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Determination of fluperlapine in human plasma by capillary gas chromatography*

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Abstract: A sensitive and simple gas chromatographic method was developed for the determination of fluperlapine in human blood plasma using amitriptyline as an internal standard. The compounds were extracted into diethyl ether, the extract chromatographed on a dimethyl silicone capillary column, and detected by nitrogen phosphorus selective flame ionization detector. The NP–FID responses, as peak area ratios of the compound and internal standard, were linearly correlated to their plasma concentrations between 10 and 1000 ng/ml. The limit of detection in plasma was 0.5 ng/ml. The relative recovery of fluperlapine was about 85%.

Keywords: *Fluperlapine determination; human plasma; capillary gas chromatography.*

Introduction

Fluperlapine, 3-fluoro-(4-methyl-1-piperazinyl)-11H dibenz[b,e]azepine (Fig. 1, structure 1) was developed by Sandoz Ltd. for use as a psychoactive agent [1]. According to pharmacological studies the non-classical clozapine type molecule regarding its antipsychotic effects seems to resemble clozapine but with less autonomic side effects, and with some antidepressant activity as well [2–6].

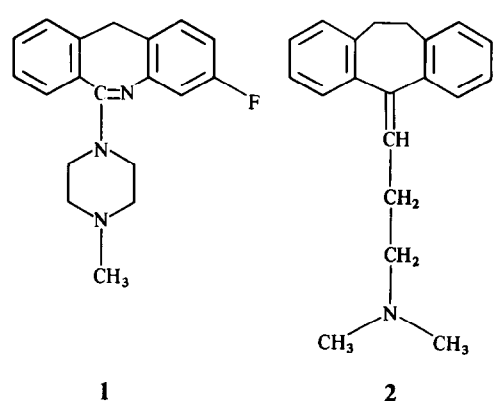

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Figure 1
Structure of fluperlapine (1) and the internal standard amitriptyline (2).

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Recently in our Institute a clinical pharmacological study was initiated by Sandoz Ltd., involving the measurement of the blood level of fluperlapine. No method was available in the literature for the determination of fluperlapine but a method for the gas chromatography of the drug was provided by Sandoz Ltd. using an OV 17 packed glass column at 250°C. However the determination of fluperlapine in biological samples demands higher selectivity and sensitivity and so a simple gas chromatographic method by which the dibenzazepine derivative could be determined in low concentrations in plasma was developed. The molecule displays very good chromatographic properties and, moreover, because of its nitrogen content, a very high sensitivity was achieved by using selective nitrogen–phosphorus flame ionisation detection. This paper describes the method and the plasma levels of fluperlapine in a patient receiving the drug during a six week period.

Experimental

Materials and reagents

Fluperlapine was donated by Sandoz Ltd. (Basle, Switzerland). Amitriptyline hydrochloride used as internal standard was obtained from EGIS Pharmaceutical Works (Budapest, Hungary). Diethyl ether, methanol and sodium hydroxide, all of them of reagent grade, were purchased from Reanal (Budapest, Hungary).

Preparation of standard solutions

Fluperlapine and the internal standard were dissolved in methanol to produce standard solutions, containing 1 g/l of these compounds each (base equivalent for amitriptyline). The solutions were further diluted with methanol to produce standard solutions containing 10 mg/l and 1 mg/l of fluperlapine and 10 mg/l of amitriptyline. The solutions were stored in the dark at 4°C for six months.

Calibration curve and quantification

Calibration curves for fluperlapine were prepared over the ranges of 0–50 ng/ml and 100–1000 ng/ml. Aliquots of blank plasma (1 ml) were spiked with 1, 2, 5, 10, 20, 50 ng and in a second 100, 200, 400, 600, 800, 900 and 1000 ng of fluperlapine, respectively, by adding 1, 2, 5 μl of a standard solution of 1 mg/l and 1, 2, 5, 10, 20, 40, 60, 80, 90 and 100 μl of a standard solution of 10 mg/l to the plasma samples. To each sample 300 ng internal standard (30 μl of standard solution of 10 mg/l) was added. Each standard plasma sample was processed according to the method described below. The ratios of the drug peak area to that of the internal standard were plotted against the known concentrations of fluperlapine in the plasma standards. Linear regression analysis gave a calibration line, found to be linear in the concentration ranges from 10–1000 ng/ml of plasma, and it could be used to calculate unknown concentrations of fluperlapine. (The standard curve was not linear at concentrations less than 10 ng/ml but the results were reproducible.) To calculate the percentage extraction, a calibration curve, a series of ten solutions with different concentrations: 10, 20, 50, 100, 200, 400, 600, 800, 900 and 1000 ng/ml of fluperlapine were prepared in methanol. The recovery was determined at all ten levels by comparing the peak areas obtained by direct injection of methanolic solutions to those obtained after the whole extraction procedure. For precision studies control samples were prepared by spiking blank plasma with fluperlapine of 100 ng/ml.

