Quantification of Metoprolol Succinate in balk and tablet formulation by HPLC: Method development and validation

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Abstract

The present paper describes a sensitive, precise and accurate HPLC method with UV detection for the quantification of metoprolol succinate in bulk and tablet formulation. Separations were carried out on Inertsil ODS-2 analytical column (150 mm x 4.6 mm, 5 µ particle size). An isocratic elution system was developed using ammonium acetate buffer: acetonitrile: acetic acid [84:15:1 *v/v/v*]. The pH of the mobile was adjusted to 3.8 with orthophosphoric acid. The elution of the analyte was achieved with a flow rate of 1.0 ml/min. Detection was by UV absorbance at a wavelength of 280 nm. The detector response was linear in the concentration of 10-50 µg/ml (R^2 = 0.9997) metoprolol succinate. The limit of detection and limit of quantitation values were found to be 0.0189 µg/ml and 0.0630 µg/ml, respectively. The method was validated following ICH guidelines. All the parameters of validation were found in the acceptance range. The method was successfully applied to the assay of metoprolol succinate in tablets.

Keywords: Metoprolol Succinate, HPLC, UV detection, Validation, Tablets

Introduction

Metoprolol succinate (MPS), chemically described as butanedioic acid;1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol, is a selective β1-adrenergic antagonist [1]. MPS competes with catecholamines, an adrenergic neurotransmitter, for binding at β1 - adrenergic receptors in the heart. This binding results in a decrease in heart rate, cardiac output and blood pressure [2]. MPS is prescribed for the treatment of angina pectoris, acute myocardial infarction and mild to moderate hypertension [3,4]. MPS may also be suggested as prophylaxis for migraine headaches [5].
MPS is officially listed in Indian Pharmacopoeia [6], British Pharmacopoeia [7] and United States Pharmacopoeia [8]. An extensive survey of literature showed that several methods have been reported for quantification of MPS. UV spectrophotometry [9] and visible spectrophotometry [10-13] methods have been developed and validated for the estimation of MPS in pharmaceutical formulations. LC-MS/MS methods was

developed and validated for the pharmacokinetics study of MPS in beagle dogs [14] and human plasma [15]. HPLC with fluorescence detection has been applied for the quantification of MPS in plasma samples of pediatric patients [16] and in human plasma & urine [17].

HPLC with UV detection is the widely used technique for the assay of drugs. Few HPLC with UV detection methods have also been reported for the assay of MPS in pharmaceutical dosage forms.

An RP-HPLC method for the determination of MPS in its dosage forms was presented by Venkateswararao et al. [18]. They used Aligent C-8, RP column and acetonitrile-water-1% ortho phosphoric acid (70:27:3 *v/v/v*) as mobile phase at a flow rate of 2.0ml/min with UV detection at 280 nm. Deepak et al. [19] determined MPS in tablets by HPLC with UV-detection at 225 nm using a Shiseido Capcellpak, CAP C-18 column and a mobile phase consisting of ACN: Buffer (pH 3.0) in a ratio of 30:70 (*v/v*). The flow rate was set at 1.0 ml/min. The method reported by Sainath et al. [20] involves the determination of MPS using stability indicating reversed phase liquid chromatography using Waters X-Terra RP 18 anlaytical column and acetonitrile: 0.05 M phosphate buffer, pH 3.0 (25:75 v/v) as the mobile phase at a flow rate of 0.8 ml/min. Quantitation of MPS was achieved with ultraviolet detection at 240 nm. The method reported by Naveen et al. [21] describes the quantification of MPS in pharmaceutical dosage form using an Inertsil ODS-2 column with a 60:5:35 (*v/v/v*) mixture of phosphate buffer (pH 6.8), methanol and acetonitrile as mobile phase. The flow rate was 1.0 ml/min with UV detection at 223 nm.

The reported HPLC with UV detection methods, however, are either poorly sensitive [18,19,21], less precise and accurate [18-21] or have narrow linear concentration ranges [19]. The flow rate of mobile phase in Venkateswararao et al. method [18] and the retention time of drug in the method of Naveen et al. [21] is more. These two factors may lead to increased utilization of solvents and cost of analysis. All the reported HPLC methods were not fully validated. Validation parameters such as system suitability [18], selectivity [18], ruggedness [19,20], limit of detection & quantification [20] were not reported.

