Journal of Chromatography, 345 (1985) 365—371
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2794

DETERMINATION OF OXPRENOLOL IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, IN COMPARISON WITH GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY—MASS SPECTROMETRY

J. GODBILLON*, M. DUVAL and G. GOSSET

Ciba-Geigy, Centre de Recherche Biopharmaceutique, B.P. 308, 92506 Rueil-Malmaison Cedex (France)

(First received April 5th, 1985; revised manuscript received July 15th, 1985)

SUMMARY

A high-performance liquid chromatographic method for the quantitative assay of oxprenolol in human plasma is described. After addition of alprenolol as internal standard, the compounds are extracted from plasma at alkaline pH into an organic phase and back-extracted into an acidic aqueous phase. Separation of the plasma components and metabolites was achieved on a reversed-phase column. Concentrations down to 66 nmol/l (20 ng/ml) can be determined with UV detection at 222 nm. This technique compares favourably with gas chromatographic and gas chromatographic—mass spectrometric methods.

INTRODUCTION

Several methods for the assay of oxprenolol in plasma have been described [1—15]. Those based on gas chromatography (GC) [1–7], thin-layer chromatography [8] or double radioisotope derivatization [9] involve one or three extraction steps followed by a derivatization procedure. Concentrations down to 33 nmol/l (10 ng/ml) can be determined. The GC methods [3, 4, 7] and the double radioisotope derivative technique [9] generally used in our laboratories appeared to be laborious and time-consuming when used for the analysis of a large number of samples.

The high-performance liquid chromatographic (HPLC) methods reported for oxprenolol [10, 11] and other β-blocking drugs [12, 13] offer the advantage of a direct detection of oxprenolol without requiring a derivatization step. The method reported by Plavsic [11], which involves an isolation step by the solid-

0378-4347/85/$03.30 © 1985 Elsevier Science Publishers B.V.

phase extraction technique, has a limit of quantitation of 100 nmol/l (30 ng/ml). Tsuei et al. [10] described a simple and rapid HPLC method allowing 33 nmol/l to be assayed using 3-ml samples. A simple procedure has been also reported by Lefebvre et al. [13], who investigated the best conditions of extraction and detection. Using 1 ml of plasma, 66 nmol/l oxprenolol can be determined. However, both these papers [10, 13] present very little validation data for precision, accuracy, limit of quantitation and stability of samples. The specificity was assessed without any of the metabolites.

The present paper describes a similar HPLC method for selectively assaying oxprenolol in plasma. Comparisons with a GC [7] and a gas chromatographic—mass spectrometric (GC—MS) method [16], done on actual samples, are also presented.

EXPERIMENTAL

Chemicals

Oxprenolol and alprenolol (internal standard) hydrochlorides, and oxprenolol metabolites, were supplied by Ciba-Geigy (Basle, Switzerland). All solvents and reagents were of analytical-reagent grade. Solution A for the mobile phase was made up of 1 mmol of sodium octanesulphonic acid (234 mg), 1 mmol of anhydrous sodium acetate (82 mg), 470 ml of water, 550 ml of methanol and 0.6 ml of glacial acetic acid.

Chromatography

Chromatography was performed on Hewlett-Packard instruments (Models 1082A or 1081B) equipped with a variable-wavelength detector (Kratos, Model SF 773) set at 222 nm and connected to a recorder—integrator (Shimadzu, Model C-R3A). A prepacked column (25 cm × 4.7 mm I.D.) filled with 10-μm LiChrosorb RP-8 (Merck, ref. 50518, batch 319880) was used. The degassed mobile phase, acetonitrile—solution A (30:70), was used at a flow-rate of 1.5 ml/min. The mobile phase and the column were at room temperature.

The peak areas were given by the integrator—recorder and peak heights were measured manually. The retention times were ca. 8.4 and 10.6 min for oxprenolol and alprenolol, respectively. More recent prepacked columns from Merck may give shorter retention times with the mobile phase composition described above. In this event, the percentage of solution A should be increased.

