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HIGH-PERFORMANCE LIQUID AND GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF CICLOPROLOL

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SUMMARY

Two methods, one based on high-performance liquid chromatography (HPLC) and the other on gas chromatography (GC), were developed for the quantification of the partial adrenergic receptor antagonist cicloprolol. In the GC method, samples are cleaned up by back-extraction, then derivatized with heptafluorobutyric anhydride and separated on a capillary cross-linked methylsilicone column. This GC method is time-consuming but, with electron-capture detection, cicloprolol can be quantified at levels down to 1 ng/ml. The HPLC method, using a reversed ODS stationary phase and fluorimetric detection, is less sensitive (5 ng/ml) but, with a single-step extraction, is faster and simpler. The determination of cicloprolol in human blood samples by the two methods gave comparable results. Routine monitoring of cicloprolol can be done easily with the HPLC method, whereas the time-consuming GC method may be reserved for pharmacokinetic studies where late-sampled tubes, with low concentrations, must be analysed.

INTRODUCTION

Cicloprolol, a partial β_1-adrenergic receptor antagonist, modulates heart rate and contractility [1–4]. It attenuates exercise-induced tachycardia and improves the ejection fraction, both at rest and during exercise, in patients with altered left ventricular function.

Two methods for determining blood levels of cicloprolol were developed, one based on high-performance liquid chromatography (HPLC) and the other on gas chromatography (GC). The latter was chosen because in the early phases

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of development of a drug the highest possible sensitivity is needed for the definition of the pharmacokinetic profile. In contrast, for long-term clinical studies and therapeutic monitoring a simpler and shorter HPLC method may be preferred.

EXPERIMENTAL

Materials

For the HPLC method a Constametric II G pump (LDC Milton Roy, Riviera Beach, FL, U.S.A.), a WISP automatic injector (Waters Assoc., Milford, MA, U.S.A.) and a Jasco FP210 fluorimetric detector (Japan Spectroscopic Co., Tokyo, Japan) were used. Integration was performed with an SP4270 integrator (Spectra-Physics, San Jose, CA, U.S.A.) or by means of the LAS (Laboratory Automation Systems Hewlett-Packard) program implemented on an HP 1000 computer (Hewlett-Packard, Palo Alto, CA, U.S.A.).

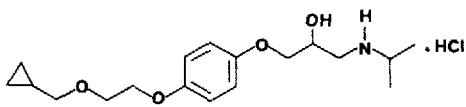
For the GC method, a Hewlett-Packard 5880A chromatograph, equipped with an HP 7672A automatic injector and an electron-capture detector (^{63}Ni), both from Hewlett-Packard (Avondale, PA, U.S.A.) was used.

Standards and reagents

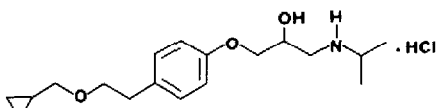
Analytical-reagent grade chemicals were used, unless indicated otherwise.

Cicloprolol, 1-[4-[2-cyclopropylmethoxy)ethoxy]phenoxy]-3-[(1-methylethyl)amino]-2-propanol hydrochloride, and the internal standard betaxolol, 1-[4-2-(cyclopropylmethoxy)ethyl]phenoxy]-3-[(methylethyl)amino]-2-propanol hydrochloride, were synthesized in the L.E.R.S. Chemistry Department (Fig. 1).

Potassium dihydrogenphosphate, ethyl acetate and hexane were obtained from Merck (Darmstadt, F.R.G.), hydrochloric acid and acetonitrile (HPLC grade) from J.T. Baker (Deventer, The Netherlands), sodium hydroxide



CICLOPROLOL



BETAXOLOL

Fig. 1 Structures of cicloprolol and the internal standard, betaxolol.

(Rectapur) from Prolabo (Paris, France), methanol (RPE-ACS) and diethyl ether (RPE) from Carlo Erba (Milan, Italy) and heptafluorobutyric anhydride (HFBA) from Pierce (Rockford, IL, U.S.A.).

Stock solutions

Stock solutions (0.1 mg/ml) of cicloprolol base and betaxolol base were prepared by dissolving precisely weighed amounts of their hydrochlorides in methanol. The salt/base ratio for both compounds was 1.11.

