Journal of Chromatography, 183 (1980) 99—103
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 581

Note

Sensitive gas chromatographic method for the determination in plasma of FM 24, 1-(2-*exo*-bicyclo[2,2,1]hept-2-ylphenoxy)-3-[(1-methylethyl)amino]-2-propanol, a new β-adrenoceptor blocking agent

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(First received December 17th, 1979; revised manuscript received February 22nd, 1980)

FM 24, 1-(2-*exo*-bicyclo[2,2,1]hept-2-ylphenoxy)-3-[(1-methylethyl)amino]-2-propanol, is a new and very potent β-adrenoceptor blocking agent whose duration of action in animal experiments has been shown to be two to five times longer than that of propranolol [1—3]. The evaluation of the pharmacokinetic profile of such a long acting drug appeared important for the better understanding of its mechanism of action, and for more efficient therapeutic application. Thus, in the course of clinical investigation of this drug, it became necessary to develop a highly sensitive and specific method for the determination of plasma levels of FM 24.

EXPERIMENTAL

Standards and reagents

FM 24 and FM 25 used as internal standard were generously supplied by Pharmindustrie (Gennevilliers, France). Their structural formulae are shown below.

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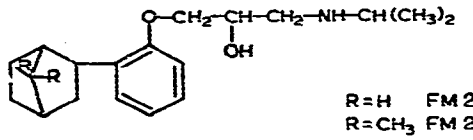

The following reagents (all purchased from Merck, Darmstadt, G.F.R.) were used: toluene, *n*-hexane, methanol, ethyl acetate, 1 *N* sodium hydroxide, 0.1 *N* hydrochloric acid (the organic solvents were distilled before use, then washed with sodium hydroxide and hydrochloric acid). Dimethylchlorosilane (DMCS) and pentafluoropropionic anhydride (PFPA) were obtained from Pierce (Rockford, IL, U.S.A.).

Glassware

All glassware was cleaned in chromic acid. Conical and round-bottomed glass centrifuge tubes were silanized with 5% DMCS in toluene, then rinsed with methanol.

Gas chromatographic conditions

Analyses were performed under isothermal conditions on a Hewlett-Packard Model 5710A gas chromatograph equipped with a ^{63}Ni linear electron-capture detector. The glass column (2 m × 3 mm I.D.) was packed with Chromosorb W HP (100—120 mesh) coated with 3% OV-17 (Pierce), and conditioned for 1 h at 260°C (argon—methane (90:10) carrier gas flow-rate, 30 ml/min), 3 h at 320°C (no gas flow) and 24 h at 270°C (carrier gas flow-rate 40 ml/min). The column temperature was 215°C, injection port temperature 250°C, detector temperature 300°C and carrier gas (argon—methane) flow-rate 40 ml/min.

Mass spectrometric conditions

A Micromass VG organic 305 gas chromatograph—mass spectrometer with a VG Data System M8 3R was used. Spectra were obtained under the following conditions in the electron-impact mode: electron energy 70 eV, trap current 200 μA, accelerating voltage 4 kV, ion source temperature 200°C and molecular separation temperature 260°C. In gas chromatographic—mass spectrometric (GC—MS) analyses, the same column was used as described under gas chromatographic conditions, except that the helium flow-rate was reduced to 20 ml/min.

Sample preparation

Extraction from plasma. In a glass-stoppered centrifuge tube was placed 0.5 ml of internal standard solution (1 μg/ml in methanol) and evaporated to dryness under a gentle stream of nitrogen, then 1 ml of plasma sample, 0.5 ml of 1 *N* sodium hydroxide and 7 ml of toluene were added. The tube was placed on a rotating mixer (60 rpm) for 15 min and then centrifuged at 4°C for 15 min at 1200 *g*. The organic phase (6.5 ml) was transferred to another centrifuge tube and back-extracted with 5 ml of 0.1 *N* hydrochloric acid by shaking for 15 min. After centrifugation (10 min), 4.5 ml of acidic aqueous layer were transferred to another centrifuge tube, alkalinized with 1 ml of 1 *N* sodium hydroxide and extracted with 6 ml of hexane by shaking for 15 min. After

centrifugation (10 min), 5 ml of organic phase were transferred to a conical test-tube and evaporated to dryness in a water-bath at 50—60°C under nitrogen. The residue was submitted to derivatization followed by analysis.

