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Determination of amosulalol in human plasma using solid-phase extraction combined with liquid chromatography and ultraviolet detection

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Abstract

Amosulalol is an antihypertensive drug with selective postsynaptic alpha 1 and non-selective beta blocking effects. A simple solid-phase extraction and high-performance liquid chromatographic (HPLC) method has been developed and validated for the quantitative determination of amosulalol in human plasma. A reversed phase C18 column was used for the separation of amosulalol and ethyl paraben (internal standard) with a mobile phase composed of 0.025 M phosphate buffer (pH 6.0)·acetonitrile (73:27, v/v) at a flow rate of 1.5 mL/min. The ultraviolet detector was operated at the 272 nm wavelength. Intra- and inter-day precision and accuracy were acceptable for all quality control samples including the lower limit of quantification of 30 ng/mL. Recovery of amosulalol from human plasma was >95.6%. Amosulalol was stable in human plasma under various storage conditions. This method was used successfully for a pharmacokinetic study in plasma after oral administration of a single 20 mg dose of amosulalol hydrochloride to 16 healthy volunteers.

Keyword: Amosulalol

1. Introduction

Amosulalol, (±)-5-[1-hydroxy-2-[[2-(*O*-methoxyphenoxy)ethyl]amino]ethyl]-2-methylbenzenesulfonamide hydrochloride (Fig. 1A), is an antihypertensive drug with selective postsynaptic alpha 1 and non-selective beta blocking effects [1]. It was known to be four times more potent than phentolamine in blocking alpha 1 adrenoceptors and three times less potent than propranolol in blocking beta 1 adrenoceptors [2]. Compared to labetalol, it showed a greater separation between neuronal uptake blocking properties and alpha receptor blocking properties [3]. It was also reported that amosulalol reduced arterial pressure without inducing tachycardia in rats and dogs [2,3].

In the earlier studies, extraction with organic solvent followed by derivatization to increase the sensitivity of high-performance liquid chromatography (HPLC) was mainly used to evaluate the amosulalol kinetics in humans. Of various labeling agents, 5-di-*n*-butylaminonaphthalene-1-sulphonyl chloride has been used in combination with HPLC to determine amosulalol [1,4]. However, the extraction of amosulalol from plasma was dependent on pH and derivatizing reaction was also highly dependent on temperature and time. Also, it required long time to get a result. In this study, we investigated a simple and rapid method for determining amosulalol levels in plasma and demonstrated the applicability of the method by analysis of plasma samples after oral administration of a single 20 mg dose in healthy male volunteers.

2. Experimental

2.1. Equipment

The HPLC system consisted of a pump (Model SCL-100, Samsung, Suwon, Korea) with a detector (Model LC 90, Perkin-Elmer, Norwalk, CT, USA) set at 272 nm

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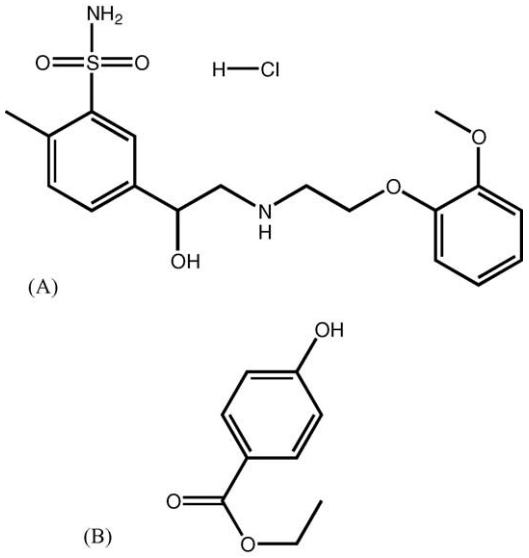

Fig. 1. Chemical structures of amosulalol hydrochloride (A) and ethyl paraben (B).

and an integrator (Model 4290, Varian, Palo Alto, CA, USA).

2.2. Materials and reagents

Amosulalol hydrochloride was supplied by Jeil Pharma. Co. Ltd. (Seoul, Korea). Ethyl paraben was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Solid-phase extraction cartridges (Strata C18-U, 3 mL, 500 mg) and an octadecylsilane column (Luna C18, 4.6 mm × 150 mm, 5 μm) were purchased from Phenomenex (Torrance, CA, USA). Acetonitrile was of HPLC grade and other reagents used were of analytical grade.

