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# Determination of sotalol in human cardiac tissue by high-performance liquid chromatography

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## Abstract

A sensitive and quantitative reversed-phase HPLC method for the analysis of D,L-sotalol in human atria, ventricles, blood and plasma was developed. Sotalol was determined in about 100 mg of human right atria, left ventricles, and in 500 $\mu$l of blood and plasma samples of patients undergoing coronary bypass surgery or heart transplantation. Patients were taking 80–160 mg of sotalol as an antiarrhythmic agent. Atenolol was used as an internal standard certifying high precision of measurement. Sotalol blood and plasma concentrations correlated linearly to the obtained signals from 26.5 ng/ml to 2.12 $\mu$g/ml. Sotalol tissue concentrations showed linearity between 0.27 ng/mg and 10.6 ng/mg wet weight. The limit of quantitation was 0.27 ng/mg at a signal-to-noise ratio of 10. Sotalol was extracted from homogenized tissue with a buffer solution (pH 9) and the remaining pellet was extracted with methanol. The methanol extract was evaporated under nitrogen and reconstituted in buffer (pH 3). The whole extract was cleaned by solid-phase column extraction, eluted with methanol, evaporated again, reconstituted in the mobile phase (acetonitrile–15 m*M* potassium phosphate buffer pH 3, 17:83, v/v) and injected onto the HPLC column (Spherisorb $C_6$ column, 5 $\mu$m, 150×4.6 mm I.D.). For the detection of sotalol, the UV wavelength was set to 230 nm. Recoveries of sotalol and atenolol in atria and ventricles were 65.6 and 75.0%, respectively. Intra- and inter-assay coefficients of variation for tissue concentrations were 3.38 and 6.14%, respectively. Intra- and inter-assay accuracy for determined tissue sotalol concentrations were 94.9±6.3 and 99.6±4.1%.

*Keywords:* Sotalol

## 1. Introduction

Cardiac arrhythmias occur with a high incidence in Western countries. However, CAST-1 and -2 studies have shown that class-I antiarrhythmic agents [1] do not exert beneficial effects on mortality and morbidity in patients following myocardial infarction [2,3]. In contrast, there is evidence that $\beta$-adrenoceptor blockers and class-III antiarrhythmic drugs are currently the best choice [4]. For instance, sotalol reduced mortality in patients with ventricular and supraventricular arrhythmias [5].

Generally, a correlation is assumed between serum sotalol concentrations and prolongation of the QTc

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interval [6,7]. However, some authors reported cases of torsade de pointes following sotalol therapy in the presence of therapeutic sotalol dosage or so called therapeutic serum concentrations [8,9]. Hence, plasma levels might be a poor predictor of clinical effects and knowledge of sotalol tissue concentrations might in part explain side effects. Furthermore, one could assume that there is a strong correlation between the therapeutic effect of sotalol and sotalol concentrations at its site of action, namely the human heart. No data are available about sotalol concentrations in the human heart. Pharmacokinetic data on tissue distribution of sotalol are only available in rat heart and plasma [10].

Given the clinical importance and widespread use of sotalol, we developed a sensitive and quantitative HPLC method with high accuracy and precision to measure sotalol concentrations in about 100 mg of human cardiac tissue samples. We employed an internal standard to control the experiment and used solid-phase column extraction to clean up sample extracts for good separation conditions.

## 2. Experimental

### 2.1. Reagents

HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All other chemicals were analytical reagent grade.

### 2.2. Drug standards and solutions

D,L-Sotalol (sotalol) was supplied by Bristol-Myers Squibb (Munich, Germany) and atenolol by Zeneca (Planckstadt, Germany). Human plasma was provided by the Abteilung für Transfusionsmedizin, Universitäts-Krankenhaus Eppendorf (Hamburg, Germany). Stock solutions of 5 $\mu$g/ml for sotalol and atenolol were made up in water. Aliquots of these stock solutions were added to drug-free plasma and to tissue, thus obtaining standard curves in the range as indicated in Table 1 in the presence of appropriate amounts of internal standard. Further stock standard solutions were divided into small aliquots and stored at −80°C until assay. They remained stable for at least six months.

### 2.3. Instrumentation and chromatographic conditions

A HPLC system of a LC Workstation Class LC10 was used (Shimadzu, Kyoto, Japan) consisting of a SIL-10A autoinjector, a LC-10AT liquid chromatograph, a SPD-10A UV-Vis detector and software provided by the manufacturer.

