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ENANTIOSELECTIVE GAS CHROMATOGRAPHIC ASSAY WITH ELECTRON-CAPTURE DETECTION FOR *dl*-FENFLURAMINE AND *dl*-NORFENFLURAMINE IN PLASMA

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SUMMARY

An enantioselective gas chromatographic assay utilising electron-capture detection has been developed for the simultaneous quantitation of enantiomers of fenfluramine and norfenfluramine in plasma. The assay involves the conversion of the enantiomers of both fenfluramine and norfenfluramine into their corresponding diastereomeric amide derivatives by an acylation reaction with *n*-heptafluorobutyryl-*S*-prolyl chloride under Schotten–Baumann conditions prior to gas chromatographic separation on an achiral polar OV-225 capillary column. Linear and reproducible standard curves were obtained over the concentration ranges 4.30–86 3 ng/ml per enantiomer and 1.25–42.25 ng/ml per enantiomer for the enantiomers of fenfluramine and norfenfluramine, respectively. The method was applied to a single-dose pharmacokinetic study in a healthy adult subject. Stereoselective differences were observed in the plasma concentration versus time profiles of the enantiomers of both fenfluramine and norfenfluramine. The area under the plasma concentration versus time curve values obtained for the *l*-isomers of fenfluramine or norfenfluramine were higher than the values of their corresponding *d*-antipodes.

INTRODUCTION

Fenfluramine, N-ethyl-α-methyl-*m*-(trifluoromethyl)phenethylamine hydrochloride, is marketed as a racemic mixture of *d*- plus *l*-isomers which is widely used as an appetite suppressant in the treatment of obesity. Fenfluramine is structurally related to the amphetamines although it has little or no central nervous system stimulant properties [1]. Fenfluramine undergoes N-deethylation to form norfenfluramine which is also pharmacologically active. A major inactive biotransformation product, *m*-trifluoromethylphenyl 1-propane-1,2-diol glucuronic acid conjugate [2] has also been reported. Experiments in animals have shown that at low doses fenfluramine releases serotonin from serotonergic neurons and prevents its reuptake, so that on chronic treatment it depletes brain

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serotonin [3–7]. Recently, flenfluramine has been investigated for its potential use in the treatment of children with autism [8–11] and adults with schizophrenia [12,13] since sub-populations of autistic children and schizophrenic adults are associated with high blood levels of serotonin.

Fenfluramine has been shown to undergo stereoselective disposition in rats [14] with higher levels of *d*-fenfluramine and *l*-norfenfluramine compared to their corresponding antipodes in plasma, red blood cells and brain. In humans, following single doses of racemic fenfluramine, there were no significant differences between the plasma levels of the enantiomers of fenfluramine or norfenfluramine although after repeated administration of racemic fenfluramine, the levels of *l*-fenfluramine and *l*-norfenfluramine were significantly higher than those of their corresponding *d*-isomers [15].

The quantitation of fenfluramine and norfenfluramine isomers in the biological samples obtained in rats [14] or humans [15] were carried out by a stereoselective analytical method utilizing gas chromatography (GC) with electron-capture detection (ECD) [16]. Although fenfluramine and norfenfluramine isomers showed good resolution on an OV-225 column [16], the optimisation of the stereoselective methodology was not clearly stated nor was the lower sensitivity limit of the quantitative assay reported. Enantioselective analytical methods need to be optimised in order to demonstrate that the derivatization reaction is complete and that the reaction rates of the two enantiomers are constant. For example, a correction factor had to be applied for the assay of propranolol enantiomers since the chiral derivatizing agent trifluoroacetyl-*S*-prolyl chloride was not enantiomerically pure [17].

In this paper a new sensitive, reliable and completely optimised enantioselective GC–ECD procedure for the quantitation of fenfluramine and norfenfluramine isomers in plasma is reported. The application of the method to a single-dose pharmacokinetic study in a human adult following an oral dose of racemic fenfluramine is also described.

EXPERIMENTAL

Materials

Pure reference standards of isomers of fenfluramine, racemic fenfluramine and norfenfluramine (Robins Research Labs., Richmond, VA, U.S.A.) and chlorphentermine hydrochloride (Parke Davis, Scarborough, Canada) were kindly donated. Heptafluorobutyric anhydride, *S*-proline, thionyl chloride and *l*-adamantanamine were purchased from Aldrich (Montreal, Canada) and glass-distilled-grade acetone, dichloromethane, benzene, *n*-pentane and amyl acetate from BDH (Toronto, Canada). All other chemicals used were of analytical grade. Disposable borosilicate culture tubes were used for extractions (16 mm × 125 mm) and evaporations (16 mm × 100 mm).