Derivatization. A 50-μl volume of PFPA (1:1 solution in ethyl acetate) was then added to the dry residue. The tube was capped and heated at 65—70°C for 1 h, then the excess of reagent was evaporated to dryness at 50—60°C under a gentle stream of nitrogen. When the residue was dried, the nitrogen flow-rate, was increased for a further 5 min in order to remove compounds derived from the reaction mixture which would appear as interfering peaks on the chromatogram. After dissolution in 150 μl of hexane, 5 μl of this solution were injected into the gas chromatograph.

Standard curves

Varying quantities of FM 24 (5—500 ng) and 500 ng of the internal standard were added to 1 ml of human plasma control and carried through the analytical procedure. The ratio of the peak areas of FM 24 to FM 25 were plotted against the concentration of FM 24.

RESULTS AND DISCUSSION

Typical chromatograms obtained with blank plasma before and after spiking with a known amount of FM 24 and with a plasma sample in a patient receiving FM 24 by the oral route are shown in Fig. 1. The peaks of the two compounds were well resolved with retention times of 4.5 and 6.8 min for FM 24 and FM

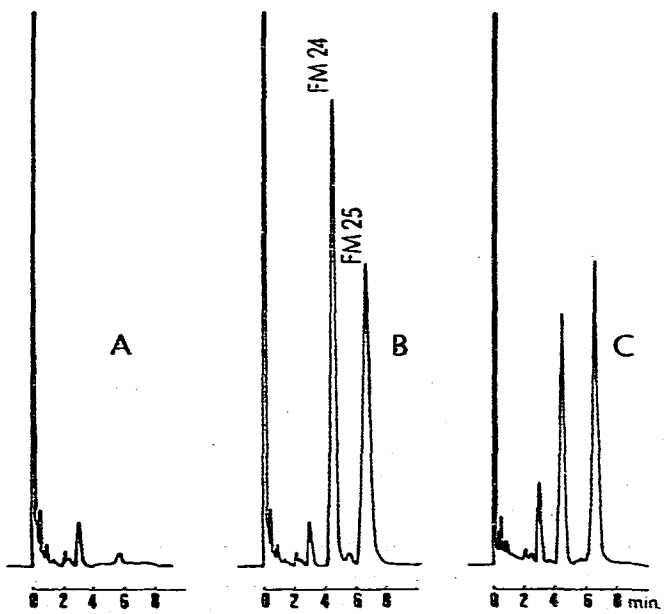

Fig. 1. Gas chromatograms of plasma extracts. (A) Plasma free from drug; (B) plasma spiked with FM 24 (100 ng/ml) and FM 25 (500 ng/ml); (C) plasma from a patient receiving 40 mg of FM 24 orally.

