Journal of Chromatography, 311 (1984) 199—205
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2224

Note

Simple method for routine determination of betaxolol in blood and urine by automated high-performance liquid chromatography with fluorimetric detection

H. CAQUERET and G. BIANCHETTI*,*

LERS-Synthélabo, 58, rue de la Glacière, 75013 - Paris (France)

(First received March 2nd, 1984; revised manuscript received April 30th, 1984)

Betaxolol, (±)-1-{4-[2-(cyclopropylmethoxy)ethyl]phenoxy}-3-isopropylamino-2-propanol hydrochloride (Kerlon®), is a new adrenoceptor blocking agent, approved for the treatment of hypertension in many European countries. In two previously published reports, the quantification of betaxolol in biological fluids with gas—liquid chromatography (GLC) and electron-capture detection was described [1,2]. These methods are time-consuming because several extraction steps are necessary in order to obtain clean chromatograms. Moreover, derivatization with heptafluorobutyric anhydride is needed to allow the quantification of concentrations as low as 0.5 ng/ml betaxolol. These GLC methods are specially suitable for pharmacokinetic studies. Betaxolol has a long terminal half-life (16—22 h), and following oral administration of a 20-mg dose the peak blood concentration ranges between 30 and 60 ng/ml. Hence, it is absolutely necessary to have a method sensitive enough to follow betaxolol blood concentrations down to 1—2 ng/ml with good precision in order to measure elimination half-lives.

For therapeutic drug monitoring the emphasis is not on sensitivity; what is required is a fast, simple and reliable method. If compliance is good, betaxolol steady-state concentrations are never below 10 ng/ml with a dose of 10 mg/day. The present report describes a high-performance liquid chromatographic (HPLC) method with fluorimetric detection and automatic injection for the determination of betaxolol in clinical setting, and gives some examples of drug monitoring in a group of hypertensive patients treated for at least one year with the drug.

*Correspondence address: LERS-Synthélabo, 23—25, Avenue Morane Saulnier, 92360 Meudon la Foret, France.

0378-4347/84/$03.00

EXPERIMENTAL

Standard and reagents

Betaxolol hydrochloride was synthesized in the Chemistry Department of Synthélabo (Bagneux, France); metoprolol hydrochloride was kindly supplied by Dr. Cavero from the Cardiovascular Department of Synthélabo (Paris, France).

Diethyl ether, acetic acid and sodium hydroxide, all analytical reagent grade, were obtained from Carlo Erba (Milan, Italy). Acetonitrile (HPLC grade) was purchased from E. Merck (Darmstadt, F.R.G.).

Stock solutions

Standard solutions of betaxolol (1 μg/ml) and metoprolol (1 μg/ml) were prepared in methanol and kept at 4°C. Under these conditions the solutions were stable for several weeks.

Equipment

Chromatography was carried out on a Micromeritics 7000 liquid chromatograph connected to an automatic injector (Micromeritics 725) and a fluorimetric detector (Kontron SFM 23 B). The fluorimetric detector was set with an excitation wavelength of 275 nm and emission wavelength of 305 nm.

Chromatographic conditions

The mobile phase was acetonitrile—acetate buffer 0.03 *M*, pH 5.6 (40:60 v/v) pumped at a flow-rate of 1.0 ± 0.01 ml/min through a stainless-steel column (15 cm × 4.6 mm I.D.) packed in our laboratory [3] with Spherisorb CN, 5 μm (Batch 17/143) (Phase Separations, Queensferry, U.K.). Acetate buffer was prepared by adding 1.8 ml of glacial acetic acid to 1 l of distilled water and adjusting the pH to 5.6 with 10 *M* sodium hydroxide.

Extraction procedure

A 1-ml volume of blood and 1 ml of distilled water were added to a conical tube containing 50 ng of the internal standard metoprolol (50 μl of a 1 μg/ml solution in methanol). This mixture, made alkaline (pH 11) with 200 μl of 2 *M* sodium hydroxide, was shaken on a Vortex mixer and then extracted with distilled diethyl ether (7 ml) on a rock-and-roll shaker for 15 min. Following centrifugation (1000 *g*, 5 min at 4°C), 6.5 ml of the upper organic phase were transferred to a second tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The dry extract was then dissolved in 880 μl of 0.03 *M* acetate buffer by agitation on a Vortex mixer. This solution was transferred to an injection vial with a volume of 880 μl [4,5], and injected onto the column by means of an automatic injector equipped with a 500-μl loop.

