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Short communication

# Direct high-performance liquid chromatography resolution on a chiral column of dexfenfluramine and its impurities, in bulk raw drug and pharmaceutical formulations

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**Abstract**

High-performance liquid chromatography (HPLC) was employed for the determination of dexfenfluramine together with the impurities L-fenfluramine (enantiomeric impurity), *ortho*-(D,L)-fenfluramine and *para*-(D,L)-fenfluramine in bulk powder and pharmaceutical formulations. The separation of the enantiomers was accomplished, without any derivatization, on a chiral column containing cellulose carbamate (Chiralcel OF) coupled with an achiral column. The effect of diethylamine and 2-propanol, added to the mobile phase was studied. The HPLC method gave good performances, allowing determination of the enantiomeric purity of dexfenfluramine together with the related impurities *ortho*- and *para*-(D,L)-fenfluramine. The method is capable of determining a minimum limit of 0.5% of L-isomer in commercial samples containing dexfenfluramine.

*Keywords:* Enantiomer separation; Dexfenfluramine; Fenfluramine

## 1. Introduction

Fenfluramine, a racemic secondary amine, has been available for over twenty years for the treatment of obesity. The therapeutic activity of this anorectic agent, in man, lies mainly with the D-isomer which is now marketed in several countries worldwide. Dexfenfluramine is effective in promoting weight loss in the short term, in addition, it appears to be well tolerated, with a lower potential for drug interactions than the racemate [1]. In fact, dexfenfluramine shows appetite suppressant activity without the stimulant and addictive properties associated with other anorectic agents. In contrast to other amphetamine derivatives, fenfluramine causes drowsiness and does not interfere with REM (rapid eye movement) sleep.

Enantiomers of amphetamines were resolved as amide derivatives [2,3] or, without any derivatization, on cellulose-based columns [4,5], or by means of a $\beta$-cyclodextrin-chiral stationary phase [6].

Amphetamine, methamphetamine and several ring-substituted analogs were separated by capillary electrophoresis (CE) using native and derivatized $\beta$-cyclodextrin as the chiral additives [7].

The enantiomeric purity of (D,L)-fenfluramine, in biological fluids and final pharmaceutical products,

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was assessed in the presence of its isomeric impurities [*ortho*- and *para*-(D,L)-fenfluramine], after derivatization with 3,5-dinitrophenylisocyanate, utilizing (*R*)- and (*S*)-1-(1-naphthyl)-ethylurea-based columns [8,9].

Capillary electrophoresis (CE) was employed for the enantiomeric separation of (D,L)-fenfluramine in pharmaceutical products [10].

The enantiomers of (D,L)-fenfluramine were resolved with gas chromatography (GC), as amide diastereoisomers, after derivatization with *n*-heptafluorobutyryl-S-propyl chloride [11].

The literature appears to lack any HPLC method for the determination of the enantiomeric purity of underivatized (D,L)-fenfluramine. As part of our laboratory's ongoing activity in the development of assays for the stereochemical purity of commercial drugs, we developed an isocratic HPLC method for the simultaneous separation, identification and measurement of L-fenfluramine, *ortho*- and *para*-(D,L)-fenfluramine (Fig. 1) in bulk powder and pharmaceutical formulations containing dexfenfluramine, with the aid of tandem-chromatography.

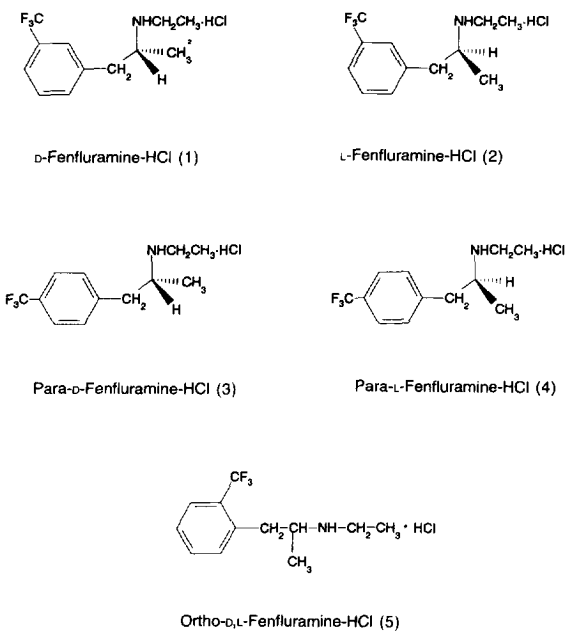


Fig. 1. Structures of dexfenfluramine HCl and its impurities.

