Journal of Chromatography, 230 (1982) 199—202
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1231

Note

High-performance liquid chromatographic assay for determination of a new β-blocking agent FM 24

M.A. LEFEBVRE, B. JULIAN and J.B. FOURTILLAN*

Centre d'Études et de Recherche CEROM, 4 Rue des Gaillards, 86000 Poitiers (France)

(First received November 10th, 1981; revised manuscript received January 19th, 1982)

FM 24, 1-(2-*exo*-bicylco-2,2,1-hept-2-yl phenoxy)-3-(1-methylethyl)amino-2-propanol, (Fig. 1) is a new β-blocking agent whose activity has been demonstrated previously [1, 2]. Recently, a gas chromatographic method was reported by Bernard et al. [3], including threefold extraction from plasma, derivatization with pentafluoropropionic anhydride and electron-capture detection. The related technique presents critical steps such as derivatization and detection, and is too time-consuming.

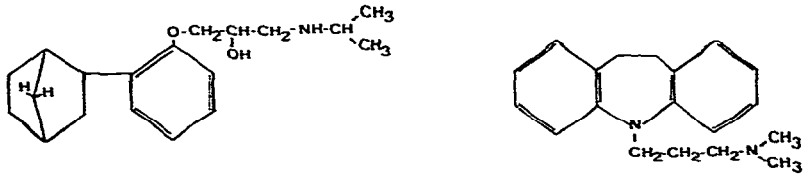

Fig. 1. Chemical structure of FM 24 and imipramine.

The aim of the present paper is to describe a simple and rapid technique allowing plasma concentration measurement as low as 5 ng/ml, suitable with FM 24 plasma levels reached following therapeutic doses. The use of a cation-exchange resin before extraction with hexane avoids any overlap in chromatograms which could be due to endogenous components. High-performance liquid chromatography is, in other respects, a reliable technique easy to carry out and suitable for the analysis of many samples a day.

0378-4347/82/0000—0000/$02.75

MATERIALS AND METHODS

Reagents and chemicals

FM 24 was generously supplied by Pharmindustrie (Gennevilliers, France) and imipramine, used as internal standard, was obtained from Ciba-Geigy (Basel, Switzerland). Hexane and acetonitrile were purchased from Burdick and Jackson (Interchim, Montluçon, France). Sodium hydroxide and acetic acid were suprapur reagents from E. Merck (Paris, France). Sodium sulfate and boric acid were analytical grade reagents. In order to pair amines such as FM 24 or imipramine in the reversed-phase liquid chromatographic mode, 1-heptanesulfonic acid, obtained from Eastman Kodak (Interchim), was used as counter-ion. Carboxymethyl Sephadex (40—120 μm) was used as the resin (Sigma, Interchim).

Apparatus

Cation-exchange separation was realized on standard econo-columns, 10 × 0.5 cm, from Bio-Rad (Touzart et Matignon, Vitry, France).

High-performance liquid chromatography was performed on a Waters instrument equipped with a 6000 A pump as delivery system, and a WISP 710 A automatic injector (Waters Assoc., Paris, France). Detection was carried out with a 970 FS Schoeffel fluorometer (Cunow, Paris, France) with an excitation wavelength of 230 nm and without any emission cut-off filter. Separation was achieved with an isocratic solvent system of water—acetonitrile—acetic acid (48:47:5, v/v) with a flow-rate of 1.4 ml/min, using a μBondapak C_{18} column (Waters Assoc.). The chart speed was set at 0.25 cm/min on the Houston instrument chart recorder (Waters Assoc.). The eluent was degassed under reduced pressure before use and the chromatographic system was operated at ambient temperature. All glassware was washed with hydrochloric acid (2 *M*) and rinsed with deionised water.

Extraction procedure

Blood samples were collected in heparinized tubes then centrifuged at 400 *g* for 10 min. A 2-ml plasma aliquot, taken for analysis, was supplemented with 50 μl of a 20 μg/ml imipramine solution. Econo-columns were filled with CM-Sephadex (to a resin height of 4 cm). Then 2 ml of plasma were placed in the column and the resin was washed twice with 5 ml of water. Using first 2 ml of 0.1 *N* sodium hydroxide in 0.1 *M* sodium sulfate solution, then twice 2 ml of borate buffer (pH 9), FM 24 and imipramine (internal standard) were eluted. The aqueous phases were placed with 9 ml of ethyl acetate in 20-ml screw-capped tubes which were shaken for 10 min. The tubes were then spun at 600 *g* for 15 min, and the organic layer evaporated to dryness under a gentle stream of nitrogen. The residue was then dissolved in 200 μl of mobile phase and 100-μl aliquots were directly injected into the chromatograph. Plasma peaks were identified by comparing their retention times, and quantitative analysis was performed on peak heights using a standard curve.

Calibration curves

A standard calibration curve was established by adding known amounts of

imipramine (0.5 μg/ml) and FM 24 to plasma reference samples, in order to obtain standard concentrations ranging from 0.0625 to 1 μg/ml. Quantitation was performed by drawing the baseline and measuring the peak height of the interesting compounds. The peak height ratio of FM 24/imipramine was calculated and a standard curve was constructed by plotting the peak height ratios against the added amounts (Fig. 2). Reproducibility trials were tested by assaying extracts of ten plasma samples supplemented with 0.05 μg/ml and 0.5 μg/ml FM 24.