For the quantification of betaxolol in urine, the same procedure was followed, but, owing to the higher concentrations of the drug in this medium, the volume analysed was scaled down to between 0.1 and 0.5 ml.

A calibration curve prepared with blood (or urine) spiked with 10, 20, 40 and 80 ng (from a methanolic solution of 1 μg/ml) and 50 ng of metoprolol as internal standard, was run with each series of samples. Each point in the calibration curve was prepared in duplicate.

Quantification

All determinations were performed by calculating the peak area ratios of betaxolol to the internal standard. The integration of the peaks and the calculations of the concentrations were performed by a Sigma 10 Perkin Elmer integrator after the definition of the response factor, between betaxolol and metoprolol.

Patients

The patients whose blood betaxolol concentrations are reported were participating in a multicentre long-term study to assess the efficacy and tolerance of betaxolol. All were outpatients, suffering at the beginning of the study from essential hypertension with a diastolic pressure > 100 mm Hg. The dose could vary from 10 to 40 mg/day, taken as a single oral administration. These patients were, in principle, on monotherapy. In these ambulatory patients blood was sampled to monitor the betaxolol concentration at the same time as the periodic clinical control. The full results of this two-year study will be reported elsewhere.

The present report also includes a second group of twelve hypertensive patients, from another long-term study, to whom betaxolol was administered as polytherapy. This makes it possible to check for possible analytical interferences.

RESULTS AND DISCUSSION

Two chromatograms obtained from a blank blood extract and from a spiked (20 ng) blood extract are presented in Fig. 1. The blank blood extract showed no endogenous interfering peaks. The retention times of metoprolol and betaxolol were 6.4 and 7.9 min, respectively. The absolute sensitivity (signal-to-noise ratio = 3) of this method, checked using standard solutions, was 0.5 ng/ml. However, from a practical point of view, the minimum quantifiable level is 1 ng/ml of blood.

The precision of the method was determined by spiking ten 1-ml aliquots of drug-free blood with four different amounts of betaxolol, i.e. 5, 50, 80 and 500 ng. After the addition of 50 ng of internal standard, the samples were processed as previously described. The coefficient of variation ranged from 11% to 3% for the concentrations 5—500 ng (Table I).

The recovery of the method was not calculated in the present study, but it is estimated to be equal to that reported in the previously described methods in which the same extraction procedure was utilized [2].

Interfering peaks from other cardiovascular drugs which can be administered concomitantly with this beta-blocker were checked. Guanethidine, furosemide, lidocaine, quinidine and diltiazem did not interfere with either betaxolol or metoprolol.

This method is suitable for an automatic sample processing and data acquisition system. Under these conditions 40 samples a day can easily be analysed, the rate-limiting step remaining the preparation of the samples for injection.

The blood concentrations observed in the patients on monotherapy are reported in Table II, and a representative chromatogram of a patient's blood sample appears in Fig. 2.

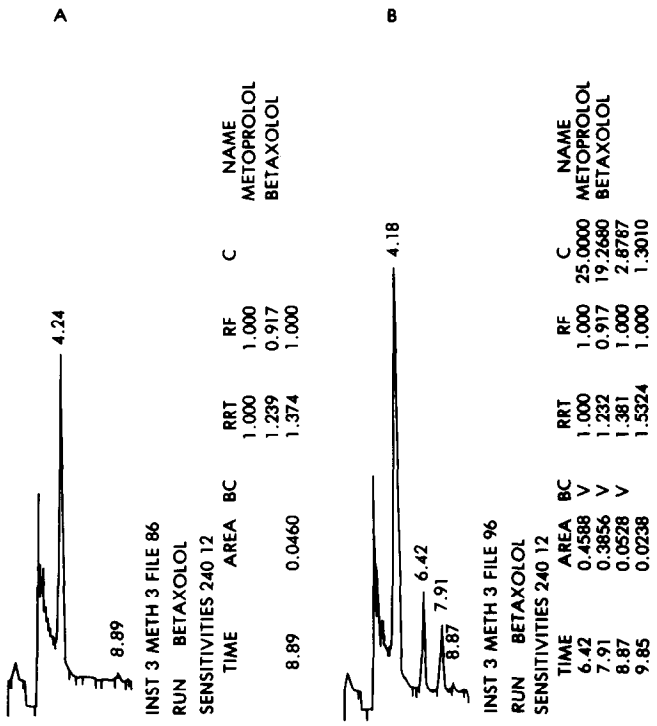

Fig. 1. Chromatograms of (A) 1 ml of blank blood extract, and (B) 1 ml of spiked blood extract containing 20 ng of betaxolol and 50 ng of internal standard (metoprolol). RRT = relative retention time, RF = response factor, C = concentration.