## 2. Experimental

### 2.1. Conditions

#### 2.1.1. Materials

Stainless-steel Chiralcel OF (250 × 4.6 mm I.D.) (Daicel Chemicals, Tokyo, Japan) and Nucleosil-$NH_2$ (200 × 4.0 mm I.D.) (Macherey-Nagel, Düren, Germany) columns were used in the method finally developed. The other chiral stationary phase columns investigated were 250 × 4.6 mm I.D. Chiralcel OD, OD-R, OC, OG and Chiralpak AD. HPLC-grade solvents were purchased from Carlo Erba (Milan, Italy). Diethyl amine (DEA) Uvasol-grade was obtained from Fluka Chemie (Buchs, Switzerland); (D,L)-fenfluramine hydrochloride and related impurities were from Servier Technologie (Paris, France).

#### 2.1.2. Apparatus

Chromatography was performed using a Perkin-Elmer Model 200 pump (Perkin-Elmer, Norwalk, CT, USA), a Waters Model U6K injector and a Model 991 programmable multi-wavelength diode array detector (Waters, Milford, MA, USA).

#### 2.1.3. Operating conditions

The following chromatographic conditions were used with Chiralcel OF: mobile phase, *n*-hexane–DEA (99.9:0.1, v/v) degassed with an ultrasonic bath before use; flow-rate, 0.5 ml/min; column temperature, 25°C; volume injected, 100 $\mu$l and detector wavelength, 265 nm. The column was easily regenerated at the end of every day by washing it with ca. 100 ml of *n*-hexane–2-propanol (90:10, v/v) at a flow-rate of 0.2 ml/min. In this way, no efficiency loss was observed throughout our work. After cleaning, the column was reconditioned with ca. 100 ml of the mobile phase. The same experimental conditions, as described above, were used with the coupled system consisting of $NH_2$-Chiralcel OF columns, but the flow-rate used was 1.0 ml/min.

#### 2.1.4. Linearity of detector response vs. concentration of standards

Calibration graphs were obtained by injecting the impurities L-fenfluramine and the *ortho* and *para*

isomers in the concentration range 0.2–25 μg/ml and dexfenfluramine in the range 4–60 μg/ml.

### 2.1.5. Sample preparation

*Capsules*

Three different commercial samples were examined with the following procedure.

The content of five capsules of each kind of pharmaceutical sample (previously weighed) was mixed and, taking into account the average mass, an amount of powder corresponding to 10 mg of dexfenfluramine was weighted exactly. The powder was suspended in 2-propanol (3 × 4 ml) and stirred for 10 min. The solvent was then centrifuged and concentrated to 10 ml with a gentle stream of nitrogen; a 1-ml sample of this solution was dried and the residue was dissolved in 25 ml of mobile phase. Following the extraction procedures described above, we obtained recoveries of 96.0% based on the calibration curve of D-fenfluramine.

## 3. Results and discussion

Currently HPLC and GC methods are employed for the determination of dexfenfluramine and its impurities in bulk products and pharmaceutical formulations. These methods, however, need a prior derivatization of the amine and its impurities to obtain chiral recognition with the stationary phase.

The aim of this work was the quantification of the L-enantiomer of fenfluramine, together with its *ortho* and *para* impurities, in bulk products and pharmaceutical formulations without any derivatization.

Methylphenylcarbamate of cellulose-based CPS (Chiralcel OF) was successfully employed to separate directly the above-mentioned compounds. Authentic reference materials of pure D- and L-fenfluramine were used to determine the elution order of the enantiomers. However, it was not possible to determine the order of elution of the *para* isomer because of a lack of a single enantiomer. Both racemic mixtures of (D,L)-fenfluramine and its *para* isomer were efficiently resolved on a Chiralcel OF column; no resolution was observed for the *ortho* isomer (Fig. 2).