Extraction procedure for plasma samples

To a plasma sample of 1 ml were added 300 ng of amitriptyline (30 μl of the standard solution of 10 mg/l), 200 μl of 1 M NaOH and 3 ml of diethyl ether in a test tube. After mechanical shaking for 20 min, the sample was centrifuged at 3000 **g** for 10 min. The upper organic phase was removed by aspiration into a sample tube. The solvent was evaporated with a gentle stream of nitrogen. The residue was dissolved in 100 μl of methanol and aliquots of 3 μl were injected into the gas chromatograph.

Standard plasma samples were prepared daily by adding 100 ng fluperlapine and 300 ng of internal standard (10 and 30 μl of the standard solutions of 10 mg/l) to 1 ml of blank plasma. The reference samples were analysed together with the other samples. The peak area ratios of fluperlapine to the internal standard were used for quantitative evaluation.

Gas chromatography

The gas chromatograph employed was a Hewlett–Packard model 5840A equipped with a 18835B capillary inlet system and a nitrogen phosphorus selective flame ionization detector. The capillary column was 25 m × 0.20 mm i.d. fused silica tubing coated with dimethyl silicone film of 0.33 micrometer (Hewlett–Packard, USA). Nitrogen of high purity was used as both the carrier and auxiliary gas. The temperature programme was from 100°C at 30°C/min to 260°C. Column pressure was 0.196 MPa and the auxiliary gas flow rate was 30 ml/min. Hydrogen and air flow rates were 3 and 50 ml/min, respectively. Temperature settings were for capillary injection port in split mode (250°C) with a split ratio of 10:1, for NP–FID system (270°C). The alkali metal salt bead heating voltage was 17.1 V. The retention times for fluperlapine and amitriptyline were 8.43 and 6.67 min, respectively.

Results and Discussion

Extraction procedure for plasma

To determine the optimum extraction conditions, investigations were made with different solvents, and with the optimal solvent at different pH values.

For liquid–liquid extraction from plasma of 1 ml containing 100 ng fluperlapine, *n*-heptane, benzene, toluene, chloroform, ethyl acetate and diethyl ether were examined at pH 9. The best recoveries were obtained with diethyl ether. Diethyl ether extraction of the drug from plasma at pH 1, 3, 5, 7, 9, 10, 11 showed that maximum recoveries were achieved at pH 11 with a ratio of plasma to solvent of 1:3.

Standard curve, accuracy and reproducibility

Known amounts of fluperlapine (1, 2, 5, 10, 20, 50, 100, 200, 400, 600, 800, 900 and 1000 ng) were added to 1 ml of drug free plasma; the concentration of internal standard was 300 ng/ml in each plasma sample. The calibration curve was linear over the range 10–1000 ng/ml of fluperlapine.

The linear correlation coefficient was calculated by the least-squares method, Y representing the ratio of peak area of fluperlapine to the peak area of the internal standard, and X the concentration of fluperlapine:

$$Y = 0.0186X - 0.1664, \qquad r = 0.992.$$

The accuracy and the reproducibility of the method were determined by processing blank plasma samples spiked with several concentrations of fluperlapine.

Precision studies of control plasma samples gave acceptable coefficients of variation. Within run precision: coefficient of variation for plasma spiked with 100 ng/ml of fluperlapine was 0.78% (mean: 95.2 ± 0.891, n = 12). Between run reproducibility: control samples containing 100 ng/ml of fluperlapine were measured over a period of 3 months to assess the stability of solutions. The mean day to day reproducibility expressed as coefficient of variation was 1.89% (mean: 92.4 ± 2.43 ng/ml fluperlapine, n = 30). Storage of samples for at least 3 months at −18°C was considered to be acceptable.

Recovery and detection limit

The extraction efficiency for fluperlapine was determined at ten levels (between 10–1000 ng/ml, see above) by measuring the peak areas of the compound after extraction from spiked plasma. These peak areas were compared to those obtained by direct injection of the solutions with the corresponding standard concentrations prepared in methanol. The recovery was about 85% and practically independent of concentration. Results are given in Table 1.

The lower limit of detection of fluperlapine based on a signal-to-noise ratio of 2:1 was estimated at 10 pg by direct injection of the compound from the standard solution which is equivalent to about 0.5 ng/ml detection limit for plasma extracts.

Application to clinical studies

The proposed method was used in a clinical pharmacological investigation of the plasma of schizophrenic patients who had received varying doses of fluperlapine. Results of the analyses of plasma samples of a schizophrenic patient receiving fluperlapine over a period of six weeks are given in Table 2. Figure 2 shows the chromatograms of the extract of a drug free plasma sample and of a plasma sample of a patient who had received fluperlapine.

