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Short communication

Development an ion-pair liquid chromatographic method for determination of sotalol in plasma using a monolithic column

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Abstract

A rapid and sensitive ion-pair HPLC method using a monolithic column and fluorescence detection has been developed for quantification of sotalol in plasma. The assay enables the measurement of sotalol for therapeutic drug monitoring with a minimum quantification limit of 10 ng ml^{-1}. The analytical method involves simple, one-step protein precipitation and no extraction procedure is needed. Sample preparation is fast and the analytical recovery was complete. The separation was carried out in reversed-phase conditions using a Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column at ambient temperature. The mobile phase was 10% acetonitrile, 0.001 M heptane sulfonic acid, 0.02 M sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 5.5 at a flow rate of 1.8 ml/min. The excitation wavelength was set at 235 nm, emission at 300 nm. The calibration curve was linear over the concentration range 20–1500 ng ml^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 7%. The method has been applied to the determination of sotalol in plasma from 12 subjects dosed with racemic sotalol.

Keywords: Sotalol; Plasma; Ion-pair; Monolithic column; HPLC

1. Introduction

Sotalol is a hydrophilic non-selective β-receptor blocker, which is used for the treatment of hypertension, supraventricular and ventricular arrhythmias. Sotalol is virtually completely absorbed from the gastro-intestinal tract and peak plasma concentrations are obtained about 2–4 h after a dose. The plasma elimination half-life is about 10–20 h. Sotalol has low lipid solubility and binding to plasma proteins is reported to be low. Very little is metabolized and it is excreted unchanged in the urine [1,2]. The measurement of sotalol in plasma, offers useful information for clinical studies of the drug in cases of intoxication, in controlling the therapy compliance of the patients and also in the study of possible pharmacokinetic interactions with other drugs [3]. For this purpose, a sensitive method is required to determine plasma sotalol concentrations in clinical studies. Several high-performance liquid chromatography methods have been reported for the determination of sotalol in biological fluids [4–11]. Generally, C_{18} reversed-phase packings with mobile phases that contain methanol as modifier are used. Detection is achieved with ultraviolet [4–6] or fluorescence [7–11] detectors. Some of these methods employed solid-phase extraction (SPE) procedures for elimination of endogenous plasma interferences [4,5,11] and the other methods [7–9] involved an extraction of buffered plasma samples with ethyl acetate or 1-pentanol-chloroform before chromatographic separation. However, these reported methods required tedious liquid–liquid or solid-phase extraction procedures and therefore sample preparation is time-consuming, complex or both. Moreover, most of aforementioned methods need long chromatographic elution time for analysis of sotalol in plasma and were not suitable in all conditions. Two HPLC procedures have been also presented by Badaloni et al. [12] and Saul et al. [13] for the analyses of sotalol based on MS–MS detection by using tandem mass spectrometry. Both of the methods are very sensitive, having low quatitation limits. However, these methods are not available for most laboratories because of their specialty requirement and financial reasons. Moreover, some purification steps have

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been used before the samples are injected to chromatographic system as liquid–liquid extraction, solid-phase extraction, etc. Recently, monolithic stationary phases have attracted considerable attention in liquid chromatography due to their simple preparation procedure, unique properties and excellent performance, especially for separation of drugs in biological samples. As opposed to individual particles packed into chromatographic columns, monolithic supports are cast as continuous homogenous phases. They represent an approach that provides high rates of mass transfer at lower pressure drops as well as high efficiencies even at elevated flow rates. Therefore, much faster separations are possible and the productivity of chromatographic processes can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. This enhances the speed of the separation process and reduces backpressure and unspecific binding without sacrificing resolution [14]. The purpose of this study is to present a simpler, more rapid and sensitive LC method for the determination of sotalol in plasma using a monolithic column with fluorescence detection. The method enables the determination of sotalol with good accuracy at low drug concentrations in plasma using single-step extraction procedure. Separation was performed on a reversed-phase monolithic column, which has lower separation impedance comparing to the particulate packings, and therefore it allows easy optimizing chromatographic conditions to obtain desirable resolution in a short time. The sample preparation only involves protein precipitation and no evaporation step is required. This reduces both sample preparation time and the volume of solvents used, and provides for improved accuracy and precision. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Experimental

2.1. Chemicals

Sotalol hydrochloride and phenol were supplied by Kimidarou Pharmaceuticals (Tehran, Iran). Sotalol is available as oral tablet containing 80 mg of sotalol and other inactive ingredients. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany). Water was obtained by double distillation and purified additionally with a Milli-Q system.