The types and amounts of solvents that can be used in the mobile phase, without damaging the chiral stationary phase of the Chiralcel OF column, are limited. We obtained baseline separations of the (D,L)-enantiomers of fenfluramine and its *para* isomer using a mobile phase consisting of *n*-hexane spiked with DEA. The presence of DEA in the mobile phase was critical for obtaining resolution of the enantiomers of (D,L)-fenfluramine and its *para*

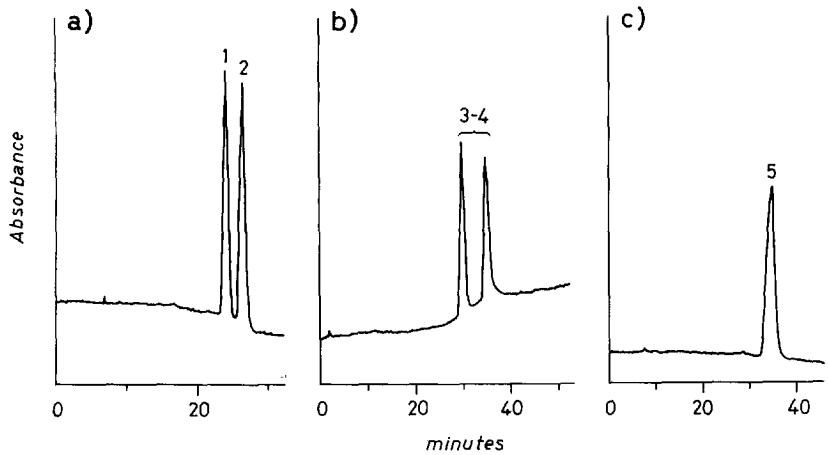


Fig. 2. HPLC of compounds shown in Fig. 1. (a) Compounds 1 and 2; (b) compounds 3 and 4; (c) compound 5. Eluent, *n*-hexane–DEA (99.9:0.1, v/v); flow-rate, 0.5 ml/min; column, Chiralcel OF; wavelength, 265 nm; temperature, 25°C.

isomer. However the enantioselectivity factors ($\alpha$), obtained with the Chiralcel OF column, were slightly affected by the DEA over the range of concentrations used (0.05–0.3%); the highest values of enantioselectivity ($\alpha$) and resolution ($R$) were observed at a concentration of 0.1% DEA (Table 1). Small amounts of DEA (lower than 0.05%) added to the mobile phase resulted in an elution time that was too long, whereas poor enantioseparation of (D,L)-fenfluramine and its *para* isomer was obtained with a DEA content in the mobile phase higher than 0.3%. Small amounts of 2-propanol (0.1%) added to the mobile phase consisting of *n*-hexane–DEA (99.9:0.1, v/v) gave rise to a co-elution of D- and L-fenfluramine enantiomers.

Attempts to separate the enantiomers of (D,L)-fenfluramine and its *ortho* and *para* isomers with different columns (Chiralcel OD, OD-R, OC and Chiralpak AD) did not succeed, whereas limited success was obtained using a Chiralcel OG column (Table 1). The Chiralcel OF column was more effective in terms of separation of the (D,L)-fenfluramine and *para*-(D,L)-fenfluramine enantiomers, whereas the racemic mixture of *ortho* isomers of fenfluramine did not give rise to any separation under the same conditions as those described for the other compounds.

However, when all isomers were combined, the chromatographic requirements became more complicated and a tandem-chromatography system, consisting of an $NH_2$-based column coupled with a Chiralcel OF column, was necessary to separate the dexfenfluramine from the related impurities (L-fenfluramine and its *para* and *ortho* isomers) (Fig. 3). Attempts to separate the above-mentioned compounds by coupling a silica-based column with a Chiralcel OF did not succeed. It is worth stressing that the amounts of *ortho*- and *para*-(D,L)-fenfluramine contained in the bulk products and pharmaceutical formulations tested were under the detection limits of the method (0.5% for each enantiomer of *para*-fenfluramine and 0.5% for *ortho*-fenfluramine).

The method is capable of accurately determining 0.5% of the L-enantiomer in final pharmaceutical products containing dexfenfluramine. It is worth noting that the content of the main impurity, L-fenfluramine, in commercial samples analysed, ranged between 1.0 and 2.0%. The detection limits obtained with this chromatographic system, using the Chiralcel OF column, were approximately 20 ng for each isomer of fenfluramine (signal-to-noise ratio greater than three), and they appeared adequate for application to industrial products (Fig. 4).

