Journal of Chromatography, 527 (1990) 436–440
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5197

Note

Determination of fluparoxan (GR50360) in plasma by gas chromatography

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(First received October 3rd, 1989; revised manuscript received December 28th, 1989)

Fluparoxan (GR50360, Fig. 1), a putative antidepressant, is a potent and highly selective antagonist at central noradrenergic α-2 receptors [1,2]. A method for the determination of fluparoxan in plasma, suitable for human pharmacokinetic studies, was required. This would involve measurement of plasma levels over a wide range of concentrations. A simple high-performance liquid chromatographic method with ultraviolet detection had been used by this laboratory to monitor fluparoxan in preliminary animal pharmacokinetic and toxicology studies. However, this method was insufficiently sensitive for the assay of human samples. This paper describes a rapid and sensitive gas chromatographic (GC) analysis of fluparoxan in plasma, which is suitable for such studies.

Fig. 1. Structures of fluparoxan (I) and the internal standard GR32462 (II).

0378-4347/90/$03.50

EXPERIMENTAL

Instrumental

A Perkin Elmer (Perkin Elmer, Beaconsfield, U.K.) 8500 gas chromatograph equipped with a 925-MBq ^{63}Ni electron-capture detector, on-column injector and linked to a Trilab 2000 integration system (Trivector, Sandy, U.K.) was used for analysis. The column was 12 m × 0.53 mm I.D. fused-silica BP-1 (SGE, Ringwood, Australia) which was connected to a 1 m × 0.2 mm I.D. deactivated fused-silica pre-column (SGE). The chromatographic conditions were as follows: initial oven temperature, 78 °C for 0.5 min, followed by a temperature gradient of 30 °C min^{-1} to 230 °C for 3.5 min; detector temperature, 350 °C; carrier gas, helium at 24 kPa; make-up gas, argon–methane at 290 kPa.

Mass spectral (MS) analysis was performed on a Finnigan (San Jose, CA, U.S.A.) MAT 4500 quadrupole mass spectrometer (electron beam, 70 eV) linked with a INCOS data system (software revision 5.5E) and interfaced to a Finnigan 9610 gas chromatograph. GC operating conditions were as given above.

Reagents and solutions

Stock standards of fluparoxan and internal standard (GR32462, II, Fig. 1), both synthesised at Glaxo Group Research (Greenford, U.K.) were prepared by weighing 12.3 and 10.0 mg of the hydrochloride salt of each compound, respectively, and dissolving in 10 ml of distilled water. The working standards of 1.0 $\mu g\ ml^{-1}$ fluparoxan and 0.83 $\mu g\ ml^{-1}$ II were made by appropriate dilution of the stock standards with blank plasma and distilled water, respectively. Both stock and working solutions were stored in the dark at 4 °C and were stable for at least one month.

Heptafluorobutyrylimidazole (HFBI) was diluted 100 times with cyclohexane and prepared just prior to each set of analyses.

All other solvents and chemicals were of analytical quality (BDH, Poole, U.K.).

Method

Calibration standards (range 10–1000 ng ml^{-1}) were prepared in glass screw-capped tubes by appropriate dilution of the fluparoxan working standard with blank plasma to give a final volume of 1 ml. Each 1-ml aliquot of plasma (standards and samples) was mixed with internal standard working solution (50 μl). All samples were then extracted (10 min) with diethyl ether (6 ml) on a rotary mixer. After centrifugation (800 *g*, 5 min) the organic layer was transferred to separate tubes and evaporated to dryness by heating (40 °C). Each extract was derivatised by adding HFBI solution (0.5 ml) and heating (70 °C, 1 h). Following a wash with 1 *M* sodium hydroxide (10 ml) to remove excess

derivatising reagent, an aliquot of the organic layer (1–2 μl) was injected onto the chromatographic column.

Clinical pharmacokinetic study

Two young healthy males (aged between 18 and 24 years) received a single 8-mg oral dose of fluparoxan following overnight fasting. Venous blood samples were drawn into heparinised tubes prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 20, 24, 28, 32 and 48 h. The samples were centrifuged (1800 *g*, 10 min) and the plasma obtained was stored at −20 °C prior to assay. Concentrations of fluparoxan in all samples were determined as described above. Pharmacokinetic parameters of fluparoxan (distribution, elimination and clearance) were determined using a non-linear regression method.

