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DETERMINATION OF β-ADRENERGIC BLOCKING DRUGS AS CYCLIC BORONATES BY GAS CHROMATOGRAPHY WITH NITROGEN-SELECTIVE DETECTION

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SUMMARY

A gas chromatographic method with nitrogen-selective detection has been developed that permits the sensitive and simple determination of β-adrenergic blocking drugs, including alprenolol, bufetolol, bupranolol, carteolol, nadolol, oxprenolol, pindolol and propranolol. The drugs were derivatized with *n*-butylboronic acid or phenylboronic acid to form their cyclic boronates. The derivatives were readily formed at room temperature, and were stable for at least 3 days. The cyclic boronates formed gave symmetrical peaks, and showed high responses with minimum detectable amounts in the range 1.5–4 pg, corresponding to $6–14 \cdot 10^{-16}$ mol/sec.

Propranolol was extracted with *n*-hexane containing 1.5% of isoamyl alcohol from alkaline plasma by a single-extraction procedure, with bufetolol as the internal standard, and derivatized with phenylboronic acid. Accurate determinations were possible in the concentration range 1–500 ng/ml, and the minimum detectable concentration in plasma was 0.5 ng/ml, permitting the pharmacokinetic study of propranolol under therapy. Pindolol was also determined in the range 2–500 ng/ml, and the minimum detectable concentration was 1 ng/ml.

These results suggest the general applicability of the method to the determination of the unchanged β-blocking drugs in plasma and other biological samples.

INTRODUCTION

The β-adrenergic blocking drugs are in clinical use in the treatment of angina and hypertension. It has been observed for several of these drugs that the response of individuals to the same dose varies considerably, which is attributed in part to individual differences in the pharmacokinetic properties of these drugs. In order to achieve a better understanding of the pharmacokinetics of these drugs, a sensitive and simple assay method for the unchanged drug in plasma is necessary.

The β-blocking drugs have been determined by several analytical methods, of which fluorometric[1,2] and electron-capture gas chromatographic (GC–ECD)[3–5] methods are generally used. The former is limited by specificity[5], sensitivity and a highly

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variable assay blank[5]. The latter, consisting of the formation of perfluoroacyl derivatives, is highly sensitive and specific but involves tedious procedures, including a back-extraction and chemical derivatization. Recently, another GC–ECD method for the determination of alprenolol has been reported, with the 2,3-dichlorophenylboronate of propane-1,3-diamine as the reagent[6]. The method utilizes the selective reaction of boronic acid with the side-chain of the drug to form cyclic boronate. The procedure is simple and the cyclic boronate has good GC properties, but the chromatographic separation requires a very long time (more than 25 min) because of the presence of thermal decomposition products of the reagent.

For practical performance of the pharmacokinetic study, a simple assay procedure would be preferable. This paper describes a GC method with nitrogen-selective detection. The method, based on a single-extraction procedure and the formation of cyclic boronates with *n*-butylboronic acid (BBA) and phenylboronic acid (PBA), is reproducible and sensitive for the determination of β-blocking drugs.

EXPERIMENTAL

Chemicals and reagents

Alprenolol, bufetolol, bupranolol, carteolol, oxprenolol and propranolol were obtained as their hydrochlorides, and pindolol was obtained as the free base from commercial sources. Nadolol was the free base was a gift from Squibb Institute (Princeton, NJ, U.S.A.). Their purity was checked by thin-layer chromatography. The molecular structures are shown in Table I.

BBA and PBA were purchased from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals used were of analytical reagent grade.

The BBA or PBA solution was prepared by dissolving 100 mg of the boronic acid in a mixture of 95 ml of ethyl acetate and 5 ml of anhydrous sodium sulphoxide.

All centrifuge tubes, pipetes and flasks were silanized as described previously[7].

Instrument

GC was carried out using a Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen–phosphorus selective detector (NPD). A silanized glass column (1.2 m × 2 mm I.D.) was packed with 2% OV-17 on Gas-Chrom Q (80–100 mesh). Helium was used as carrier gas at a flow-rate of 30 ml/min, and air and hydrogen as detector gases at 70 and 3 ml/min, respectively. The voltage applied to the NP collector was 18–19 V which was about 2–3 V higher than the usual operating voltage. The column, injector and detector temperatures were 265, 320 and 300°C, respectively.

For the determination of pindolol, a glass column packed with 2% Dexsil 410GC was used and operated at 260°C. Other conditions were the same as described above.

