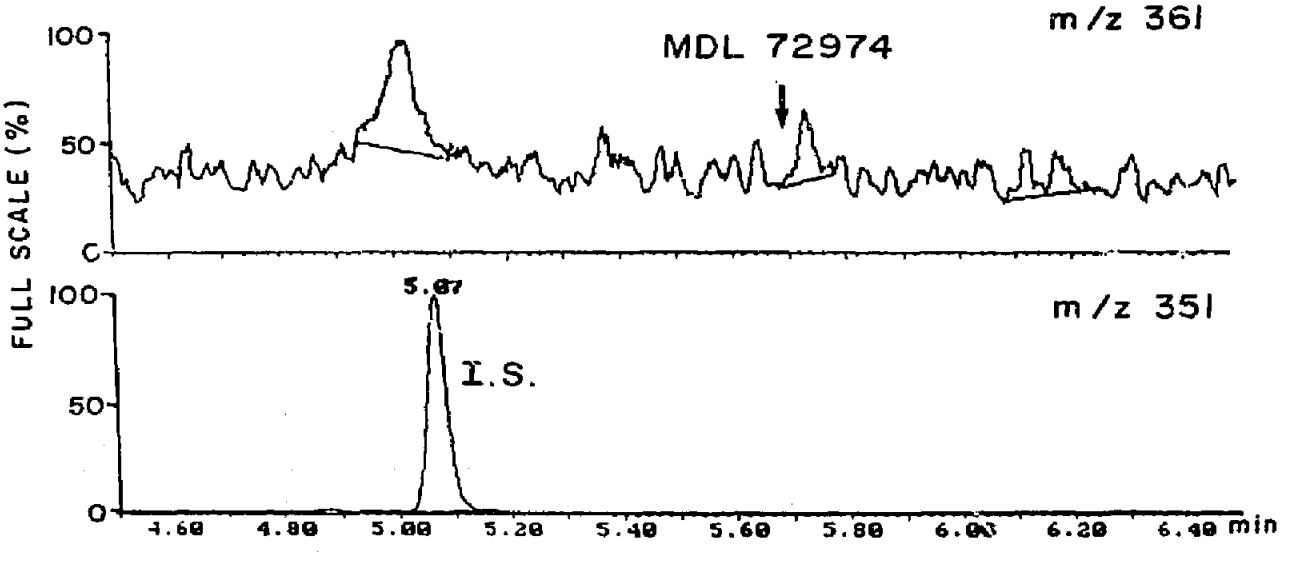


Fig. 2. Typical mass chromatogram obtained from a control human plasma sample spiked with 50 pmol/ml I.S.