Chromatographic separation was performed on a Spherisorb $C_6$ column (5 $\mu$m, 150×4.6 mm I.D. Chromatographie Service, Langerwehe, Germany) with a Sperisorb $C_6$ guard column (5 $\mu$m, 17×4 mm I.D.) at ambient temperature. The mobile phase consisted of acetonitrile–15 m*M* potassium phosphate buffer, pH 3, 17:83 (v/v) for separation of plasma samples and 10:90 (v/v) for tissue and blood samples. For the detection of sotalol, the UV wavelength was set to 230 nm. The flow-rate was 1 ml/min. Aliquots of the extracts (50 $\mu$l) were injected onto the HPLC system. Sotalol was quantified by relating the peak-height ratio of sotalol and the internal standard atenolol in the unknown sample to the peak-height ratio of a known standard concentration.

A Vac-Elut vacuum manifold and Bond-Elut columns, packed with $C_8$-bound silica particles of 40 $\mu$m (100 mg/ml of column volume) (Analytichem

Table 1
Linearity of sotalol signals in the sotalol plasma and ventricle tissue assay

| Sotalol in matrix | Linearity range | Recovery of sotalol (mean±S.D., $n$=6) (%) | C.V. (%) | Recovery of internal standard (mean±S.D., $n$=6) (%) | C.V. (%) |
|---|---|---|---|---|---|
| Sotalol in plasma (ng/ml) | 26.5–2120 | 77.1±5.9 | 7.7 | 80.3±6.9 | 8.6 |
| Sotalol in tissue (ng/mg) | 0.27–10.6 | 65.6±3.9 | 3.9 | 75.0±5.9 | 7.9 |

Recovery was calculated as mean over all obtained concentrations.

International, Harbor City, CA, USA) were used for clean up of samples.

*2.4 Plasma, blood and tissue samples*

Cardiac atria, blood and plasma samples were received from patients undergoing coronary bypass surgery. Intraoperatively, right atrial tissue samples of about 100 mg were frozen immediately into liquid nitrogen and kept frozen at −80°C until further analysis. From the same patient blood and plasma were obtained. Part of the blood was centrifuged at 2000 *g* for 15 min to separate plasma. Blood and plasma samples were stored at −80°C until further analysis. Ventricle samples were received from explanted human hearts subsequent to orthotopic heart transplantation. Sotalol plasma concentrations were obtained in a healthy volunteer 1, 2, 3, 4, 6, 8, 12 and 24 h after oral ingestion of 160 mg sotalol. The study was approved by the local ethics committee (No. 925).

*2.5. Plasma and blood extraction*

Sotalol was extracted from plasma and blood as described earlier with some modifications [11]. In brief, solid-phase extraction cartridges (Bond-Elut $C_8$ columns) were activated by washing with methanol and water under vacuum with the Vac-Elut system. Plasma samples together with the internal standard atenolol and an appropriate amount of 0.05 *M* borate buffer, pH 9, were applied to the columns. After washing with water, acetonitrile was applied to displace the remaining water. Sotalol was eluted with methanol, the solution was evaporated to dryness and reconstituted in 400 μl of mobile phase; 50-μl aliquots was injected onto the HPLC system.

*2.6. Tissue extraction*

Frozen tissue samples (about 100 mg) were put into liquid-nitrogen-cooled Teflon tubes. To each sample, 100 μl of atenolol stock solution and 400 μl of water were added. Simultaneously sotalol free cardiac tissue was spiked with known sotalol standard and internal standard concentrations. Frozen samples were homogenized in a micro-dismembrator (Braun, Melsungen, Germany), transferred into glass tubes and thawed. Teflon tubes were rinsed twice with 250 μl of 1 *M* potassium phosphate buffer pH 3. After centrifugation at 2740 *g* for 20 min at 20°C supernatant was transferred into a second glass tube (S1). The remaining pellet was extracted with 1 ml methanol for 15 min under sonication. After centrifugation for 10 min at 20°C, the supernatant was evaporated to dryness under a stream of nitrogen at 40°C. The residues were reconstituted in 500 μl 15 m*M* potassium phosphate buffer pH 3, added to S1 and centrifuged again. Samples were sucked slowly through Bond-Elut columns under vacuum. Columns were washed twice with water. Finally, elution of the compounds was performed with 200 μl methanol twice, evaporated again to dryness under a stream of nitrogen at 40°C and reconstituted in 500 μl mobile phase. A 50-μl aliquot was injected onto the HPLC column.

