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## Measurement of dexfenfluramine metabolism in rat liver microsomes by gas chromatography–mass spectrometry

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### Abstract

A specific and useful method was developed for the determination of dexfenfluramine metabolism by microsomal systems utilising GC–MS. The synthesis of two metabolites 1-(3-trifluoromethylphenyl)propan-2-ol ('alcohol') and 1-(3-trifluoromethylphenyl)-1,2-propanediol ('diol') via straightforward routes, were confirmed by MS and NMR spectra. The conditions for extraction from alkalinised microsomal mixtures of the metabolites nordexfenfluramine, 1-(3-trifluoromethylphenyl)propan-2-one ('ketone'), alcohol and diol, their conversion to trifluoroacetate derivatives and analysis by GC–MS–SIM are described. Calibration curves were constructed between 48 and 9662 nM and fitted to quadratic equations ( $r^2 > 0.999$ ). The method precision was good over low (121 nM) medium (2415 nM) and above medium (9662 nM) concentrations for all metabolites; the within- and day-to-day coefficients of variation ranged between 2.5–12.4% and 6.7–17.5%, respectively. The accuracy, measured as bias, was very good both within- and day-to-day (range: –0.4–12.6%, 0.8–18.9%). For most metabolites, the C.V. for the assay and bias increased at 121 nM. Dexfenfluramine metabolism by rat liver microsomes was investigated using the assay method and showed a concentration dependent increase in nordexfenfluramine and ketone metabolites over the substrate range of 5–200  $\mu$ M.

**Keywords:** Dexfenfluramine

### 1. Introduction

Dexfenfluramine, the (+)-enantiomer of fenfluramine, is a widely prescribed drug for appetite suppression in the treatment of obesity. Little is known about the metabolic disposition of dexfenfluramine as most information has arisen from work with ( $\pm$ )-fenfluramine and a few *in vivo* studies of dexfenfluramine in rats [1], primates [2] and humans [3]. The proposed pathways of metabolism, based on

plasma and urinary metabolites, are shown in Fig. 1. ( $\pm$ )-Fenfluramine may be deethylated to form norfenfluramine or undergo deamination to form the 1-(3-trifluoromethylphenyl)propan-2-one ('ketone') intermediate which gives rise to 1-(3-trifluoromethylphenyl)propan-2-ol ('alcohol'), 1-(3-trifluoromethylphenyl)-1,2-propanediol ('diol'), 1-(3-trifluoromethyl) benzoic acid and hippurate derivatives [4–7]. Serotonergic activity resides with fenfluramine and norfenfluramine only. Almost nothing is known about the *in vitro* metabolism of fenfluramine except for an early study using guinea pig liver fractions which reported a nitron derivative, an

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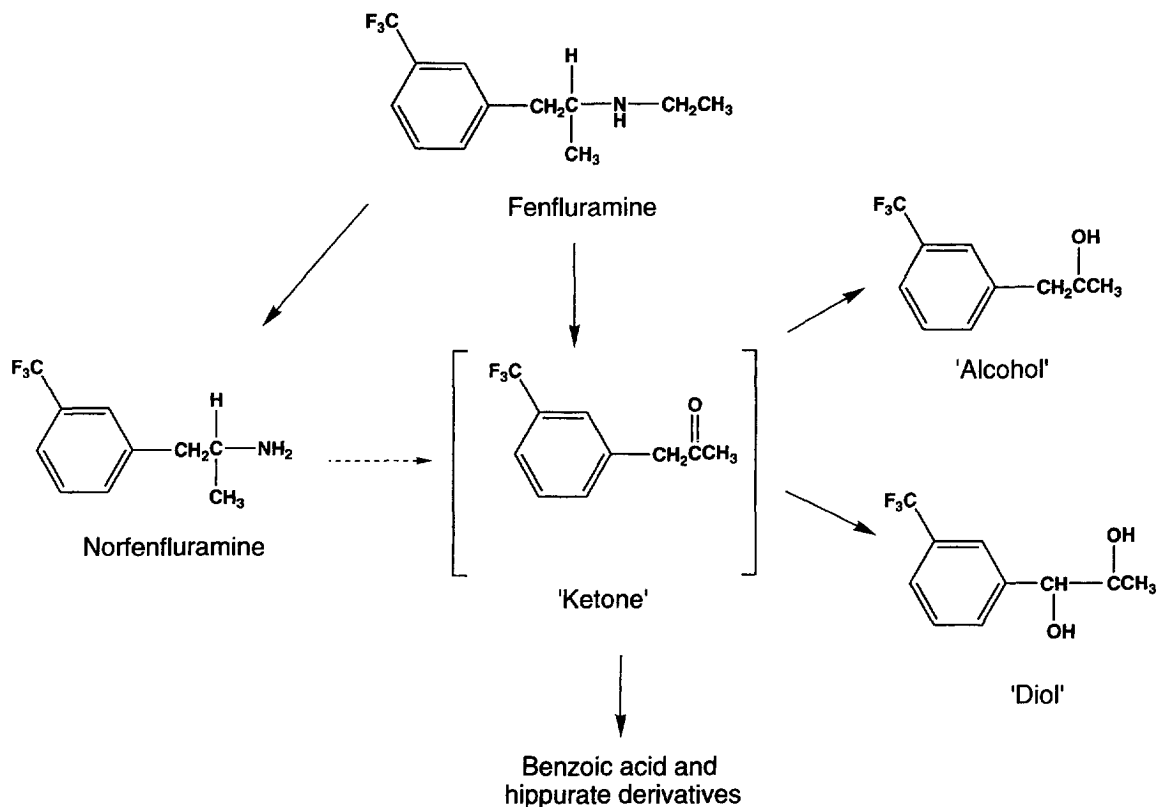