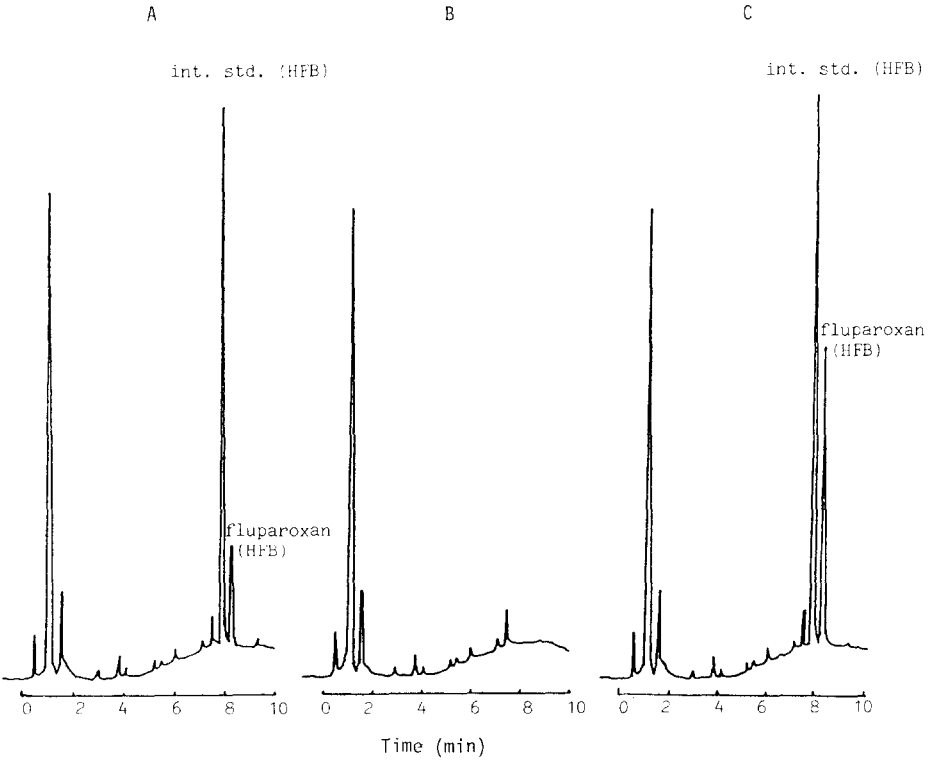

Fig. 2. Chromatograms of (A) a calibration standard containing 315 ng ml^{-1} internal standard (II) and 50 ng ml^{-1} fluparoxan (HFB derivatives), (B) a drug-free blank plasma sample and (C) a plasma sample taken from a human volunteer 16 h after receiving a single oral dose of 8 mg fluparoxan.

RESULTS

Evaluation of method

Under the conditions described for chromatography, derivatised fluparoxan and II gave two well resolved and well defined peaks with retention times of approximately 8.2 and 8.0 min, respectively (Fig. 2). No interfering peaks were observed in blank (drug-free) plasma. Extraction was maximal (70%) at plasma pH for both fluparoxan and II. Peak-height ratio expressed relative to

