RESEARCH ARTICLE

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ATENOLOL IN HUMAN PLASMA BY LC-MS

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Abstract: A rapid and sensitive liquid chromatography-mass spectrometry (LC-MS) method has been developed and validated for quantification of atenolol in human plasma. The analyte was extracted from human plasma by solid-phase extraction technique by using samprep SPE columns C_{18} (50µg, 70A), followed that simple isocratic chromatographic condition with mobile phase acetonitrile: 0.5% formic acid and mass spectrometric detection that enables detection at nanogram levels. Pantoprazole was used as the internal standard. A phenomenex C_{18} column provided chromatographic separation of the analyte which was followed by detection with mass spectrometry. The mass transition ion-pair was followed as m/z 267.00 for atenolol and 384.00 for pantoprazole. The retention times were 3.808 and 7.143 minutes for atenolol and pantoprazole respectively. The proposed method has been validated with linear range of 200-12000 ng/mL for atenolol. The precision and accuracy values are within 5 %. The overall recovery of atenolol was 98.55%.

Keywords: Atenolol, LC-MS, human plasma.

INTRODUCTION

Atenolol is a synthetic, beta-selective (cardio selective), adrenoreceptor blocking agent. It is chemically described as benzene acetamide. 4-[2'-hydroxy-3'-[(1-methyl ethyl) amino] propoxy] benezeneacetamide. It is indicated in the management of hypertension. The analytical methods available for the estimation of atenolol are official in IP, BP, USP where as the reported methods for the estimation of atenolol in the literature by high performance liquid chromatography (HPLC) [1, 2, 3, 4, 5] and gas chromatography (GC) [6, 7] and UV spectrophotometry methods [8]. Methods of measuring drugs in biologic media are increasingly important problems related to bioavailability and bioequivalence studies, new drug development, drug abuse, clinical pharmacokinetics and drug research are highly dependent on accurately measured drugs in biological fluids. For the estimation of the drugs present in the biological fluid, LC-MS method is consider to the more suitable since this is a powerful and rugged method. The present study describes development and validation of a simple, specific, rapid and sensitive LC-MS method for the determination of atenolol in human plasma.

MATERIALS AND METHODS

Materials

The reference standard of atenolol and pantoprazole were obtained from Micro labs, Bangalore. High purity water was prepared in-house using a milli-Q water purification system obtained from Millipore (India) Pvt Ltd. HPLC grade acetonitrile, methanol, and formic acid AR grade were purchased form S.D fine chemicals. Drug free (blank) heparinized human plasma was stored at -70 ºC prior to use.

Instrumentation and chromatographic conditions

The HPLC system (Shimadzu LC-10AD) consisting of a binary pump, auto sampler (SIL-HTc) detection was performed by an applied biosystems. Sciex (API 2000) mass spectrometer using atmospheric pressure chemical ionization for ion production was used, which was controlled by LC-MS solution data station software. The column used was phenomenex C_{18}, 100 x 4.6mm i.d., 5µ. The mobile phase was prepared by mixing acetonitrile and 0.5 % formic acid in the ratio of 60:40 %v/v. Chromatographic study was performed at ambient temperature at flow rate of 0.5 mL/minute. The compound ionized in the positive atmospheric pressure chemical ionization (APCI) mode of mass spectrometer. Analysis was performed in selective ion monitoring (SIM) mode. Atenolol and pantoprazole were detected at m/z 267.00 and m/z 384.00 respectively.

Preparation of the stock solution, calibration curve solutions and quality control samples

Stock solution of atenolol (1000 µg/mL) is prepared in water. Series of working standard solutions with concentrations of 4000.00, 6000.00, 12000.00, 25000.00, 60000.00, 100000.00, 200000.00, and 240000.00 ng/mL were prepared by dilution of aliquots of stock with mobile phase. Working internal standard solution (50 µg/mL) was prepared in the mobile phase. Low, medium, and high quality control solutions (14000, 16000 and 200000 ng/mL respectively) were also prepared in mobile phase. Linearity was prepared by spiking blank plasma at concentration of 200, 300, 600, 1000, 3000, 5000, 10000 and 12000 ng/mL of atenolol; quality control is 700.00, 8000.00 and 11000.00 ng/mL. Stock solutions and plasma samples were stored at -70±2 ºC until processing.