Instrumentation

A Model 5840A gas chromatograph equipped with a ^{63}Ni electron-capture de-

tector and a Model 5840A integrator (Hewlett Packard, Edmonton, Canada) and a 30 m×0.33 mm I.D. OV-225 capillary column (Terochem Labs., Edmonton, Canada) were used.

Gas chromatographic conditions

The initial column oven temperature (190°C) was held for 14 min and thereafter increased at a rate of 15°C/min to a final temperature of 250°C, which was held for 5 min. The injection port temperature was 280°C, the detector temperature 300°C, the argon–methane (carrier gas) flow-rate 1 ml/min, the argon–methane (make up gas) flow-rate 60 ml/min, the split vent flow-rate 28 ml/min, the septum vent flow-rate 2 ml/min and the head pressure on the column 15 p.s.i.

Preparation of standards and reagents

Stock solutions of *dl*-fenfluramine (10 μg/ml), *dl*-norfenfluramine (10 μg/ml) and chlorphentermine (10 μg/ml), prepared weekly by dissolving their hydrochloride salts in double-distilled (deionized) water, were stored at 4°C in the dark. Appropriate dilutions were made as required. Sodium hydroxide (0.1 *M*) and 0.1 *M* hydrochloric acid solutions were prepared in double-distilled water fresh every week. *N*-Heptafluorobutyryl-*S*-prolyl chloride (S-HPC, 0.02 *M*) was prepared according to the reported procedure [18].

Extraction and derivatization

To a borosilicate culture tube were added 1 ml plasma and 50 μl internal standard solution containing 41.8 ng of chlorphentermine free base. The sample was mixed (Vortex genie, Fisher Scientific, Edmonton, Canada) and 0.5 ml of 0.1 *M* sodium hydroxide was then added. The sample was mixed again and 5 ml of *n*-pentane were added. The tubes were capped and the contents mixed at 1600 rpm for 10 min on a rotating-type mixer (Ika, Vibrax VXR, Fisher) and then centrifuged (Model TJ-6 centrifuge, Beckmann Instruments, Palo Alto, CA, U.S.A.) at 1720 *g* for 5 min at 4°C. The upper organic phase was transferred by pasteur pipette to a borosilicate tube containing 1 ml of 0.1 *M* hydrochloric acid. The sample tube was capped, the contents were mixed (1600 rpm for 10 min) and centrifuged (1720 *g*, 5 min). The organic layer was discarded. To the aqueous layer were added 1.2 ml of 0.1 *M* sodium hydroxide and the contents were mixed (5 s). S-HPC (50 μl) was then added and after mixing (1600 rpm for 10 min) the sample was allowed to stand for 45 min at room temperature, after which 5 ml of *n*-pentane were added and the contents mixed (1600 rpm for 10 min) and centrifuged (1720 *g* for 5 min). The organic layer was transferred into another tube and the solvent was evaporated at 65°C. The residue was dissolved in 20–30 μl of amyl acetate and suitable aliquots (1–2 μl) were injected into GC–ECD system.

Effect of the amount of S-HPC on reactivity

dl-Fenfluramine (250 ng/ml per enantiomer) or *dl*-norfenfluramine (200 ng/ml per enantiomer) was allowed to react with various volumes of 0.02 *M* S-HPC (20, 30, 40, 50 and 60 μl) as described earlier. *l*-Adamantanamine hydrochloride, previously derivatized with S-HPC (500 ng/ml) in amyl acetate, was added to

the residue as an external standard and the samples were analysed by GC–ECD thereafter. Each set of reaction conditions was studied in duplicate.

Effect of reaction time on the formation of the diastereomers

dl-Fenfluramine (250 ng/ml per enantiomer) or *dl*-norfenfluramine (200 ng/ml per enantiomer) was each reacted in duplicate with 50 μl of 0.02 *M* S-HPC. The reaction was stopped at various times (10, 20, 30, 45 and 60 min) by extraction of diastereomers with *n*-pentane. *l*-Adamantanamine, previously derivatized with S-HPC (500 ng/ml) in amyl acetate, was added to the residue as an external standard, after which the samples were analyzed by GC–ECD.