*2.7. Data analysis and statistics*

Data are given as arithmetic means±standard deviation (S.D.). Time course of plasma concentrations were calculated using Topfit 2.0 software [12]. Precision was calculated as percent coefficients of variation (C.V., %). Accuracy was expressed as percent of sotalol measured in each sample relative to the known amount of sotalol added.

## 3. Results

*3.1. Plasma and blood samples*

Sotalol and atenolol were separated in plasma using a mobile phase of acetonitrile–15 m*M* potassium phosphate buffer, pH 3, 17:83 (v/v). No interferences were found from endogenous plasma at the retention times of sotalol (5.8 min) or the internal standard (4.4 min) (chromatograms not shown). Fig. 1 shows typical chromatograms of drug-free blood (Fig. 1A), and blood spiked with 1.06 μg/ml sotalol and 1.09 μg/ml internal standard atenolol (Fig. 1B). To avoid significant interferences with endogenous blood constituents the mobile phase was changed to 10:90 (v/v) in the sotalol blood assay. Then, good separation of sotalol and the internal standard from endogenous blood peaks was achieved (A vs. B).

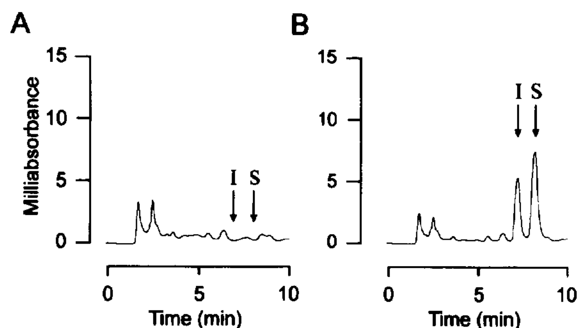


Fig. 1. Representative chromatograms of sotalol in blood samples. (A) Blood sample without drug. (B) Spiked blood containing 1.06 μg/ml sotalol (S) and 1.09 μg/ml internal standard atenolol. The arrows in A indicate the anticipated elution times of sotalol (S) and the internal standard (I), the arrows in B indicate the actual sotalol and internal standard peak.

The absolute recovery of sotalol in plasma was measured following addition of varying standard amounts ($n$=6). Peak heights after plasma extraction were compared to those after direct injection of aequeous solutions of the standard and the internal standard. The absolute recoveries for sotalol (77.1±5.9%) were comparable to those of the internal standard (80.3±6.9%) (see Table 1). Linearity of the standard curves was determined from 26.5 to 2120 ng/ml.

### 3.2. Cardiac tissue samples

A typical chromatogram of drug-free cardiac tissue is shown in Fig. 2A. No interferences were found from endogenous tissue at the retention times of sotalol (7.8 min) and the internal standard (6.8 min). Representative chromatograms of spiked cardiac tissue in Fig. 2B–F demonstrate good separation of varying sotalol amounts. Linearity of the standard curves was obtained from 0.27 ng/mg wet weight (Fig. 2B) to 10.6 ng/mg wet weight (Fig. 2F).

Fig. 3B shows a typical chromatogram of a ventricle from a patient medicated with sotalol. The patient sotalol tissue concentration of 0.64 ng/mg wet weight is in the linear range of the sotalol tissue assay (0.27 to 10.6 ng/mg).