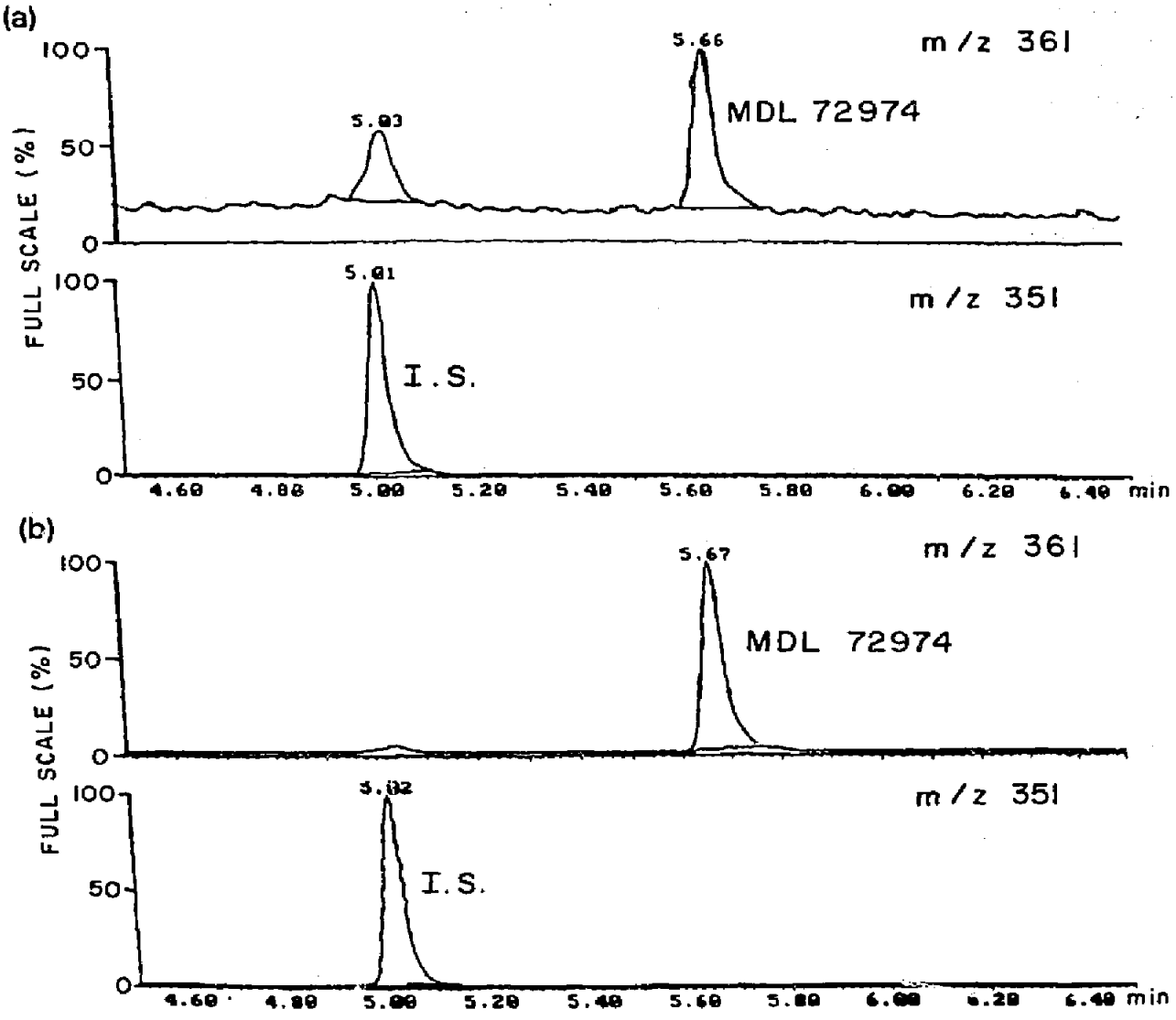


Fig. 3. Selected-ion monitoring traces obtained from a control plasma sample spiked with (a) 5 pmol/ml I and 50 pmol/ml I.S. and (b) 50 pmol/ml I and 50 pmol/ml I.S.

The plasma concentration–time course obtained on day 10 for the human volunteer is shown in Fig. 5. The observed $C_{max}$ value was 43.1 pmol/ml, which was attained at 1 h ($t_{max}$). The concentration of I in the urine was 176.5 pmol/ml, corresponding to a 24-h urinary excretion of 0.37% of the dose.

## CONCLUSION

The present GC–MS assay represents a specific and sensitive analytical method for the measurement of I at the picomole level in plasma and urine samples of human origin. The N-PFP derivative of the compound is stable and yields high

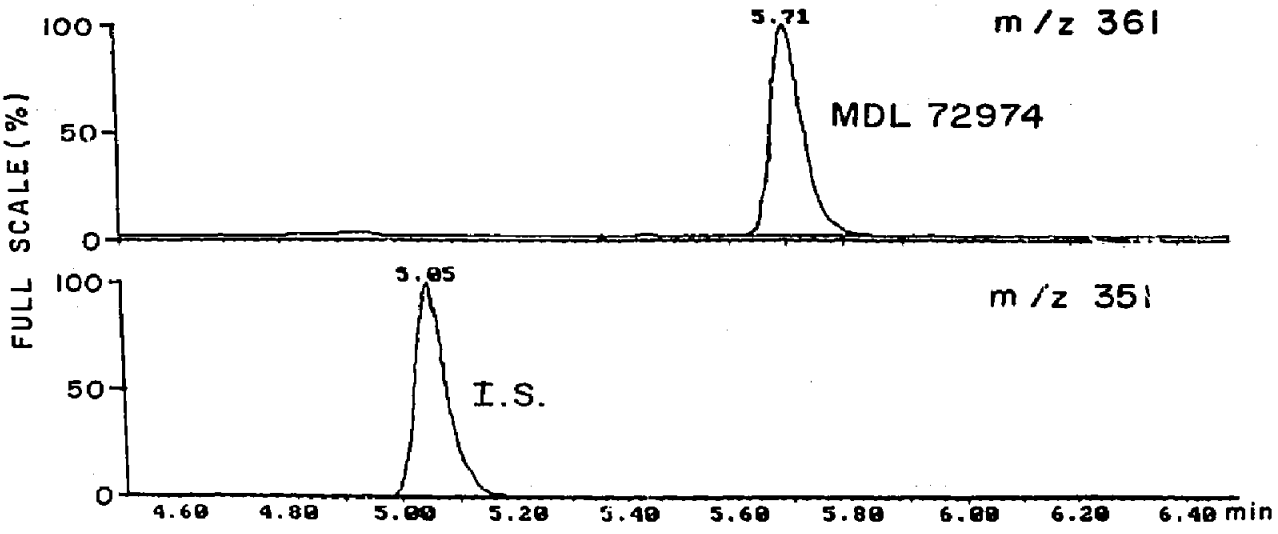


Fig. 4. Selected-ion monitoring traces obtained from a urinary extract (50 $\mu$l) obtained on day 10 with 50 pmol/ml I.S. added.

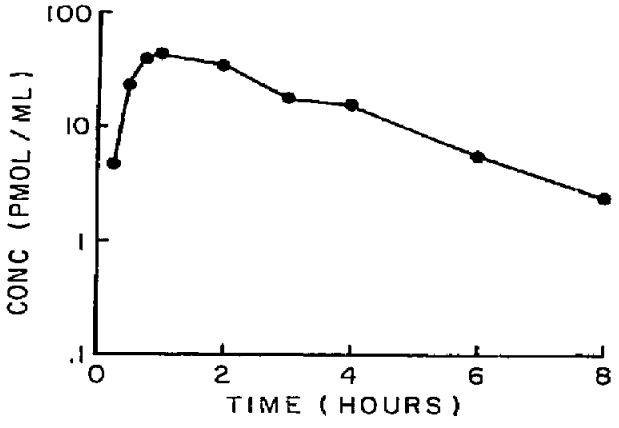


Fig. 5. Plasma concentration–time course of I after the tenth dose of 12 mg per day of I administered orally to a healthy volunteer.

sensitivity with no endogenous interferences. The reproducibility demonstrates that precision and accuracy of this technique are suitable for routine GC–MS analysis of I. The method has been successfully applied to the quantification of I in human plasma and urine.

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