Sample preparation

A 50-μl aliquot of internal standard solution (added amount 925 pmol or 264 ng) and a 1-ml aliquot of human plasma are introduced into a 10-ml polypropylene tube and mixed for a few seconds on a vortex mixer. Then, 1 ml of 1 *M* sodium hydroxide and 5 ml of ethyl acetate—diethyl ether (4:1) are added. The stoppered tube is shaken on a rotating shaker (Infors) for 10 min at 300 rpm and centrifuged at 2000 *g*. The organic phase, transferred into a 5-ml polypropylene tube, and 250 μl of 0.005 *M* sulphuric acid are mixed mechanically for 10 min at 300 rpm and centrifuged at 2000 *g*. A 50-μl aliquot of the aqueous phase is injected.

Calibration curves

Calibration samples were prepared by adding 50 μl of reference aqueous oxprenolol solution and 50 μl of aqueous alprenolol solution to 1 ml of plasma. The added amounts corresponded to concentrations ranging from 66 to 3310 nmol/l (20 to 1000 ng/ml). The samples were then worked up according to the procedure described above.

RESULTS AND DISCUSSION

Plasma interferences

As shown in Fig. 1, oxprenolol is well separated from the plasma components. The analysis of plasma from fourteen volunteers did not reveal any interference. There were no UV-absorbing compounds appearing late in the chromatogram.

Selectivity

Oxprenolol is extensively metabolized in man [14, 17—20]. Conjugates

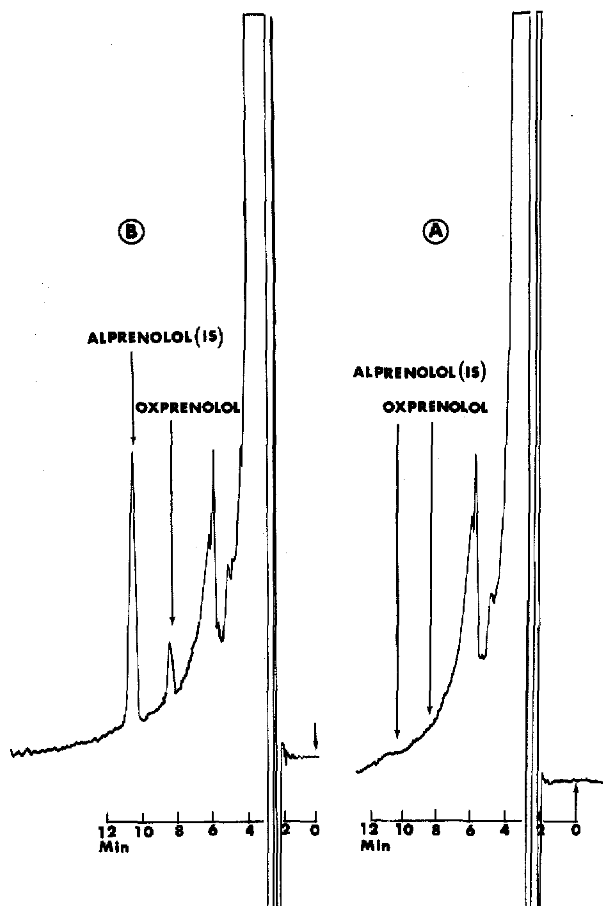

Fig. 1. Chromatogram of (A) an extract of a blank human plasma and (B) an extract of 1 ml of human plasma spiked with 132 pmol of oxprenolol and 925 pmol of alprenolol.

of the unchanged drug, the main plasma metabolites [21], will not be extracted. As expected from the chromatographic conditions used, N- and O-dealkylated metabolites, their oxidation and acylation derivatives, and a ring-hydroxylated metabolite eluted ahead of the parent drug and, thus, did not interfere.

Potential interferences from other drugs commonly prescribed together with oxprenolol have not been evaluated. Interferences from furosemide or chlorthalidone (diuretics) or from isosorbide dinitrate, nitroglycerine or hydralazine (vasodilators) are very unlikely, owing to the physicochemical properties of these compounds.

Linearity

The calibration curves were established as described above. A linear relationship was found between the peak-area or peak-height ratio and the concentration of oxprenolol in the range 66—3310 nmol/l. The equation of the calibration curve was calculated by the least-squares method using weighted linear regression, with a weighting factor of $(\text{concentration})^{-2}$.