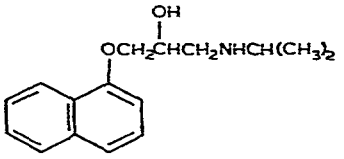
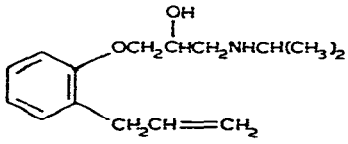
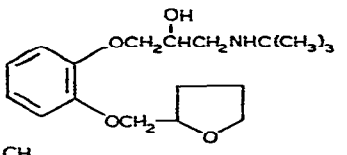
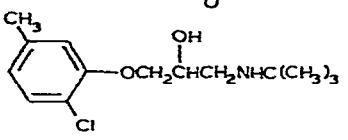
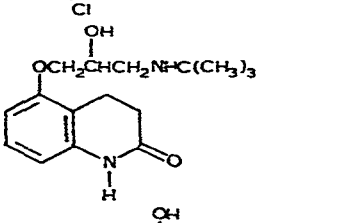
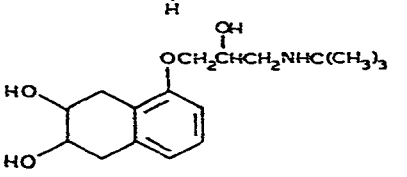
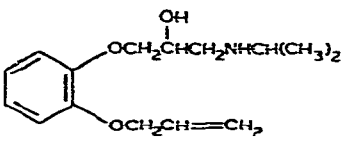
Preparation of derivatives

Pindolol and nadolol were dissolved in ethyl acetate, and other drugs were extracted with ethyl acetate from their hydrochlorides and dried with anhydrous sodium sulphate. The drug solution was mixed with BBA or PBA solution, and dilutions in reagent solution were analysed in triplicate by GC–NPD to determine the detector responses of the drugs.

TABLE I

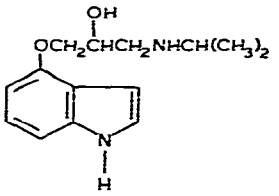
STRUCTURES RETENTION INDICES AND NPD RESPONSES FOR CYCLIC BORONATES OF β-ADRENERGIC BLOCKING DRUGS

A glass column (1.2 m × 2 mm I.D.) packed with 2% OV-17 on Gas-Chrom Q, 80–100 mesh, was used.

Generic	*Structure*	*Retention index*		*Minimum detectable amount**	
		*BBA***	*PBA****	*pg*	*Mol/sec* ($\cdot 10^{-16}$)
Propranolol		2706	3238	1.9	7.1
Alprenolol		2303	2749	2.0	8.1
Bufetolol		2935	3408	3.7	11.5
Bupuranolol		2455	2870	2.4	9.0
Carteolol		3275	3759	4.0	13.7
Nadolol		3171	4167	3.2	10.4
Oxprenolol		2426	2876	2.3	8.6

(Continued on p. 612)

TABLE I *(continued)*

Generic	*Structure*	*Retention index*		*Minimum detectable amount**	
		*BBA***	*PBA****	*pg*	*Mol/sec (· 10^{-16})*
Pindolol		2896	3447	1.5	6.1

* Minimum detectable amount expressed in picograms is valid at t_R = 1–2 min.
** *n*-Butylboronic acid.
*** Phenylboronic acid.

Determination of propranolol in plasma

To 1 ml of plasma sample was added 0.2 ml of an aqueous solution of the internal standard (bufetolol, 1 μg/ml) and 2 ml of 1 *N* sodium hydroxide solution in a glass-stoppered 15-ml centrifuge tube. The tube was shaken with 5 ml of *n*-hexane containing 1.5% of isoamyl alcohol for 10 min and centrifuged for 5 min. The organic layer (4 ml) was transferred into another tube and evaporated to dryness under a gentle stream of air at 50°C. The residue was dissolved in 50 μl of PBA solution, and a 2-μl aliquot of the solution was injected into the column.

Samples (1 ml) of the control plasma containing 1–500 ng of propranolol were treated as described above. Peak-area ratios of propranolol to the internal standard (bufetolol) were measured and plotted against the amount of propranolol added.

Determination of pindolol in plasma

The procedure was essentially the same as for propranolol but the extraction was with ether. Propanolol was used as the internal standard.

RESULTS AND DISCUSSION

Cyclic boronates of β-blocking drugs

The β-blocking drugs were readily derivatized at room temperature with BBA or PBA to form their cyclic boronates (Fig. 1). The reaction was not affected by temperature (20–60°C) or time (0–2 h) under excess boronic acid.

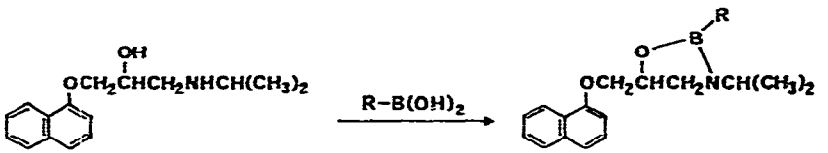


Fig. 1. Reaction of β-blocking drug, propranolol, with boronic acid. R = n-C_4H_9: *n*-butylboronic acid; R = C_6H_5: phenylboronic acid.

The retention indices for BBA and PBA derivatives of eight β-blocking drugs examined are shown in Table I. The PBA derivatives have larger retention indices than the BBA derivatives (*ca.* 400–500 units). Only nadolol showed prolongation of retention index (*ca.* 1000 units) because of the introduction of two cyclic boronate groups into the molecule, at the hydroxyamine and diol sites. The results permit the choice of most suitable derivative for GC analysis in order to obtain the well separated peaks of interest with regard to biological samples.