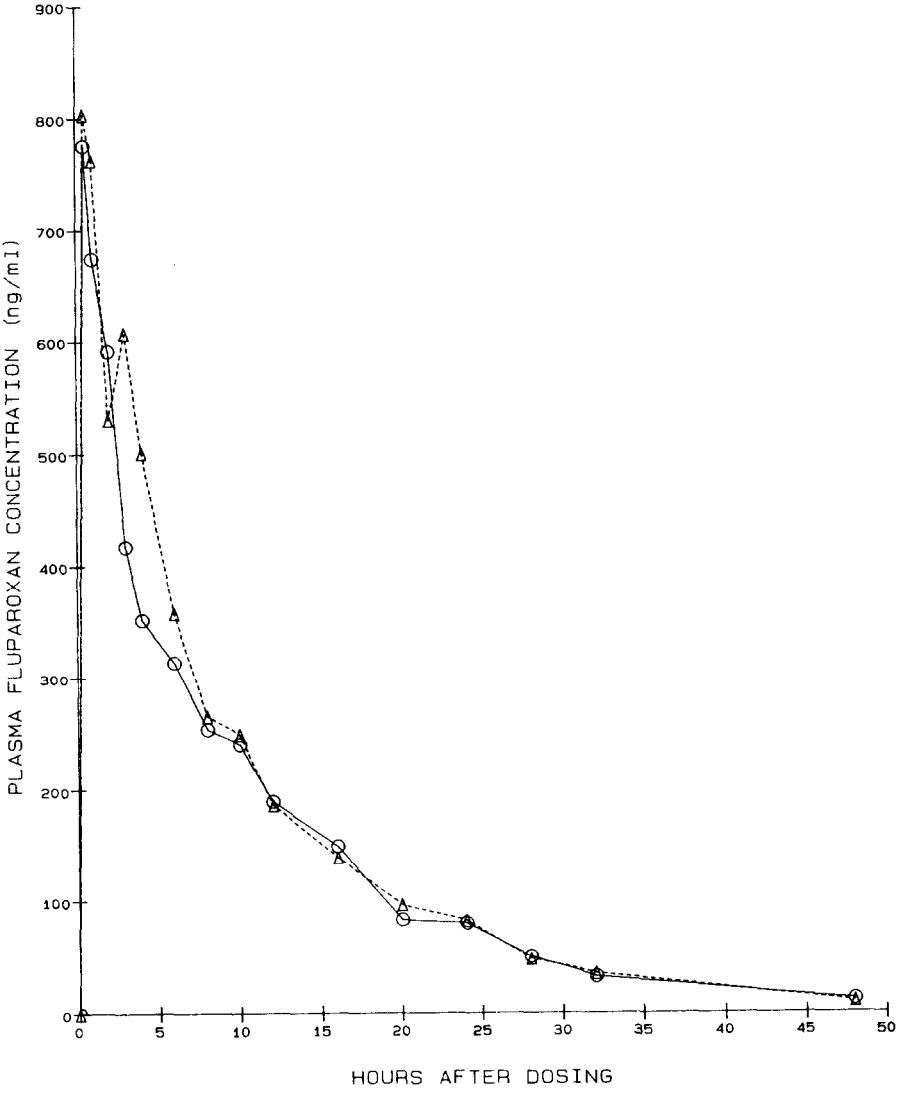

Fig. 3. Plasma fluparoxan concentrations in the two human volunteers.

plasma fluparoxan concentration gave a linear plot of slope $3.6 \cdot 10^{-3}$ ml ng^{-1} and intercept $6.2 \cdot 10^{-3}$. Replicate samples ($n=6$) of standards containing 10, 50, 200, 500 and 1000 ng ml^{-1} fluparoxan yielded within-day coefficients of variation of 7.9, 2.0, 5.8, 2.7 and 3.8%, respectively. The between-day coefficient of variation for the slope of calibration curves run on ten different days was 7.8%. The limit of detection for fluparoxan was taken as the concentration of the bottom standard (10 ng ml^{-1}). The presence of fluparoxan in the plasma of dosed human volunteers was confirmed by comparing the electron-impact mass spectra of the derivatised product with that of a pure sample.

Pharmacokinetic results

Plasma fluparoxan concentrations in the two volunteer subjects are shown in Fig. 3. The mean pharmacokinetic parameters for fluparoxan obtained for the two volunteers were: elimination half-life, 7.8 h; apparent volume of distribution, 13.4 l; plasma clearance, 19.7 ml min^{-1}.

DISCUSSION

The described method allows for the sensitive and reliable quantitation of fluparoxan in human plasma and is suitable for the routine analysis of clinical samples. Drug-free plasma does not contain any interfering endogenous substances, and this eliminates the necessity for clean-up procedures. The sensitivity achieved by the method has been found to be adequate for all pharmacokinetic studies undertaken. However, there is scope for lowering the limit of detection simply if required. This could be achieved by using standards covering a narrow and lower range of concentrations than described and also by decreasing the volume of HFBI solution added at the derivatisation stage. More recently this method has been successfully adapted for use in the split and programmable temperature vaporisation injection modes which allow the use of an autoinjector.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. J. Ayrton, Mr. M.J. Daniel and Dr. H. Hassani for their helpful suggestions and Miss P.A. Williams for her help in preparation of the manuscript.

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