RESULTS AND DISCUSSION

Fig. 2 illustrates a chromatogram obtained when FM 24 was injected as an extract of human plasma. Using the mobile phase described in the method section, FM 24 and imipramine had a mean retention time of 5.8 and 4.5 min, respectively. The total time required for the analysis of each sample was 10 min. Peaks were well separated and no interference from endogenous compounds was observed. The extraction recovery of FM 24 was 85.2 ± 3.4%. The standard calibration curve was generated by plotting peak height ratios against the FM 24 concentration. The relationship was linear in the concentration range 0.0625—1 μg/ml, with an excellent correlation coefficient of 0.999 ($y = 2.458x + 0.003$, where y is the peak height ratio and x is the FM 24 concentration). The limit of detection for FM 24 was 5 ng/ml. Reproducibility of the assay was tested by ten replicate analyses of plasma fortified with 0.05 or 1 μg of FM 24; the coefficient of variation was 13.5 and 3.9%, respectively.

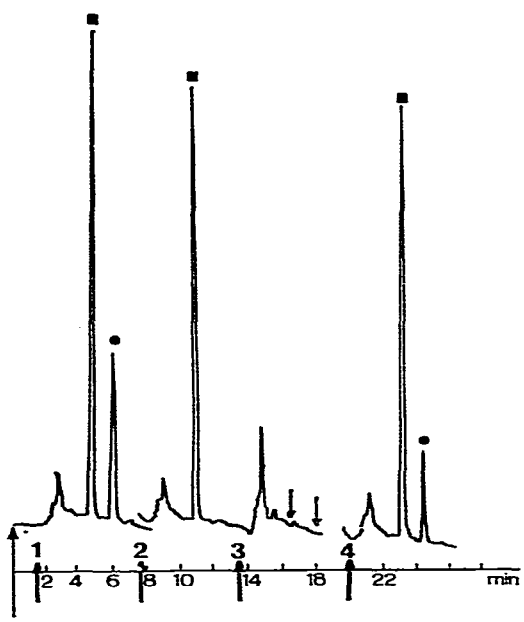

Fig. 2. Typical chromatograms of plasma samples supplemented with (1) 1 μg of imipramine (▪) and 0.125 μg of FM 24 (•); and (2) 1 μg of imipramine. (3) Blank plasma; (4) patient's plasma containing 0.088 μg/ml FM 24, supplemented with 1 μg of imipramine (internal standard).

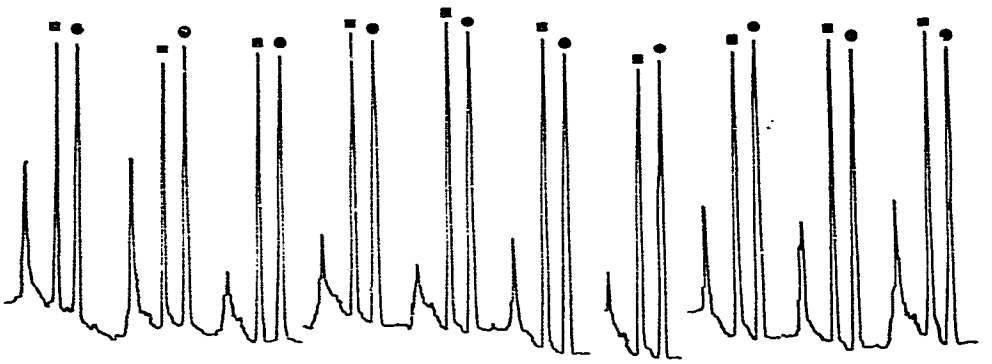

Fig. 3 Chromatogram obtained in reproducibility assay performed with 0.5 μg/ml FM 24. (■) Imipramine, (●) FM 24.

This method had been compared to gas chromatography—mass spectrometry (GC—MS) in the laboratory. The limit of the assay with GC—MS, 0.5 ng/ml, allowed the FM 24 plasma level to be followed over 48 h. Although the detection limit of the HPLC technique was not sufficient to detect such concentrations, it could measure FM 24 plasma concentrations as well as urine concentrations for 12 h, after an 80-mg oral administration. In addition, HPLC did not require a derivatization step, and the time needed for the assay allowed the analysis of a large number of samples a day.

In conclusion, despite the fact that FM 24 plasma levels could not be followed as far as necessary to define pharmacokinetic parameters after single 80-mg oral doses, the described technique appears to possess a sufficient degree of specificity, sensitivity and reliability to be employed for experimental or clinical pharmacological studies.

REFERENCES

1 G. Le Fur, J.J. Paillard, C. Rougeot and A. Uzan, Eur. J. Pharmacol., 67 (1980) 413.
2 C.T. Dollery, H.J. Dargie, E.J. Rowlands, J. Sassard, G. Cuisinaud and N. Bernard, Abstract 8th International Congress of Pharmacology, Japanese Pharmacological Society and Science Council of Japan, 1981, Abstr. No. 1644.
3 N. Bernard, G. Cuisinaud, C. Jozefczak, M. Seccia, N. Ferry and J. Sassard, J. Chromatogr., 183 (1980) 99.