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# Study of human urinary metabolism of fenfluramine using gas chromatography–mass spectrometry

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## ABSTRACT

The metabolism of (±)fenfluramine, 1-(*m*-trifluoromethylphenyl)-2-N-ethylpropane, an anoretic agent, was investigated in humans. The analysis method was based on the use of ion-exchange resin extraction, solid-phase purification on the Bond Elut™ $C_8$ cartridge, gradient elution high-performance liquid chromatography, enzymic hydrolysis of conjugates, further purification by Bond Elut $C_8$ cartridge, derivatisation and capillary column gas chromatography–mass spectrometry (GC MS). After administration of a 1 mg $kg^{-1}$ oral dose, four metabolites plus unchanged fenfluramine were recovered in the 0–24 h urine from human volunteers and characterised by GC–MS. In the unconjugated form, fenfluramine, norfenfluramine and *m*-trifluoromethylhippuric acid were detected by GC–MS. In the aglycone form, the major metabolite, 1-(*m*-trifluoromethylphenyl)-1,2-propane diol (fenfluramine diol), was monitored using GC–MS. The mass spectral characteristics of the *m*-trifluoromethylhippuric acid methyl ester, 1-(*m*-trifluoromethylphenyl)-1,2-propane ditrifluoroacetate derivatives and the norfenfluramine and fenfluramine free base obtained under electron-impact ionization are presented. The metabolism of fenfluramine is discussed including a metabolic pathway in man accounting for the formation of its biotransformation products.

## INTRODUCTION

1-(*m*-Trifluoromethylphenyl)-2-N-ethylpropane (fenfluramine) has been shown to induce the serotonergic system through combined actions of the drug to induce release and block the re-uptake of serotonin from nerve terminals resulting in anorectic activity. (±)-Fenfluramine has shown appetite suppressant activity without stimulant and addictive properties associated with other anorectic agents. More recently the dextro enantiomer has been developed which when used at lower doses improves patient acceptability. Although (±)fenfluramine metabolism has been studied extensively, using various species [1] and *in vitro* [2] techniques, the main objective of this work was to perform a definitive study in man, using [$^{14}$C](±)-fenfluramine where metabolite quantitation and characterisation included the specificity of gas chromatography–mass spectrometry (GC–MS).

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## EXPERIMENTAL

### *Chemicals and reagents*

(±)-Fenfluramine hydrochloride and various metabolite reference standards were supplied by Technologie Servier (Orleans, France). [$^{14}$C](±)-Fenfluramine, labelled in the β-carbon of the molecule, was supplied by Commissariat à l'Energie Atomique (Gif-sur-Yvette, France) with a specific activity of 14 mCi mmol$^{-1}$ and a radiochemical purity of >97%. Trifluoroacetic anhydride and N-methyl-N'-nitro-N-nitrosoguanidine (used to generate diazomethane) were obtained from Aldrich (Poole, U.K.). Bond Elut cartridges were obtained from Jones Chromatography (Mid-Glamorgan, U.K.). β-Glucuronidase, type VII, from *Escherichia coli* (EC 3.2.1.31) was supplied by Sigma (Poole, U.K.). All other chemicals and solvents were of Analar grade and supplied by BDH (Poole, U.K.).

### *Urine samples*

Fenfluramine metabolites were isolated from pooled 0–24 h urine samples collected from four human volunteers (three male and one female) after oral administration of [$^{14}$C](±)-fenfluramine hydrochloride (1 mg kg$^{-1}$, 30 μCi) to two male subjects and (±)-fenfluramine hydrochloride to the remaining two subjects (1 mg kg$^{-1}$).

### *Thin-layer chromatography (TLC)*

TLC analysis was performed on silica gel plates (Merck 60 $F_{254}$, 0.25 mm thickness) developed in a dichloromethane–methanol–ammonia sp.g. 0.88 (70:30:1, v/v) solvent system. The position of the reference compounds was visualised using ultraviolet (UV) light, and the position of the radioactive drug plus metabolites was located using a TLC linear analyser (Berthold Model LB2842) or by autoradiography on X-ray film (Agfa Gavert, Osray M3).