2.2. Instruments and chromatographic conditions

The chromatographic apparatus consisted of a model Wellchrom K-1001 pump, a model Rheodyne 7125 injector and a model RF10AXL fluorescence detector connected to a model Eurochrom 2000 integrator, all from Knauer (Berlin, Germany).

The separation was performed on Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column from Merck (Darmstadt, Germany). The excitation wavelength was set at 235 nm, emission at 300 nm. The mobile phase was 10% acetonitrile, 0.001 M heptane sulfonic acid, 0.02 M sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 5.5 at a flow rate of 1.8 ml/min. The mobile phase was prepared daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

2.3. Standard solutions

Stock solutions (6 mg ml^{-1}) of sotalol were prepared in water and 60 and 6 μg ml^{-1} solutions were made by dilution in water. Then 20, 50, 300, 600, 900, 1200 and 1500 ng ml^{-1} working standards were prepared in plasma from the 6 μg ml^{-1} solution and stored at +4 °C.

2.4. Sample preparation

To 500 μl of plasma in a glass-stoppered 15 ml centrifuge tube were added 50 μl of phenol as internal standard (10 μg ml^{-1}) and 50 μl of 48% perchloric acid aqueous solution. After mixing (30 s), the mixture centrifuged for 10 min at 6000 rpm. Then 40 μl of supernatant was injected into liquid chromatograph.

2.5. Biological samples

Twelve male healthy volunteers were included in this study. The study protocol was approved by the Ethics Committee of Shaheed Beheshti University of Medical Sciences and written informed consent was obtained from the volunteers. Sotalol was administered in a single dose of 160 mg to the volunteers after over night fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at −20 °C until assayed.

2.6. Stability

The stability of sotalol was assessed for spiked plasma samples stored at −20 °C for up to 2 months and at ambient temperature for at least 12 h. The stability of stock solutions stored at −20 °C was determined for up to 1 month by injecting appropriate dilutions of stocks in distilled water on day 1, 15 and 30 and comparing their peak areas with fresh stock prepared on the day of analysis. Samples were considered to be stable, if the assay values were within the acceptable limits of accuracy and precision (RSD <10%).

2.7. Plasma standard curve

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at −20 °C. After thawing, stock solution of sotalol was added to yield final concentrations ranging from 20 to 1500 ng ml^{-1}. Internal standard solution was added to each of these samples to yield a concentration of 1000 ng ml^{-1}. The samples were then prepared for analysis as described above.

2.8. Selectivity and specificity

Control human plasma, obtained from 12 healthy volunteers, was assessed by the procedure as described above and com-

pared with respective plasma samples to evaluate selectivity of the method. Verapamil and some β-receptor blockers like propranolol, atenolol and metoprolol were also tested for potential interferences.

2.9. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of sotalol (50, 500 and 1000 ng ml^{-1}) to pool plasma (quality control samples). For intra-day precision and accuracy five replicate quality control samples at each concentration were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days.

2.10. Limit of quantification (LOQ) and recovery

For the concentration to be accepted as LOQ, the percent deviation from the nominal concentration (accuracy) and the relative standard deviation must be ±10% and less than 10%, respectively, considering at least five times the response compared to the blank response [15]. The relative analytical recovery for plasma at three different concentrations of sotalol (50, 500 and 1000 ng ml^{-1}) was determined. Average recovery of sotalol was determined by comparing AUC obtained after injection of the processed QC samples with those achieved by direct injection of the same amount drug in distilled water at different concentrations (six samples for each concentration level).

2.11. Pharmacokinetic analysis

Sotalol pharmacokinetic parameters were determined by non-compartmental methods. Elimination rate constant (K) was estimated by the least-square regression of plasma concentration–time data points in the terminal log-linear region of the curves. Half-life was calculated as 0.693 divided by K. The area under the plasma concentration–time curve from zero to the last measurable plasma concentration at time t (AUC_{0-t}) was calculated using the linear trapezoidal rule. The area was extrapolated to infinity ($AUC_{0-\infty}$) by addition of Ct/K to AUC_{0-t} where Ct is the last detectable drug concentration. Peak plasma concentration (C_{max}) and time to peak concentration (T_{max}) were obtained directly from the individual plasma concentration versus time curves.