Fig. 1. Proposed metabolic scheme for (±)-fenfluramine in humans and metabolites that have been identified in plasma and urine [4–7].

oxime and *N*-hydroxyfenfluramine as the *in vitro* metabolites of (±)-fenfluramine [8].

A range of analytical methods have been employed for the investigation of (±)-fenfluramine and norfenfluramine in plasma and urine. Midha et al. [6] reported the MS of trifluoromethyl acetate (TFA) derivatives of urinary metabolites from volunteers including two previously unknown metabolites, the 'alcohol' and 'diol'; the latter compound was identified by rationalisation of the MS as no standard was available. Marchant et al. [5] compared, by TLC and HPLC, the plasma and urinary products in humans, dogs, mice and rats given radiolabelled dexfenfluramine. Eleven products were isolated, including mono- and diglucuronide conjugates, but few structures were identified. Richards et al. [7] developed a sensitive GC-based method for dexfenfluramine and nordexfenfluramine in plasma samples using nitro-

gen-phosphorous detection. Gross et al. [9] reported a sensitive method for the detection of dexfenfluramine and nordexfenfluramine in urine by reverse phase HPLC with UV detection.

To date, however, no method has been established for the extraction and detection of nordexfenfluramine, ketone, alcohol and diol metabolites simultaneously. Such an investigation would necessitate the synthesis of alcohol and diol metabolites which are not freely available and an adequately sensitive and selective assay method for as many metabolites as possible. We report a straightforward synthesis for fenfluramine metabolites diol and alcohol, and analysis of dexfenfluramine metabolites using GC-MS. In addition to the selectivity of using GC-MS with selected ion monitoring (SIM) this technique has the added advantage of detecting and tentatively identifying potential new metabolites

formed *in vitro*. Our assay has been applied to the preliminary analysis of dexfenfluramine metabolism in rat liver microsomes.

## 2. Experimental

### 2.1. Materials

1-(3-Trifluoromethyl)phenylpropan-2-one ('ketone', 97%) and ethylmagnesium bromide solution in tetrahydrofuran (1 M) were purchased from Aldrich (Milwaukee, WI, USA), mephentermine hemisulfate was obtained from Sigma (St. Louis, MI, USA), *m*-chloroperbenzoic acid (70%) was obtained from Fluka (Buchs, Switzerland), trifluoroacetic anhydride (TFAA) was obtained from Alltech (Deerfield, IL, USA), sodium borohydride ( $\text{NaBH}_4$ ) was purchased from BDH (Poole, England) and glucose 6-phosphate dehydrogenase and the disodium salts of glucose 6-phosphate and NADP were obtained from Boehringer-Mannheim (Germany). Dexfenfluramine hydrochloride and nordexfenfluramine hydrochloride were gifts of Technologie Servier. All solvents were of analytical grade and used without further processing except diethyl ether which was purified by passage through activated alumina prior to use. All glassware was silanised with 10% dichloromethylsilane (Alltech) in hexane to deactivate the surfaces.

### 2.2. Metabolite synthesis

#### 2.2.1. 1-(3-Trifluoromethylphenyl)propan-2-ol ('alcohol')

1-(3-Trifluoromethyl)phenylpropan-2-one was reduced with  $\text{NaBH}_4$  in 95% ethanol to give 1-(3-trifluoromethylphenyl)propan-2-ol. The MS of the TFA-derivatised alcohol gave the following peaks at  $m/z$  281 (10%), 186 (100%), 159 (85%), 141 (19%), 117 (30%), 113 (19%), 109 (18%), 69 (73%), matching that reported by Midha et al. [6].

