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Note

High-performance liquid chromatographic assay of sotalol: improved procedure and investigation of peak broadening

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Sotalol, 4-[hydroxy-2-(isopropylamino)ethyl]methanesulphonanilide, is a non-selective β-adrenergic receptor blocker and an antiarrhythmic agent with a broad spectrum of activity in supraventricular and ventricular tacharrhythmias [1]. The therapeutic range of serum sotalol concentration for the suppression of cardiac arrhythmias is 1–3 mg/l [1]. Several high-performance liquid chromatographic (HPLC) methods for the measurement of sotalol concentration in biological fluids have been published [2–7]. However, most of the reported assays have disadvantages such as use of large sample volumes ($\geqslant$ 1 ml) [2,4–7], complicated and lengthy extraction procedures [3,6], expensive disposable extraction columns [2,4], long retention times [4,5,7] and broad peaks [3–5] during HPLC analysis. Some methods also require equipment that is not commonly present in a clinical laboratory, *e.g.* fluorescence HPLC detector [3,4] and HPLC phenyl column [7].

We report here an improved HPLC assay which uses liquid–liquid extraction, the readily available C_{18} reversed-phase analytical column and UV detection at 226 nm. The assay offers considerable advantages in performance and speed of chromatographic analysis as compared to previous methods. In addition, the peak broadening of sotalol and the internal standard atenolol which is observed with some of the published methods [3–5] has been shown to be a function of the extraction procedure and was eliminated by modification of the extraction protocol.

EXPERIMENTAL

Chemicals

Sotalol hydrochloride was extracted from pharmaceutical tablets, (Sotacor 160 mg, Astra Pharmaceuticals, North Ryde, Australia), recrystallized from etha-

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nol–water and dried in vacuum. The purity of the crystalline solid was determined by HPLC and its melting point (202–204°C; lit. [8] 205–206°C). Atenolol (internal standard) was obtained from pharmaceutical tablets (Tenormin, ICI Australia Operations, Melbourne, Australia). Dichloromethane and acetonitrile were HPLC grade (Millipore, Lane Cove, Australia); isopropyl alcohol (analytical grade) and 1-octanesulphonic acid sodium salt (Unichrom) were purchased from Ajax Chemicals (Auburn, Australia); disodium tetraborate, potassium dihydrogenphosphate and sulphuric acid were analytical grade (B.D.H., Kilsyth, Australia).

Apparatus

The liquid chromatographic system consisted of a Model 8500 solvent delivery system and a Vari-Chrom UV–VIS detector (Varian Instrument Division, Palo Alto, CA, U.S.A.). The 150 mm × 4.5 mm column was slurry packed with 5-μm ODS Hypersil. The mobile phase consisted of 10 m*M* phosphate buffer pH 3.2–acetonitrile 4:1 (v/v) containing 3 m*M* 1-octanesulphonic acid sodium salt. A flow-rate of 1 ml/min was used and the absorbance was measured at 226 nm with an absorbance range setting of 0.02.

Standard solutions

Sotalol standard solutions containing 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/l were prepared daily by the addition of appropriate amounts of aqueous solution containing 28.3 mg/l sotalol hydrochloride (corresponding to 25.0 mg/l sotalol free base) to a sotalol-free commercial quality control serum (Gibcotrol Serum Low, Laboratory Services, Auckland, New Zealand). The internal standard solution was prepared by dissolving atenolol in distilled water at a concentration of 25 mg/l.

Buffer solutions

Borax buffer was prepared by making a saturated solution of disodium tetraborate and adjusting the pH to 9 with 6 *M* hydrochloric acid. Phosphate buffer was prepared by dissolving 1 36 g of potassium dihydrogenphosphate in 1000 ml of distilled water and adjusting the pH to 3.2 by 0.2 *M* phosphoric acid.

Assay procedure

To a 10-ml screw-cap glass tube were added 500 μl of serum (or plasma), 50 μl of internal standard solution, 100 μl of saturated borax buffer pH 9.0 and 5 ml of dichloromethane–isopropyl alcohol (3:1). The contents were mixed by inversion on a rotator at 40 rpm for 5 min and centrifuged at 800 *g* for 3 min. The upper layer was aspirated and discarded, the organic layer was transferred to a tapered-bottom glass tube and evaporated to dryness at 55°C under a stream of nitrogen. The residue was dissolved in 100 μl of 0.05 *M* sulphuric acid by vortex-mixing for 15 s prior to injection of an aliquot (30–50 μl) onto the HPLC column.