TABLE I

GRADIENT HPLC CONDITIONS

Spherisorb ODS1 semipreparitive column (25 cm × 5 mm I.D.): flow-rate, 2 ml min$^{-1}$. A = Methanol; B = methanol–0.05 *M* orthophosphoric acid (5:95); C = water–methanol (95:5); D = methanol–water (70:30).

| Time (min) | % A | % B | % C | % D |
|---|---|---|---|---|
| 0 | 30 | 70 | 0 | 0 |
| 20 | 0 | 0 | 0 | 100 |
| 25 | 70 | 30 | 0 | 0 |
| 70 | 70 | 30 | 0 | 0 |

*High-performance liquid chromatography (HPLC)*

The reversed-phase HPLC system used for metabolite isolation consisted of a Waters WISP 712 injector, Waters 600 multi-solvent delivery system and, for detection, a Perkin Elmer LC-90 spectrophotometric detector linked in series to a Ramona 5 (Raytest, Sheffield, U.K.) radiochromatographic detector.

The semi-preparative gradient analysis was performed using a Spherisorb ODS1 column (25 cm × 5 mm I.D.) with a flow-rate of 2 ml $min^{-1}$; details of the gradient solvent system are shown in Table I.

*Metabolite extraction*

Fenfluramine and its metabolites were isolated using the scheme outlined in Fig. 1. The gradient HPLC conditions used were those stated previously in Table I. Metabolite fraction 2 was methylated and fraction 3 was trifluoroacetylated using the derivatisation conditions described in Table II.

*Gas chromatography–mass spectrometry*

GC–MS analysis was carried out employing the conditions stated in Table III.

## RESULTS AND DISCUSSION

The recovery of radioactivity in human urine was greater than 90% after oral administration of [$^{14}$C](±)-fenfluramine hydrochloride (1 mg $kg^{-1}$), indicating that the drug was well absorbed with fenfluramine and its biotransformation products being predominantly renally excreted. A mean recovery of 45% of the dose was excreted in the 0–24 h urine sample and was representative of the total excreted.

The HPLC profile of the methanol eluate from the XAD-2 resin extraction is shown in Fig. 2. Metabolite fraction 2 was found to co-elute with the *m*-trifluoromethylhippuric acid standard. Metabolite fraction 3 was reduced in intensity after enzyme hydrolysis, using a glucuronide-specific enzyme, with a corresponding increase in the fenfluramine diol standard peak. Metabolite fraction 4 showed an increase in the intensity of fenfluramine after enzyme hydrolysis indicating the possible presence of a fenfluramine conjugate.

The capillary column GC–MS electron-impact spectra for diazomethylated metabolite fraction 2 and the *m*-trifluoromethylhippuric acid methyl ester standard, with an indication of its fragmentation pattern, are shown in Fig. 3. The molecular ion $M^+$ (*m/z* 261) and the major characteristic fragment ions are present in both standard and metabolite fraction 2. The mass spectra for the trifluoroacetylated aglycone fraction of metabolite fraction 3 and the fenfluramine diol ditrifluoroacetate standard, with its fragmentation scheme, are shown in Fig. 4. The $[M-F]^+$ (*m/z* 393) and major fragment ions are evident in the standard and hydrolysed metabolite fraction 3. The spectra for the metabolite fractions 4 and 5, plus the norfenfluramine and fenfluramine free base standards with their frag-