#### 2.2.2. 1-(3-Trifluoromethylphenyl)-propan-1,2-diol ('diol')

3-Trifluoromethylbenzaldehyde (1.75 g, 100 mmol) was reacted with the Grignard reagent ethylmagnesium bromide (110 mmol) in tetrahydrofuran;

subsequent work-up gave 1-(3-trifluoromethyl)-phenylpropan-1-ol. The MS had  $m/z$  204 (3%), 185 (9%), 175 (100%), 173 (8%), 147 (9%), 145 (11%), 127 (74%), 77 (11%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) gave 0.9 ppm ( $-\text{CH}_3$ , t, 3H); 1.75 ppm ( $-\text{CH}_2-\text{CH}_3$ , m, 2H); 2.45 ppm ( $-\text{CH}-\text{OH}$ , s, 1H); 4.65 ppm ( $-\text{CH}-\text{OH}$ ; d of d, 1H); 7.35–7.6 ppm (aromatic-H, m, 4H).  $^{13}\text{C NMR}$  9.76 ( $-\text{CH}_3$ ); 31.85 ( $-\text{CH}_2-$ ); 75.22 ( $-\text{CH}-\text{OH}$ ); 122.65, 124.05, 129.73, 129.30 (aromatic-C), 130.2 ( $\text{F}_3\text{C}-\text{C}$ ); quartet 116.1, 121.46, 126.88, 132.29 ( $\text{F}_3\text{C}$ ).

This alcohol (200 mg, 1 mmol) was dehydrated, then purified by chromatography on activated alumina to give 1-(3-trifluoromethylphenyl)propene of MS at  $m/z$  186 ( $\text{M}^+$ , 80%) 167 (10%) 165 (17%) 159 (11%) 117 (100%) 115 (42%) 91 (11%) 69 (11%) as previously reported [10].

Finally the propene was converted to the diol metabolite by the method of Fringuelli et al. [11] followed by chromatography of the oil on activated alumina to give pure 1-(3-trifluoromethylphenyl)-propan-1,2-diol. The MS of the *bis*-TFA derivative gave  $m/z$  393 (7%) 298 (22%) 271 (70%) 243 (28%) 185 (16%) 155 (14%) 141 (43%) 113 (16%) 69 (100%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ) gave 0.95 ppm ( $-\text{CH}_3$ , d, 3H); 2.85 ppm ( $-\text{CH}-\text{OH}$ , s, 2H); 3.95 ppm (C2,  $-\text{CH}-\text{OH}$ , m, 1H); 4.7 ppm (C1,  $-\text{CH}-\text{OH}$ ; d, 1H); 7.35–7.6 ppm (aromatic-H, m, 4H).  $^{13}\text{C NMR}$  16.7 ( $-\text{CH}_3$ ); 71.1 (C2,  $-\text{CH}-\text{OH}$ ); 76.6 (C1,  $-\text{CH}-\text{OH}$ ); 123.6, 124.4, 124.8, 128.6, 129.9, 130.6 ( $\text{F}_3\text{C}-\text{C}$ ), 141.2 ( $-\text{C}-\text{CH}-\text{OH}$ ); quartet 115.9, 121.7, 126.8, 132.1 ( $\text{F}_3\text{C}$ ) which are consistent with the structure of the 'diol'.

### 2.3. Sample preparation

The I.S. mephentermine (50  $\mu\text{l}$ , 600 nM final concentration) was added to 1 ml of incubation mixture sample prior to extraction with diethyl ether–dichloromethane (3:2, 2.5 ml) with vortex mixing for 2 min. Centrifugation at 1500 g for 5 min separated the two layers, the upper layer was removed to a fresh tube and the extraction repeated. The organic layers were combined and reduced in a dry nitrogen stream with the addition of ethyl acetate (1 ml) to prevent drying. TFA derivatives of dexfenfluramine and metabolites were prepared by the

addition of TFAA (50  $\mu$ l) and heated at 55°C for 30 min. Excess TFAA was removed in a dry nitrogen stream and samples were reconstituted to 1 ml with ethyl acetate prior to analysis.

