Journal of Chromatography, 427 (1988) 181–187
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4107

Note

Improved high-performance liquid chromatographic method for the analysis of serum sotalol

GIFFORD L. HOYER*

Cardiovascular Analysis Laboratory, University of Arizona Medical Center, Tucson, AZ 85724 (U.S.A.)

(First received October 13th, 1987; revised manuscript received December 28th, 1987)

Sotalol, a non-cardioselective β-adrenergic blocking agent [1,2], is currently under review for approval by the Food and Drug Administration as an antiarrhythmic drug. Many studies have determined blood levels of sotalol that are effective in suppressing cardiac arrhythmias. The range of efficacious trough concentrations was 0.34–3.5 μg/ml [3] with most finding between 1.0 and 3.0 μg/ml [4–6]. It has been shown that sotalol is eliminated primarily by the kidneys without prior metabolism [7]. Patients with chronic renal failure [8] or increasing serum creatinine [9] display decreased elimination of sotalol. Decreased clearance will result in increased serum sotalol concentrations; therefore, it is desirable to monitor serum sotalol levels in patients.

Several techniques are available for quantitating patient blood levels of sotalol. A spectrofluorometric method [9] and modifications of the method [8] were developed first but lacked specificity. A sensitive and specific gas chromatographic (GC) method for determining sotalol levels in human plasma was developed; however, it required laborious extraction and derivatization steps [10]. Several high-performance liquid chromatographic (HPLC) techniques [11–14] exist for quantitating sotalol in serum; however, some require fluorescence detectors [12,13] or require excessive sample preparation [11–14]. The reversed-phase HPLC technique of Gallo [15] lacked reliability. An internal standard (I.S.) was employed [16] to increase the method's reliability; however, the chromatographic run times were long (>8 min). The method presented in this paper is

*Address for correspondence: Department of Internal Medicine, Section of Cardiology, University of Arizona, Tucson, AZ 85724, U.S.A.

0378-4347/88/$03.50

faster (decreased retention times), has a simple extraction procedure and has low inter- and intra-assay variation.

EXPERIMENTAL

Chemicals

Sotalol hydrochloride and the internal standard (I.S., MJ-6564-1) were supplied by Bristol Meyers Pharmaceutical Research and Development Section. Potassium phosphate dibasic (K_2HPO_4) grade V was purchased from Sigma (St. Louis, MO, U.S.A.). Nonylamine (98%) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Ethyl acetate (HPLC grade) was obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Acetonitrile was supplied by Burdick & Jackson via American Scientific Products Distribution Center (McGaw Park, IL, U.S.A.). Phosphoric acid (85% analytical reagent) was obtained from Mallinckrodt via American Scientific Products Distribution Center. All water used in this assay was distilled, treated by reverse osmosis, charcoal-filtered and subsequently filtered through a 0.45-μm pore Nylon filter.

Instrumentation and chromatographic conditions

The liquid chromatographic system consisted of a Rheodyne 7010 manual injection valve and a Hewlett-Packard 1090 liquid chromatograph with a diode-array detector. A Hewlett-Packard 85B personal computer controlled the chromatograph and detector and also recorded and analyzed spectrographic data on the internal standard and sotalol. Chromatograms and integration of absorbance data from the diode-array detector were obtained from a Hewlett-Packard 3392A integrator. An Altex 25$\times$0.4 cm reversed-phase 5-μm octadecylsilane column (ODS C_{18}) was used for the chromatographic separation.

The mobile phase consisted of 0.01 *M* potassium phosphate dibasic buffer at pH 2.4 (using phosphoric acid) containing 0.002 *M* nonylamine. The flow-rate was held constant at 2.0 ml/min with a back-pressure maintained near 210 bar. The column temperature was kept at 40°C. Mobile phase was pumped through the column for 30 min prior to analytical runs to allow equilibration. In addition, a conditioning injection of 100 μg each of pure sotalol and internal standard were applied to the column prior to analytical runs. The diode-array detector was set to detect absorbance at 235 nm.