TABLE I

REPRODUCIBILITY OF THE HPLC METHOD FOR THE MEASUREMENT OF BETAXOLOL AT DIFFERENT CONCENTRATIONS IN BLOOD

Theoretical concentration (ng/ml)	Number of measurements	Mean concentration obtained (ng/ml)	Standard deviation	Coefficient of variation (%)
5	10	4.7	0.5	11
50	11	48.8	3.1	6
80	10	79.4	2.8	3.5
500	11	508.5	17.0	3

It is interesting to observe the consistency of the blood concentrations of betaxolol, within the same subjects, monitored over intervals of two to four months.

The very narrow intra-subject fluctuation of the betaxolol blood concentration has been already observed in many studies, which also showed minimal (two to three times) inter-individual variation of the blood betaxolol concentrations for the same given dose [6—8]. In subjects reported in this study, the inter-individual differences for betaxolol blood concentrations may appear greater. However, it must be considered that different doses were administered, that the sampling was done either before or after the drug intake, and that the subjects studied were outpatients.

TABLE II

CONCENTRATIONS OF BETAXOLOL IN BLOOD OF HYPERTENSIVE PATIENTS UNDERGOING CHRONIC TREATMENT WITH THE DRUG AT DIFFERENT DOSES

Subject	Weight (kg)	Dose (mg)	Date	Time from last dose (h)	Betaxolol plasma conc. (ng/ml)
B.M.	108	40	30.07.82	28.0	27.8
		40	29.11.82	1.5	42.4
B.E.	47	10	28.06.82	1.5	29.1
		10	04.10.82	4.5	33.9
		10	07.02.83	1.5	33.7
V.M.	79	10	20.09.82	3.0	16.1
		10	24.01.83	2.0	17.2
C.M.	80	40	21.06.82	1.5	100.7
		40	25.10.82	1.5	99.3
		40	28.02.83	2.0	136.5
M.T.	48	20	28.06.82	24.0	61.7
		20	04.10.82	24.0	73.2

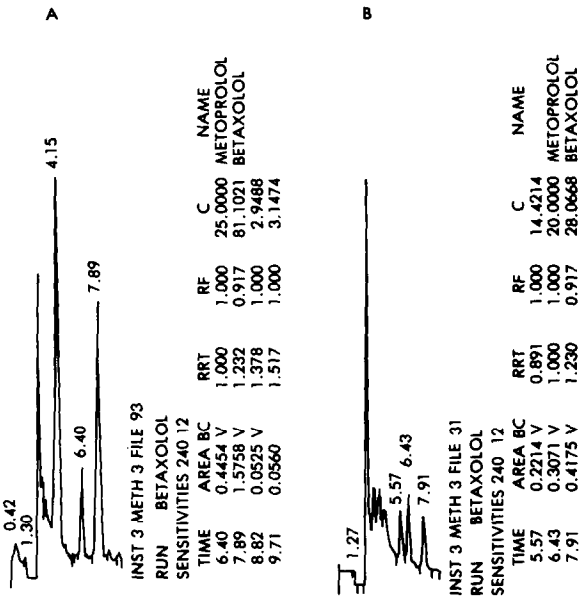

Fig. 2. Chromatograms of (A) 1 ml of blood from a patient receiving betaxolol 20 mg/day, and (B) 0.1 ml of urine from a patient receiving betaxolol 20 mg/day. Abbreviations as in Fig. 1.

For the second group of twelve patients, the individual blood concentrations are not reported because they include subjects sampled both after the first dose and after 16—30 days of treatment, which gives no comparable data. The most important observation concerns the fact that they were on polytherapy, and that clonidine, verapamil, pentobarbital, digoxin, chlortalidone, clofibrate, reserpine, caffeine, triamterene, cyclophosphamide and trimetonine were

associated with betaxolol. None of these drugs appeared to interfere with betaxolol quantification.

