Journal of Chromatography, 336 (1984) 313—319
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2312

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SOTALOL IN BIOLOGICAL FLUIDS

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(First received April 10th, 1984; revised manuscript received August 3rd, 1984)

SUMMARY

A sensitive, selective and reproducible reversed-phase high-performance liquid chromatographic method is described for the quantification of sotalol in human serum and urine. Sotalol and the internal standard, atenolol, were extracted from alkalinized serum and urine (pH 9.0) into 1-butanol—chloroform (20:60, v/v). The organic phase was evaporated, and to the residue was added 0.1 *M* sulphuric acid (serum analysis) or mobile phase (urine analysis). The mobile phase consisted of 0.01 *M* phosphate buffer (pH 3.2) and acetonitrile (20:80, v/v) containing 3 m*M* n-octylsodium sulphate. The flow-rate was 1.5 ml/min. The retention times of atenolol and sotalol were 7 and 10 min, respectively. Ultraviolet detection at 226 nm made it possible to achieve a detection limit of 0.03 μmol/l.

INTRODUCTION

The pharmacological properties of sotalol were first described in 1965 by Lish et al. [1]. These and subsequent studies showed that sotalol hydrochloride is a non-selective beta-blocking drug, devoid of intrinsic sympathomimetic and membrane-stabilizing activity [2]. Sotalol seems to differ from other beta-antagonists in general use in that it also exerts amiodarone-like effects of the group III antiarrhythmic drugs [3] and furthermore prolongs, in a concentration-dependent manner, the action potential duration and QTc interval in the electrocardiogram [4]. Although sotalol hydrochloride has been used in the treatment of cardiovascular diseases for about a decade, there are few data about its quantitative measurement in biological fluids. In most of the pharmacokinetic and pharmacodynamic studies where the concentrations of the drug have been measured [5—12], the assay method has been the spectrofluorometric procedure described by Garret and Schnelle [13], or a slight

0378-4347/84/$03.00

modification of it [12]. However, the spectrofluorometric assay needs large volumes of plasma and lacks specificity.

In view of the clinical use of sotalol, a sensitive and reproducible high-performance liquid chromatographic (HPLC) method has been developed for its quantitation in serum and urine. The method involves the structurally related compound atenolol as internal standard, and a relatively simple extraction procedure prior to chromatography.

EXPERIMENTAL

Chemicals and glassware

Sotalol hydrochloride was purchased from Lääke-Farmos (Turku, Finland) and the internal standard, atenolol, from Oy Star Ab (Tampere, Finland). Chloroform, 1-butanol, orthophosphoric acid, potassium dihydrogen phosphate and sulphuric acid were obtained from E. Merck (Darmstadt, F.R.G.) and were of analytical grade. Acetonitrile, HPLC grade, was purchased from Rathburn Chemicals (Walkerburn, U.K.). Only double-glass-distilled water was used. All glassware was silanized before use with a 10% solution of dimethyldichlorosilane in toluene. After 15 min in this solution, the glassware was washed with toluene and methanol.

Apparatus and HPLC procedure

The liquid chromatographic system consisted of an M-45 solvent delivery system, a Model U6K liquid chromatographic injector (Waters Assoc., Milford, MA, U.S.A.), and an LKB 2238 Uvicord SII detector with ultraviolet lamp of wavelength 226 nm (Bromma, Sweden). The absorbance range was 0.05 for serum analysis and 0.1 for urine analysis. The column was a reversed-phase μBondapak™ Phenyl column (30 cm × 3.9 mm I.D., 10 μm particles size; Waters Assoc.). Pre-column filter and packing material, Bondapak C_{18}/Corasil, were from Waters Assoc. A one-channel recorder 2210 LKB (Bromma) was used (chart speed 2 mm/min, sensitivity 20 mV).

The mobile phase for the separation of sotalol and internal standard consisted of a mixture of 0.01 *M* phosphate buffer, pH 3.2—acetonitrile (80:20, v/v), containing 3 m*M* *n*-octylsodium sulfate (Merck). The mobile phase was filtered through a Millipore filter unit equipped with Millipore filter, HATF 04700, pore size 0.45 μm (Millipore, Molsheim, France), 0.5 h after *n*-octylsodium sulphate had been added, and then degassed ultrasonically (Sonicor). The flow-rate was 1.5 ml/min.

Calibration graphs

Calibration graphs were prepared by adding sotalol to drug-free serum and urine to provide varying concentrations. These samples were then processed through the analytical procedure described. The internal standard was diluted with 0.5 *M* Tris—HCl buffer (pH 9.0) to give the final concentration of 2.0 μmol/l for serum extractions and 50.0 μmol/l for urine extractions.

The evaluation of the chromatograms was based on the peak height ratios of sotalol and atenolol. The calibration graphs for sotalol in human serum ranged from 0.1 to 4.0 μmol/l, and in urine from 3.25 to 200 μmol/l. The concentration of the stock solution was 1 mmol/l.

