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Note

Simplified method for the determination of oxprenolol and other β-receptor blocking agents in biological fluids by gas–liquid chromatography

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Owing to the great interest and the intensive research in the field of adrenergic β-receptor blocking agents, the quantitative determination of these compounds in biological material has been demanded more and more frequently.

For this reason, previously reported methods had to be revised to increase their efficiency.

The procedure described here is a simplified and modified version of the method reported by Jack and Riess[1]. Probably the most important modification is the replacement of water with sodium acetate solution in the washing step following the re-extraction of oxprenolol from the alkaline aqueous phase. This step has been found to be critical in different laboratories where water of pH <7 had been used and the operators were not aware of the fact that at a pH of 6.5 virtually 100% of the oxprenolol will remain in the aqueous phase. By using a 5% sodium acetate solution instead of water, this problem is overcome, while the same result is achieved, namely removal of trace amounts of sodium hydroxide from the organic phase prior to derivatization.

It was found that the final organic extract can be evaporated without additional drying with calcium chloride. By omitting the drying step, the overall recovery is increased, because during drying some of the oxprenolol and internal standard are adsorbed on the calcium chloride.

Finally, the gas–liquid chromatographic conditions were slightly modified. The use of shorter columns with a small inner diameter provides shorter times of analysis. The procedure described can also be used to determine pindolol, propranolol, alprenolol and metoprolol.

EXPERIMENTAL

Gas–liquid chromatography

A Pye-Unicam Series 104 gas chromatograph, equipped with a ^{63}Ni electron-capture detector operated at a pulse interval of 150 μsec and a glass column (1.5 m × 2 mm I.D.) packed with 3% JXR on Chromosorb G (80–100 mesh), was used. The temperatures were: column oven, 200°; injector, 220°; and detector 350°. The carrier gas (nitrogen) flow-rate was 30 ml/min.

TABLE I

CALIBRATION FOR THE COMPLETE PROCEDURE FOR DETERMINING OXPRENOLOL IN PLASMA BY GAS CHROMATOGRAPHY

The samples containing 0–100 ng of oxprenolol were dissolved in 0.5 ml of toluene, and the samples containing 200 and 300 ng of oxprenolol were dissolved in 1 ml of toluene; 5 μl of each sample were injected.

Oxprenolol concentration in plasma (ng/ml)	*Value of F_x* for a single determination*
50	0.53
	0.57
100	0.87
	0.97
200	1.66
	1.61
300	2.19
	2.10
	2.23

* F_x is the ratio of the peak area of oxprenolol to that of the internal standard.

Reagents and materials

The internal standard used was metoprolol (CGP 2175, Ciba-Geigy Ltd., Basle), 1-(2-propylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol.

Trifluoroacetic anhydride was obtained from Fluka (Buchs, Switzerland). This compound and the organic solvents used were distilled prior to use.

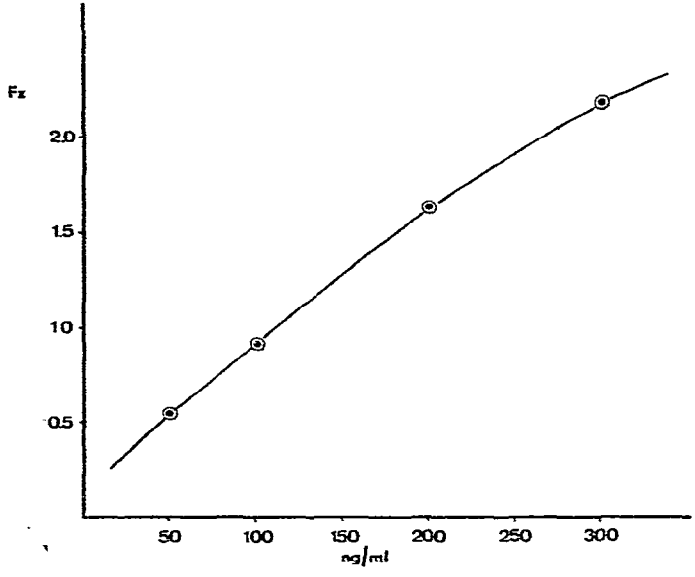

Fig. 1. Quantitative analysis for unchanged oxprenolol: calibration graph for complete analytical procedure. F_x is as defined in Table I, and the volumes of solutions and injections are also as in Table I.