The calibration curve was reproducible over one month. However, for routine analysis, it would be advisable to establish a curve once a week and to analyse validation samples every working day.

Accuracy, precision and limit of quantitation

Plasma from several volunteers was used to prepare samples spiked with oxprenolol. These samples were analysed several times. The data summarized in Table I show that the described method permits the accurate and precise determination of oxprenolol in plasma at concentrations down to 66 nmol/l (20 ng/ml). Despite the marked tailing of the solvent front (Fig. 1), the peak height corresponding to this limit (ca. 6 mm) could be precisely measured.

TABLE I

ACCURACY, PRECISION AND LIMIT OF QUANTITATION FOR THE HPLC ASSAY OF OXPRENOLOL IN SPIKED PLASMA SAMPLES

Concentration added		Concentration found		*n*	Coefficient of variation (%)	Mean accuracy* (%)
nmol/l	ng/ml	nmol/l	ng/ml			
66	20**	63.8	19.3	10	6.3	96.4
102.6	31**	93.5	28.3	5	7.1	91.4
132.4	40**	127.6	38.6	6	7.1	96.0
205.2	62**	197.0	59.6	15	5.4	95.7
1230	372	1179	356.6	15	6.5	95.9
1322	400	1363	412.2	6	2.6	103
2460	744	2413	729.9	10	5.7	97.9
Overall accuracy (mean ± S.D.) (%)						96.6 ± 3.4

*Mean of the individual values.
**Peak-height ratios, otherwise peak areas were used.

Stability

The aqueous solutions of internal standard alprenolol and of oxprenolol for calibration are stable for at least one month when stored at +4°C. Once prepared in 250 μl of 0.005 *M* sulphuric acid, the samples may be stored for one night in the refrigerator before injection. No change in concentration of oxprenolol was observed in frozen plasma samples after one-year storage.

Comparison with other methods

The present HPLC method was compared to the GC method [7] in use for

TABLE II

MAIN FEATURES OF THE HPLC, GC [7] AND GC—MS [16] METHODS FOR THE ASSAY OF UNCHANGED OXPRENOLOL IN PLASMA

Feature	GC	GC—MS	HPLC
Tubes	Silanized glass tubes	Silanized glass tubes	Disposable polypropylene tubes
Plasma volume (ml)	1	1	1
Internal standard	Propranolol	[$^{13}C_3$]Oxprenolol	Alprenolol
Extraction	1. Extraction into dichloromethane—diethyl ether 2. Back-extraction into acidic aqueous phase 3. Re-extraction into dichloromethane—diethyl ether 4. Evaporation	1. Extraction into dichloromethane—diethyl ether 2. Evaporation	1. Extraction into ethyl acetate—diethyl ether 2. Back-extraction into acidic aqueous phase
Derivatization	1. HFBA, 1 h at room temperature 2. Extraction at pH 5 into hexane	1. HFBA, 15 min at room temperature 2. Extraction at pH 5 into hexane	None
Injection	3 μl of organic phase Manual	1—2 μl of organic phase Manual	50 μl of aqueous phase Automatic
Detection	Electron capture	Chemical ionization, negative ions	UV 222 nm
Time of analysis (min)	13	2	11
Maximum number of samples per day	20	50	60
Calibration curve	Valid for one month, calibration samples reinjected every week range 33—3310 nmol/l (10—1000 ng/ml)	Valid for one week, calibration samples reinjected every day Range 20—1500 nmol/l (6—450 ng/ml)	Valid for one week Range 66—3310 nmol/l (20—1000 ng/ml)
Limit of quantitation (nmol/l; ng/ml)	33; 10	20; 6	66; 20
Complexity of equipment	+	+++	+
Cost: instrument	+	+++++	++
solvents	++	+	+++
reagents	+	++	