#### 2.4. Gas chromatography–mass spectrometry

Samples (2  $\mu$ l) were injected into a Shimadzu QP-2000 GC/MS (Kyoto, Japan) equipped with a 30 m $\times$ 0.25 mm column coated with 0.25  $\mu$ m phase DB-17 (J&W Scientific, Folsom, CA, USA) using a Shimadzu AOC-1400 autosampler. The temperature program for the GC–MS was as follows: oven initial temperature 80°C held for 4 min increasing to 150°C at 7°C/min, then to 200°C at 15°C/min with the injection port, interface and ion source maintained at 250°C. Splitless injections were made with a valve wait time of 1 min. The carrier gas was ultra high purity helium at a linear velocity of 35 cm/s. Positive ion MS were acquired at 70 eV between 50 and 600 amu at 1 s per decade, between 5.4 and 15 min. Quantitation of dexfenfluramine metabolites was achieved by selected ion monitoring (SIM); the ion pairs monitored and retention times of components were: *bis*-TFA derivatised diol (271, 298; 5.6 min), TFA-derivatised alcohol (186, 159; 6.4 min), ketone (*m/z* 183, 159; 8.8 min), TFA-derivatised nordexfenfluramine (140, 280; 11.7 min), TFA-derivatised dexfenfluramine (12.7 min) and TFA-derivatised mephentermine (110, 232; 13.8 min).

#### 2.5. Assay validation

Stock solutions of diol, alcohol, ketone and nordexfenfluramine mixtures were freshly prepared in acetone (241.5, 48.3 and 2.4  $\mu$ M) for addition to denatured incubation mixtures with I.S. (600 nM final concentration). Calibration curves were prepared by dilution of the stock solutions to give final concentrations of 48, 121, 241, 483, 2415, 4831 and 9662 nM for each component. Precision and accuracy of the assay was assessed at low (121 nM), medium (2415 nM) and above medium (9662 nM) concentrations in incubation mixtures containing dexfenfluramine (20 000 nM) and internal standard (600 nM). Samples were processed as described and quantitation of diol, alcohol, ketone and nordexfenfluramine metabolites was attained by plotting peak

areas versus added metabolite concentration. Each calibration curve was then fitted to a two-stage quadratic equation. The day-to-day variability (C.V.) was measured by calculating the mean (and S.D.) metabolite recovery at the three concentrations over 8 days and the within-day variability was measured by comparison of metabolite measurements in 7 replicate determinations on the same day at the 3 concentration levels. The percentage difference between the mean values for each of the assessed concentrations and the nominal value were calculated (bias).

#### 2.6. Rat microsomal metabolism

Male Sprague–Dawley rats were anaesthetised, their livers removed and perfused with ice-cold 0.1 M phosphate buffer containing 0.15 M KCl, pH 7.4. The livers were homogenised in 4 vol of 0.1 M phosphate buffer and centrifuged at 10 000 g for 20 min at 4°C, the supernatant removed and centrifuged at 100 000 g for 1 h to obtain the microsomal pellet. The pellets were resuspended in 0.1 M phosphate buffer pH 7.4 containing 20% glycerol and stored at –80°C until required. Microsomal protein and cytochrome P450 content were measured by the methods of Lowry et al. [12] and Omura et al. [13], respectively.

Duplicate microsomal incubations were carried out in 0.1 M phosphate buffer pH 7.4 at 37°C in a shaking water bath and contained rat microsomes (ca. 1 mg/ml), NADPH-regenerating system consisting of 2.5 mM MgCl<sub>2</sub>, 50 mM KCl, 1.5 mM glucose 6-phosphate, 0.3 mM NADP<sup>+</sup> and 2.5 IU glucose 6-phosphate dehydrogenase in a final volume of 1 ml. After a preincubation of 2.5 min, reactions were initiated by the addition of dexfenfluramine–HCl in buffer (at 5, 10, 20, 40, 50, 80, 160 and 200  $\mu$ M final concentration) and were incubated for 10 min. Reactions were terminated by the addition of saturated Na<sub>2</sub>CO<sub>3</sub> solution (250  $\mu$ l) with vortex mixing and placement of the tubes on ice.

The linear formation of metabolites from dexfenfluramine (at 250  $\mu$ M) was determined in duplicate incubations with increasing rat microsomal protein concentration (0.43 to 2.15 mg/ml), and an incubation time of 10 min. In separate experiments, the linearity of formation of metabolites from dex-

fenfluramine (at 250  $\mu\text{M}$ ) was investigated between 5 and 20 min in duplicate samples using a microsomal protein concentration of 1 mg/ml.