The calibration curves (five points for the impurities and dexfenfluramine) showed good linearity with a correlation coefficient of 0.9995 for *para*-(D,L)-fenfluramine ($c = 573493.7A - 10.8$) (the

Table 1
Chromatographic data for (D,L)-fenfluramine and *para*-(D,L)-fenfluramine on Chiralcel OF and Chiralcel OG[a] columns

| Compound | Column | $k_1'$[b] | $\alpha$[c] | $R_s$[d] | Eluent[e] |
|---|---|---|---|---|---|
| (D,L)-fenfluramine | Chiralcel OF | 4.19 | 1.17 | 1.95 | A |
| | | 2.70 | 1.17 | 2.03 | B |
| | | 2.36 | 1.14 | 1.91 | C |
| | | 1.59 | 1.11 | 1.01 | D |
| | Chiralcel OG | 5.40 | 1.08 | 0.95 | A |
| *para*-(D,L)-fenfluramine | Chiralcel OF | 5.26 | 1.13 | 1.55 | A |
| | | 3.18 | 1.22 | 2.75 | B |
| | | 2.80 | 1.12 | 1.68 | C |
| | | 1.92 | 1.07 | 0.92 | D |
| | Chiralcel OG | 5.99 | 1.11 | 1.39 | A |

[a]Chromatographic conditions are as described in Fig. 2.
[b]The capacity factor of the first eluted enantiomer.
[c]The enantioselectivity factor.
[d]The resolution factor.
[e]Eluents employed are: A = *n*-hexane–diethyl amine (99.95:0.05, v/v), B = *n*-hexane–diethyl amine (99.9:0.1, v/v), C = *n*-hexane–diethyl amine (99.8:0.2, v/v) and D = *n*-hexane–diethyl amine (99.7:0.3, v/v).

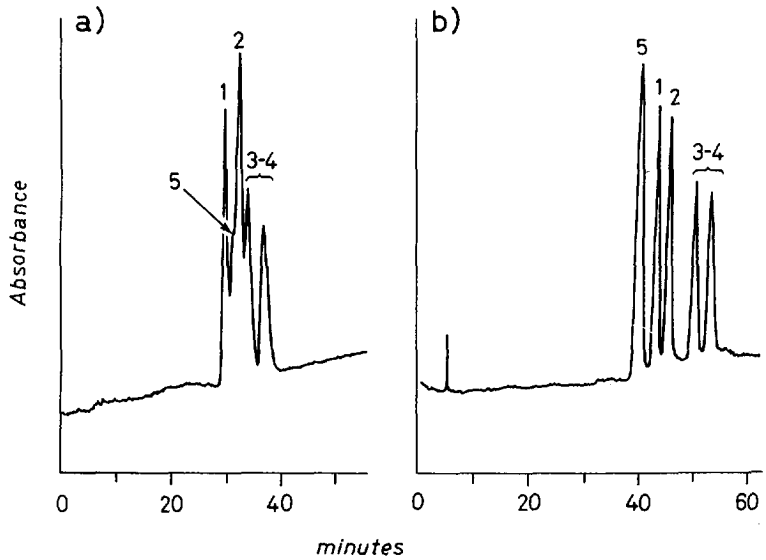


Fig. 3. HPLC of the compounds shown in Fig. 1. (a) Compounds 1–5; eluent, *n*-hexane–DEA (99.9:0.1, v/v); flow-rate, 0.5 ml/min; column, Chiralcel OF; wavelength, 265 nm; temperature, 25°C. (b) Compounds 1–5; eluent, *n*-hexane–DEA (99.9:0.1, v/v); flow-rate, 1.0 ml/min; tandem arrangement, Nucleosil-$NH_2$–Chiralcel OF coupled in series; wavelength, 265 nm; temperature, 25°C.