Effect of the amount of S-HPC and the reaction time on the enantiomeric ratios of fenfluramine and norfenfluramine isomers

The enantiomeric ratios (*l*/*d*) for the isomers of fenfluramine and norfenfluramine were calculated for the various volumes (20–60 μl) of S-HPC added. Similar calculations were made for the various times (10–60 min) when the reaction was stopped.

Stability of diastereomeric S-HPC derivatives of dl-fenfluramine and dl-norfenfluramine

Duplicate samples of *dl*-fenfluramine (21.6–86.3 ng/ml free base per enantiomer) and chlorphentermine hydrochloride (50 μl) or *dl*-norfenfluramine (21.13–84.5 ng/ml free base per enantiomer) and chlorphentermine hydrochloride (50 μl) were each allowed to react with 50 μl of 0.02 *M* S-HPC for 45 min as described above. The residues obtained after evaporation were stored for various lengths of time (one, four, five and eight days), after which they were analyzed by GC–ECD.

Standard curves for isomers of fenfluramine and norfenfluramine

A series of plasma samples from healthy volunteers were spiked with both *dl*-fenfluramine (86.3–4.3 ng/ml free base per enantiomer) and *dl*-norfenfluramine (42.25–1.25 ng/ml free base per enantiomer). Internal standard (50 μl chlorphentermine hydrochloride stock solution) was added. Separate standard curves for the enantiomers of fenfluramine and norfenfluramine were constructed after chromatographing the spiked plasma standards and plotting the peak-height ratio of each enantiomer to that of the internal standard against the concentration of each enantiomer.

Intra- and inter-assay variation

Intra-assay variability in the analysis of the enantiomers of fenfluramine and norfenfluramine was determined by analysing a series of plasma samples (replicates) spiked with fenfluramine (10.3–86.3 ng/ml free base per enantiomer) and norfenfluramine (4.25–42.25 ng/ml free base per enantiomer) within a day. Inter-assay variability was determined similarly except that samples were analysed on four separate days.

Human study

A healthy male volunteer weighing 67.5 kg, after an overnight fast, was given orally 60 mg fenfluramine hydrochloride (three 20-mg tablets, Pondimin®). A predose blood sample (15 ml) was obtained immediately before ingestion of the drug. Further blood samples (15 ml each) were drawn into heparinized evacuated tubes (Vacutainers) at 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 6.5, 8.0, 12.0, 24.0, 32.0, 48.0, 72.0, 96.0 and 168.0 h. Care was taken to avoid contact of the blood with the rubber stoppers of the Vacutainers during collection of venous blood. The blood samples were immediately centrifuged and the plasma separated and stored at −20°C until analysis.

Pharmacokinetic calculations

Areas under the plasma concentration versus time curves (AUC_0^t) were calculated by linear trapezoidal rule. Areas to infinity (AUC_0^∞) were obtained by adding to AUC_0^t the quotient of the last plasma concentration measured and the terminal slope value [19]. Plasma elimination half-life values ($t_{1/2}$) for the enantiomers were estimated from the terminal slope of the plasma concentration–time curves. Apparent oral clearance values (Cl_0) for the isomers were calculated according to standard procedure [19].

RESULTS AND DISCUSSION

There are two requirements which must be satisfied in the development of an enantioselective analytical method on the formation of diastereomeric derivatives. Firstly, the chiral reagent used should be of high enantiomeric purity and secondly, there must be no racemisation during the derivatisation reaction or during chromatography. In the present study S-HPC was used as the chiral derivatizing agent since it met with the above criteria and had been successfully used in the development of enantioselective assays for *dl-threo*-methylphenidate [20,21]. Thus enantiomers of fenfluramine and norfenfluramine were converted into their respective diastereomeric derivatives by reaction with S-HPC under Schotten–Baumann conditions. The Schotten–Baumann reaction was carried out in an aqueous alkaline medium, which had the advantage that excess of the acylating reagent was destroyed and thus did not interfere with the chromatography.