3. Results and discussion

Under the chromatographic conditions described, sotalol and the internal standard peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Owing to the polar nature of sotalol, the first attempts for assay development, were made using weak eluents. This resulted in poor separation of endogenous substances and accumulation of these substances on the enrichment columns. To overcome these problems, heptanesulfonic acid as added to the analytical eluent as an ion-pairing reagent to retain the analyte for a longer period. After optimizing the mobile phase composition and pH, the average retention times of sotalol and phenol were 3.9 and 5.0 min,

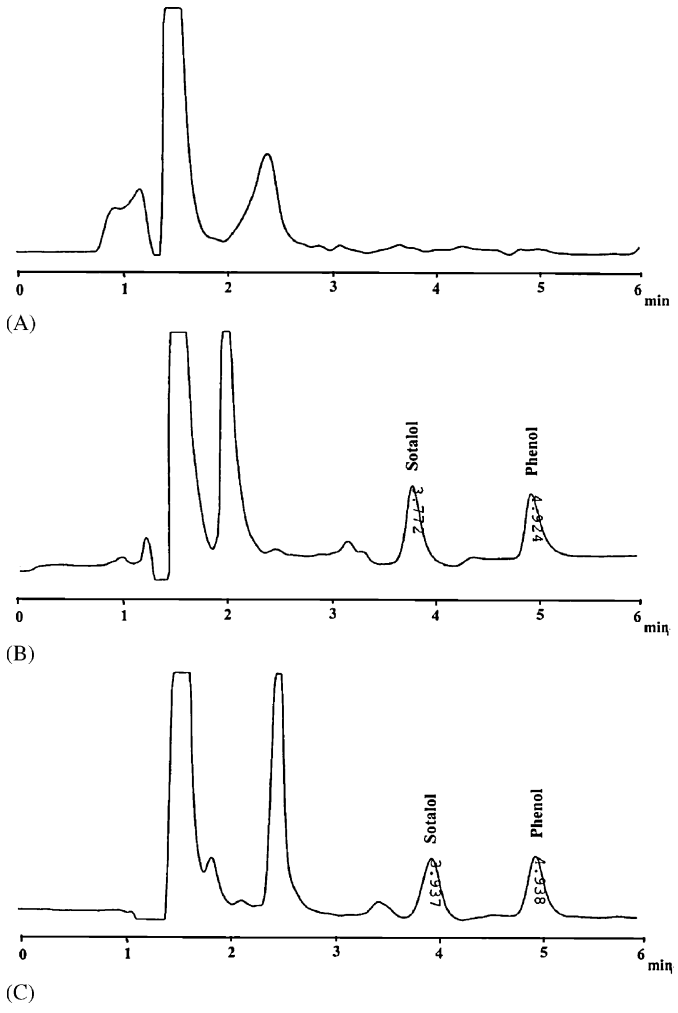

Fig. 1. Chromatograms of: (A) blank plasma; (B) blank plasma spiked with 900 ng ml^{-1} sotalol and 1000 ng ml^{-1} phenol (internal standard); (C) plasma sample from a healthy volunteer 1 h after oral administration 160 mg of sotalol.

respectively. Also each single run can be completed in 6 min, which is considerably less than the previously reported HPLC methods [4,5,8]. Fig. 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. Verapamil and some β-receptor blockers like propranolol, atenolol and metoprolol did not interfere with analytes peaks as well. The calibration curve for the determination of sotalol in plasma was linear over the range 20–1500 ng ml^{-1}. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.999.The relative standard deviation (RSD) values of the slope were equal to or better than 5%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (Table 1). The relative analytical recovery for plasma at three different concentrations of sotalol was determined. Known amounts of sotalol were added to drug-free plasma in concentrations ranging from 50 to 1000 ng ml^{-1}. The internal standard was added and the relative recovery of sotalol was calculated by comparing the peak areas for extracted sotalol from spiked plasma

Table 1
Assay linearity

	Coefficient of the linear regression analysis (r)	Slope ± S.D.	Intercept ± S.D.
Intra-assay ($n=6$)	$(0.999 \pm 2) \times 10^{-3}$ (RSD = 0.2%)	$(0.0021 \pm 1) \times 10^{-4}$ (RSD = 4.8%)	$(0.0071 \pm 4) \times 10^{-4}$
Inter-assay ($n=9$)	$0.999 \pm 6) \times 10^{-3}$ (RSD = 0.6%)	$(0.0023 \pm 1) \times 10^{-4}$ (RSD = 4.3%)	$(0.0068 \pm 3) \times 10^{-4}$