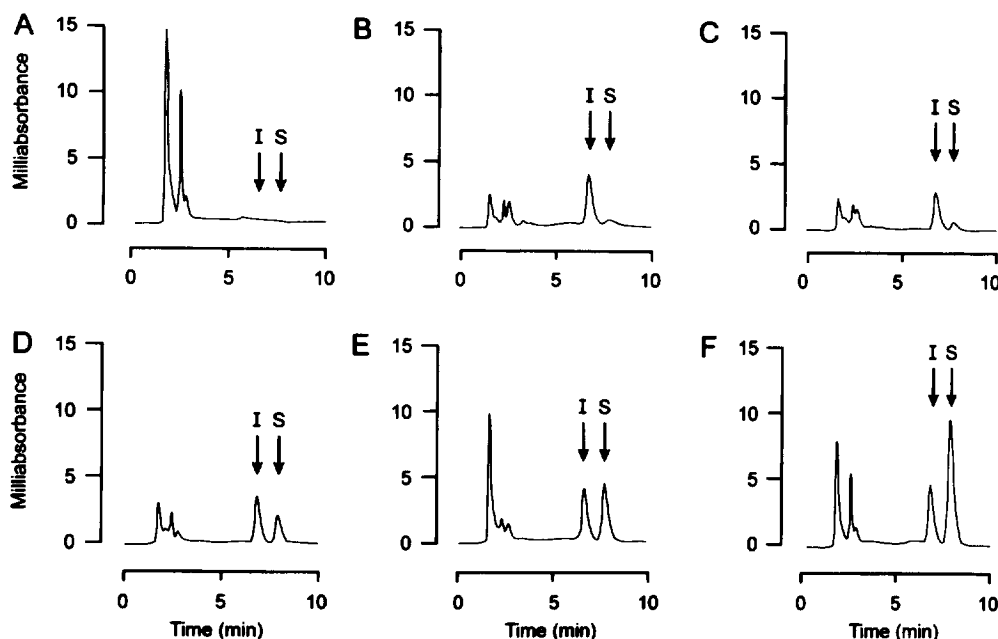


Fig. 2. Representative chromatograms of human ventricle samples without drug (A) and with varying amounts of sotalol spiked human ventricle: (B) 0.27, (C) 0.53, (D) 2.65, (E) 5.3 and (F) 10.6 ng sotalol/mg wet weight. The arrows in A indicate the anticipated elution times of sotalol (S) and the internal standard (I), the arrows in B–F indicate the sotalol and the internal standard peak.

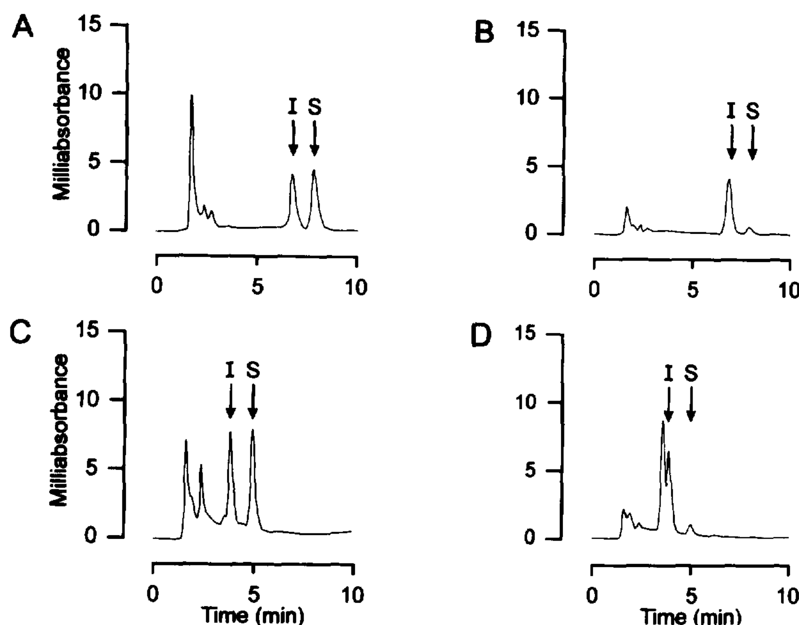


Fig. 3. Representative chromatograms of a human ventricle sample following separation in different mobile phases. (A, C) Human ventricle sample spiked with sotalol (S) and the internal standard (I). (B, D) Tissue spiked with internal standard (I) of a patient treated with sotalol. The mobile phase in A and B consisted of acetonitrile–15 m*M* potassium phosphate buffer, pH 3 (10:90, v/v). Sotalol eluted at a retention time of 7.8 min (S), the internal standard (I) at 6.8 min. The mobile phase in C and D consisted of acetonitrile–15 m*M* potassium phosphate buffer, pH 3 (20:80, v/v). Sotalol retention time shortened to 5.0 min and atenolol retention time to 4.2 min (C). The same shift in retention times for sotalol and atenolol occurred in the patient sample (D). This indicated peak identity of sotalol in the patient cardiac tissue.