2.3. Preparation of standard solutions

Working stock solutions of amosulalol hydrochloride and ethyl paraben (internal standard, IS, Fig. 1B) were prepared in methanol at a concentration of 1 mg/mL. Prior to use, these two stock solutions were further diluted with water to obtain working solutions at the concentration of 10 μg/mL. An appropriate dilution of the working solution with drug free plasma from healthy volunteers gave a concentration range between 30 and 1000 ng/mL of amosulalol hydrochloride. To 1 mL of the prepared plasma were added 100 μl of ethyl paraben at a 2 μg/mL solution in water and 2 mL of 0.1 M acetate buffer (pH 6.0). Acetate buffer (0.1 M) was prepared with 13.61 g of sodium acetate trihydrate dissolved in 1 L of water and then the pH was adjusted with glacial acetic acid.

Seven calibration samples (30, 50, 100, 300, 500, 700 and 1000 ng/mL) were prepared by spiking blank plasma with appropriate volumes of the working solutions. Quality control samples (30, 50, 100, 500 and 1000 ng/mL) and stability samples (100 and 1000 ng/mL) were independently prepared in the same manner.

2.4. Sample preparation

Solid-phase extraction cartridge columns were activated before use by washing successively with 4 mL of methanol, 2 mL of water and 2 mL of 0.1 M acetate buffer (pH 6.0). Plasma samples (1.0 mL) was mixed with 100 μl of the working IS solution (2 μg/mL) and was applied to a 3 mL activated cartridge column, and passed slowly through the column under mild vacuum (100 mmHg). The column was then washed with 2 mL of a mixture of water–acetonitrile (8:2, v/v) and drained completely after the wash. To elute amosulalol and IS from the column, 2 mL of methanol was applied to each column. The eluate was evaporated to dryness at 45 °C under a stream of nitrogen gas. The residue was reconstituted with 200 μL of mobile phase and then 60 μL was injected directly into the HPLC system.

2.5. Chromatographic conditions

The reversed phase C18 column was eluted with a mixture of 0.025 M phosphate buffer (pH 6.0) and acetonitrile (73:27, v/v) at a flow rate of 1.5 mL/min. Phosphate buffer was prepared with 3.403 g of potassium dihydrogen phosphate dissolved in 1 L of water and then the pH was adjusted with ortho-phosphoric acid. The UV detector was set at 272 nm. All analyses were performed at room temperature.

2.6. Method validation

2.6.1. Specificity

The degree of interference by endogenous plasma constituents with amosulalol and IS was evaluated by inspection of chromatogram derived from processed blank and spiked plasma samples, and also from processed blank samples injected during each analytical run.

2.6.2. Calibration curve

Calibration standards at the concentrations of 30, 50, 100, 300, 500, 700 and 1000 ng/mL were extracted and assayed as mentioned above. The calibration curve was constructed based on peak area ratio of the drug and IS.

2.6.3. Accuracy and precision

Intra-day accuracy and precision of the method were estimated by assaying five replicate plasma samples at five different concentrations, in five analytical runs. The overall mean precision was defined by the percentage of relative standard deviation (R.S.D.) of five standards at five different concentrations analyzed on the same day. Inter-day variability was estimated from the analysis of the five standards on five separate days during method validation.

2.6.4. Extraction recovery

Recovery of amosulalol after the solid-phase extraction was determined by comparing observed amosulalol peak area

in extracted plasma, to those of non-processed standard solutions.

2.6.5. Stability

The freeze–thaw stability of amosulalol hydrochloride in plasma was evaluated over three freeze–thaw cycles. Stability control plasma samples in triplicate at the levels of 100 and 1000 ng/mL were immediately frozen at −70 °C, and thawed at room temperature three consecutive times. After that, the samples were processed and assayed. The stability of amosulalol hydrochloride in quality control samples stored at room temperature for 24 h and at −70 °C for 4 weeks was also assessed. The mean values of amosulalol were compared with the initial ones, which were assayed immediately after preparation of stability control plasma samples. The stability was expressed as a percentage of the initial value.