Sample preparation

At the time of analysis, the plasma samples were removed from the deep freezer and kept in the room temperature and allowed to thaw. Samprep SPE C_{18} columns (50 µm, 70A) 100mg/1mL cartridges were conditioning by passing 1mL of methanol through them. To prepare samples for loading, 0.5mL plasma was mixed with 250 µL of 50 µg/mL of internal standard. Sample was vortexed to mix, centrifuged for 5 minutes at 15000 rpm and loaded on cartridge. Samples were washed with 2 mL water. Analyte and internal standard were eluted from the cartridge using 0.5 mL of methanol. 10 µL of sample was injected onto LC-MS.

Validation procedures

Linearity

Linearity tests were performed in the concentration range of 200.00-12000.00 ng/mL of atenolol containing 50 µg/mL internal standard. These solutions were analyzed and the peak areas, response factors were calculated. The calibration curve was plotted using response factor vs. concentration of the standard solutions. The calibration curves were constructed on six different days over period of two weeks to determine the variability of the slopes and intercepts.

Recovery

Extraction recoveries of atenolol from spiked samples were determined by comparing the peak areas obtained by extraction of freshly prepared plasma extracts at low, medium and high concentration levels, with those found by direct injection of an aqueous standard solution at equivalent concentrations (n=6).

Precision

The precision of the method was determined by intra-day precision and inter-day precision. The intraday precision was evaluated by analysis of blank plasma samples containing atenolol at three different concentrations of LQC, MQC, HQC using nine replicate determinations on same day. The interday precision was similarly evaluated over two-week period. The mean concentration, mean % bios, standard deviation and % CV were calculated.

System suitability

The parameters namely column efficiency, resolution, peak asymmetry factor and capacity factor for the standard solutions were calculated.

Table 1. Recovery studies

Level	Concentration of drug added (ng/ml)	Amount of drug recovered (ng/ml) in plasma sample	Recovery (%)	Amount of Drug recovered (%) in Mobile phase	Relative Recovery (%)
Level-I	700.00	696.42 ± 1.56	Mean : 97.09 CV : 1.65 N : 6	Mean : 98.61 CV : 1.03 N : 6	98.45
Level-II	8000.00	79624 ± 0.98	Mean : 96.12 CV : 0.89 N : 6	Mean : 98.33 CV : 2.69 N : 6	97.75
Level-III	11000.00	9952.03± 1.33	Mean : 97.62 CV : 1.52 N : 6	Mean : 99.14 CV : 0.94 N : 6	99.47

Table 2. Precision studies (ng/ml)

	Nominal Concentration (ng/mL)		
S.N	LQC	MQC	HQC
	700.00	8000.00	11000.00
1	699.48	7045.12	11475.27
2	640.04	8068.08	11248.63
3	670.22	7894.11	11762.03
4	600.63	7740.62	10780.55
5	585.56	7987.63	10751.72
Mean	639.186	7747.112	11203.64
S.D (+/-)	47.3326	410.8871	438.9884
C.V. (%)	7.41	5.30	3.92
% Nominal	91.31	96.84	101.85
n	5	5	5
	Nominal Concentration (ng/mL)		
S.N	LQC	MQC	HQC
	700.00	8000.00	11000.00
1	701.96	7664.32	10264.11
2	659.89	7520.06	9578.02
3	680.11	6482.92	9942.22
4	510.72	7283.74	10424.09
5	587.07	7341.08	10620.88
Mean	627.95	7258.424	10165.864
S.D (+/-)	78.4735	458.8258	411.9920
C.V. (%)	12.50	6.32	4.05
% Nominal	89.71	90.73	92.42
n	5	5	5
	Nominal Concentration (ng/mL)		
S.N	LQC	MQC	HQC
	700.00	8000.00	11000.00
1	678.25	7045.12	11475.27
2	590.78	8068.08	11248.63
3	542.93	7894.11	11762.03
4	498.02	7740.62	9045.87
5	561.63	7987.63	10751.72
Mean	574.322	7747.112	10856.704
S.D (+/-)	67.1573	410.8871	1077.7275
C.V. (%)	11.69	5.30	9.93
% Nominal	82.05	96.84	98.70
n	5	5	5

RESULTS AND DISCUSSION

Estimation of atenolol in human plasma was carried out using optimized chromatographic conditions. Validation parameters such as accuracy, precision, linearity and range, system suitability, sensitivity (limit of detection and limit of quantitation), robustness and ruggedness were evaluated.

Accuracy

The percentage recovery values for atenolol ranging from 97.75 to 99.47 and given in Table 1. The coefficient of variation (%) of these values was less than 10%. It is indicative that the developed method is accurate and reliable.

Precision

The optimized method for the estimation of atenolol was found to be precise and this was evident from the coefficient of variation values, which were less than 10.00 % at all concentrations and given in Table 2.