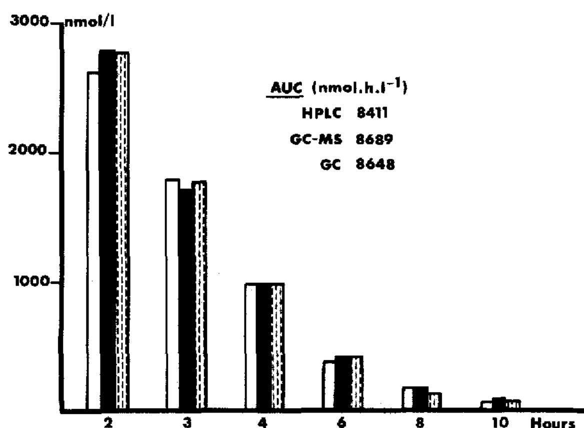

Fig. 2. Plasma concentrations of oxprenolol after administration of a single 160-mg oral dose to a healthy volunteer, measured by HPLC (□), GC—MS (▪) and GC (▨) methods. (To convert into ng/ml, multiply the data by 0.302.)

several years in our laboratories and to a GC—MS technique recently developed in our centre [16]. Their main features are summarized in Table II.

The GC method [7] appeared to be laborious and time-consuming because it requires conditioning of the glassware and implies three extraction steps, one derivatization step and a purification step before chromatography. With manual injection, a maximum of twenty samples can be worked up daily by an experienced technician. The GC—MS method [16] requires costly instrumentation and the preparation of an internal standard labelled with a stable isotope. With an analysis time of only 2 min, up to fifty samples can be analysed daily by a technician familiar with MS.

The HPLC method offers the advantage of direct detection of oxprenolol without requiring a derivatization step. Commercially available tubes can be used without any previous conditioning. The sample preparation is simpler than that of the other two techniques. Although higher than that of GC or GC—MS methods, its limit of quantitation appeared to be suitable for pharmacokinetic investigations in man.

Oxprenolol concentrations in plasma samples from one healthy volunteer given a single oral dose of 160 mg of oxprenolol hydrochloride were determined by the three methods. The data displayed in Fig. 2 indicate a good agreement between the three methods for the assay of oxprenolol in plasma. Data obtained by any of the techniques could be reliably compared.

REFERENCES

1 T. Nambara, S. Akiyama and M. Kuruta, Yakugaku Zasshi, 93 (1973) 439.
2 T. Walle, J. Pharm. Sci., 63 (1974) 1885.
3 D.B. Jack and W. Riess, J. Chromatogr., 88 (1974) 173.
4 P.H. Degen and W. Riess, J. Chromatogr., 121 (1976) 72.
5 D. de Bruyne, H. Kinsun, M.A. Moulin and M.C. Mignot, J. Pharm. Sci., 68 (1979) 511.
6 T. Yamaguchi, Y. Morimoto, Y. Sekine and M. Hashimoto, J. Chromatogr., 239 (1982) 609.
7 A. Sioufi, D. Colussi and P. Mangoni, J. Chromatogr., 278 (1983) 185.

8 M. Schäfer and E. Mutschler, J. Chromatogr., 164 (1979) 247.
9 W. Riess, Ciba Found. Symp., 26 (1974) 139.
10 S.E. Tsuei, J. Thomas and R.G. Moore, J. Chromatogr., 181 (1980) 135.
11 F. Plavsic, Acta Pharm. Jugosl., 32 (1982) 137.
12 B.R. Patel, J.J. Kirschbaum and R.B. Poet, J. Pharm. Sci., 70 (1981) 336.
13 M.A. Lefebvre, J. Girault and J.B. Fourtillan, J. Liq. Chromatogr., 4 (1981) 483.
14 W. Dieterle and J.W. Faigle, J. Chromatogr., 259 (1983) 301.
15 K. Kawashima, Jpn. J. Pharmacol., 31 (Suppl.) (1981) 76P.
16 D. Gaudry, D. Wantiez, J.P. Metayer and J. Richard, Biomed. Mass Spectrom., 12 (1985) 269.
17 W.L. Nelson and T.R. Burke, J. Med. Chem., 22 (1979) 1082.
18 W. Riess, M. Huerzeler and F. Raschdorf, Xenobiotica, 4 (1974) 365.
19 G.A. Leeson, D.A. Garteiz, W.C. Knapp and G.J. Wright, Drug Metab. Dispos., 1 (1973) 565.
20 D.A. Garteiz, J. Pharmacol. Exp. Ther., 179 (1971) 354.
21 W. Dieterle, personal communication.