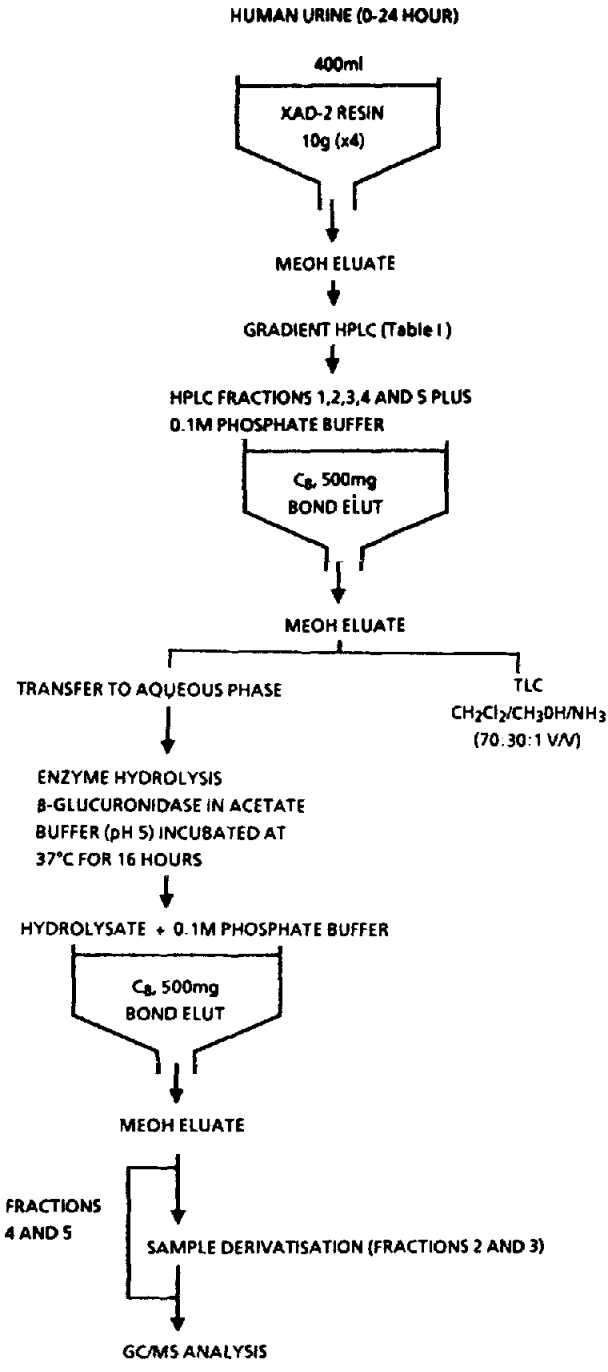


Fig. 1. Metabolite isolation.

TABLE II

SAMPLE DERIVATISATION

| Derivatisation | Conditions |
|---|---|
| Methylation | Ethereal diazomethane (1 ml) for 1 h at room temperature |
| Trifluoroacetylation | Dry ethyl acetate (50 μl) plus trifluoroacetic anhydride (500 μl) heated for 1 h at 55°C. The derivative was washed with ammonia solution (0.1 *M*, 1ml) and extracted into organic solvent (dichloromethane–diethyl ether, 11:14, v/v) |

TABLE III

GAS CHROMATOGRAPHY–MASS SPECTROMETRY

Analyses by GC–MS were performed on a DELSI Di700 gas chromatograph coupled to a NERMAG R10-10C mass spectrometer controlled by a Sidar Series 2026 data system. All data were archived on to a TK-50 magnetic tape streamer device.

| Method | Conditions |
|---|---|
| Gas chromatography | DB-1 (dimethyl polysiloxane) fused-silica capillary column (30 cm × 0.252 mm I.D.; 25 μm film thickness) coupled directly to the mass spectrometer ion source. Column temperature of 80°C for 1 min, 80 to 150°C at 40°C/min, then up to 300°C at 30°C/min. |
| Mass spectrometry | Helium carrier gas: 0.7 bar and a linear velocity of 0.7 m/s<br>Injection solvent: Methanol or dichloromethane–diethyl ether<br>Injection method: Adapted OCI-3 on-column injector<br>Ionisation mode: Electron impact (EI) or ammonia chemical ionisation (CI)<br>Source temperature: 100°C<br>Electron energy: 70 or 90 eV<br>Indicated source pressure: 13.3 Pa (for CI)<br>Scanned mass range: 40–750 (EI) or 60–750 (CI) |

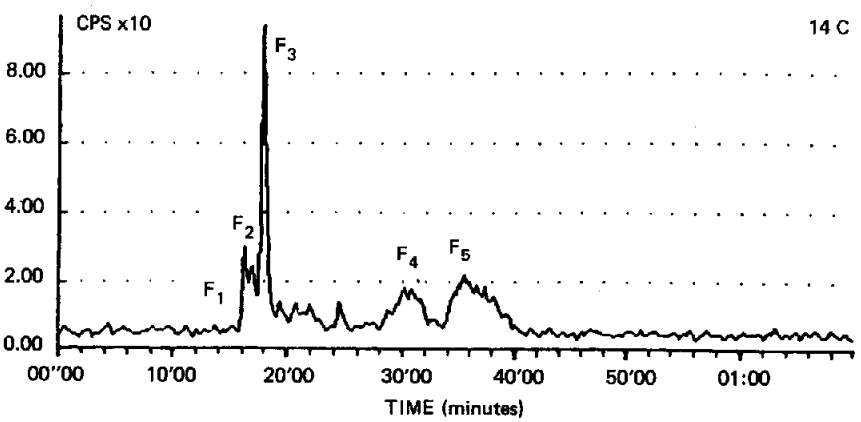


Fig. 2. HPLC profile of 0–24 h urine extract. $F_1$–$F_5$ = metabolite fractions 1–5.