Fig. 1 shows the chromatograms of the S-HPC-derivatized isomers of fenfluramine and norfenfluramine and internal standard. It can be noted from this figure that all the peaks were well separated and there were no interfering peaks from endogenous plasma constituents. The assignments of *l*-norfenfluramine and *d*-norfenfluramine peaks were tentatively based on the previous report of the resolution of pure reference standards on an OV-225 column [16].

In the present study, the S-HPC-derivatized fenfluramine or norfenfluramine isomers showed excellent separation when injections of each diastereomeric pair were made on a column packed with a polar OV-225 stationary phase (cyanopropylmethyl–phenylmethyl silicone). However, when S-HPC-derivatized *dl*-fenfluramine and *dl*-norfenfluramine were injected together, the peak corresponding to the *d*-enantiomer of norfenfluramine overlapped with that of the *l*-enantiomer

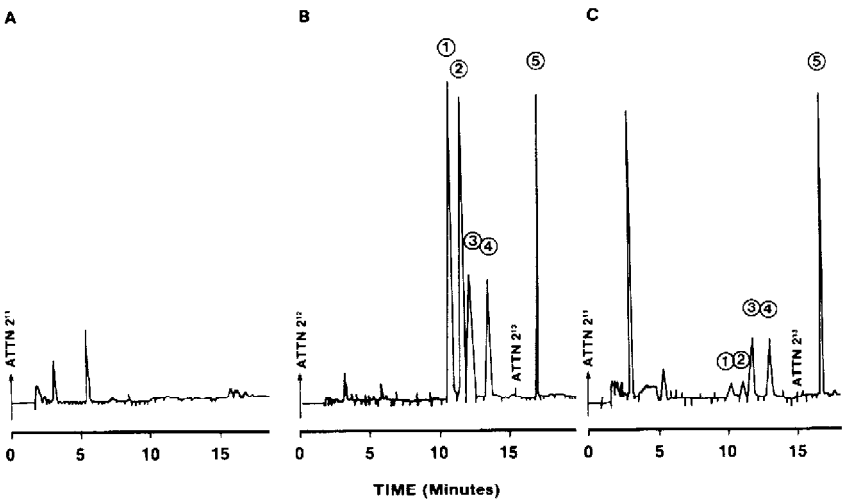
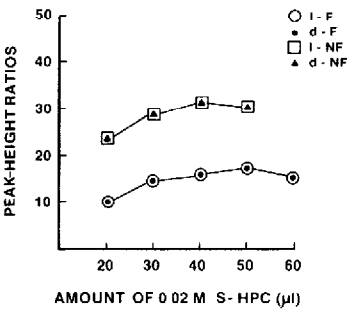


Fig 1. GC–ECD of S-HPC-derivatised extracts of (A) blank plasma; (B) spiked plasma standard containing 42.25 ng/ml norfenfluramine per enantiomer and 43.15 ng/ml fenfluramine per enantiomer; (C) 3.0-h plasma from a healthy adult volunteer dosed with 60 mg fenfluramine hydrochloride. Peaks (S-HPC derivatives): 1 = *l*-norfenfluramine; 2 = *d*-norfenfluramine; 3 = *l*-fenfluramine; 4 = *d*-fenfluramine; 5 = chlorphentermine (internal standard).

of fenfluramine with the column oven set at 230 °C or above. Good baseline separation of the latter two peaks was obtained when the column oven temperature was decreased to 190 °C (Fig. 1). Thus initial column oven temperature was held at 190 °C until the diastereomeric derivatives of *d*- and *l*-norfenfluramine and *d*- and *l*-fenfluramine were separated and thereafter the temperature was increased at 15 °C/min to 250 °C and then held at this temperature for 5 min. The higher temperature facilitated the faster elution of the internal standard and also removed any high-boiling impurities.

During the development of the enantioselective methodology, care was taken to optimise firstly the amount of S-HPC reagent to be added and secondly the reaction time for the conversion of the isomers to their corresponding diastereomers. The possibility of racemization associated with either synthesis of the chiral reagents and/or the formation of diastereomers has been well documented [22–25]. In the present work S-HPC was synthesized with care according to the reported procedure [18]. Fig. 2 shows the effect of the amount of S-HPC on the acylation reaction in which the diastereomeric derivatives of fenfluramine and norfenfluramine enantiomers were formed. Thus 50 μl of S-HPC were found to be adequate for the acylation of both the isomers of fenfluramine and norfenfluramine. Fig. 3 shows the effect of reaction time on the acylation reaction. It required only 10 min for the completion of the acylation reaction and there were no changes in the amounts of diastereomers of either fenfluramine or norfenfluramine formed beyond 10 min. Neither the amount of S-HPC nor the reaction time had any effect on the enantiomeric ratios (*l*/*d*) of the diastereomeric derivatives of fenfluramine and norfenfluramine. The enantiomeric ratios for both *dl*-fenfluramine and *dl*-norfenfluramine remained constant suggesting that the re-