The stock solutions were diluted with methanol to give 10, 1 and 0.1 μg/ml solutions of cicloprolol and a 1 μg/ml solution of betaxolol. The solutions were stored in a refrigerator at 4°C, and fresh solutions were prepared regularly every week.

HPLC method

About 500 μl of blood (or plasma) in a conical tube were accurately weighed, 50 μl of internal standard solution (1 ng/μl) added and the mixture was vortex-mixed. The pH was adjusted to 12 by adding 100 μl of 2 *M* sodium hydroxide solution. After extraction with 7 ml of unstabilized (or distilled) diethyl ether on a rotary shaker for 20 min, the organic phase, separated by centrifugation at 900 *g* for 7 min at −20°C, was evaporated to dryness under a gentle stream of nitrogen at 37°C. The dry extract was dissolved in 300 μl of 0.05 *M* KH_2PO_4 aqueous solution adjusted to pH 2.5 with phosphoric acid, and 150 μl were injected onto the column.

The chromatographic system consisted of a stainless-steel column (150 mm×4.6 mm I.D.) packed with Spherisorb ODS-1, 5 μm (SFCC, Gagny, France). The mobile phase was acetonitrile–phosphate buffer (0.05 *M*, pH 2.5) (35:65, v/v). The flow-rate was 1 ml/min. The excitation and emission wavelengths were set at 285 and 325 nm, respectively.

GC method

The first part of the extraction was the same as that for the HPLC method except that the organic phase from the first extraction was transferred into another tube and vortex-mixed with 2 ml of 0.1 *M* hydrochloric acid for 20 s for back-extraction (the mixture may also be shaken on a rotary shaker for 10 min). After centrifugation at 900 *g* for 5 min at −20°C, the organic phase was discarded and the aqueous phase, after thawing in a water-bath at 37°C, was again vortex-mixed for 10–20 s with 4 ml of unstabilized diethyl ether.

The aqueous phase was separated by centrifugation at 900 *g* for 5 min at −20°C and thawed in a water-bath at 37°C; 300 μl of 2 *M* sodium hydroxide solution (final pH ranging from 11 to 13) were then added for the final extraction with 7 ml of diethyl ether; the mixture was vortex-mixed for 10–20 s and centrifuged at 900 *g* at −20°C for 5 min. The organic phase was dried under a gentle stream of nitrogen at 37°C.

The resulting extract was derivatized by adding 200 μl of a 10% solution of HFBA in ethyl acetate and heated for 30 min in a water-bath at 55°C (not exceeding 60°C). The tubes were cooled to room temperature, opened and the excess of HFBA was eliminated by heating in a water-bath at 60°C under a gentle stream of nitrogen. Any remaining HFBA was eliminated by adding 2 ml of hexane–ethyl acetate (90:10, v/v) and again evaporated to dryness at 60°C under a gentle stream of nitrogen.

After the tubes had cooled to room temperature, the dry extract was dissolved in 100 μl of hexane–ethyl acetate (90:10, v/v) and 2 μl of this solution were injected into the chromatograph. The GC system consisted of a cross-linked methylsilicone column (Hewlett-Packard) (25 m×0.22 mm I.D.) with a film thickness of 0.11 μm and an electron capture-detector operating at 300°C. The injection temperature was 300°C; the temperature gradient for the column oven was from 210 to 275°C at 15°C/min. Argon–methane (95:5) was used as the carrier gas and as the make-up gas. The column flow-rate was 5 ml/min and the splitting ratio at the injector was 1:20.

Integration and calculation

Quantification was obtained with the LAS program on the HP 1000 for the HPLC method or from the level 4 HP method for the GC method (both systems from Hewlett-Packard).

Validation

Linearity. The linearity of the GC and HPLC methods was evaluated at concentrations up to 250 ng/ml with spiked samples.

Accuracy and precision. The accuracy and precision of the method were assessed by using quality controls of 20 and 250 ng/ml which, in addition to monitoring variability in the method, gave results on the long-term stability of the product under normal storage conditions for clinical samples.

Comparison of the two methods. Plasma samples, collected from a healthy volunteer who had received chronic treatment with 50 mg of cicloprolol per day and 0.25 mg of digoxin per day, were analysed by three analysts using both methods.