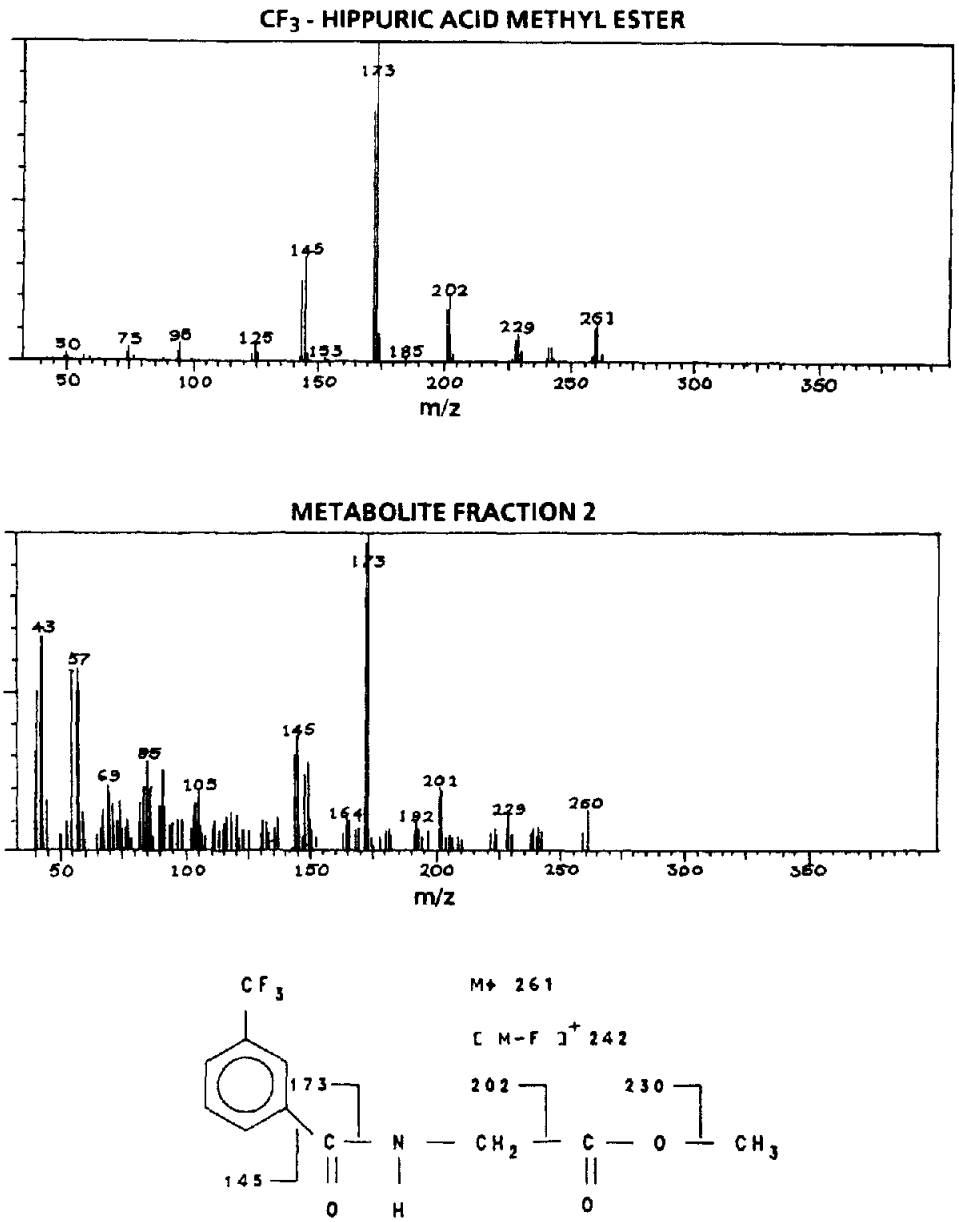


Fig. 3. Electron-impact mass spectra of methylated metabolite fraction 2 and *m*-trifluoromethylhippuric acid methyl ester standard.

mentation patterns, are shown in Figs. 5 and 6, respectively. The molecular ions [$M^+$ (*m/z* 203) and $M^+$ (*m/z* 231), respectively] and their characteristic fragment ions are present in the standards and metabolite fractions 4 and 5.