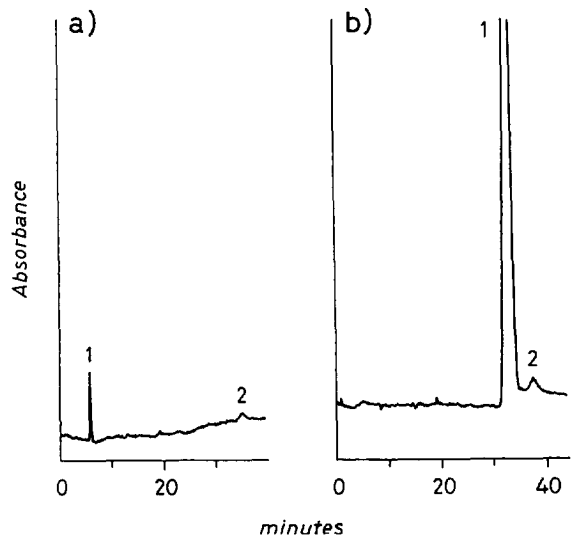


Fig. 4. (a) Minimum amount of L-fenfluramine detectable, 20 ng; eluent, *n*-hexane–DEA (99.9:0.1, v/v); flow-rate, 0.5 ml/min; column, Chiralcel OF; wavelength, 265 nm; temperature, 25°C. (b) HPLC of pharmaceutical formulation (capsules): 4 $\mu$g of D-fenfluramine, 44 ng L-fenfluramine; eluent, *n*-hexane–DEA (99.9:0.1, v/v); flow-rate, 0.5 ml/min; column, Chiralcel OF; wavelength, 265 nm; temperature, 25°C.

calibration curve was obtained by adding the area of the two enantiomers), 0.9999 for the *ortho*-(D,L)-fenfluramine ($c$ = 282972.2$A$ + 0.4), 0.9986 for the L-fenfluramine ($c$ = 216561.4$A$ + 1.7); 0.9985 for the dexfenfluramine ($c$ = 223562.8$A$ + 3.8) ($c$ = sample amount in ng, $A$ = area counts). The calibrations curves were obtained by using the tandem-chromatographic system described above. Table 2 shows the accuracy and precision data at each individual standard concentration.

The HPLC method described, was designed for quality assurance purposes for monitoring bulk products and pharmaceutical formulations. It appears to be easy to use and reproducible, the main advantage is that no derivatization is necessary to separate the drug from its enantiomeric and isomeric impurities. Therefore, the drawbacks due to racemization, or different rates of reaction of the single enantiomer, with the derivatizing agent, were avoided.

In conclusion, with the increasing interest in enantiomerically pure drug formulations from both pharmaceutical industries [12] and regulatory authorities [13–16], a chromatographic method capable of determining chiral impurities is quite suitable for quality control in pharmaceutical formulations.

Table 2
Inter-day precision and accuracy of *ortho*, *para* and (D,L)-fenfluramine standards

| Compound | Parameter[a] | Amounts of sample injected (ng/100 μl) | | | | |
|---|---|---|---|---|---|---|
| *ortho* | | 854 | 427 | 213 | 106 | 53 |
| | M | 855 | 424 | 215 | 104 | 55 |
| | R.S.D.(%) | 0.6 | 0.9 | 1.4 | 2.0 | 1.3 |
| | R.E.(%) | +0.1 | −0.7 | +0.9 | −1.9 | +3.8 |
| | *n* | 3 | 3 | 3 | 3 | 3 |
| *para* | | 2440 | 1220 | 490 | 240 | 120 |
| | M | 2422 | 1269 | 467 | 234 | 121 |
| | R.S.D.(%) | 0.2 | 0.8 | 2.4 | 6.4 | 11.6 |
| | R.E.(%) | −0.6 | +4.0 | −4.7 | −2.5 | +0.8 |
| | *n* | 3 | 3 | 3 | 3 | 3 |
| *levo* | | 243 | 162 | 81 | 40 | 20 |
| | M | 243.6 | 159.8 | 83.5 | 39.8 | 20.4 |
| | R.S.D.(%) | 3.5 | 1.5 | 6.9 | 7.9 | 7.4 |
| | R.E.(%) | +0.2 | −1.4 | +3.0 | −1.6 | +0.5 |
| | *n* | 3 | 3 | 3 | 3 | 3 |
| *dex* | | 6110 | 4075 | 2037 | 1018 | 407 |
| | M | 6121 | 4069 | 2022 | 982 | 425 |
| | R.S.D.(%) | 1.0 | 1.3 | 1.1 | 2.5 | 3.7 |
| | R.E.(%) | +0.2 | −0.2 | −0.7 | −3.5 | +4.4 |
| | *n* | 3 | 3 | 3 | 3 | 3 |

[a]M = mean; R.S.D. = relative standard deviation; R.E. = relative error: *n* = number of replicates.

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