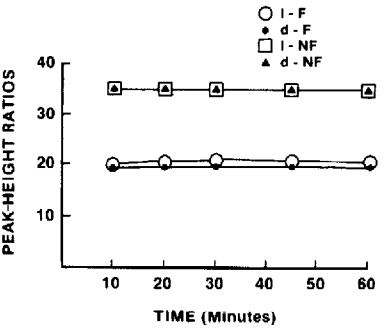


Fig. 2. Effect of the amount of S-HPC on the formation of the diastereomeric amide derivatives of the enantiomers of fenfluramine (F) and norfenfluramine (NF).

Fig. 3. Effect of the reaction time on the formation of S-HPC derivatives of *dl*-fenfluramine (F) and *dl*-norfenfluramine (NF).

TABLE I

STABILITY OF *n*-HEPTAFLUOROBUTYRYL-*S*-PROLYL-*dl*-FENFLURAMINE AS A FUNCTION OF STORAGE TIME AT ROOM TEMPERATURE

Spiked concentration (ng/ml per enantiomer)	*n*	Actual concentration after analysis (mean ± S.D.) (ng/ml per enantiomer)							
		One day		Four days		Five days		Eight days	
		l	*d*	*l*	*d*	*l*	*d*	*l*	*d*
86.30	3	87.7 ±5.33	85.03 ±5.79	84 87 ±9.06	86.33 ±7.31	84.77 ±1.74	86.83 ±1.08	88.62 ±6.32	87.61 ±3.99
48 15	3	43.5 ±1.18	42.16 ±0.93	41 36 ±1.59	40.62 ±1.04	39.65 ±3.18	40.49 ±4.60	44.91 ±1.08	43.64 ±1.39
21 60	3	22.1 ±0.71	21.41 ±0.75	21.47 ±2.16	22.36 ±1.94	22.57 ±0 25	24.03 ±0.99	24.86 ±0.61	22.00 ±0.30

actions were complete and that kinetic resolution was not a problem. Tables I and II show the stability of the derivatives of the isomers of fenfluramine and norfenfluramine as a function of storage time. The S-HPC derivatives of both isomers of fenfluramine and norfenfluramine were found to be stable for at least eight days at room temperature.

The assay based on the measurement of peak-height ratios of the analyte and internal standard versus concentration was linear ($r^2 = 0.99$) in the concentration range 4.3–86.3 ng/ml for each enantiomer fo fenfluramine and 1.25–42.25 ng/ml for each enantiomer of norfenfluramine (Tables III and IV). A mean slope value of 0.005 was obtained in case of each fenfluramine isomer, whereas the corresponding values obtained for the isomers of norfenfluramine were 0.0547 (*l*-norfenfluramine) and 0.0531 (*d*-norfenfluramine). The lower limit of detection of the analysis was found to be 0.5 and 2.0 ng/ml for the enantiomers of norfen-

TABLE II

STABILITY OF *n*-HEPTAFLUOROBUTYRYL-*S*-PROLYL-*dl*-NORFENFLURAMINE AS A FUNCTION OF STORAGE TIME AT ROOM TEMPERATURE

Spiked concentration (ng/ml per enantiomer)	*n*	Actual concentration after analysis (mean ± S.D.) (ng/ml per enantiomer)							
		One day		Four days		Five days		Eight days	
		l	*d*	*l*	*d*	*l*	*d*	*l*	*d*
84.50	3	86.16 ± 3.57	85.62 ± 6.01	86.71 ± 7.29	84.13 ± 5 13	84.30 ± 4 31	86.54 ± 2.36	83.55 ± 1.48	86.87 ± 1.89
42.25	3	42.82 ± 0.94	41.94 ± 1.06	38 80 ± 0.92	39.27 ± 2 37	39.47 ± 2 63	42.36 ± 1.30	43.28 ± 0.69	43.22 ± 2.93
21.13	3	21.68 ± 2.60	21.87 ± 1.03	24.04 ± 0.80	22.63 ± 0.44	22.43 ± 0.42	22.63 ± 0.71	20.14 ± 0.83	21.83 ± 0.90