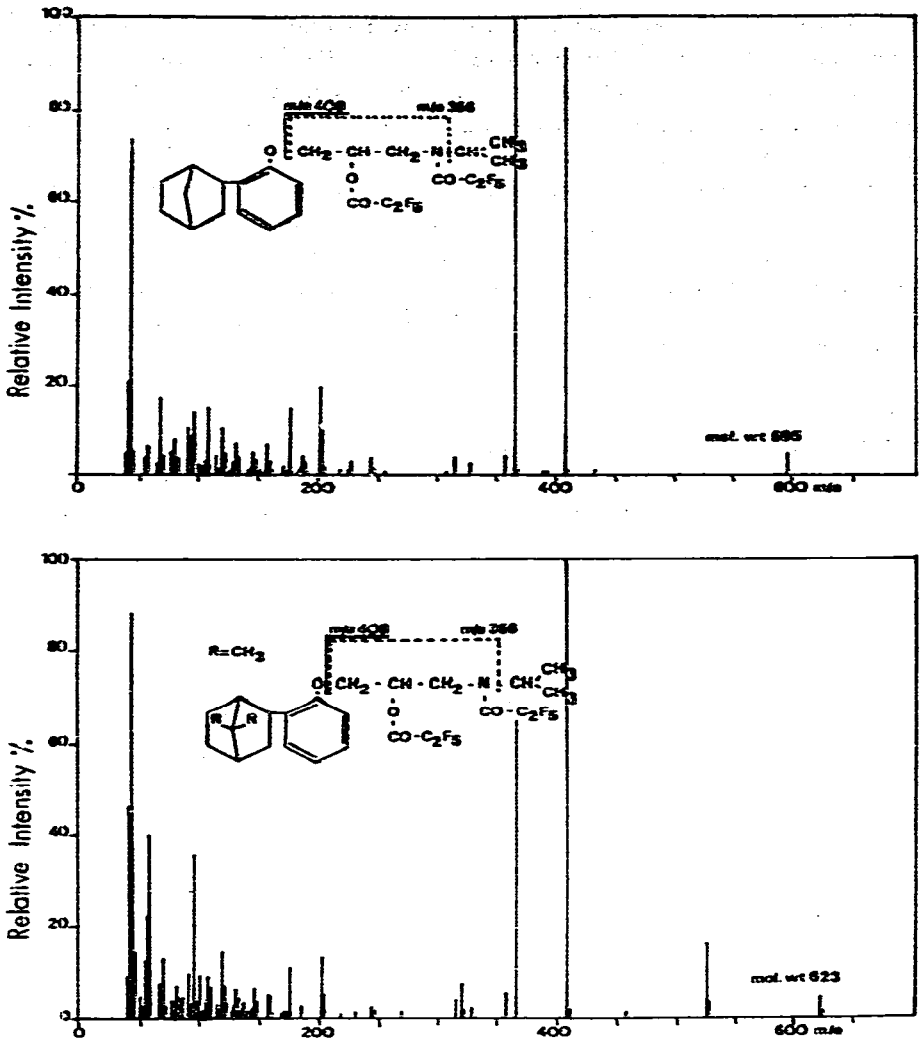

Fig. 2. Mass spectra obtained during GC—MS analyses for dipentafluoropropionates of FM 24 (top) and FM 25 (bottom).

25, respectively. No interferences were observed from endogenous substances.

The GC—MS analysis confirmed the identity of the GC peaks. Those due to the reaction products of FM 24 and FM 25 with PFPA showed molecular ions at *m/e* 595 and 623 respectively in the mass spectra corresponding to the formation of the dipentafluoropropionates (Fig. 2). Their structure was confirmed by the observation of ions at *m/e* 408 and *m/e* 366, the formation of which from molecular ion is specific for a β-blocker aryloxy group [4].

A standard calibration graph obtained after extraction of FM 24 from plasma was linear in the range of concentration studied ($y = 0.0042\,x + 0.00645$; $r = 0.9998$). The reproducibility of the method is reported in Table I. The recovery of the extraction procedure determined with ^{14}C-labelled FM 24 was found to be 72.5%.

TABLE I

ACCURACY OF DETERMINATION OF FM 24 IN HUMAN PLASMA

Amount added (ng/ml)	Average of 6 assays ± S.D. (ng/ml)	Coefficient of variation (%)
5	6.0 ± 0.6	9.9
10	10.8 ± 0.5	4.6
20	19.7 ± 0.9	4.6
50	51.1 ± 1.2	2.3
100	101.4 ± 2.5	2.5
200	194.2 ± 4.6	2.4
500	503.7 ± 13.3	2.6

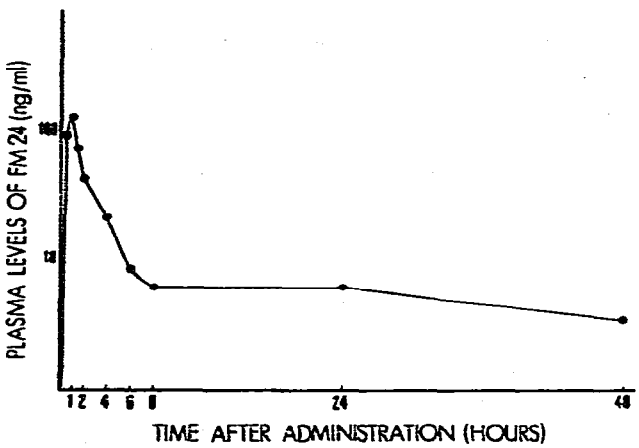

Fig. 3. Plasma level—time curve obtained for FM 24 following a single oral administration of 40 mg of the drug to a patient.

The absolute sensitivity of the electron-capture detector was about 30 pg for FM 24; this means that under the prescribed conditions, it is possible to detect 1 ng/ml of FM 24 in plasma.

The method has been applied to the determination of FM 24 in plasma of patients treated with this β-blocker. Fig. 3 illustrates the time course of plasma levels of FM 24 observed in a patient after a single oral administration of 40 mg of the drug. The observation of the last part of the curve clearly indicates that the method described above allows the pharmacokinetic study of FM 24 even in the case of a single oral administration of a very small dose in man.

ACKNOWLEDGEMENTS

The authors thank Mr. D. Deruaz (Mass Spectrometry Center of the Faculty of Pharmacy) for the mass spectrometric analyses, and Dr. A. Uzan (Pharmindustrie) for the generous gift of FM 24 and FM 25.

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