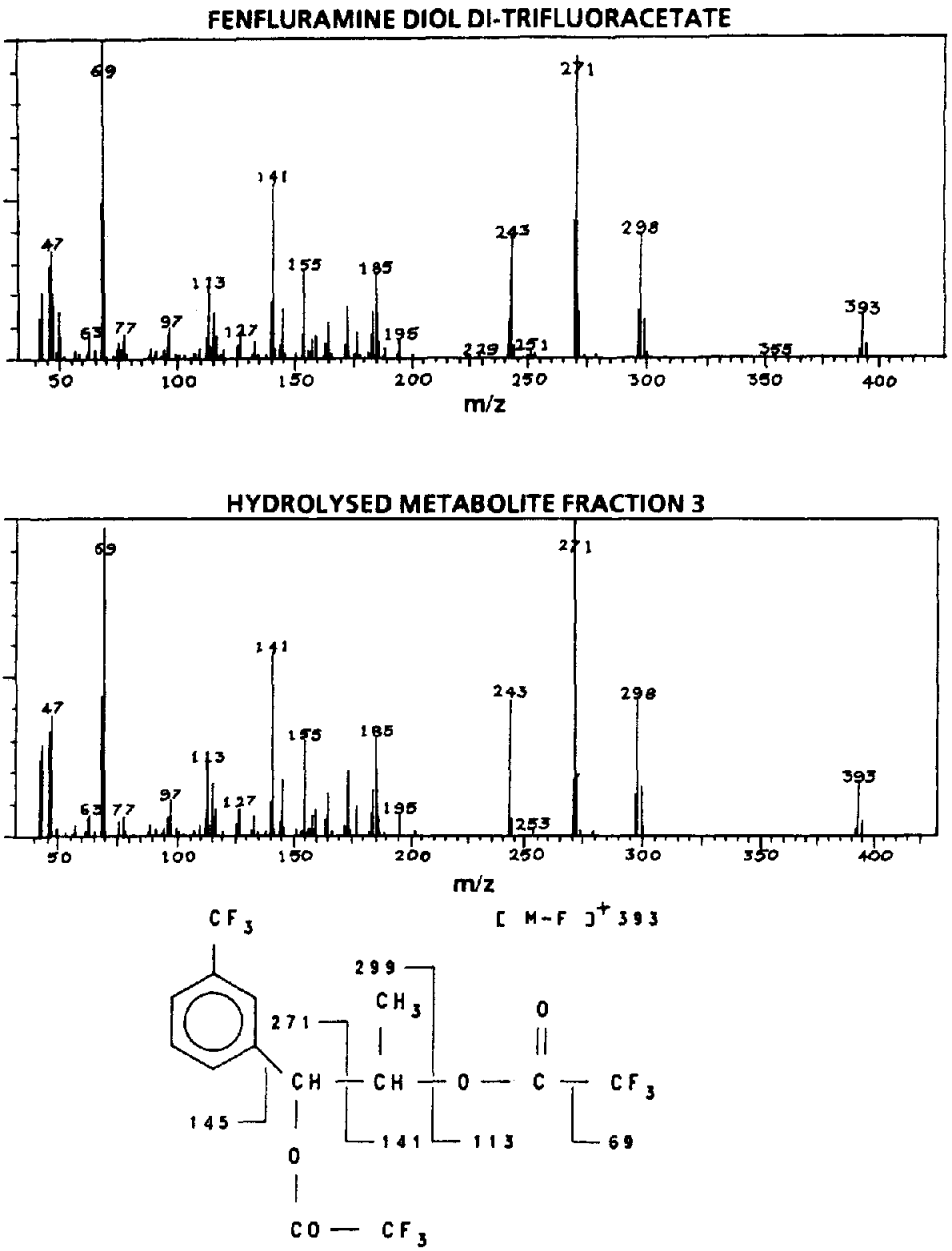


Fig. 4. Electron-impact mass spectra for trifluoroacetylated aglycone of metabolite fraction 3 and fenfluramine diol di-trifluoroacetate standard.