TABLE III

STANDARD CALIBRATION CURVE DATA FOR FENFLURAMINE ENANTIOMERS

Concentration (ng/ml)	*n*	Peak-height ratios derivatized fenfluramine enantiomer/internal standard (mean ± S.D.)	
		d-Fenfluramine	*l*-Fenfluramine
86.30	3	0.4402 ± 0.011	0.4643 ± 0.026
43.15	3	0.1770 ± 0 012	0.1845 ± 0.010
17.20	3	0.0779 ± 0.006	0.0793 ± 0.007
8.60	3	0.0428 ± 0.005	0.0443 ± 0.005
4.30		0.0215 ± 0.002	0.0211 ± 0 002
r^2		0.99	0.99
Intercept		−0.009	−0.011
Slope		0 005	0.005

fluramine and fenfluramine, respectively. Studies on inter-assay and intra-assay variation were carried out to show that quantitation of *dl*-fenfluramine and *dl*-norfenfluramine were reproducible and accurate (Tables V and VI). A mean coefficient of variation of less than 10% for each derivatised enantiomer demonstrated that the assay had good reproducibility. The accuracy studies demonstrated that the concentration values obtained after analysis were within 20% of the theoretical values.

The new enantioselective analytical method was applied to the quantitation of fenfluramine and norfenfluramine isomers in biological samples. Fig. 4 shows the plasma concentration–time curve of the enantiomers of fenfluramine and norfenfluramine in an adult following a 60-mg oral dose of racemic fenfluramine. Table VII shows simple pharmacokinetic parameters calculated from the plasma concentration–time profile. The times to peak concentration (t_{max}) for the iso-

TABLE IV

STANDARD CALIBRATION CURVE DATA FOR NORFENFLURAMINE ENANTIOMERS

Concentration (ng/ml)	n	Peak-height ratios derivatized norfenfluramine enantiomer/internal standard (mean ± S.D.)	
		l-Norfenfluramine	*d*-Norfenfluramine
42.25	3	2.3650 ± 0.191	2.297 ± 0.094
21 13	3	1.1400 ± 0.023	1.192 ± 0.034
8.50	3	0.571 ± 0.005	0.586 ± 0.027
4 25	3	0.274 ± 0.007	0.281 ± 0.006
1.25	3	0.0795 ± 0.002	0.089 ± 0.003
r^2		0.99	0.99
Intercept		0.0395	0.0380
Slope		0.0547	0.531

TABLE V

STATISTICAL EVALUATION OF THE ACCURACY AND REPRODUCIBILITY OF THE ASSAY FOR FENFLURAMINE ENANTIOMERS IN PLASMA

No.	Concentration of each enantiomer (ng/ml)	n	Concentration of each enantiomer after analysis (mean ± S.D.) (ng/ml)	
			l	*d*
Intra-assay variability				
1	86.30	5	85 62 ± 3.55	83.16 ± 3.37
2	43.15	5	44.18 ± 1.64	43.16 ± 0 84
3	21.60	4	21 86 ± 1.10	20.90 ± 0.72
4	10.83	4	9.93 ± 0.94	9.73 ± 0.94
Overall coefficient of variation (%)			5 59	4.78
Inter-assay variability				
1	86.30	5	84.04 ± 3.04	86.01 ± 1.90
2	43.15	5	43.96 ± 2 89	45.22 ± 2.11
3	21.60	5	21.96 ± 1.65	22.10 ± 0.85
4	10.83	5	9.09 ± 1.02	9.40 ± 0.95
Overall coefficient of variation (%)			7 22	5.21

mers of fenfluramine were 8.0 h whereas the corresponding values for the enantiomers of norfenfluramine were 32.0 h. The peak plasma concentration values (C_{max}) were higher for fenfluramine isomers than the corresponding norfenfluramine isomers. AUC_0^t values were calculated up to 96.0 h for the fenfluramine enantiomers and up to 168.0 h for norfenfluramine enantiomers. The apparent Cl_0 values calculated for *l*-isomers of both fenfluramine and norfenfluramine for the one subject studied were smaller than the corresponding values for *d*-isomers. The $t_{1/2}$ value for *l*-fenfluramine (28.87 h) was observed to be longer than the