### 3. Results and discussion

#### 3.1. Metabolite synthesis and analysis

Authentic standards of drug metabolites are required for accurate identification and measurement of rates of metabolism, in addition to validation of assay methods. A procedure for the synthesis of the diol, the major human urinary metabolite, has not been previously reported. The method described in this report has few steps, is quick to perform and does not require specialist skills or equipment. The product is recovered in reasonable overall yield. The

MS spectrum of the *bis*-TFA derivative of the synthesised diol metabolite matches the spectrum reported as the putative *bis*-TFA derivative of the diol extracted from urine by Midha et al. [6]. The procedure for reduction of the ketone to the alcohol using  $\text{NaBH}_4$  is rapid, quantitative and does not require further purification of the product.

There was excellent peak shape and chromatographic separation of dexfenfluramine metabolites from co-extracted compounds in the incubation mix as shown by the sample chromatogram and SIM profiles (Fig. 2). The peak areas for extracted metabolite standards (at 9662 nM) showed different intensities in the mass spectrometer, the descending order of intensity was nordexfenfluramine > alcohol > diol > ketone indicating that TFA derivatives have increased signal intensity for the relevant metabolites.

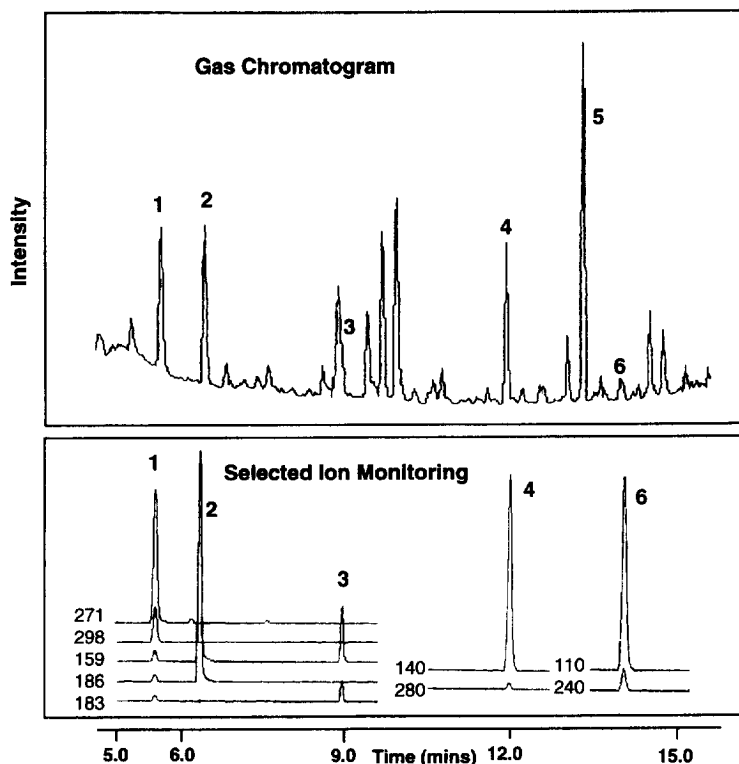


Fig. 2. Total ion chromatogram obtained between 50–600 amu in scan mode and SIM profiles of TFAA treated diol (1), alcohol (2), ketone (3) and nordexfenfluramine (4) (added at 9662 nM) extracted from incubation mixtures containing dexfenfluramine (20  $\mu\text{M}$ , 5) and internal standard mephentermine (600 nM, 6).

### 3.2. Sample preparation

In the initial method development, solid-phase  $C_{18}$  Sep-Pak cartridges (Waters, USA) were used for extraction of dexfenfluramine and metabolites from incubation mixtures. However, the conditions developed for the recovery of metabolites from the cartridge (methanol elution, evaporation of solvent to dryness) were found to be too harsh for volatile metabolites, such as the ketone and alcohol, and resulted in poor recovery. The current method employing liquid–liquid extraction of dexfenfluramine and metabolites followed by evaporation of the solvent in a dry nitrogen stream was developed to enable the recovery of these metabolites. The physicochemical properties of dexfenfluramine metabolites vary widely. There is a large difference in  $pK_a$  values for the metabolites and several products have high vapour pressures which require careful selection of extraction and derivatisation conditions. Volatile solvents were more suitable for extraction of metabolites from incubation mixtures as concentration of fractions used mild conditions. Extraction of incubation mixtures with purified diethyl ether alone resulted in highly variable recoveries of metabolites whilst with the use of the 3:2 diethyl ether–dichloromethane mixture, recovery of metabolites was more

consistent. The choice of 3:2 diethyl ether–dichloromethane as the extraction solvent allowed optimal recovery of metabolites.