Importance of the type of spectrophotofluorimeter

A marked difference both in sensitivity and selectivity was observed in relation to the type of spectrofluorimeter used (Kontron versus filter fluorimetric detector). While running samples from a patient who should have been on monotherapy, we observed an unknown interference eluting with betaxolol. This interference was present only in the chromatogram obtained with the filter fluorimetric detector (Schoeffel GM 970 monochromator). The

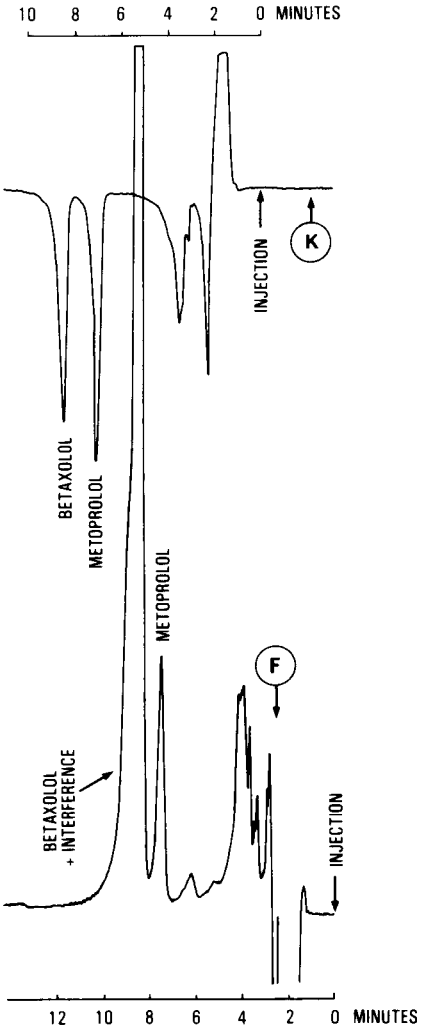

Fig. 3. Chromatograms obtained from the plasma of a patient supposed to be on monotherapy. The trace obtained with the filter fluorimetric detector (F) shows a peak interfering with betaxolol. On the other hand, the trace obtained with the Kontron (K), which is working in series with the other detector, does not show any interfering peak. The working conditions were: (A) Schoeffel filter detector, excitation 220 nm, no emission filter; (B) Kontron, excitation 275 nm, emission 305 nm.

chromatogram obtained from the Kontron detector, which was working in series with the other fluorimeter, was, however, perfectly clean (Fig. 3).

We presumed that the lack of selectivity was due to the configuration of the filter fluorimetric detector: excitation filter at 220 nm and no emission filter, this configuration being that giving the best sensitivity. By adding an emission filter at 320 nm we reduced the sensitivity of the detector by factor of two. By changing the excitation wavelength using a 275-nm filter, with or without the emission filter at 320 nm, in order to have the same configuration as the Kontron detector, the sensitivity was decreased by a factor of five. Nevertheless, the addition of the emission filter did not improve the specificity of the detector, and the interference could not be separated from betaxolol. It is likely that the intrinsic geometry of the double-monochromator detector (Kontron) gives more sensitivity and selectivity than the filter detector.

CONCLUSION

The method described is suitable for routine monitoring of betaxolol. The time required for the preparation of 40 samples for the injection is less than 2 h. Moreover, using automatic injection and an appropriate data system, manual intervention by the operator is minimal. The steady-state concentrations of betaxolol, during chronic treatment, usually do not fall below 10 ng/ml, even if the patient is treated with 10 mg of the drug. The sensitivity of the proposed method should therefore be more than adequate for the quantification of the drug. Moreover, a certain number of drugs which can be associated with a beta-blocker like betaxolol, do not seem to interfere with its analytical quantification. For interferences which cannot be separated with HPLC, it is always possible to use the gas chromatographic method with capillary column [2].

REFERENCES

1 G. Bianchetti, J. Ganansia and P.L. Morselli, J. Chromatogr., 176 (1979) 134.
2 J. Ganansia, G. Gillet, P. Padovani and G. Bianchetti, J. Chromatogr., 275 (1983) 183.
3 M. Broquaire, J. Chromatogr., 170 (1979) 43.
4 M. Broquaire and P.R. Guinebault, J. Liquid Chromatogr., 4 (1981) 2039.
5 P.R. Guinebault and M. Broquaire, J. Chromatogr., 217 (1981) 509.
6 P.J. Cadigan, D.R. London, B.L. Pentecost, G. Bianchetti, R. Gomeni, J.R. Kilborn and P.L. Morselli, Brit. J. Clin. Pharmacol., 9 (1980) 569.
7 J.F. Giudicelli, M. Chauvin, C. Thuillez, C. Richer, G. Bianchetti, R. Gomeni and P.L. Morselli, Brit. J. Clin. Pharmacol., 10 (1980) 41.
8 G. Bianchetti, C. Blatrix, R. Gomeni, J.R. Kilborn, J. Larribaud, P.W. Lucker, P.L. Morselli, J.J. Thebault and S. Trocherie, Arzneim.-Forsch., 30 (1980) 1912.