Table 2
Reproducibility of the analysis of sotalol in human plasma ($n=5$)

Concentration added (ng ml^{-1})	Concentration measured (mean ± S.E.)	
	Intra-day	Inter-day
50	50.1 ± 3.1 (6.2)	49.2 ± 3.2 (6.5)
500	496.7 ± 20.9 (4.2)	495.3 ± 22.8 (4.6)
1000	980.5 ± 38.2 (3.9)	982.4 ± 35.4 (3.6)

Values in parentheses are coefficients of variation (%).

and a standard solution of sotalol in water containing internal standard with the same initial concentration. The average recovery was 96.5 ± 1.3% ($n=6$). The limit of quantification (LOQ), as previously defined, obtained 10 ng ml^{-1} by analyzing this amount of sotalol in a drug-free plasma sample. This is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of sotalol. As shown in Table 2, coefficients of variation were less than 7%, which is acceptable for the routine measurement of sotalol. Stability was determined for spiked plasma samples under the conditions as previously described. The results showed that the samples were stable during the mentioned conditions. The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic sotalol research. This method is well suited for routine application in the clinical laboratory because of the speed of analysis and simple extraction procedure. Owing to use of the monolithic column, which has lower separation impedance comparing to the particulate packings, much faster separations are possible the productivity of chromatographic processes can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. Accordingly, the chromatographic elution step is undertaken in a short time (less than 6 min) with high resolution. The sample preparation only involves protein precipitation and no evaporation step is required. Also, the use of a smaller sample volume provides an advantage as compared with some previous methods that require 1–2 ml of plasma for analysis of sotalol. Over 350 plasma samples were analyzed by this method without any significant loss of resolution. No change in the column efficiency and back pressure was also observed over the entire study time, thus proving its suitability. In this study plasma concentrations were determined in 12 healthy volunteers, who received 160 mg of sotalol each. Fig. 2 shows the mean plasma concentration–time curve of sotalol: plasma concentration reached a maximum 2.50 ± 0.47 h after dosing with a level of 1.41 ± 0.05 μg ml^{-1}. The pharmacokinetic parameters were summarized in Table 3. These parameters are in good agreement with that found previously [5,16].

Table 3
Mean pharmacokinetic parameters (±S.D.) of sotalol following administration of 160 mg sotalol ($n=12$)

Parameter	Result
C_{max} (μg ml^{-1})	1.41 ± 0.05 (3.5)
T_{max} (h)	2.50 ± 0.47 (18.8)
AUC_{0-48} (μh ml^{-1})	21.48 ± 1.65 (7.7)
$AUC_{0-\infty}$ (μh ml^{-1})	22.91 ± 1.82 (7.9)
$T_{1/2}$ (h)	12.27 ± 0.27 (2.2)
K_{el} (h^{-1})	0.056 ± 0.001 (2.2)

Values in parentheses are coefficients of variation (%).

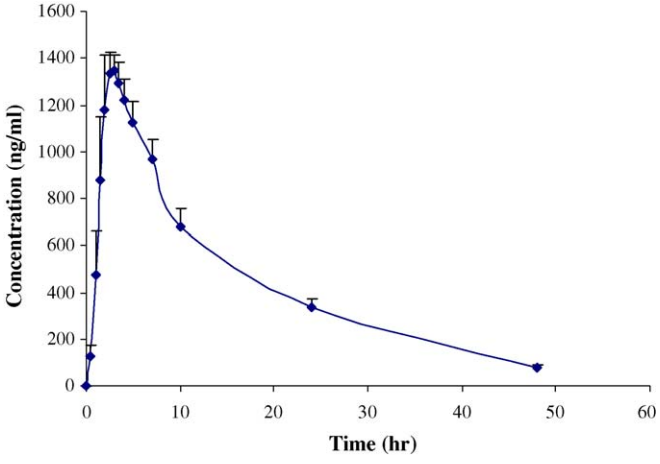

Fig. 2. Mean plasma concentration–time profile of sotalol in healthy volunteers ($n=12$) after a single 160 mg sotalol.

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