Various derivatisation procedures were investigated with the aim of finding a universal agent for optimum peak shape and sensitivity for nordexfenfluramine, alcohol, diol and mephentermine but which did not interfere with the measurement of other metabolites. Conversion of metabolites to TFA derivatives with TFAA resulted in the highest sensitivity and best response for all metabolites in comparison with derivatisation to trimethylsilyl derivatives, or conversion to acetates or no derivatisation. The use of an alternative TFA-derivatising agent, trifluoroacetylimidazole, gave inconsistent results with the metabolite standards. The degree of derivatisation by this agent varied with temperature of reaction and was not equivalent for all metabolites.

### 3.3. Calibration curves, precision and accuracy

The calibration curve parameters were obtained for dexfenfluramine metabolites extracted from microsomal incubation mixtures throughout the range 48 to 9662 nM. Representative calibration curves for each of the metabolites are given in Fig. 3.

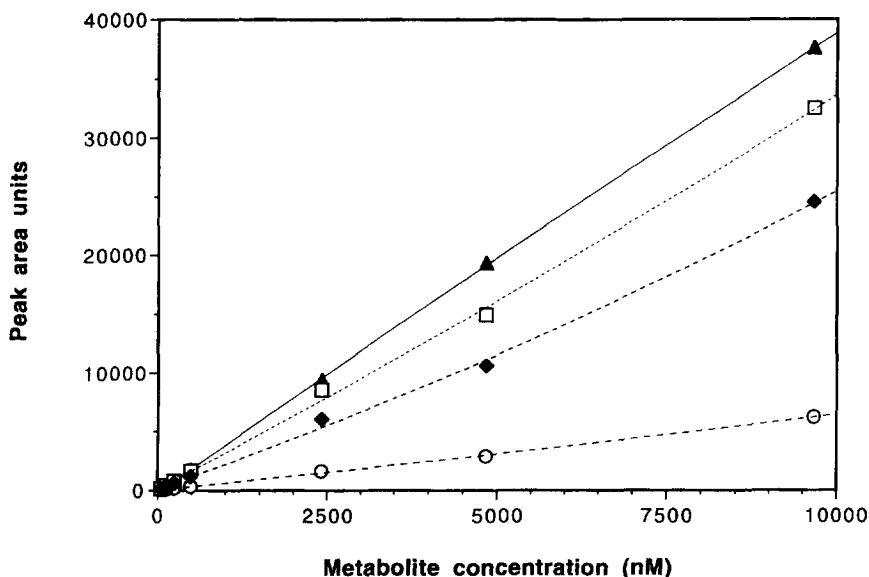


Fig. 3. Representative calibration curves for diol (solid diamonds), alcohol (open squares), ketone (open circles) and nordexfenfluramine (solid triangles) constructed over the range 48 to 9662 nM.

Table 1  
Within-day assay accuracy and precision for dexfenfluramine metabolites extracted from denatured microsomal incubation mixtures ( $n=7$ )

Metabolite	Concentration added (pmol/ml)	Concentration determined (mean and S.D.) (pmol/ml)	C.V. (%)	Bias (%)
Diol	9662	9741±318	3.3	0.8
	2415	2718±77	2.8	12.6
	121	121±6	4.7	4.9
Alcohol	9662	9623±239	2.5	-0.4
	2415	2672±84	3.1	10.7
	121	117±7	6.4	-3.6
Ketone	9662	10035±253	2.5	3.9
	2415	2643±75	2.9	9.4
	121	132±7	5.6	8.9
Nordexfenfluramine	9662	9998±604	6.0	3.5
	2415	2545±316	12.4	5.4
	121	125±7	6.0	3.5

The use of two stage quadratic equations improved the description of the calibration curves compared with linear regression analyses as they accounted for slight curvatures at the highest concentration level (9662 pmol/ml). Correlation coefficients for all curves were excellent ( $>0.999$ ) and variation in the  $x$ -value was low to moderate for the analytes over 5 curve determinations.

The mean ( $\pm$ S.D.) recovery of mephentermine, the internal standard, from incubation mixtures was 91.2 ( $\pm$ 7.3) % at 600 nM when compared with the signal for mephentermine prepared directly in ethyl acetate. The derivatising agent, TFAA, was found to be susceptible to hydrolysis by residual water in incubation extracts but since mephentermine is ana-

lysed as its TFA derivative, it also serves as a useful indicator of the efficiency of derivatisation.

