Determination of betaxolol in human plasma by high-performance liquid chromatography with fluorescence detection

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Abstract

Objective. To present a liquid chromatography with fluorescence detection method for the determination of betaxolol in human plasma without internal standard. *Method.* A reversed-phase column was used with a fluorescence detector at 227 nm (excitation) and 301 nm (emission). The mobile phase was consisted of acetonitrile, methanol and phosphate buffer at a flow rate of 1.0 ml/min. *Results.* The standard curve was linear over a range of 2.5 – 80 ng/ml. The recovery from human plasma was found to be 88% of betaxolol. Validation results showed the linearity, specificity, accuracy, precision and stability, as well as on application to the analysis of plasma samples 1 hour after oral administration of 40 mg of betaxolol in healthy volunteers. *Conclusion.* Liquid chromatography with fluorescence detection is an accurate, precise and reliable method to determine the betaxolol hydrochloride in human plasma. [Life Science Journal. 2007; 4(3): 30 – 33] (ISSN: 1097 – 8135).

Keywords: fluorescence detection; betaxolol; bioavailability

1 Introduction

Betaxolol, (±)-1-p-2-(cyclopropylmethoxy)ethylpheno xy-3-(isopropylamino)-2-propanol hydrochloride [63659-19-8] (Figure 1), is a new β-adrenoceptor blocking agent which is highly β1-selective, equipotent with propranolol after intravenous administration in human[1]. It has the lower first-pass loss[1], and produc a potency on the β1-receptors which is about 6 times that of propranolol after oral administration to human[2] and leading to highly reproducible intersubject blood levels and a predictable dose/blood level relationship. The drug is 100% absorbed in the gut[3]. It is used for the treatment of hypertension and glaucoma[4].

The assay of betaxolol by gas chromatography (GC) with electron-capture detection (ECD) and GC-mass spectrometry (MS) have been reported in the literature[5–8]. Chromatographic analysis of betaxolol in body fluids such as plasma, urine and ophthalmic solution was mainly based on high-performance liquid chromatography (HPLC) combined with ultraviolet or fluorescence detection[9 – 14].

This paper describes a sensitive and specific liquid chromatography method with fluorescence detection for betaxolol quantification in human plasma without internal standard and its application for bioavailability research.

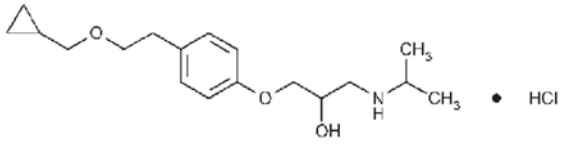

Figure 1. Molecule structure of betaxolol

2 Materials and Methods

2.1 Reagents

Betaxolol hydrochloride standard powder with purity of 99.7% was provided by New Drug R & D Centre of Zhengzhou University (Henan, China). Betaxolol hydrochloride tablets for test (New Drug R & D Centre of Zhengzhou University, China), betaxolol hydrochloride tablets as control (Lorex Pharmaceuticals, Belgium), HLPC-grade quality acetonitrile and methanol (Tida, USA) were used and other chemicals were analytical grade.

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2.2 Chromatography

The HPLC was comprised of a solvent delivery system (Agilent 1100) and a fluorescence detector (Agilent G1321A) and a DVD detector (Agilent G1321A). The analytical column was a stainless steel column packed with 5 μm ODS C18 (4.6 × 250 mm, Hypersil). The mobile phase was consisted of 0.2% potassium dihydrogen phosphate (pH 3.0) : acetonitrile : methanol (60 : 37 : 3, v/v) with a flow rate of 1.0 ml/min at ambient temperature. The fluorescence excitation and emission wavelengths were 227 nm and 301 nm, respectively.

2.3 Preparation of standard

Standard solutions of betaxolol were prepared with concentrations of 0.25, 0.5, 1, 2, 4, 6 and 8 mg/L and stored at 4 ºC. This solution was stable for several weeks.

2.4 Sample preparation

200 μ1 of 0.2 M sodium hydroxide was added to 1 ml blank unhaemolysed blood. The mixture was extracted with 4 ml of cyclohexane and centrifugated at 3000 rpm for 10 minutes. 3.5 ml of the upper organic phase was evaporated to dryness at 37 ºC under a gentle stream of nitrogen. The rest was dissolved in 100 μ1 of mobile phase by agitation on a vortex mixer. 20 μ1 of the solution was injected into the chromatograph by an automatic injector.