It is well known that the cyclic boronate derivatives are readily hydrolysed or solvolysed[8,9]. In the case of β-blocking drugs, the derivatives were stable at room temperature for at least 3 days. However, 7 days after derivative formation, the PBA derivatives were almost decomposed but BBA derivatives were not.

The cyclic boronates of the drugs have good GC properties and give high responses on NPD. The detector responses of BBA and PBA derivatives were almost the same, and the minimum detectable amount, givings a signal three times greater than the background noise level, were in the range $6–14 \cdot 10^{-16}$ mol/sec, corresponding to 1.5–4 pg under the GC conditions used (Table I). The described valves were about 10–30 times more sensitive than those of NPD when operated as usual, because of the higher collector voltage.

Determination of propranolol in plasma

Propranolol was extracted from plasma by a single-extraction procedure and derivatized with PBA, as described in the Experimental section. Bufetolol, used as the internal standard, was added to plasma before extraction. The procedure permits analysis of 30 or more samples per day on an instrument. The calibration graph obtained with 1–500 ng of propranolol in 1 ml of plasma is shown in Fig. 2. The graph is rectilinear within a 500-fold range and passed through the origin. The minimum detectable concentration was *ca.* 0.5 ng/ml. The relative standard deviation after eight

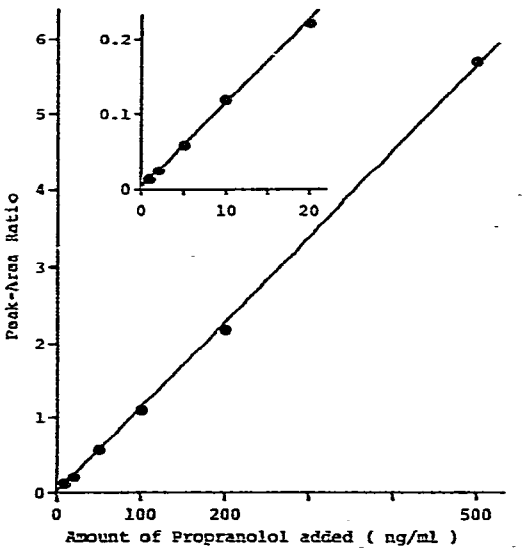
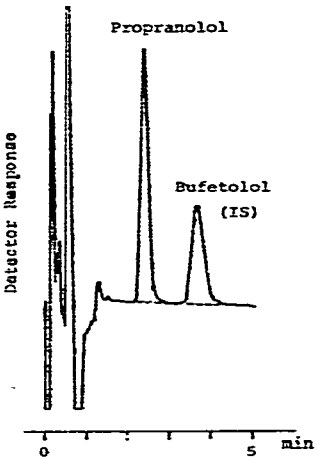


Fig. 2. Calibration graph for propranolol in plasma. Plots are means of three determinations.



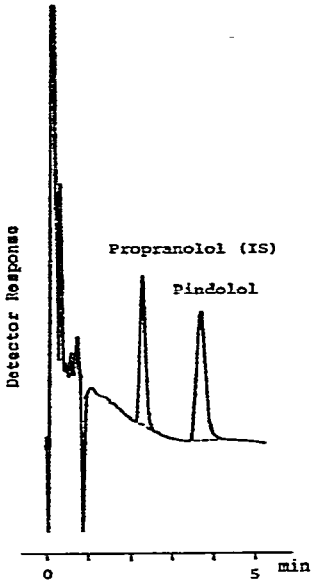


Fig. 3. A typical chromatogram of propranolol in plasma, propranolol corresponding to a plasma concentration of 100 ng/ml. Broken lines represent the background from control plasma. Gas chromatographic conditions as described in the text.

Fig. 4. A typical chromatogram of pindolol in plasma, pindolol corresponding to a plasma concentration of 100 ng/mL Broken lines represent the background from control plasma. Gas chromatographic conditions as described in the text.

determinations was 6.6% at the 10 ng/ml level and 4.1% at the 100 ng/ml level. Fig. 3 shows a typical chromatogram of a plasma sample containing 100 ng of propranolol.

Compared with other methods for the determination of propranolol, the described method was 10–20 times more sensitive than the fluorometric method[1]. This method was simpler and speedier than the GC–ECD method[4,5], which includes a back-extraction procedure and derivatization with trifluoroacetic or heptafluorobutyric anhydride, and the sensitivity was about the same. The results indicate that the proposed method would permit the pharmacokinetic study of propranolol under therapy.

Determination of pindolol in plasma

Pindolol was also determined by the same method but the extraction was with ether. The calibration graph obtained with 2–500 ng of pindolol was rectilinear and passed through the origin. The minimum detectable concentration was 1 ng/ml. A typical chromatogram containing 100 ng of pindolol is shown in Fig. 4. The method was about three times more sensitive than the fluorometric method[2].

CONCLUSION

The results indicate that the described method is generally applicable for the determination of the unchanged β-blocking drugs in plasma and other biological samples. The high sensitivity and relative simplicity of the method would permit

analysis of small samples, and permit pharmacokinetic studies, particularly after low doses, resulting a better understanding of the therapeutic effects of β-blocking drugs.

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