2.7. Single-dose pharmacokinetic study

The assay was applied to pharmacokinetic studies of amosulalol following 20 mg single dose. Eight male and eight female healthy volunteers aged between 19 and 25 years were selected for the study. All subjects gave their written informed consents, and the clinical protocol was approved by the Ethics and Review Committee. The volunteers were judged to be healthy by physical examination and were not receiving any medications 1 week before and during the study period. Amosulalol hydrochloride (Rowgan® Tablets, Jeil Pharm.Co., Ltd., Seoul, Korea) was administered orally with 240 mL of water in the morning (8:30 am) after 12 h overnight fast. Any food and drink were withheld for at least 4 h after dosing. Lunch and dinner of beef soup with rice were served four and 10 h after dosing. Ten milliliters of blood samples were collected in green-top vacutainers (containing sodium heparin) via an in-dwelling cannula placed on the forearm before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after drug ingestion. The blood samples were centrifuged at 3000 × *g* for 15 min at room temperature, and the plasma was transferred to separate plasma tube. The separated plasmas were stored at −70 °C until analysis. Comparison of peak area ratios from the unknown samples with those from the calibration curve permitted quantitation of the samples.

3. Results

3.1. Specificity

Fig. 2 shows the well-resolved chromatographic peaks of amosulalol and ethyl paraben at 3.1 min of amosulalol and 9.9 min, respectively. The blank plasma after extraction consistently contains no significant interfering peaks.

3.2. Linearity

The relation between amosulalol hydrochloride concentrations and peak area ratio of amosulalol to IS was linear from 30 to 1000 ng/mL ($y = 0.7353x - 0.0031$, $r^2 = 0.9996$).

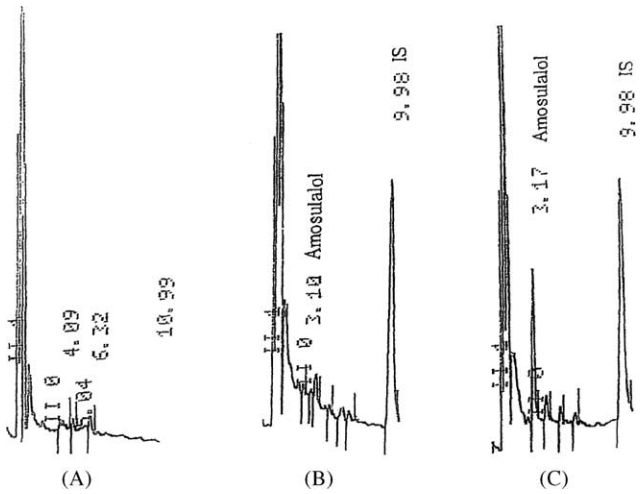

Fig. 2. Chromatograms for control human plasma (A), control plasma spiked with 30 ng/mL of amosulalol hydrochloride and 2 μg/mL of IS (B), and control plasma spiked with 500 ng/mL of amosulalol hydrochloride and 2 μg/mL of IS (C).

3.3. Accuracy, precision and recovery

The limit of quantitation (LOQ) of amosulalol hydrochloride was determined as the sample concentration of amosulalol hydrochloride resulting in peak heights of 10 times baseline noise. The LOQ was found to be 30 ng/mL. Based on three times peak height of baseline noise, the limit of detection was calculated to be 7 ng/mL. The intra- and inter-day precisions of the methods were determined by the assay of five samples of drug-free plasma containing known concentrations of amosulalol hydrochloride. As described in Table 1, the intra- and inter-day R.S.D. (%) was within 10.8%, which were acceptable for all quality control samples including the LOQ. The accuracy of amosulalol hydrochloride ranged between 95.1 and 103.7%. All the batches met the quality control acceptance criteria [5].

The extraction recovery of amosulalol hydrochloride at concentrations of 100, 500 and 1000 ng/mL was 99.5 ± 0.5, 96.2 ± 0.8 and 95.6 ± 4.3% ($n = 3$), respectively, while for IS at concentration of 200 ng/mL it was 100.5 ± 1.1% ($n = 3$). These results suggested that there was no difference in

Table 1
Intra- and inter-day precision and accuracy of the determination of amosulalol hydrochloride in plasma

Concentration (ng/mL)	R.S.D. (%)		Accuracy (%)
	Intra ($n = 5$)	Inter ($n = 5$)	
30	10.80	10.00	103.66
50	6.63	9.55	95.12
100	4.98	7.92	95.16
500	5.74	5.82	95.90
1000	6.44	2.49	97.57

Table 2
Stability of amosulalol hydrochloride in plasma

Concentration (ng/mL)	Treatment	Percentage of initial value
100	Three freeze–thaw cycles	97.1 ± 6.3
	Stored at room temperature for 24 h	93.1 ± 4.7
	Stored at −70 °C for 4 weeks	102.6 ± 1.5
1000	Three freeze–thaw cycles	96.2 ± 2.1
	Stored at room temperature for 24 h	93.6 ± 5.8
	Stored at −70 °C for 4 weeks	96.3 ± 1.8

extraction recovery at different concentrations of amosulalol hydrochloride.