The main objective of this investigation was to develop a simple, sensitive, accurate and precise HPLC with UV detection method for quantitative analysis of MPS in bulk and in its tablet dosage forms and to validate the method in accordance with ICH guidelines [22].

Materials and methods

Instrumentation

HPLC analysis was performed with an Aligenet HPLC system, Model A1100 equipped witha programmable variable wavelength UV-visible detector, autosampler and LC Ezchrome software. Schimadzu UV-Spectro photometer Model 2489 was used for spectral measurements. pH measurements were done with Elico, Hydrogen Electrode ph meter model LI 127. Samples and chemicals are weighed using Mettler Toledo Analytical balance AB265-S/FACT.

Chemicals and Reagents

All the solvents and chemicals used were of HPLC and analytical reagent grade, respectively. Ammonium acetate, orthophosphoric acid, acetonitrile, methanol, acetic acid, triethyl amine was from purchased from Merck specialities private limited,

Mumbai, India. Milli-Q water (Merck specialities private limited, Mumbai, India) was used throughout the analysis.

Chromatographic conditions

Chromatographic separation was achieved on an Inertsil ODS-2 (150 x 4.6 mm, 5µ) analytical column maintained at 27 ± 1ºC. The mobile phase was an 840:150:10 (*v/v/v*) mixture of ammonium acetate buffer-acetonitrile-acetic acid. The flow rate was fixed as 1.0 ml/min and UV detection was performed at 280 nm. The injection volume was 20 µl.

Preparation of mobile phase

3.9 gm of ammonium acetate was dissolved in 840 ml of HPLC grade water. To this 10 ml of acetic acid, 140 ml of acetonitrile and 2 ml of triethylamine was added. The contents were mixed well. The pH of the final solution was adjusted to 3.8 with orthophosphoric acid. Before use, the mobile phase was filtered through 0.45 µm nylon filter.

Preparation of standard solutions

MPS reference drug was obtained as gift sample from Mylan Laboratories, Hyderabad, India. Mobile phase was used as diluent for the preparation of stock and working standard solutions. A stock solution of MPS (1000 µg/ml) was prepared in the mobile phase. Working standard solutions were prepared by apt dilution of the stock solution with the mobile phase to get solution in the concentration range from 10 to 50 µg/ml MPS.

General Procedures

Calibration curve

Twenty µl of working standard solutions (10, 20, 30, 40 and 50 µg/ml MPS) was injected automatically into the column in triplicate under the described chromatographic conditions. The chromatograms were recorded. The calibration curve was prepared by plotting the mean peak area versus concentration of MPS in µg/ml.

Procedure for assay of MPS in tablets

Metocard XL 12.5 tablet dosage forms, labeled to contain 12.5 mg of MPS, manufactured by Torrent pharmaceutical limited, India, were procured from the local pharmacy market. Ten tablets were accurately weighed and crushed into a fine powder. An amount of the powder equivalent to 100 mg of MPS was weighed and dissolved in 50 ml of mobile phase by shaking in ultrasonic bath for about 20 min for the complete dissolution of MPS. The solution was filtered through 0.45 µm nylon filter into a 100 ml calibrated flask. The volume was completed with mobile phase and mixed well. This solution was appropriately diluted with the mobile phase to get a concentration of 30 µg/ml MPS. Twenty µl of tablet sample solution was injected automatically into the column in triplicate under the described chromatographic conditions. The chromatograms were recorded. The concentrations of MPS in tablet was calculated from the calibration curve or from the regression equation derived.

Results and discussion

HPLC method optimization

The chromatographic conditions (analytical column, composition of the mobile phase, its pH, its flow rate and detection wavelength) were optimized through several trials to achieve the better sensitivity and good symmetric peak shape for MPS. Different combination ratios of ammonium acetate buffer at different pH, acetic acid and acetonitrile were tested. The best chromatographic separation was achieved on Inertsil ODS-2 column (150 mm × 4.6 mm i.d., particle size 5 µm) using a mobile phase composed of ammonium acetate buffer (pH 3.8): acetonitrile: acetic acid (84:15:1, *v/v/v*) pumped with a flow rate of 1 ml/min. The column temperature was kept constant at 27±1°C. The better sensitivity for MPS was achieved when the UV detector was set at 280 nm. Under the above described chromatographic conditions, MPS was detected at retention time of 11.288 min. The representative chromatogram of MPS is shown in Figure 1.