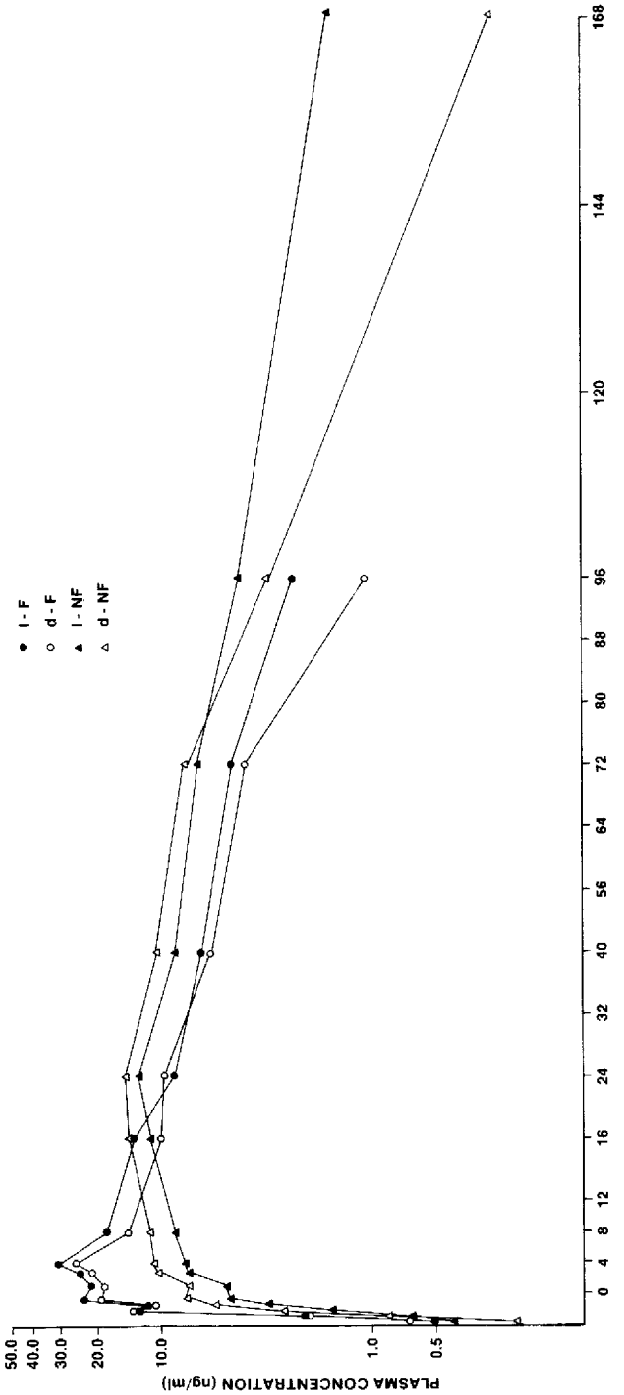


Fig. 4. Plasma concentration versus time profiles of the enantiomers of fenfluramine (F) and norfenfluramine (NF) in a healthy subject after a single oral dose of 60 mg racemic fenfluramine hydrochloride.

TABLE VI

STATISTICAL EVALUATION OF THE ACCURACY AND REPRODUCIBILITY OF THE ASSAY FOR NORFENFLURAMINE ENANTIOMERS IN PLASMA

No.	Concentration of each enantiomer (ng/ml)	n	Concentration of each enantiomer after analysis (mean ± S.D.) (ng/ml)	
			l	*d*
Intra-assay variability				
1	42.25	4	42.61 ± 2.75	42 01 ± 2.00
2	21.13	4	20.86 ± 0.99	20.81 ± 1.47
3	8.50	5	7.83 ± 0.34	7.53 ± 0.26
4	4.25	5	5.07 ± 0.20	5.11 ± 0.18
Overall coefficient of variation (%)			4.87	4.70
Inter-assay variability				
1	42.25	5	42.91 ± 0.27	42.16 ± 0.49
2	21.13	5	20.90 ± 0.81	20.19 ± 0.73
3	8.50	5	8.07 ± 0.41	7.83 ± 0.42
4	4.25	5	5.01 ± 0.18	5.06 ± 0.27
Overall coefficient of variation (%)			3.32	3.86