RESULTS

HPLC method

Under the conditions described, cicloprolol and the internal standard, betaxolol, had retention times of 8.8 and 11.2 min, respectively, without any interference from endogenous substances in the blood; this is shown by the chromatograms of a blank sample, a spiked sample and a routine clinical sample (Fig. 2).

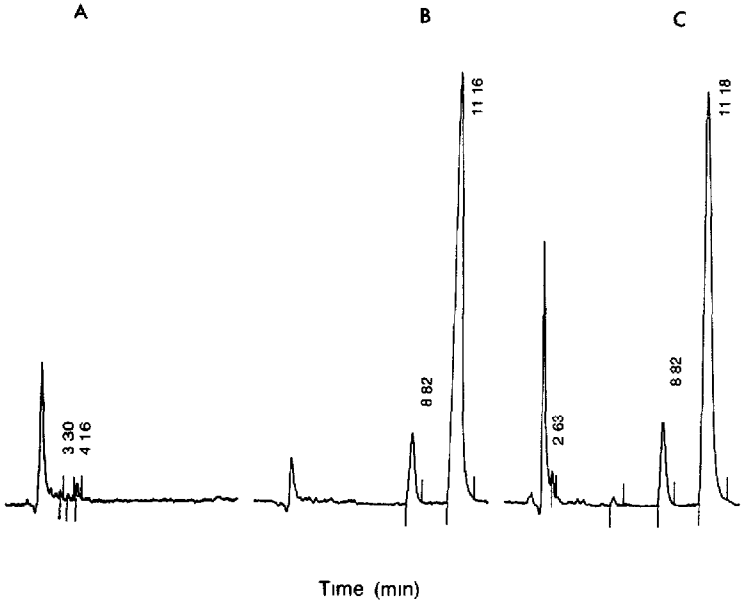


Fig. 2. Typical chromatograms for the determination of cicloprolol obtained by HPLC. (A) Blank blood (not spiked); (B) blood spiked with 25 ng/ml cicloprolol; (C) blood sample obtained from a healthy volunteer 36 h after the last dose of a chronic treatment with 100 mg of cicloprolol per day The measured concentration was 31 ng/ml.

TABLE I

CALIBRATION FOR CICLOPROLOL BY THE TWO METHODS

Theoretical concentration (ng/ml)	Observed concentration (ng/ml)	Deviation (%)
HPLC method		
0	0	0
5	5.8	16
10	11	10
25	25	0
25	25	0
50	53	6
100	101	1
250	260	4
GC method		
0	0	0
1	0.9	10
2.5	2.5	0
5	5.0	0
10	9.8	2
25	25	0
100	103	3
250	228	9

Table I gives the spiked and calculated values for the calibration graph; these values show that the method is linear in the range 5–250 ng/ml. The limit of determination was 5 ng/ml with a deviation from the theoretical value of 16%. The deviation for the other values ranged from 10% for 10 ng/ml to 4% for 250 ng/ml concentrations.

GC method

Under the conditions described, cicloprolol and the internal standard, betaxolol, had retention times of 12.5 and 8.8 min, respectively, without any in-