2.5 Calibration curve

The calibration curve was prepared by plotting the peak area of betaxolol to the concentration of betaxolol and used for the determination of betaxolol levels in samples. Calibration graphs for betaxolol were prepared by adding 10 μl of each standard solution to 1 ml unhaemolysed blood, respectively. These blood samples were prepared as section 2.4. Calibration graphs were established for blood concentrations of betaxolol up to 80 μg/ml.

2.6 Sample collection

In this reseach, eighteen healthy male adult volunteers were fasted for at least twelve hours before administration and four hours after administration. Four tablets (each containing 10 mg of betaxolol) and 200 ml of distilled water were administered to each subject. Blood samples of 3 ml were collected in heparinized vacuum tubes by venipuncture at 0 (pre-dose), 1, 2, 3, 4, 5, 7, 9, 12, 24, 36, 48 and 60 hours after administration. The plasma was separated by centrifugation immediately after collection and was stored frozen at – 20 °C until analysis.

2.7 Validation

To measure the validation of the method, the parameters of accuracy, precision, limit of quantification (LOQ), limit of detection (LOD), linearity and selectivity were investigated.

The recovery of betaxolol was determined by comparison of peak area values of a spiked human plasma standard (60 ng/ml) with a standard solution in water at the same concentration.

To evaluate accuracy and precision, standard solutions in human plasma at three concentration levels over the calibration curve, were analyzed. The concentration levels were 2.5, 20 and 60 ng/ml of betaxolol. Each of these samples was analyzed five times within that day and in 5 different days thereafter, to determine the intra-day and inter-day accuracy and precision.

Precision was calculated as relative standard deviation (RSD), the accuracy was percentage deviation of found concentration from added concentration.

The selectivity was described as the ability of the method to resolve betaxolol from baseline and other peaks.

3 Results and Discussion

3.1 Analysis of betaxolol in human plasma

In the presented method, betaxolol were accurately separated on Hypersil C18 column (4.6 × 250 mm, 5 μm) using a mobile phase consisting of acetonitrile : methanol : 0.2% potassium dihydrogen phosphate (37 : 3 : 60, v/v) after optimization of chromatographic conditions, adjust with 0.025 M phosphoric acid to pH 3.0. The optimum flow-rate was 1.0 ml/min. The selection of a suitable detection procedure is also important for optimizing an HPLC method. For betaxolol, essentially two types of detection can be used: UV-visible spectrometry and fluorescence. The optimum wave-lengths of betaxolol in UV-visible spectrometry were λ_{max} = 220 nm and λ_{max} = 273 nm, and fluorescence detectors were λ_{ex}= 227 nm and λ_{em} = 301 nm. After direct injection of betaxolol standard into the HPLC system with UV-visible or fluorescence detectors, detection limits with a signal-to-noise ratio of 3 were 25 ng/ml and 0.5 ng/ml, respectively. But optimization of chromatographic conditions with UV-visible detector was acetonitrile-phosphate buffer (20 : 80, v/v), phosphate buffer includes 0.1% tetrebutylammonium and 0.29% monobasic potassium phosphate and adjusts with 0.025 M phosphoric acid to pH 3.0.

In HPLC-UV-visible method, the retention time of betaxolol was 10 minutes. Additionally, interfering peaks from matrix occurred in the chromatogram of a drug-free human plasma sample with UV-visible detection. Although, betaxolol was well separated from interfering

peaks, UV-visible detection response was about 25-fold lower than fluorescence detection response. Because of these observations, fluorescence detection was used in the research.

In HPLC-fluorescence detection, the retention time of betaxolol was 7.5 minutes (Figure 2a). After extraction of drug-free human plasma sample with cyclohexane, all extra peaks were eliminated (Figure 2b). Betaxolol peaks were well-resolved in the human plasma sample obtained from male adult volunteers (Figure 2c).

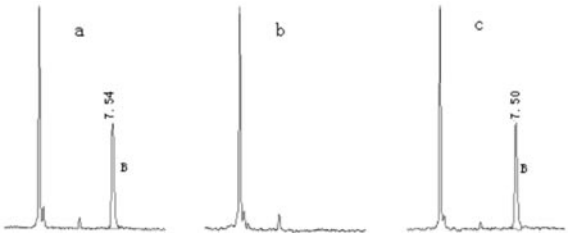

Figure 2. Representative chromatograms of (a) betaxolol standard in mobile phase after extraction of human plasma sample spiked with betaxolol; (b) after extraction of drug-free plasma; (c) plasma sample containing betaxolol obtained from a volunteer injected into the HPLC–fluorescence detection system. B: betaxolol.