TABLE VII

SIMPLE PHARMACOKINETIC PARAMETERS OF ENANTIOMERS OF FENFLURAMINE AND NORFENFLURAMINE IN AN ADULT (67.5 kg) DOSED WITH 60 mg RACEMIC FENFLURAMINE HYDROCHLORIDE

Compound	t_{max} (h)	C_{max} (ng/ml)	AUC_0^t (ng/ml/h)	AUC_0^∞ (ng/ml/h)	$t_{1/2}$ (h)	Cl_o (l/kg/h)
l-Fenfluramine	8.0	30.91	876.68	973.32	28.87	0.91
d-Fenfluramine	8.0	25.45	746.56	772.18	17.24	1.15
l-Norfenfluramine	32.0	13.18	1010.22	1113.41	43.86	0.80
d-Norfenfluramine	32.0	14.58	9 45.12	950.04	19.8	0.94

corresponding value for *d*-fenfluramine (17.24 h) in this one subject although Caccia et al. [15] calculated similar $t_{1/2}$ values for *l*-fenfluramine (24.3 ± 1.8 h) and *d*-fenfluramine (24 ± 8 h) isomers. However, it should be recognized that calculations of half-life in the earlier study was based on the measurement of plasma concentration over 48.0 h, whereas, in our study the concentrations of *d*-fenfluramine and *l*-fenfluramine were determined up to 96.0 h. The *l*-enantiomer of fenfluramine in this subject appeared to be eliminated more slowly than its *d*-antipode, which is also reflected in the Cl_o values (0.91 l/kg/h for *l*-fenfluramine and 1.15 l/kg/h for *d*-fenfluramine). The AUC_0^∞ calculated for *l*-fenfluramine was 1.26 times greater than the corresponding value for *d*-fenfluramine indicating a mild enantioselectivity in the disposition of fenfluramine isomers which was supported by the fact that plasma levels of *l*-fenfluramine were significantly higher

than those of *d*-fenfluramine in a paired *t*-test ($t=4.4$, 13 degrees of freedom, $p<0.01$). Similarly, the $t_{1/2}$ value for *l*-norfenfluramine (43.86 h) was longer than that of *d*-norfenfluramine (19.8 h) based on the measurement of plasma concentrations up to 168.0 h. Thus *l*-norfenfluramine like *l*-fenfluramine appears to be eliminated more slowly, as further indicated by a lower Cl_o value of 0.80 l/kg/h as compared to 0.94 l/kg/h calculated for *d*-norfenfluramine. Moreover, the plasma levels of *l*-norfenfluramine were significantly greater than those of *d*-norfenfluramine in a paired *t*-test ($t=1.89$, 13 degrees of freedom, $p<0.05$) such that AUC_0^∞ calculated for *l*-norfenfluramine was 1.17 times greater than the corresponding value for the *d*-isomer.

In an earlier study [15], after single oral doses of racemic fenfluramine to human subjects, *l*-fenfluramine and *d*-fenfluramine were found to have similar t_{max}, $t_{1/2}$ and AUC_0^∞ values such that there were no significant differences in the plasma levels of the enantiomers, whereas after repeated oral doses to the same subjects, *l*-fenfluramine was demonstrated to have higher plasma levels, longer half-lives and larger AUC_0^∞ values than those of the corresponding *d*-enantiomer. The observation of a longer $t_{1/2}$ value of *l*-fenfluramine as opposed to *d*-fenfluramine in the late elimination phases, in the subject from the present study, provides a hint for the greater accumulation of *l*-isomer after chronic doses of racemic fenfluramine.

In conclusion, a new sensitive and specific enantioselective analytical method for the quantitation of both fenfluramine and norfenfluramine enantiomers in plasma has been developed. The method has been applied to a pilot pharmacokinetic study in a healthy adult and has been shown to have adequate sensitivity to follow the plasma concentration versus time curves for fenfluramine isomers up to 96.0 h and those of norfenfluramine isomers to 168.0 h, following a single oral dose. Observation of longer $t_{1/2}$ values for *l*-isomers of both fenfluramine and norfenfluramine as opposed to the corresponding *d*-antipodes further adds to the significance of the present enantioselective method which is capable of measuring low plasma levels of fenfluramine and norfenfluramine enantiomers in their late elimination stages.

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