Linearity

It was observed that the optimized methods were linear within a specific concentration range for atenolol. The calibration curves were plotted between response factor and concentration of the standard solutions. The linearity ranges were found to be 200.00–12000.00 ng/mL (Table 3) (Figure 1). The results indicated no significant interday variability of slopes and intercepts over the optimized concentrations range.

Table 3. Linearity and range

Drug Concentration (ng/ml)	Internal Standard Concentration (ng/ml)	Response Factor (RSD)
200.00	50.0	0.000
300.00	50.0	0.018
600.00	50.0	0.076
1000.00	50.0	0.148
3000.00	50.0	0.225
5000.00	50.0	0.520
10000.00	50.0	1.127
12000.00	50.0	2.254

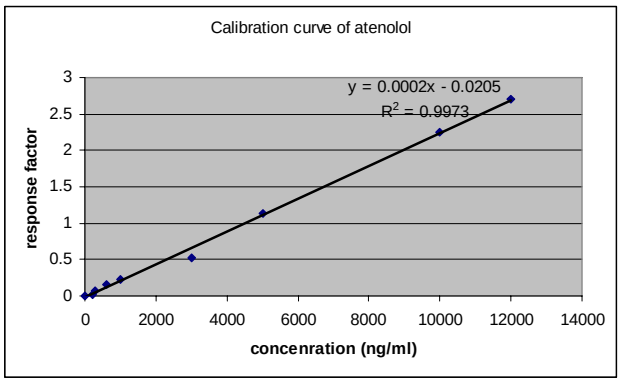

Figure 1. Calibration curve for atenolol

System suitability

The values obtained demonstrated the suitability of the system for the analysis of the atenolol in plasma (Figure 2, 3 and 4). The values were given in Table 4.

Limit of detection

The limit of detection value (LOD) was found to be 1 ng/mL and limit of quantization was 5 ng/mL. These observations indicate that the developed methods have adequate sensitivity.

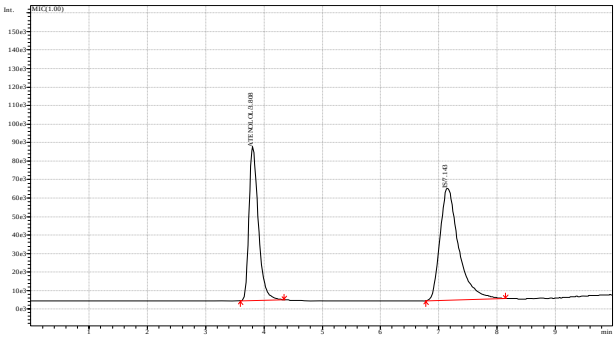

Figure 2. Chromatogram of atenolol and internal standard (standard solution)

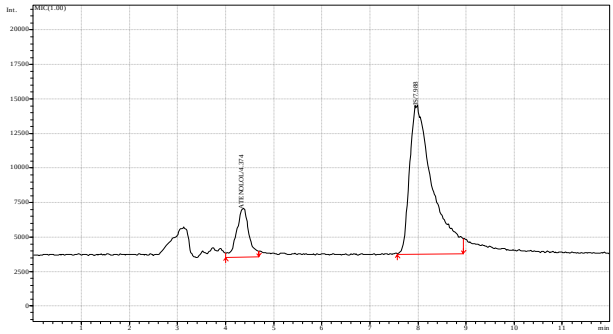

Figure 3. Chromatogram of atenolol and internal standard (sample solution)

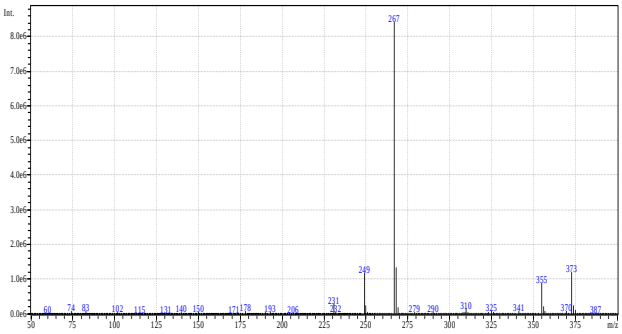

Figure 4. Chromatogram of atenolol at scan positive mode

Table 4. System suitability studies

Parameters	Internal Standard	Drug
Theoretical Plate	24872	26045
Resolution factor	1.5	
Asymmetric factor	1.00	1.05
LOD((ng/ml)	5.00	1.00
LOQ((ng/ml)	10.00	5.00

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of atenolol in human plasma. The mobile phase is simple to prepare and economical. This method can be employed for the routine analysis of drug concentrations in bioavailability and bioequivalence studies.

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