Extraction procedure

Serum. A 1-ml volume of the solution of internal standard in 0.5 *M* Tris—HCl buffer (pH 9.0) was aded to 1 ml of sample or standard in a 15-ml PTFE-lined screw-capped tube, and the mixture was gently vortexed. After the addition of 7 ml of chloroform—1-butanol (60:20, v/v), the tubes were shaken in a vertical position for 10 min, then centrifuged for 10 min at 1500 *g*. The organic phase (5 ml) was transferred to a 10-ml conical glass-stoppered test tube and evaporated to dryness under a gentle stream of nitrogen in a water bath (50°C). The residue was redissolved in 50—100 μl of 0.1 *M* sulphuric acid by vortexing for 30 sec; 20—30 μl were injected onto the column.

Urine. The extraction procedure was the same as that for serum except that 0.5 ml of urine was used, and the residue was dissolved in 250—1000 μl of the mobile phase.

Recovery and reproducibility

The absolute analytical recovery of sotalol from human serum and urine was estimated by comparing the peak heights obtained from the injection of known quantities of the compound with those obtained from the injection of extracts of serum and urine samples spiked with sotalol. Within-day reproducibility and accuracy were evaluated by analysis of serum and urine samples (n = 6) containing 4.0 and 50.0 μmol/l sotalol, respectively. Day-to-day reproducibility was determined by assaying serum standards containing 0.1, 0.25, 0.5, 1.0, 2.0 and 4.0 μmol/l on six occasions, and urine standards containing 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 μmol/l on six occasions. In addition, the influence of injection volume and the initial volume of serum and urine on the reproducibility was evaluated.

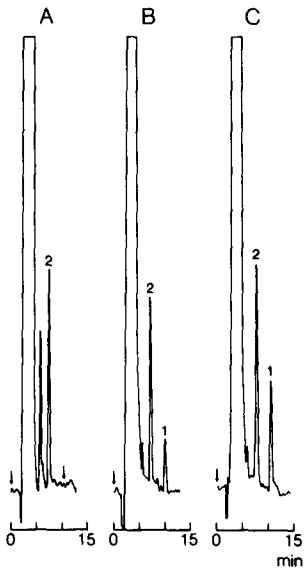
Stability and storage of sotalol

To assay the stability of frozen samples, 1-ml aliquots of pooled serum from volunteers were stored frozen at −20°C in polypropylene centrifuge tubes and subsequently assayed by the method described.

RESULTS

Typical chromatograms obtained from serum and urine analyses are shown in Figs. 1 and 2, respectively. The analysis of drug-free serum and urine samples, performed without the addition of internal standard and sotalol, did not reveal the presence of any endogenous interfering compounds. The retention times of atenolol and sotalol were 7 and 10 min, respectively. There was a linear correlation between sotalol serum and urine concentrations and the peak height ratios between the drug and internal standard over the ranges measured: 0.1—4.0 n*M* (serum) and 6.25—200 n*M* (urine). The calibration curve for sotalol in serum had a slope of 0.5539, and an ordinate intercept of −0.0156 with a correlation coefficient (r) of 0.9971 over the range of assay. In urine, the calibration curve had a slope of 0.0260, and an ordinate intercept +0.0014, with r = 0.9987. The amounts of internal standard in the serum and urine analyses were 2 and 50 nmol, respectively.

The absolute recovery of sotalol from serum was determined by comparing



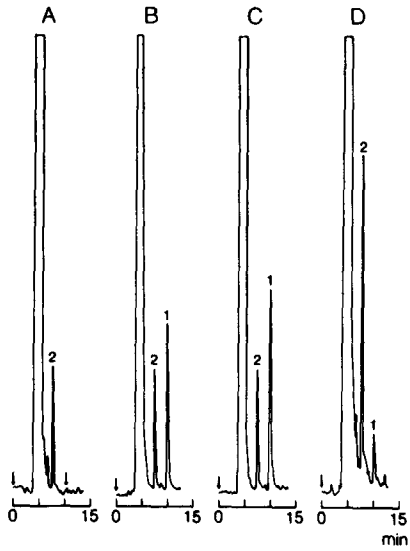


Fig. 1. Chromatograms of human serum: (A) before administration (control); (B) spiked with 0.5 nmol of sotalol; (C) sample obtained from a volunteer 24 h after oral intake of 160 mg of sotalol hydrochloride. Peaks: 1 = sotalol; 2 = internal standard (atenolol). Detector: 226 nm, 0.05 a.u.f.s. Recorder: sensitivity 20 mV, chart speed 2 mm/min.