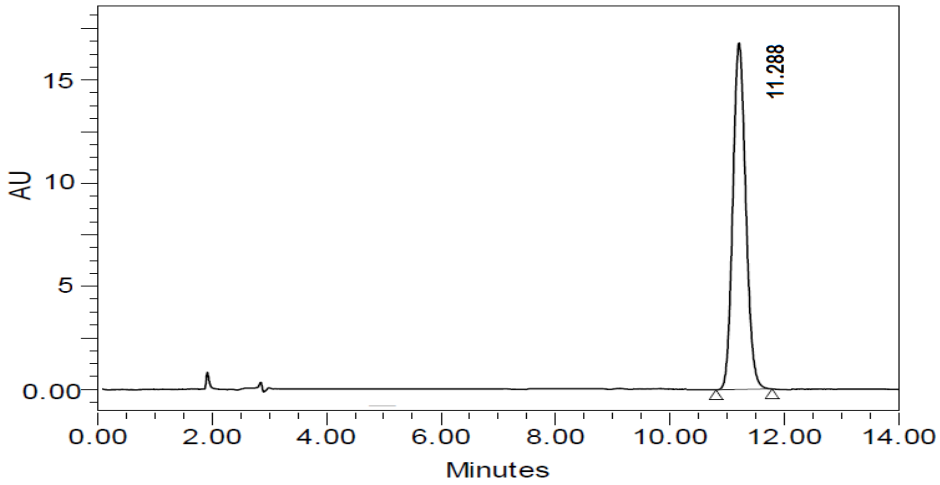

Figure 1: Chromatogram of MPS at optimized HPLC conditions

HPLC method validation

Method validation was done in accordance with ICH recommendation [22].

System suitability

Chromatographic parameters associated to the developed method must pass the system suitability limits before the analysis of sample. The injection repeatability, tailing factor and theoretical plate number for the MPS peak was evaluated using a solution containing 30 µg/ml of MPS. The percentage relative standard deviation of five consecutive injections was found to be 0.140%, indicating good injection repeatability. The tailing factor for MPS peak was found to be 1.16, indicating good peak symmetry. The theoretical plate number was found to be 5590 for the column used, thus representing satisfactory column efficiency. All the results assure the satisfactoriness of the proposed HPLC method for routine analysis of MPS.

Selectivity

The selectivity study was assessed to verify the absence of interference by the excipients in the tablet and components of mobile phase. For this study, MPS standard solution, tablet sample solution, and mobile phase blank solution were injected into the

chromatographic system. The chromatograms were recorded and are shown in Figures 2-4. The chromatogram demonstrated the selectivity of the proposed method, since there were no peaks at the retention time of MPS from excipients commonly coformulated in their tablets; the chromatogram of the tablet sample solution was same as that of the standard solution. Furthermore no peaks were seen the chromatogram of mobile phase blank.