For validation of the sotalol peak in the patient sample the mobile phase was changed from 10:90 (v/v) (A and B) to 20:80 (v/v) (C and D). Spiked tissue and patient sample were injected again into the HPLC column. Sotalol retention time was shortened from 7.8 to 5.0 min and atenolol retention time from 6.8 to 4.2 min, as demonstrated in the chromatograms of spiked tissue (A and C). The same shift in the sotalol retention time of the corresponding peaks was obtained in the patient chromatograms (B and D). This indicated peak identity of sotalol in the patient cardiac tissue.

Fig. 4 illustrates typical chromatograms of an atrial sample (Fig. 4B) from a patient treated with sotalol and the corresponding blood sample of the same patient (Fig. 4D). A sotalol tissue concentration of 1.8 ng/mg wet weight corresponded to a sotalol blood concentration of 1.1 $\mu$g/ml.

Intra-assay variation was determined by analyzing five sotalol concentrations and three replicate samples (Table 2). At all concentration levels the precision of the measurement, expressed as the C.V. values, was found to be lower than 5%, except at the lowest concentration (0.27 ng/mg) with 11.5. The accuracy expressed as percent of the concentration of sotalol measured in each sample relative to the known amount of added sotalol amounted to 92.8±6.3%. Additionally, inter-assay precision in all concentration levels was below 10% and accuracy amounted to 97.3±5.6%. Extraction efficiency in cardiac tissue of sotalol was 65.6±3.9% and was somewhat lower than the internal standard atenolol (75.0±5.9%).

## 4. Discussion

Sotalol is an effective class-II and -III antiarrhythmic agent in patients with supraventricular and ventricular arrhythmias and is, therefore, extensively used in Europe and the United States.

An amount of 160 mg orally administered sotalol

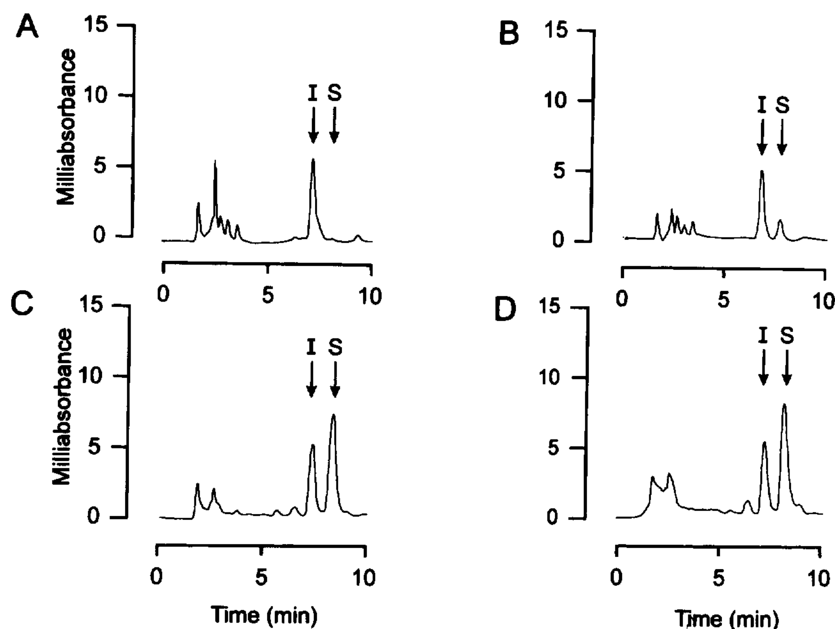


Fig. 4. Representative chromatograms of spiked right atrial and right atrial blood sample, and a blood sample of a patient treated with sotalol. (A) Atenolol (I) spiked right atrium. (B) Patient sample containing 1.8 ng sotalol/mg atrial tissue. (C) Blood spiked with sotalol (S) and internal standard (I). (D) Corresponding blood sample of the same patient containing 1.1 $\mu$g/ml sotalol. The arrows in A indicate the anticipated elution time of sotalol (S) and the peak of the internal standard (I), the arrows in B–D indicate the sotalol and the internal standard peak.

is an effective antiarrhythmic dosage which is often used [13]. Plasma sotalol concentrations following ingestion of 160 mg sotalol resulted in plasma concentrations between 0.5 and 1.5 $\mu$g/ml plasma and are in agreement with previous publications [14,15]. A correlation is suggested between serum sotalol concentrations and antiarrhythmic drug efficacy [6,7]. Consequently, sotalol plasma concentrations should correlate strongly with sotalol tissue concentrations at its site of action, namely the human heart.