Assay characteristics

Linearity studies and within-day reproducibility were evaluated by quadruplicate analyses of serum sotalol standards containing 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/l. Inter-assay precision was determined using serum standards containing 1.0, 2.0 and 4.0 mg/l on six occasions over several weeks. Absolute analytical recovery was calculated by comparing the peak heights of sotalol standards that had been taken through the extraction procedure with the peak heights resulting from the direct injection of solutions of sotalol in 0.05 *M* sulphuric acid, after appropriate volume corrections. The detection limit was defined by peak response equal to three-fold the measured background noise.

Investigation of peak broadening

Standard solutions containing sotalol and atenolol at 5 mg/l each were prepared in water, 0.05 *M* sulphuric acid or HPLC mobile phase separately. The standard solution in dilute sulphuric acid was mixed with a variety of organic solvents and solvent mixtures. When the acidic and organic layers were miscible, an aliquot of mixture was injected directly onto the HPLC column. With the water-immiscible chlorinated hydrocarbons, approximately 10% (v/v) of organic solvent was added to the acidic standard. The mixture was thoroughly mixed, the layers were separated by centrifugation and an aliquot of the aqueous solution was analysed by HPLC. The speed of the chart recorder was 20 mm/min and widths at half height for sotalol and atenolol peaks were measured.

RESULTS AND DISCUSSION

A variety of HPLC mobile phases had been used in the published sotalol assays, many of them [3,5,6] containing acetic acid which is not suitable for HPLC analysis with UV detection due to the absorbance of acetic acid at 226 nm, the λ_{max} of protonated sotalol. We found that the mobile phase consisting of phosphate buffer, acetonitrile and an ion-pairing agent, as used by Karkkainen [7] with a phenyl column, gave sharp and well resolved peaks for sotalol and the internal standard, atenolol, on a reversed-phase C_{18} column.

For the extraction of sotalol and internal standard from serum, we followed the procedure described by Poirier *et al.* [5] except that isoamyl alcohol was replaced by the less expensive isopropyl alcohol. The compounds were extracted into a mixture of isopropyl alcohol and dichloromethane (1:3) at pH 9, then back-extracted into a small volume of dilute sulphuric acid. An aliquot of the acidic layer was injected onto the HPLC column. We noticed that Poirier's extraction procedure gave substantial chromatographic peak broadening of the sotalol and internal standard. Published chromatograms from other reports using this extraction procedure [3,5] suggests similar peak broadening problems. Column efficiencies for these methods are well below 10 000 plates/m (calculated from peak width at half height) instead of the expected > 50 000 plates/m. After a

series of experiments we found that the peak broadening was caused by the alcohol component of the organic extraction solvent. The presence of an alcohol is, however, essential for a successful extraction of the zwitterionic sotalol from aqueous solution [6]. Thus, it is necessary to remove the alcohol after the extraction We overcame the problem of peak broadening by evaporation of the organic solvent and redissolution of the residue in dilute acid prior to injection onto HPLC column. Typical chromatograms of extracts from serum standard, serum blank and serum from a patient receiving sotalol are shown in Fig. 1. The retention times of the internal standard atenolol (A) and sotalol (S) were 4.0 and 5.5 min, respectively, and no interference from endogenous material in serum was