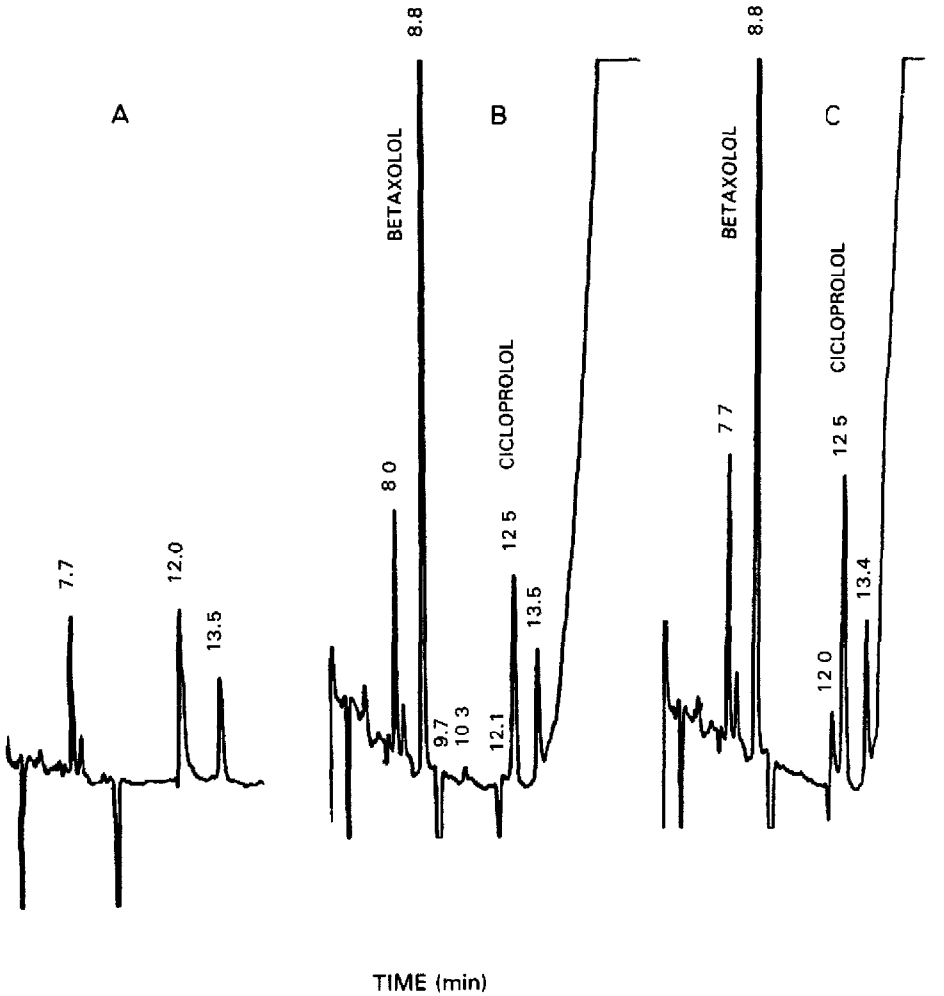


Fig. 3. Typical chromatograms for the determination of cicloprolol obtained by GC. (A) Blank blood (not spiked); (B) blood spiked with 25 ng/ml cicloprolol; (C) blood sample obtained from a healthy volunteer 12 h after the last dose of a chronic treatment with 50 mg of cicloprolol per day and 0.25 mg of digoxin per day. The measured concentration was 81 ng/ml.

TABLE II

COMPARISON OF HPLC AND GC METHODS BY ANALYSIS OF THE SAME CLINICAL SAMPLES

Sample No	Concentration (ng/ml)							Analysis of results						
	Jan. HPLC 2[a]	Jan. GC 2	Jan. GC 1	April HPLC 2	May HPLC 2	June GC 2	June HPLC 3	*n*	Mean (ng/ml)	S.D. (ng/ml)	S.E.M. (ng/ml)	C.V. (%)	Max. (ng/ml)	Min. (ng/ml)
0	27	23	22	24	–[b]	–[b]	–[b]	4	24	2	1.1	9	27	22
1	35	31	33	32	–[b]	–[b]	–[b]	4	33	2	0.9	5	35	31
2	145	147	158	146	–[b]	–[b]	–[b]	4	149	6	3.0	4	158	145
3	156	143	134	–[b]	156	–[b]	–[b]	4	147	11	5.4	7	156	134
4	134	123	138	–[b]	138	–[b]	127	5	132	7	3.0	5	138	123
5	113	102	116	–[b]	110	111	–[b]	5	110	5	2.3	5	116	102
6	91	89	90	–[b]	89	92	92	6	91	1	0.6	2	92	89
7	79	81	65	–[b]	71	81	–[b]	5	75	7	3.2	9	81	65
8	69	67	66	65	–[b]	70	72	6	68	3	1.1	4	72	65

[a]Analyst No.
[b]Insufficient sample.

terference from endogenous substances in the blood. This is shown by the chromatograms of a blank sample, of a spiked sample and of a routine sample (Fig. 3).

Table I gives the spiked and calculated values for the calibration graph. The calibration graph shows that the method is linear between 1 and 250 ng/ml, with a limit of determination of 1 ng/ml. The deviation from the theoretical value ranged from 10% for 1 ng/ml to less than 1% for 2.5 and 5 ng/ml. However, a deviation from the theoretical value of −9% was found for 250 ng/ml.

Comparison of the two methods

Nine clinical samples were analysed by the two methods; in addition, the determination by the two methods was performed by different analysts (Table II). The coefficients of variation were less than 10%. The two methods thus give the same results.