The assay precision, determined by the C.V. for the metabolites measured over low, medium and above medium concentrations from incubation mixtures is good both for within- and day-to-day determinations (Tables 1 and 2). The variability in the assay method increases with decreasing concentration for all analytes but is still acceptable at the lowest level (121 pmol/ml). The most impressive results were obtained with the ketone metabolite which has very low variability in determinations both within and between days. This is a significant result as this metabolite is the most volatile and therefore difficult to recover accurately. It is also the intermediate metabolite for

Table 2  
Day-to-day assay accuracy and precision for dexfenfluramine metabolites extracted from denatured microsomal incubation mixtures ( $n=8$ )

Metabolite	Concentration added (pmol/ml)	Concentration determined (mean and S.D.) (pmol/ml)	C.V. (%)	Bias (%)
Diol	9662	9742±855	8.8	0.8
	2415	2505±231	9.2	3.7
	121	119±18	15.6	-2.0
Alcohol	9662	9821±853	8.7	1.7
	2415	2445±239	9.8	1.3
	121	118±15	12.7	-2.4
Ketone	9662	9973±673	6.7	3.2
	2415	2453±254	10.3	1.6
	121	126±13	10.7	4.1
Nordexfenfluramine	9662	10242±1488	14.5	6.0
	2415	2514±333	13.2	4.2
	121	144±25	17.5	18.9

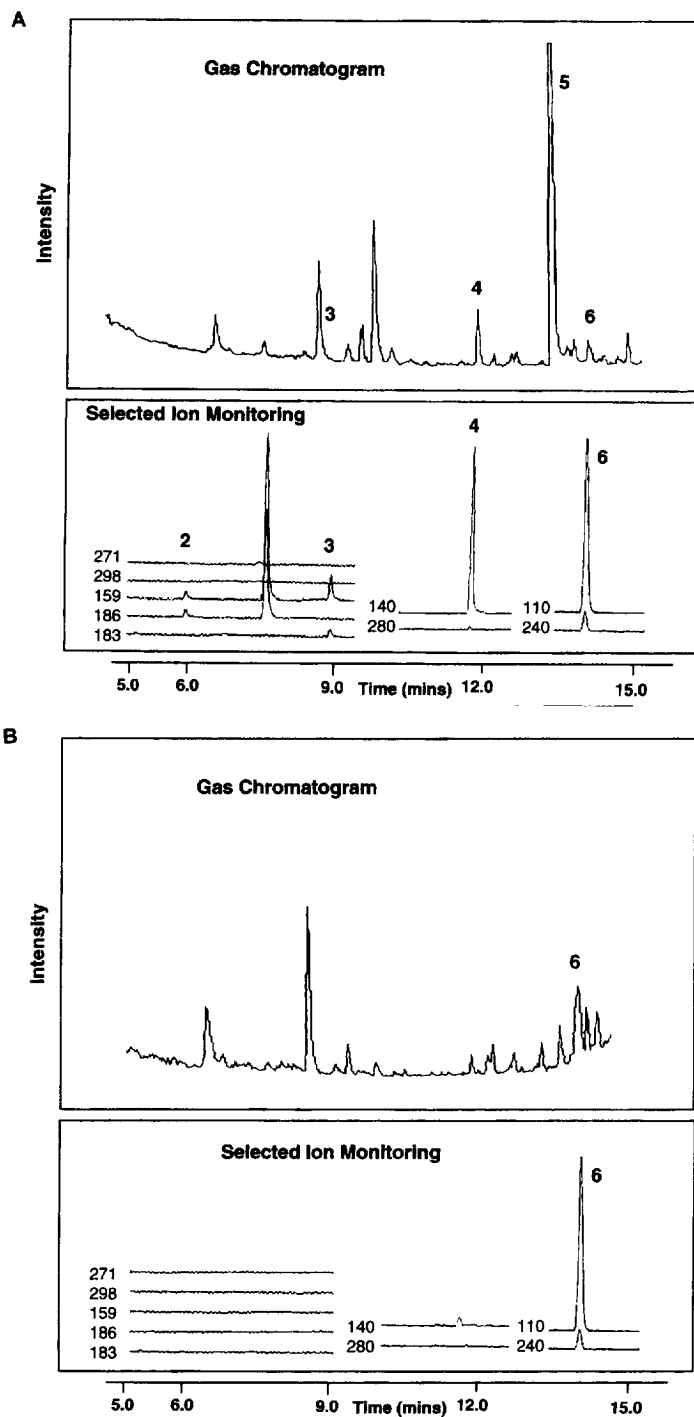


Fig. 4. Total ion chromatograms and SIM profiles of the (A) extracted products of rat microsomal metabolism of dexfenfluramine (at 160  $\mu\text{M}$ ) after 10 min incubation and (B) extracted blank microsomal mixture. Identification of the peaks by number as given in Fig. 2.