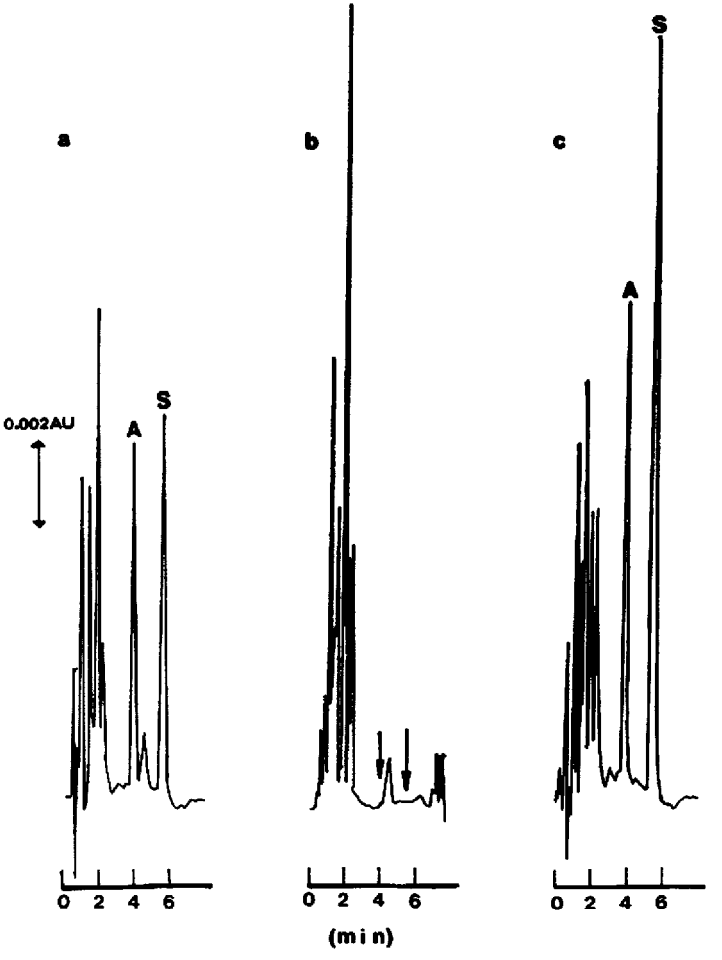

Fig 1. Chromatograms of extracts of (a) serum standard (2 mg/l), (b) blank serum and (c) serum from patient taking sotalol (2.9 mg/l) For chromatographic conditions, see Experimental section. Peaks: A = atenolol; S = sotalol.

detected. The modified sample preparation allowed an HPLC analysis time of less than 6 min, which is considerably shorter than all previously reported methods employing liquid–liquid extraction [3,5,7].

The method exhibits a good linear correlation between serum concentrations of sotalol and peak-height ratios for sotalol/internal standard over the range 0.25–8.0 mg/l. The calibration curve for sotalol had a slope of 0.563 ± 0.006 and an ordinate intercept of 0.003 ± 0.021 with a correlation coefficient of 0.9988. Intra-assay precision, expressed as the coefficient of variance (C.V.), was found to be 5.2% at 0.25 mg/l, 4 2% at 0.5 mg/l, 3.8% at 1.0 mg/l, 0.8% at 2.0 mg/l, 2 2% at 4.0 mg/l and 4.7% at 8.0 mg/l ($n = 4$). The corresponding inter-assay data were found to be 5.6% at 1.0 mg/l, 3.2% at 2.0 mg/l and 4.3% at 4.0 mg/l ($n = 6$). The absolute analytical recovery of sotalol from serum was 75%. This compares well with solid–liquid extraction (76% [2,4]) and other liquid–liquid extractions (70–77% [5,7]), and is superior to extraction involving protein precipitation (< 60% [6]). The detection limit for sotalol in serum by this assay was 0.01 mg/l which is well below the effective therapeutic level and sufficiently low for single-dose pharmacokinetic studies.

In order to elucidate the cause of the peak broadening observed with the sample extraction procedure of Poirier *et al.* [5] we prepared a variety of solutions containing sotalol and atenolol. Those solutions were injected onto the HPLC column, and peak shapes of sotalol and atenolol were examined in detail. There

TABLE I

PEAK WIDTH AT HALF HEIGHT OF SOTALOL AND ATENOLOL

Conditions: see experimental section, chart speed 20 mm/min.

No.	Solvent[a]	Peak width (mm)		Comment
		Sotalol	Atenolol	
1	Water	4 2	3.5	
2	0 05 *M* Sulphuric acid	4.3	3.5	
3	Mobile phase	4.2	3.5	
4	2 + DC	3.9	3.8	
5	2 + DC–IP (3:1)	5 4	7.5	
6	2 + CH–IA (3 1)	6.7	3.7	Tailing
7	2 + DC–IP (3:1)	6.8	10	Sotalol and atenolol at 2.5 mg/l)
8	2 + 10% IP	4.8	3.8	
9	2 + 20% IP	7.3	5.8	
10	2 + 50% IP	–	–	Cluster of peaks
11	9 + DC	5.5	4.5	

[a] DC = dichloromethane; CH = chloroform; IP = isopropyl alcohol; IA = isoamyl alcohol.

was no apparent difference between the peak widths of both compounds whether dissolved in water, dilute sulphuric acid or HPLC mobile phase (Table I, Nos. 1–3). We then mixed a solution of sotalol and atenolol in dilute sulphuric acid, the back-extraction medium used in the assay, with various organic solvents which were employed in the extraction, and reanalysed the mixture by HPLC. We observed that addition of dichloromethane produced only small effects including a slight narrowing of the sotalol peak (Table I, No. 4). However, addition of two-component solvents containing chlorinated hydrocarbons and alcohol, gave greatly different chromatograms (Table I, Nos. 5–7). Dichloromethane–isopropyl alcohol (3:1) produced serious distortion and "fronting" of the peaks (Fig. 2b) which is only partially reflected in their increased width at half height (sotalol + 26%, atenolol + 114%). Chloroform–isoamyl alcohol (3:1), the extraction solvent used by Poirier *et al.* [5], caused tailing for the sotalol peak (peak width at half height + 56%) but did not seem to affect the internal standard (Table I, No. 6). It seemed therefore that the alcohols were responsible for the detrimental chromatographic behaviour either by interfering in the chromatographic process or by increasing the solubility of the chlorinated hydrocarbons in the aqueous