3.2 Assay validation

The calibration curve of the method was linear over the range of 2.5 – 80 ng/ml (C = 0.1098A + 0.4492, r = 0.9998). The lower LOD was 0.5 ng/ml at S/N = 3 and the LOQ was 2.5 ng/ml (RSD = 3.4%, n = 5). The recovery of betaxolol from plasma was 70.1% at a concentration of 60 ng/ml (n = 1)(Table 1).

Table 1. The recovery of betaxolol hydrochoride in plasma by HPLC

Added concentration (ng/ml)	Recovery of method (%)	RSD (%)	Recovery of extraction (%)	RSD (%)
2.5	109.5	3.8	66.1	6.5
20	98.5	6.8	67.3	5.8
60	99.6	2.4	70.1	4.4

Precision and accuracy values for within-day and day-to-day studies are listed in Table 2. Precision (RSD) was less than 10.0% at selected concentrations and accuracy values ranges from 99.8% to 107.2%. These values were acceptable according to the acceptance criteria's for accuracy and precision[15].

3.3 Application to biological samples

The proposed analytical method was applied to a pilot study to compare the bioavailability of two betaxolol (hydrochloride) formulations: 40 mg tablet. This research was conducted with eighteen volunteers with single oral dose (four 10 mg tablets) and randomized crossover design. Typical plasma concentration versus time profiles is shown in Figure 3.

Table 2. The precisions of betaxolol hydrochoride in plasma by HPLC

Concentration (μg/L)	Intra-day (n = 5)		Inter-day (n = 5)	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
2.5	107.2	3.4	104.8	4.9
20	100.3	6.9	101.4	8.1
60	99.8	2.4	102.9	4.0

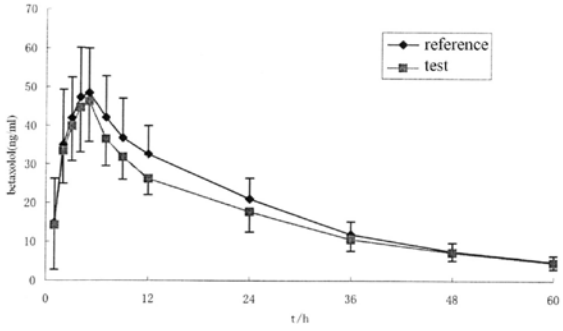

Figure 3. Mean plasma concentrations of test and reference formulations after 40 mg single oral dose (eighteen healthy volunteers).

Plasma concentrations of betaxolol were in the standard curve range and remained above the 2.5 ng/mL quantitation limit for the entire sampling period. The observed maximum plasma concentration (C_{max}) was 53.69 ± 10.58 ng/mL for the reference and 51.45 ± 9.21 ng/mL for the test. The corresponding time of maximum concentration (T_{max}) was 4.1 ± 1.1 hours for the reference and 4.6 ± 1.4 hours for the test. The value of the area under the curve from time 0 to the last sampling time (AUC_{0-t}) was 1145 ± 218 ng · h/ml as reference and 1007 ± 165 ng · h/ml for the test, and area under the curve from 0 to ∞ ($AUC_{0-\infty}$) was 1281 ± 250 ng · h/mL for the reference and 1165 ± 214 ng · h/mL for the test. The elimination half-life ($t_{1/2}$) was 16.47 ± 2.80 hours for the reference and 17.16 ± 2.27 hours for the test. The pharmacokinetic data obtained were similar to those reported by Stagni[16]. In addition, the mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ was higher than 80%, complying with the Food and Drug Administration Bioequivalence Guideline[17].

The ratio test/reference (T/R) and 90% confidence intervals (90 CIs) for overall analysis were comprised within the previously stipulated range (80% – 125%). Therefore,

the results demonstrated the relative bioavailability of the two formulations of betaxolol hydrochloride.

4 Conclusion

In conclusion, UV-visible detection response was about 25-fold lower than fluorescence detection response, so we must choose the more sensitive fluorescence detector. Liquid chromatography with fluorescence detection could be an accurate, precise and reliable measurement of betaxolol hydrochloride concentrations in human plasma for up to 60 hours after oral administration of 40 mg to healthy volunteers. The described method has proven to be fast and robust, with analysis time of each sample requiring less than 15 minutes. The assay method has significant advantage over other techniques previously described for measuring betaxolol in biological fluids. The sensitivity of the assay is sufficient to follow accurately the pharmacokinetics of this drug.

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