The HPLC method presented provides sensitive and quantitative analysis of human cardiac tissue, blood and plasma samples for the antiarrhythmic drug sotalol. Extraction of sotalol from tissue is a crucial point. Few data about sotalol extraction of tissues are available. Tissue sotalol extraction from rats were reported with a tissue sample size of 500 mg minimum [10]. The complex extraction procedure included five extraction steps before injection onto the HPLC column [10]. In preliminary experiments we were unable to reproduce a recovery of

Table 2
Intra-assay and inter-assay validation of sotalol concentrations in human cardiac tissue

| Amount of sotalol added (ng/mg tissue) | Intra-assay ($n$=3) | | | Inter-assay ($n$=7) | | |
|---|---|---|---|---|---|---|
| | Sotalol measured (mean±S.D.) | Precision (C.V., %) | Accuracy (%) | Sotalol measured (mean±S.D.) | Precision (C.V., %) | Accuracy (%) |
| 5.30 | 5.64±0.04 | 0.71 | 106 | 5.44±0.36 | 6.6 | 103 |
| 2.65 | 2.48±0.01 | 0.40 | 93.5 | 2.60±0.15 | 5.7 | 97.2 |
| 1.06 | 0.99±0.02 | 2.02 | 92.6 | 1.00±0.02 | 2.2 | 94.0 |
| 0.53 | 0.51±0.01 | 1.96 | 89.8 | 0.50±0.04 | 8.0 | 99.8 |
| 0.27 | 0.26±0.03 | 11.5 | 92.8 | 0.28±0.03 | 8.8 | 104 |

about 40% according to that protocol (data not shown). Sotalol tissue recovery showed a high S.D. (22%). No internal standard was used to control the complex experimental procedures. Consequently, it can be assumed that precision of determined sotalol concentrations was low and not useful for our purpose to correlate plasma and tissue concentrations. In addition, the chromatograms, e.g., Fig. 3 in Ref. [10] showed suboptimal separation of the sotalol peak from a broad initial elution front, indicating protein or other residues remaining in the samples. In order to use smaller tissue samples (100 mg) we had to optimize extraction procedure to avoid high background signals.

In the present assay tissue was extracted with an alkaline buffer and re-extracted with methanol. This led to a sotalol recovery of 65.5±5.9%. Precision was below 10% and the linear range was from 0.27 to 10.6 ng/mg. It allowed to measure sotalol in human ventricle and atrial samples of 100 mg (wet weight). This is important because sotalol is a therapeutic agent in ventricular and supraventricular atrial arrhythmias. Precision and accuracy of measurement was confirmed by controlling the experiment with addition of an internal standard. Furthermore, we used solid-phase column extraction to clean up sample extracts which brought about good separation conditions (Figs. 2–4) and guaranteed a high degree of reproducibility of the assay (Table 2). Validation of sotalol was performed by changing the mobile phase with a retention time shift. It remains to be elucidated in future to check peak homogeneity with the advent of a photodiode array detector. Interference by other co-medicated drugs and metabolites has to be checked systematically during the analysis procedure.

For clinical applications, the sotalol tissue assay can be used for correlation of sotalol plasma, blood and tissue concentrations of patients under antiarrhythmic therapy.

It is unknown whether sotalol accumulation differs in myocardial tissue with various etiologies of cardiovascular diseases, e.g., dilative cardiomyopathy, hypertrophic cardiomyopathy or coronary heart disease. Furthermore, no data are available whether human cardiac tissue concentrations differ in patients with short- and long-term treatment. In addition, determination of sotalol concentrations in human cardiac tissue is necessary for the validation of in vitro experiments. It is a concern that $\beta$-adrenoceptor blocker concentrations in human cardiac tissue might impair the interpretation of subsequent contraction experiments or other biochemical studies, e.g., radioligand binding studies [16]. Others have tried complicated control contraction procedures to validate data of atenolol from atria [16]. For quantification of atenolol, the sotalol tissue assay could be easily adopted.

In summary, the HPLC method reported here offers a technique to quantitate sotalol concentrations in human cardiac tissues with good accuracy and precision. This method might be extended to the quantification of other $\beta$-adrenoceptor blockers with related molecular structures.

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