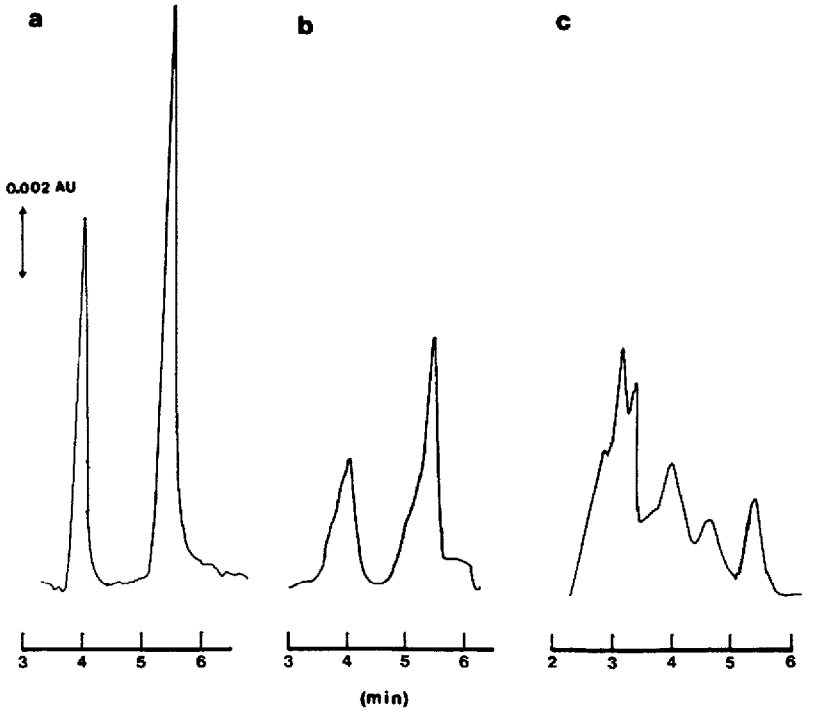

Fig. 2 Chromatograms of sotalol and atenolol dissolved in 0 05 *M* sulphuric acid (5 mg/l each) which had been mixed with (a) neat, (b) dichloromethane–isopropyl alcohol (3 1) and (c) 50% isopropyl alcohol. Conditions as for Table I Injection volumes. (a, b) 20 μl, (c) 40 μl

phase. When isopropyl alcohol alone was added in increasing amounts to the acidic aqueous solution of sotalol and atenolol, there was indeed a progressive broadening of the observed peaks (Table I, Nos. 8–10). Isopropyl alcohol concentrations greater than 20% resulted in substantial peak distortions, mainly through "fronting". It was noteworthy that the retention time of the peak maximum remained constant throughout this experiment. At 50% added isopropyl alcohol a cluster of peaks appeared with the last peak corresponding to the "normal" peak of sotalol (Fig. 2c). The acidic mixture containing 20% isopropyl alcohol was mixed with dichloromethane and an aliquot of the aqueous layer reinjected The peaks were narrower than those obtained with the parent mixture (Table I No. 11 *versus* No. 9), which perhaps reflects the lower concentration of isopropyl alcohol in the aqueous layer after mixing with dichloromethane. Furthermore it could be shown that the distortion of the peak shape depended on the ratio of solute to isopropyl alcohol. Thus, mixing an acidic solution of sotalol and atenolol at 2.5 mg/l each with dichloromethane–isopropyl alcohol (3:1) produced more extensive peak broadening than was obtained with solute concentrations at 5 mg/l (Table I, No. 7 *versus* No. 5).

Peak distortion with reversed-phase HPLC has been reported elsewhere [9,10]. In extreme circumstances multiple peaks were obtained for a single compound due to the interaction between solute, injection solvent and mobile phase [9,10]. The effects have been explained in terms of solubility parameters [9,10]. To our knowledge there are no similar reports for paired-ion chromatography [11,12], but in principle the same effects could explain the observed distortions. We have circumvented the problem of peak broadening by removing the organic solvent prior to HPLC analysis as described above. The resulting sharp peaks allow a decrease of analysis time to less than 6 min with good separation between sotalol, internal standard and endogenous material. We have no evidence of any interference from endogenous material or other drugs in samples from more than ninety patients.

In conclusion, this is a fast, reliable and accurate method for the determination of serum sotalol levels which is suitable for therapeutic drug monitoring program or pharmacokinetic studies.

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