Accuracy and precision

The results for quality control samples analysed using both methods during six months showed that the experimental values differ from the theoretical values by less than 10% (Fig. 4). The low value ranged between 18 and 21 ng/ml (mean ± S.D. = 19.0 ± 0.9 ng/ml) and the high value between 234 and 269 ng/ml (mean ± S.D. = 253 ± 9 ng/ml).

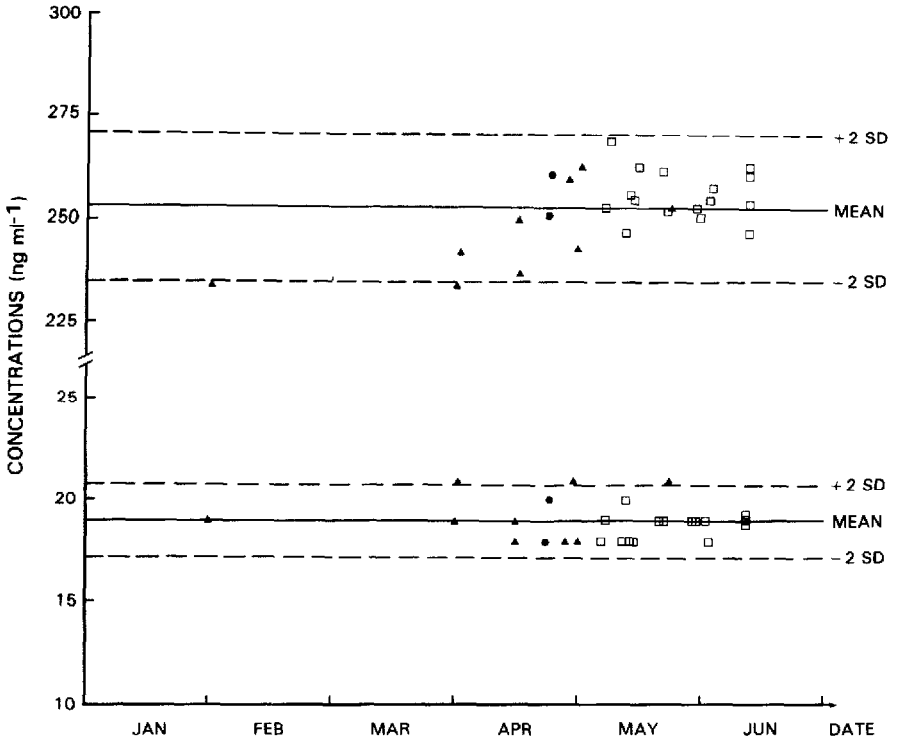


Fig. 4. Precision of the two methods, using quality control samples. ▲, analyst 1; ●, analyst 2; □, analyst 3.

DISCUSSION AND CONCLUSION

The HPLC method is simple and rapid, but its limit of determination is 5 ng/ml; the GC method has a limit of determination of 1 ng/ml, but it requires a back-extraction and a derivatization, which requires a longer time for the preparation of the sample. Nevertheless, because of the similarity of the results obtained by the two methods, the HPLC method may be used for most samples and GC may then be used to determine concentrations lower than 5 ng/ml.

Generally, the GC method may be used in kinetic studies in which late samples (48 h) have been collected and for which the blood concentrations of cicloprolol would presumably be low. In other instances, particularly for monitoring repeated-dose clinical studies, the HPLC method is preferable because of its simplicity.

REFERENCES

1 H. Pouleur, H. van Mechelen, H. Balasim, M.F. Rousseau and A.A. Charlier, J. Cardiovasc. Pharmacol., 6 (1984) 720–726.

2 P.E. Hicks, C. Cavero, Ph. Manoury, F. Lefevre-Borg and S.Z. Langer, J. Pharmacol. Exp. Ther., 242 (1987) 1025-1034.
3 J.P. Grilliat, D. Le Van, D. Mayeux, F. Regnier, J.F. Thiercelin, J.P. Thenot, B. Orofiama and J. Larribaud, Therapie, 42 (1987) 519-524.
4 B. Silker, S.P. Verma, S.K. Sharma, W. Baig, N.C. Jackson, G. Reynolds, M.A. Frais and S.H. Taylor, Int. J. Cardiol., 17 (1987) 127-136.