Fig. 2. Chromatograms of human urine: (A) before administration (control); (B) spiked with 50 nmol of sotalol; (C) sample from 12—24 h collection fraction from a volunteer receiving a 160-mg oral dose of sotalol hydrochloride; (D) sample from 60—72 h collection fraction from a volunteer receiving a 160-mg oral dose of sotalol hydrochloride. Peaks: 1 = sotalol; 2 = internal standard (atenolol). Detector: 226 nm, 0.1 a.u.f.s. Recorder: sensitivity 20 mV, chart speed 2 mm/min.

the peak heights of extracted standards to the peak heights of known concentrations injected. The absolute recoveries ranged from 70% to 75% over the concentration range 0.1—4.0 nmol. Tables I—III show that the recoveries with atenolol as the internal standard varied between 96% and 106% in serum and between 99% and 103% in urine. The sensitivity of the assay was 0.1 μmol/l when 1 ml of the sample was used and 20 μl of the final solution were injected into the column. The detection limit could be lowered by injecting a larger volume into the column and by using a larger volume of the sample in the pharmacokinetic study. Thus the absolute limit of detection was 0.03 μmol/l.

TABLE I

WITHIN-DAY ACCURACY AND PRECISION DATA FOR SOTALOL IN SERUM AND URINE (n = 6)

Sample	Amount added (nmol/ml)	Mean concentration found (nmol/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Recovery (%)
Serum	4.0	4.04	0.02	0.01	0.53	101.0
Urine	50.0	51.36	0.87	0.36	1.70	102.7

TABLE II

DAY-TO-DAY ACCURACY AND PRECISION DATA FOR SOTALOL IN SERUM (n = 6)

Amount added (nmol/ml)	Mean concentration found (nmol/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Recovery (%)
0.1	0.106	0.009	0.004	8.30	106.0
0.5	0.501	0.020	0.009	4.06	100.2
1.0	0.982	0.046	0.019	4.67	98.2
2.0	1.923	0.101	0.041	5.27	96.2
4.0	4.131	0.116	0.047	2.80	103.3

TABLE III

DAY-TO-DAY ACCURACY AND PRECISION DATA FOR SOTALOL IN URINE (n = 6)

Amount added (nmol/ml)	Mean concentration found (nmol/ml)	Standard deviation	Standard error	Coefficient of variation (%)	Recovery (%)
6.25	6.33	0.28	0.11	4.36	101.3
12.5	12.48	0.56	0.23	4.43	99.8
25.0	24.82	1.21	0.49	4.88	99.3
50.0	50.84	1.21	0.49	2.38	101.7
100.0	100.38	2.11	0.86	2.10	100.4

TABLE IV

STABILITY OF SOTALOL IN FROZEN SERUM SAMPLES

Week	Concentration found (nmol/ml)
1	1.88
	2.07
	1.95
2	2.04
	2.19
3	1.98
	2.02
4	1.99
	2.07
Mean ± S.D.	2.02 ± 0.09

The within-day accuracy and precision data for serum and urine are presented in Table I. The corresponding day-to-day data are shown in Tables II and III. These data indicate that the assay used here is sufficiently accurate and precise for pharmacokinetic studies.

No decrease in the measured sotalol concentration was detected when samples were stored for periods of up to four weeks at −20°C (Table IV).

A change in the volume of urine from 100 μl to 2 ml and of serum from 0.5 to 2 ml had no influence on the accuracy and reproducibility studies when the paired urine and serum samples were spiked with sotalol to contain 25.0 and 1.0 nmol, respectively.

DISCUSSION

The extraction of sotalol into the organic phase is pH-dependent. Sotalol has two pK_a values (8.80 and 9.80). It has been shown that the maximum extraction into an organic solvent can be effected at the maximum concentration of the neutral form in equilibrium with the zwitterion, half-way between the two pK_a values, i.e. about pH 9 [13]. When 1.0 ml of Tris—HCl buffer (pH 9.0) was added to 0.1—2 ml of urine (pH varied from 4.8 to 8.2), or to 1—2 ml of serum, the final pH was always 9.0.

Sotalol displays two ultraviolet absorption peaks depending on the pH of the medium [13]. In acidic conditions, the λ_{max} is 227 nm, and above pH 8.5 the λ_{max} is 248 nm. Since the molar absorptivity of sotalol in basic solutions is greater than in acidic conditions, a lower wavelength and an acidic mobile phase were chosen for quantitative measurement to prolong the lifetime of the column. The lower limit of detection in acidic conditions was nevertheless sufficient to be acceptable for pharmacokinetic studies.

The optimal separation of sotalol and the internal standard from endogenous material was obtained when the ratio of phosphate buffer to acetonitrile was 80:20 (v/v). Caffeine interfered with the analysis when the acetonitrile concentration was increased. Bubble formation in the mobile phase was minimized by allowing the solution to stand at room temperature for half an hour before filtration and sonication.

The principal advantages of the present HPLC method, compared to the spectrofluorometric method of Garret and Schnelle [13] and to its modifications [12], are improved sensitivity and specificity. The HPLC method recently described by Lefebvre et al. [14] is also sensitive, but no accuracy and precision studies were provided.

The HPLC method reported here has proved to be particularly useful for pharmacokinetic studies of sotalol in volunteers. The results of those studies will be reported elsewhere.

ACKNOWLEDGEMENTS

The author is grateful to Professor Heikki Vapaatalo, Head of the Department of Pharmacology, for his support of this work, and to Mrs. Kirsti Ristilä for her excellent technical assistance.

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