Table 1
Accuracy, reproducibility and recoveries for the analysis of fluperlapine in plasma. Peak area ratios of fluperlapine and the internal standard are linearly correlated to their plasma concentrations. The calibration curve is linear over the range 10–1000 ng/ml of fluperlapine with a slope of 0.019 ± 0.0001, a Y intercept of −0.166 ± 0.042 and a correlation coefficient of 0.9992

Fluperlapine concentration added (ng/ml)	Mean concentration found (ng/ml), n = 8	Precision RSD (%)†	Recovery (%)‡
10	8.3*	4.3	83
20	17.1*	2.7	85
50	41.9*	2.3	84
100	84	3.9	84
200	171	2.8	85
400	341	3.0	85
600	507	3.5	84
800	701	2.0	87
900	779	1.8	86
1000	857	1.7	86

*n = 9.
†Relative Standard Deviation.
‡Rounded data.

Table 2
Concentrations of fluperlapine measured in plasma samples of a schizophrenic patient receiving varying doses of the drug over a period of six weeks. Blood samples were taken 2 h after oral administration of fluperlapine

Day	Dosage (mg)	Fluperlapine concentration (ng/ml) mean ± SD, $n = 3$
1	200	—
3	350	118 ± 1.9
7	550	411 ± 2.1
14	650	477 ± 2.5
21	600	436 ± 0.9
28	700	505 ± 2.8
35	450	265 ± 1.4
42	450	277 ± 3.1

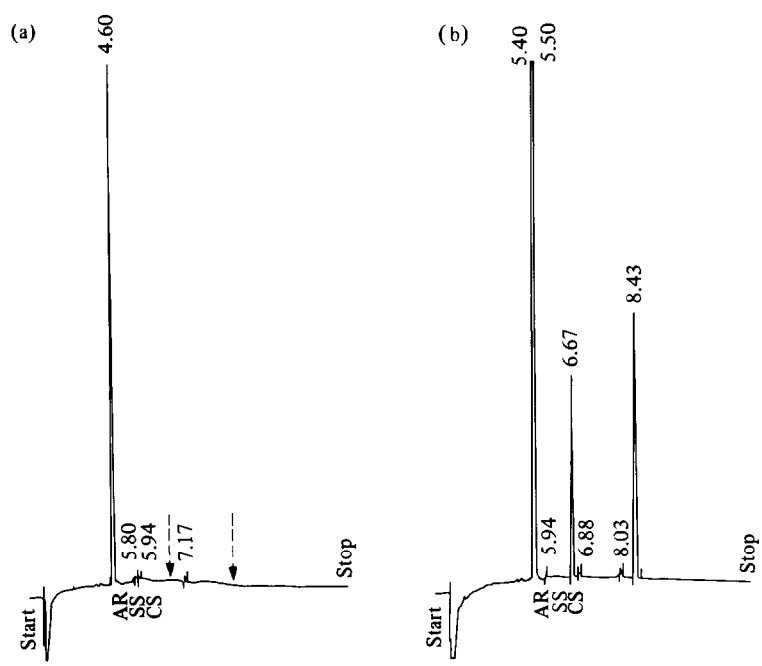

Figure 2
(a) Chromatogram of the extract from drug free plasma sample, no internal standard was added; (b) chromatogram of the extract from plasma of a patient taken 2 h after administering an oral dose of 500 mg fluperlapine. Concentration of fluperlapine is 523 ng/ml.

For checking possible chromatographic interferences the retention times of some drugs commonly used in psychiatric treatment were established under the extraction and chromatographic conditions applied. None of the drugs examined interfered with fluperlapine. Retention times are given in Table 3.

Table 3
Retention times of some drugs using the chromatographic conditions described for fluperlapine

Drug	Retention time (min)
Fluperlapine	8.43
Amitriptyline	6.67
Promazine	6.49
Chlorpromazine	7.63
Levomepromazine	8.11
Thioridazine	8.70
Trifluperidol	8.89
Haloperidol	7.12
Clozapine	11.43
Phenobarbitone	5.12
Diazepam	7.04
Medazepam	6.19
Butobarbitone	3.52
Tofizopam	14.05
Maprotilinie	6.42
Imipramine	7.17
Desipramine	7.31
Clomipramine	8.08
Nortriptyline	6.85

Conclusion

A method was developed to measure the quantity of fluperlapine in human plasma at low concentrations. The use of capillary gas chromatography and NP–FID detection provided high separation efficiency and sensitive detection of fluperlapine. A simple procedure for the determination in plasma samples containing 10 ng/ml or more of the compound without the need to purify the extracts is described. The method is quick to use for routine measurements, and sufficiently sensitive for use in pharmacokinetic studies.

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