Extraction and derivatization

A 2-ml volun e of plasma (or blood) is mixed with 0.5 ml of an aqueous solution of the internal stanoard (200 ng/ml of CGP 2175). Then 2 ml of saturated sodium chloride solution (for blood only) and 0.3 ml of 1 *N* sodium hydroxide solution are added and the mixture is shaken with 5 ml of methylene chloride–diethyl ether (1:4) for 15 min at 200 rpm on a rotary shaker, and centrifuged briefly if necessary. The organic phase is removed and shaken with 2 ml 0.1 *N* hydrochloric acid for 15 min at 200 rpm. The organic phase is discarded by aspiration, 0.3 ml of 1 *N* sodium hydroxide solution is added to the aqueous phase and the mixture is shaken with 5 ml of methylene chloride–diethyl ether mixture (1:4) for 15 min at 200 rpm.

The organic phase is removed and washed with 2 ml of 5% sodium acetate solution. After separation, the organic phase is evaporated to dryness under a stream of dry nitrogen at 45°C. Then 0.5 ml dry benzene (stored over calcium chloride) and 0.1 ml of trifluoroacetic anhydride are added, the tube is tightly stoppered and the contents are mixed and left to react at room temperature for 1 h.

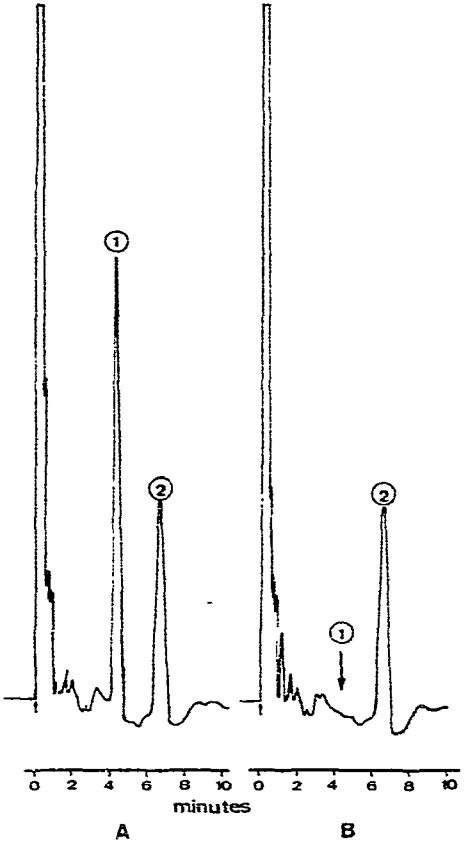

Fig. 2. Chromatograms of (A) an extract of 1 ml of plasma containing 200 ng of oxprenolol (1) and 100 ng of internal standard (2), and (B) an extract of 1 ml of plasma containing 100 ng of internal standard only. The sample was dissolved in 1 ml of toluene and 5 μl were injected into the chromatograph.

TABLE II

ANALYSIS FOR OXPRENOLOL IN SPIKED PLASMA SAMPLES

Plasma samples containing amounts of oxprenolol unknown to the analyst were analyzed in five independent determinations; each solution was injected once.

Oxprenolol present (ng/ml)	*Oxprenolol found (ng/ml)*		
	$\bar{x}$ ($n = 5$)	s (x)	*Coefficient of variation*
50	51.8	2.28	4.4
150	148.8	4.60	3.1
300	296.8	7.69	2.6

The excess of reagent and the solvent are evaporated to dryness under a stream of nitrogen at 45°. The dry residue is dissolved in 0.5–1 ml of toluene and approximately 3–5 μl are injected into the chromatograph.

RESULTS AND DISCUSSION

A calibration graph was prepared by analyzing plasma samples containing known amounts of oxprenolol. The resulting peak area ratios between oxprenolol and the internal standard are given in Table I and illustrated in Fig. 1. Typical chromatograms are shown in Fig. 2.

To test the entire procedure for accuracy and precision, test samples containing oxprenolol concentrations unknown to the analyst performing the determination were prepared and analyzed as described above. The results are given in Table II. The small differences between the found and theoretical values show that this method is adequate for application in pharmacokinetic studies. The sensitivity of the method, when applied to oxprenolol, was about 10 ng/ml.

The procedure can also be applied to the determination of the following β-blocking agents: metoprolol, alprenolol, pindolol and propranolol. In each instance one of the other compounds can serve as the internal standard.

REFERENCE

1 D. B. Jack and W. Riess, *J. Chromatogr.*, 88 (1974) 173.