3.4. Stability

Knowledge of the stability of the drug in test material is a prerequisite for obtaining valuable data [6]. The stability of amosulalol hydrochloride under various conditions is described in Table 2. Under all conditions tested, amosulalol hydrochloride was stable with detected concentrations of at least 93.1% of the initial concentration.

3.5. Pharmacokinetics

This analytical method was applied to the quantitation of plasma amosulalol concentrations in more than 200 samples from healthy volunteers in pharmacokinetic studies. The application of the method to determine the plasma level in humans is depicted in Fig. 3. After oral administration of amosulalol hydrochloride tablets at 20 mg dose, the maximum plasma concentration (C_{max}) of amosulalol was 323.1 ng/mL at 3.1 h. The mean area under the curve (AUC) and elimination half-life were calculated to be 1878.9 ± 438.9 (ng h)/mL and 3.0 ± 1.4 h, respectively. Also, the parameter estimates for total clearance (Cl/F) and volume of distribution (Vz(terminal)/F) were 10.35 ± 2.37 L/h and 0.76 ± 0.32 L/kg, respectively, which indicates that the plasma clearance of amosulalol is relatively slow and extravascular distribution of the drug is limited [1]. Apparent shoulder was observed in the absorption phase in Fig. 3, possibly due to the dose-dumping. Typical chromatograms of plasma samples in a subject at 3 and 12 h after drug administration are shown in Fig. 4.

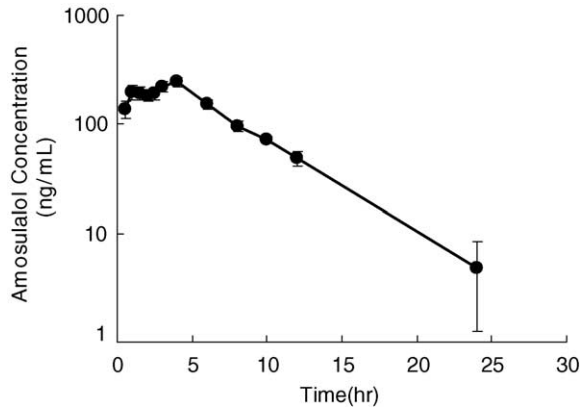

Fig. 3. Mean plasma concentration of amosulalol vs. time after an oral dose of 20 mg of amosulalol hydrochloride. Each point represents the mean ± S.D. of 16 healthy subjects.

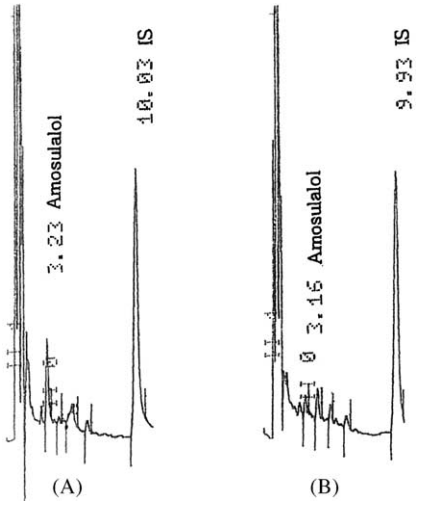

Fig. 4. Typical chromatograms of samples obtained from a healthy volunteer 3 h (A) and 12 h (B) after 20 mg oral dose of amosulalol hydrochloride.

4. Discussion

The determination of amosulalol using solid-phase extraction technique together with HPLC has proven to be simple, rapid, sensitive, specific, accurate and reproducible. There has been only one method for the analysis of amosulalol, which used fluorescence derivatization. The method, however, is time-consuming and complex, which needs to have exact pH (7.5–8.5) for extraction and temperature (60 °C) and reaction time (90 min) for derivatization. Compared to the derivatization method, this solid-phase extraction method has improved simplicity of sample preparation. The intra- and inter-day precision and accuracy were acceptable in all quality control samples including the LOQ of 30 ng/mL. Recovery evaluations showed that amosulalol was recovered at least 95.6%. Amosulalol was stable in human plasma under various storage conditions including three freeze–thaw cycles. The applicability of this method for pharmacokinetic and bioequivalence studies in human has also proved to be suitable. Therefore, this simple and validated assay could readily be used in any pharmacokinetic studies using humans.

Acknowledgements

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