Standards

Sotalol in HPLC-grade methanol was pipetted in duplicate into labeled conical centrifuge tubes in amounts of 0.15, 0.75, 1.5, 3.0, 6.0 and 7.5 μg. The methanol was then evaporated to dryness in a 40°C water bath under a stream of nitrogen gas. A 1-ml volume of pooled serum was added to each tube. Pooled serum with a known amount of sotalol added (2.0 μg/ml) was used as a control to assess the accuracy of each assay. Pooled serum with no sotalol added was used as a blank (zero) control. Duplicate 1-ml samples of the spiked control, blank control and patient serum were pipetted into labeled conical centrifuge tubes. Identification

of sotalol extracted from serum was established by comparing spectra obtained for pure sotalol and the corresponding peak in the extracted samples.

Serum extraction

Duplicate serum samples of spiked pooled serum, blank pooled serum or patient serum were extracted by solid-phase extraction [17]. A brief description of the extraction procedure: 1 ml of serum was adjusted to pH 9.3 by the addition of 0.5 ml of 0.5 *M* bicine buffer, N,N-bis(2-hydroxyethyl)glycine, pH 9.4. Internal standard (2 μg) was added prior to extraction to control for variation in extraction efficiency, and the tubes were vortexed. C_{18} reversed-phase disposable extraction columns (Baker 10 extraction system) were washed twice with 1.5 ml methanol followed by two 1.5-ml rinses with 0.17 *M* bicine buffer, pH 9.4. The serum samples were then applied to the column and allowed to feed by gravity through the packing. The columns were then rinsed twice with 1.5 ml of 0.17 *M* bicine buffer to rinse water-soluble components retained on the column. The sotalol was eluted from the column using acetonitrile–ethyl acetate (2:1) in three 1-ml aliquots. The elution solution was separated from any aqueous solution collected from the column by immersing the collection tube in a dry ice–methanol bath for 5 s. The unfrozen (organic) portion was transferred to another tube and dried under nitrogen in a water bath (40°C). The dried extract was reconstituted in 200 μl of mobile phase and 100 μl were injected onto the column.

Assay validation

Data validating the solid-phase extraction methodology has been previously published [17] and, therefore, will not be discussed here. Several methods were utilized to assess the efficiency of the chromatography. Intra-assay variation was determined by analyzing ten aliquots of the same spiked serum (3.0 μg/ml) on one day and determining the variation within the samples. Inter-assay variation was determined by comparing the results of the analysis of aliquots of spiked serum (2.0 μg/ml) for seven different assay days. Interference from several antiarrhythmics and other commonly prescribed drugs was tested by extracting and analyzing TDM serum (Fischer Scientific), a commercially prepared spiked serum.

RESULTS

A chromatogram of extracted spiked pooled serum is shown in Fig. 1 and compared to chromatograms of extracted blank pooled serum and extracted patient serum. The I.S. and sotalol elute from the column at approximately 3.4 and 4.5 min, respectively. Earlier eluting peaks represent unidentified serum components extracted from serum. The chromatogram of extracted pooled serum does not display interfering components at the times sotalol or the I.S. elute. Serum from more than thirty patients receiving sotalol showed to interfering compounds.

The diode-array detector allows the collection of UV absorption spectral data. Computer analysis showed that the spectra of the putative sotalol peak obtained from pure standard and extracted spiked serum were identical. In addition, the

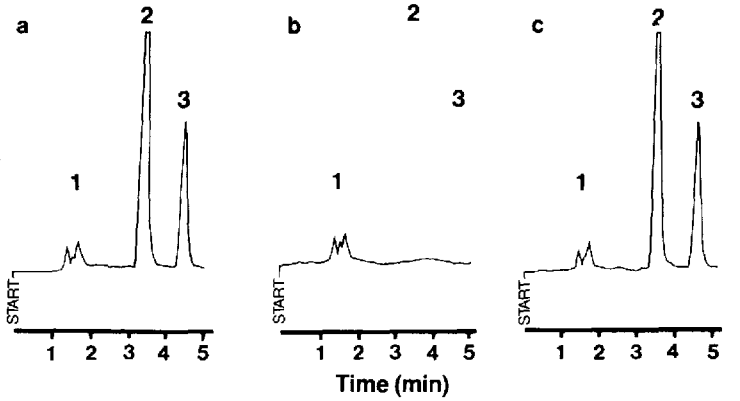

Fig. 1. Chromatograms of extracted human serum. (a) Extracted pooled serum (1 ml) spiked with 0.75 μg/ml sotalol standard; (b) extracted blank pooled serum; (c) extracted serum (1 ml) from patient receiving sotalol. Chromatographic conditions: flow-rate, 2.0 ml/min; mobile phase, 0.01 *M* potassium dihydrogenphosphate buffer pH 2.4 with 0.002 *M* nonylamine; column temperature, 40°C, column, 25×0.4 cm, 5-μm ODS packing; detector, 235 nm. Peaks: 1 = normal serum components; 2 = MJ-6564-1 (internal standard); 3 = sotalol.