Metabolite fractions 2, 4 and 5 were also subjected to ammonia chemical ionisation GC–MS analysis to confirm the metabolite molecular weight. Fractions 2, 3, 4 and 5 were subsequently co-chromatographed with the relevant

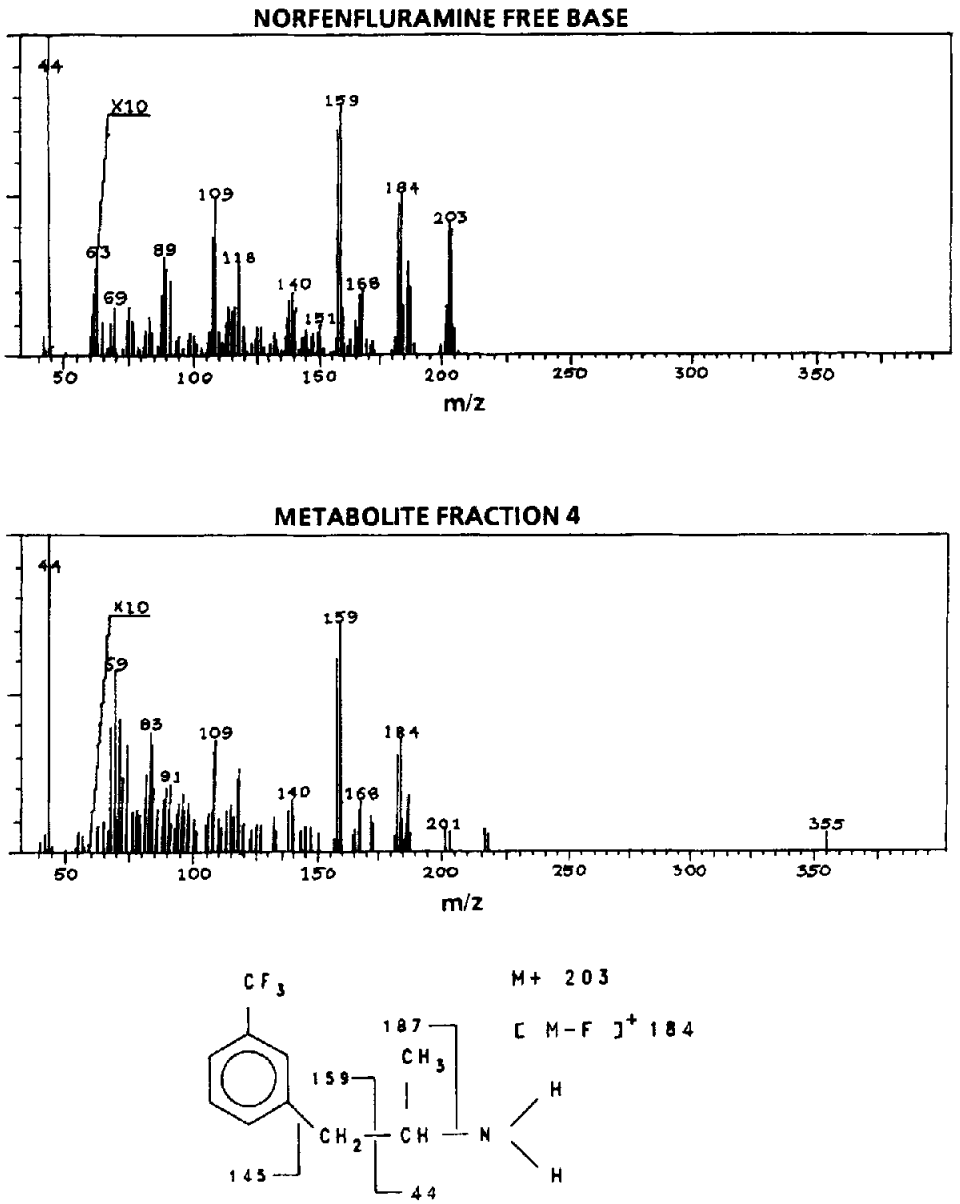


Fig. 5. Electron-impact mass spectra for metabolite fraction 4 and norfenfluramine free base standard.

standard using GC–MS to confirm that the metabolite present and reference compound were identical.