both the alcohol and diol which are major human urinary metabolites. Nordexfenfluramine gave the highest variability using the assay method; it is the most basic of the metabolites and least likely to be extracted from incubation mixtures. Previous investigators have reported day-to-day variability for nordexfenfluramine of 12.6% at 591 pmol/ml [9]. The accuracy of the analytical method, measured as the bias, is very good for all metabolites at the 3 concentration levels in the day-to-day analysis (Table 2), with the exception of nordexfenfluramine at the lowest concentration (121 pmol/ml) where the values obtained were overestimated. The bias for the within-day analysis for all concentrations of metabolites was low.

The high precision and accuracy of this method demonstrates that this is a sensitive and useful method for the measurement of dexfenfluramine metabolism. The increasing C.V. for measurement of diol, alcohol and ketone at the 121 nM level indicates this is the level of quantitation; the limit of detection was not determined for the metabolites but the *S/N* ratio for the diol, alcohol, ketone and nordexfenfluramine at 48 nM was at least 20, 22, 6 and 52, respectively.

### 3.4. Rat microsomal metabolism

In rat liver microsomes dexfenfluramine is metabolised to nordexfenfluramine, ketone and a small quantity of alcohol over a 10 min incubation period. The formation of nordexfenfluramine and ketone metabolites from dexfenfluramine were linear with increasing rat microsomal protein up to 2 mg/ml, and linear with time up to 15 min.

The major products of rat microsomal metabolism of dexfenfluramine (at 160  $\mu\text{M}$  final concentration) are shown in Fig. 4A and the final chromatogram is of an extracted blank microsomal mixture (Fig. 4B). Nordexfenfluramine and ketone were produced at 275.5 and 44.6  $\text{pmol min}^{-1} \text{nmol P450}^{-1}$ , respectively, at the dexfenfluramine concentration of 160  $\mu\text{M}$ . The sensitivity of the assay method for determination of microsomal metabolism is demonstrated by an enzyme kinetic study using rat liver microsomes. The substrate concentration versus velocity curves for production of nordexfenfluramine and ketone metabolites from dexfenfluramine at

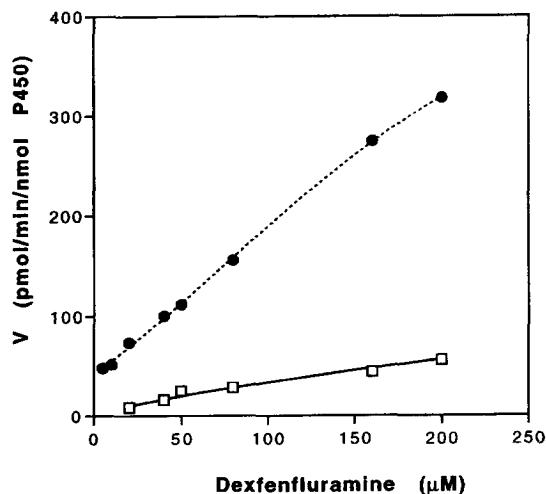


Fig. 5. Substrate concentration versus velocity curves for production of nordexfenfluramine (solid circles) and ketone (white squares) metabolites from dexfenfluramine by rat microsomes. The production of metabolites was linear with dexfenfluramine concentrations between 5 and 200  $\mu\text{M}$ .

concentrations between 5 and 200  $\mu\text{M}$  by rat liver microsomes are shown in Fig. 5. It appeared that  $V_{\text{max}}$  for the production of both nordexfenfluramine and ketone was not achieved at a substrate concentration at 200  $\mu\text{M}$ .

## 4. Conclusion

This paper describes the synthesis of the alcohol and diol metabolites of dexfenfluramine; for the diol this is the first report. A GC–MS-based method has been described and validated for the measurement of dexfenfluramine metabolism by microsomal systems which accommodated the range of physicochemical characteristics of the metabolites. Dexfenfluramine and metabolites were detected using a reliable method and sensitive GC–MS analysis of TFA derivatives after solvent extraction from alkalised incubation mixtures. In rat liver microsomes, dexfenfluramine is rapidly *N*-dealkylated to nordexfenfluramine and to a lesser extent, deaminated to form the ketone. This *in vitro* system can now be applied to the study of dexfenfluramine metabolism in human liver microsomes and heterologously expressed human cytochrome P450 enzymes with the

aim of discovering the major enzymes involved in it's metabolism.

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