tentative sotalol peaks had identical elution times. The absorbance maxima previously reported [9] are similar to the spectra we observed. Therefore, we feel confident in identifying the peak at 4.5 min in Fig. 1 as sotalol.

The linearity of this assay has been tested in the range of 0.15–7.5 μg/ml. To 1 ml, drug-free pooled serum, 0, 0.15, 0.75, 1.5, 3.0, 6.0 or 7.5 μg of sotalol was added and assayed in duplicate. The calibration curve obtained by plotting the peak-height ratio of sotalol/I.S. versus the concentrations of sotalol was linear throughout the analytical range studied. Linear regression data for seven consecutive calibration curves performed over five months are shown in Table I. It can be seen that there is a high correlation coefficient of linearity between the points for the calibration curves.

Intra- and inter-assay precision was evaluated by analyzing serum sotalol concentrations repeatedly within the same day and over a period of different assay days. Ten samples containing 3.0 μg/ml sotalol were analyzed and found to have a mean of 2.89 μg/ml with a standard deviation of 0.156 and coefficient of variation of 5.4%. Inter-assay variation was determined by the analysis of spiked serum ($n = 7$) containing 2.0 μg/ml sotalol. They were found to have a mean value of

TABLE I

LINEAR REGRESSION DATA FOR CALIBRATION CURVES

Data presented in this table were compiled from seven consecutive sotalol assays performed over a five-month period.

	Slope	y-Intercept	Coefficient
Mean	1.577	0.039	0.9989
S.D.	0.082	0.023	0.0021

TABLE II

DRUGS TESTED FOR INTERFERENCE WITH THE ASSAY OF SOTALOL

None of the compounds tested interfered with sotalol or internal standard detection or quantitation.

Antiarrhythmics	Other drugs	
Amiodarone	Acetaminophen	Primidone
Disopyramide	Amikacin	Salicylates
Lidocaine	Carbamazepine	Theophylline
Mexiletine	Ethosuximide	Tobramycin
N-Acetylprocainamide	Gentamicin	Valproic acid
Phenytoin	Lithium	Amitryptiline
Digoxin	Methotrexate	Desipramine
	Phenobarbital	Imipramine
		Nortryptiline

2.02 μg/ml with a standard deviation of 0.122 and a coefficient of variation of 6.04%.

In order to establish the accuracy of the proposed assay we carried out recovery studies, using pooled drug-free serum spiked with 0.75, 1.5 and 6.0 μg/ml sotalol. The assays were performed in triplicate and the sotalol concentrations were determined through the peak-height ratio method by referring to the respective calibration curves. The concentrations (mean $\pm$ S.D.) were 0.77 ± 0.017, 1.51 ± 0.068 and 5.97 ± 0.057 μg/ml, respectively. The range of accuracy was 98.8–102.7%. We also tested the extraction recovery by comparing peak-height ratios of extracted spiked serum containing the same amount of sotalol. It was found that the average extraction efficiency was 76%.

The detection limit for sotalol by this method, as defined by peak-height response equal to three-fold the measured background noise, was 0.02 μg/ml. The lower detection limit of the assay is well below the effective therapeutic levels seen in previous studies and is sufficiently low to follow sotalol blood levels for pharmacokinetic studies.

We tested 26 commonly administered drugs that could be present in patient serum (Table II) and cause interference under the conditions of the assay. None of the drugs investigated interfered with the detection or quantitation of sotalol or the internal standard. No interfering compounds have been noted in the analysis of twenty patient serum samples.

DISCUSSION

The method presented in this article allows the detection and quantitation of sotalol utilizing isocratic HPLC. The main advantage of this method is that it has short retention times producing a chromatographic analysis time of 5 min. Furthermore, this method requires no organic solvent to elute sotalol from the column, utilizes UV absorbance detection, and has good sensitivity and reproducibility.