Work is still in progress to identify metabolite fraction 1. The MS (molecular

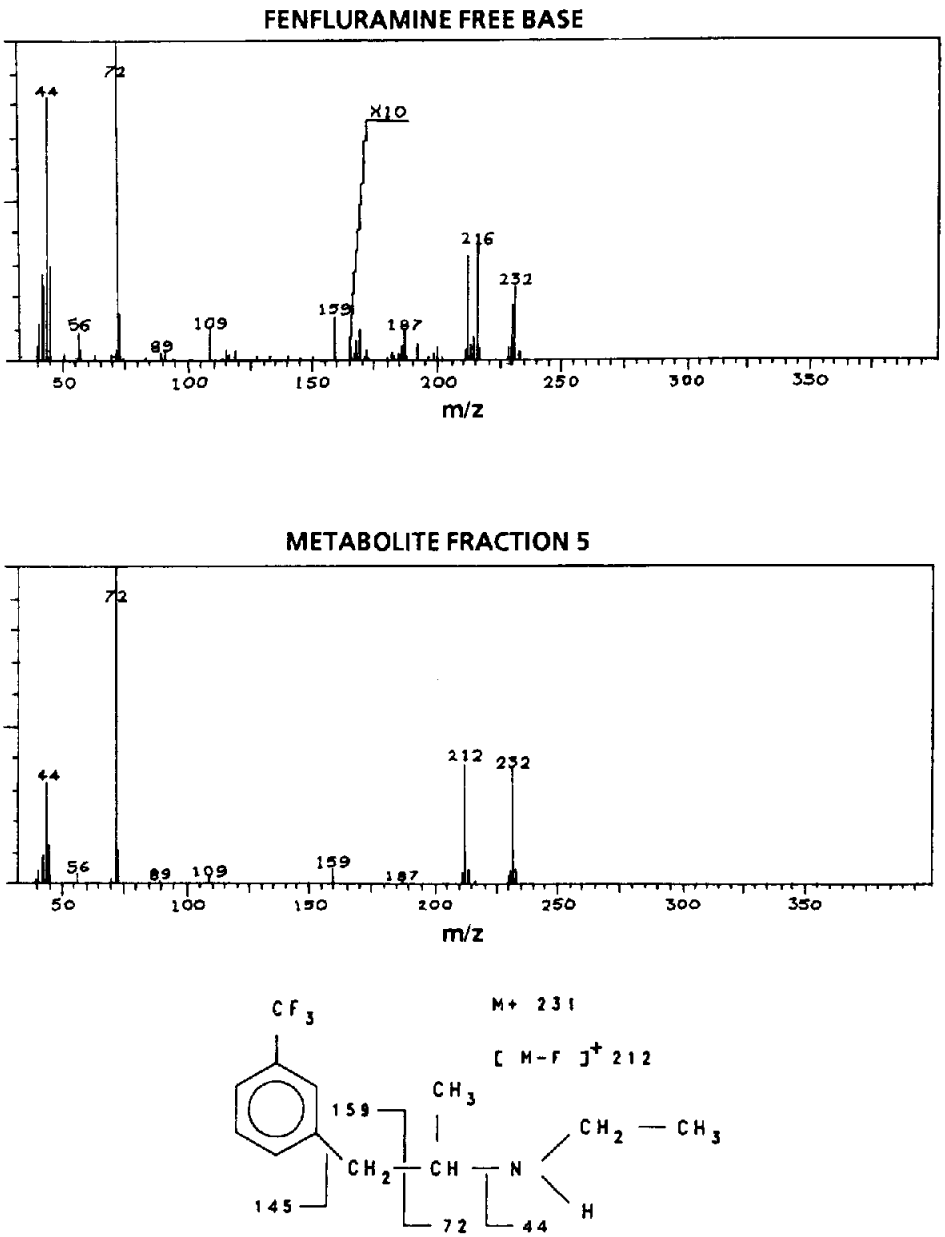


Fig. 6. Electron-impact mass spectra for metabolite fraction 5 and fenfluramine free base standard.

weight and fragmentation pattern) of the capillary column GC–MS retention time when co-chromatography of the metabolite fraction plus the standard were analysed and the comparable data from TLC and HPLC methods all indicated

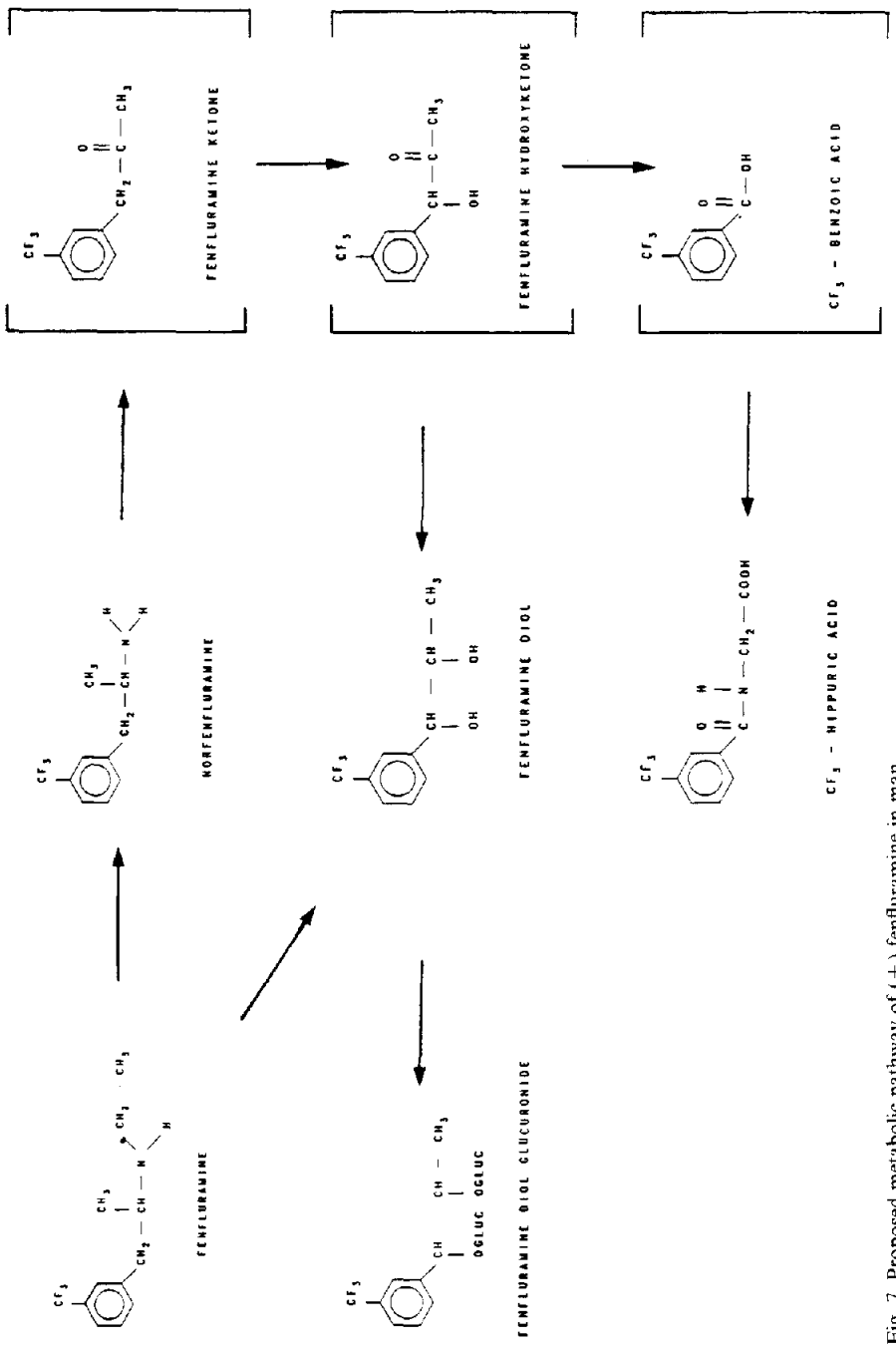


Fig. 7. Proposed metabolic pathway of (±)-fenfluramine in man.

that the metabolite characterisation and identification was specific for the components present.

From the metabolites of fenfluramine identified a metabolic pathway has been proposed (Fig. 7) in man. The fenfluramine was N-de-ethylated to norfenfluramine which possibly undergoes oxidative deamination to the fenfluramine ketone and C-oxidation to the fenfluramine hydroxyketone and fenfluramine diol. The fenfluramine hydroxyketone was further biotransformed to the trifluoromethylbenzoic acid, with phase II conjugation occurring for both components, the trifluoromethylbenzoic acid to trifluoromethylhippuric acid and the fenfluramine diol to fenfluramine diol glucuronide (the major metabolite, see Fig. 2). These results confirm those found previously by another worker in our laboratory [3]; however, at that time not all metabolites were identified using MS.

## REFERENCES

1 N. C. Marchant, S. Bass, M. A. Breen, F. A. Tucker, R. P. Richards and B. Campbell, in *Progress in Pharmacology and Clinical Pharmacology, Proceedings of the 4th International Symposium on the Biological Oxidation of Nitrogen in Organic Molecules, Munich, Sept. 17–21, 1989*, in press.
2 K. K. Midha, E. M. Howes and J. K. Cooper, *Xenobiotica*, 13 (1983) 31.
3 R. P. Richards, *Ph. D. Thesis*, University of Surrey, Guildford, 1985.