The extraction method presented uses disposable extraction columns that may be considered relatively expensive for a single use. Cleaning and reactivating the columns take time, therefore, it may be found that the extraction method [17] presented may not be cost- or time-effective. Other methods using liquid–liquid extraction of sotalol may be found to be more desirable. However, most extraction methods do not show an extraction efficiency as great as we found and may result in some interfering peaks not seen with this method. The analysis time is already short (<5 min); however, the addition of acetonitrile (1–5%) to the mobile phase or increasing the nonylamine concentration to 0.004 *M* can decrease analysis time up to 2 min. Because this method is isocratic in nature, the column does not require reequilibration after each injection. The same column that is utilized for the sotalol assay is also used in several other assays. The column was washed after any assay using acetonitrile–water (50:50). No effect on the efficiency or performance of the column for other assays has been noted.

As shown in Table I, there was good linearity and reproducibility of the calibration curves over a five-month period. Variation in elution time was also very small (0.1 min) within one day. This should allow the programming of integrator windows for automatic detection of sotalol and the internal standard.

The sensitivity of this assay is sufficient to monitor patient sotalol serum levels under normal treatment regimens. The half-life of sotalol has been reported to be between 14 and 20 h [17]. The sensitivity of this assay should permit detection of sotalol levels for five half-lives after a starting concentration of 0.64 μg/ml. In summary, the assay described in this paper has high sensitivity, is reproducible and accurate, and can be used either clinically or to study pharmacokinetics.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Gallo of Bristol-Meyers and the Bristol-Meyers Pharmaceutical Company for supplying the sotalol hydrochloride, MJ-6564-1, I.S., and personal communications necessary for the development of this assay. This study was supported in part by gifts from Mr. and Mrs. M. Weiner and Mr. and Mrs. G. Jaffin.

REFERENCES

1 B.N. Singh and E.M. Vaughan Williams, Br. J. Pharmacol., 39 (1970) 675.
2 H.C. Strauss, J.T. Bigger, Jr. and B.F. Hoffman, Circ. Res., 26 (1970) 661.
3 T. Wang, R.H. Bergstrand, K.A. Thompson, L.A. Siddoway, H.J. Duff, R.L. Woosley and D.M. Roden, Am. J. Cardiol., 57 (1986) 1160.
4 J. Senges, W. Lengfelder, R. Jauernig, E. Czygan, J. Brachmann, I. Rizos, S. Cobbe and W. Kudler, Circulation, 69 (1984) 577.
5 D.G. McDevitt and R.G. Shanks, Br. J. Clin. Pharmacol., 4 (1977) 153.
6 K. Nademanee, G. Feld, J. Hendrickson, P.N. Singh and B.N. Singh, Circulation, 72 (1985) 555.
7 T.B. Tjandramaga, J. Thomas, R. Verbeeck, R. Verbesselt, R. Verberckmoes and P.J. Schepper, Br. J. Clin. Pharmacol., 3 (1976) 259.
8 H. Sundquist, M. Antilla and M. Arstila, Clin. Pharmacol. Ther., 16 (1974) 465.
9 E.R. Garrett and K. Schnelle, J. Pharm. Sci., 60 (1971) 833.

10 T. Walle, J. Pharm. Sci., 63 (1974) 1885.
11 S. Karkkainen, J. Chromatogr., 336 (1984) 313.
12 M.A. Lefebvre, J. Girault, M.Cl. Saux and J.B. Fourtillan, J. Pharm. Sci., 69 (1980) 1216.
13 M.A. Lefebvre, J. Girault and J.B. Fourtillan, J. Liq. Chromatogr., 4 (1981) 483.
14 B. Lemmer, T. Ohm and H. Winkler, J. Chromatogr., 309 (1984) 187.
15 D.G. Gallo, Mead Johnson Pharmaceutical Division Report, Gall-DG-0777, 1979.
16 M.P. Boarman, Bristol-Meyers Pharmaceutical Research and Development Division Report, BOAR-MP-09758, 1983.
17 M.J. Bartek, Bristol-Meyers Pharmaceutical Research and Development Division Report, BART-MJ-R.P-10588, 1984.
18 M. Antilla, M. Arstila, M. Pfeffer, R. Tikkanen, V. Vallinkoski and H. Sundquist, Acta Pharmacol. Toxicol., 39 (1976) 118.