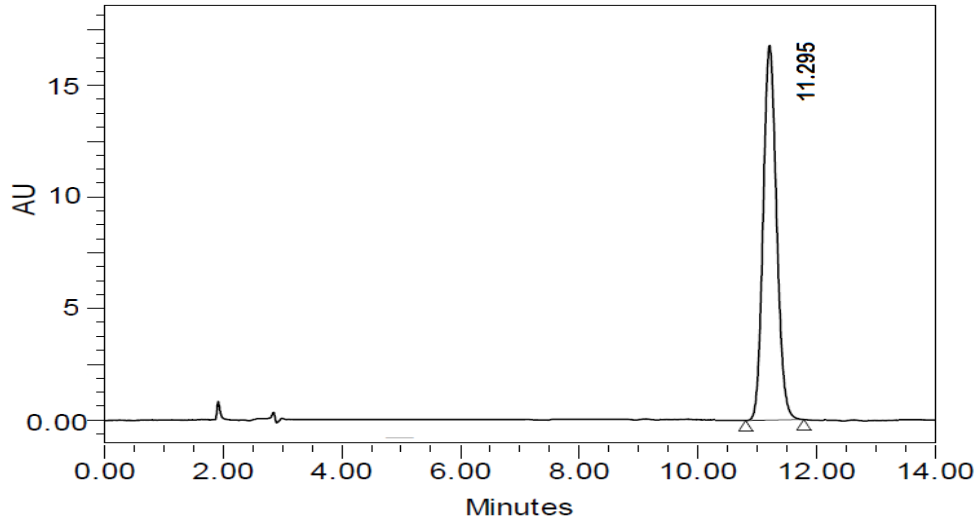

Figure 2: Chromatogram of MPS tablet sample

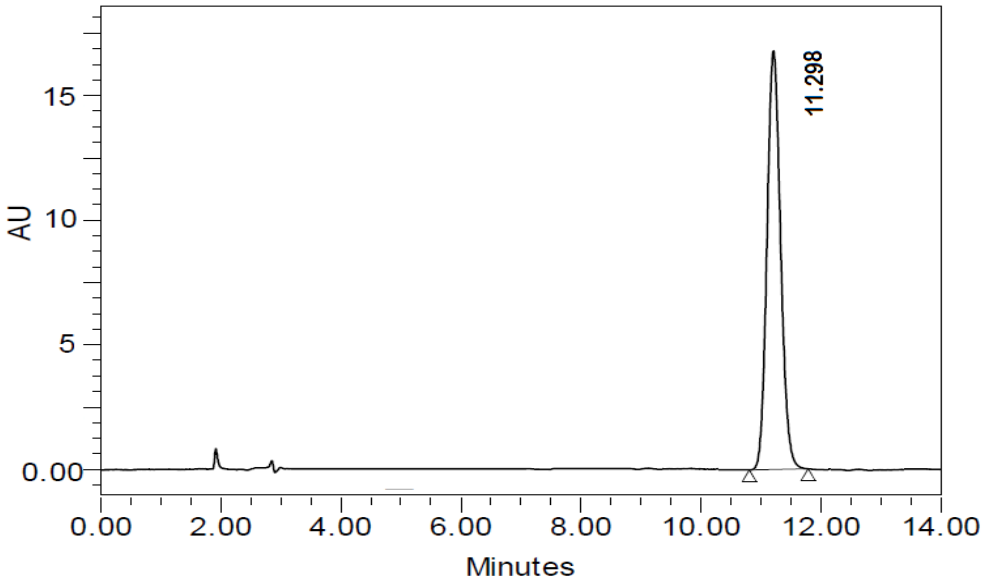

Figure 3: Chromatogram of MPS standard solution

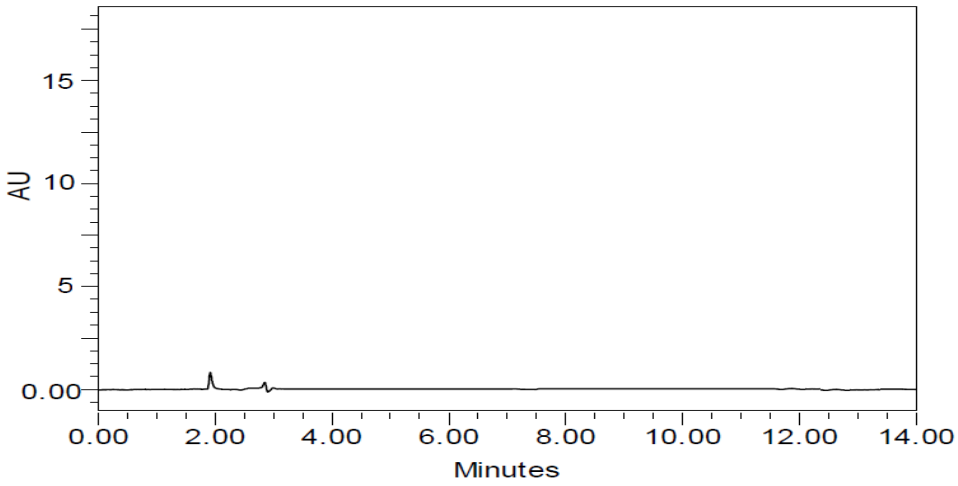

Figure 4: Chromatogram of mobile phase blank

Linearity and sensitivity

Under the optimum HPLC conditions, linear relationship with good correlation coefficients (R^2 = 0.9997, 3 repeated injections per each concentration) were found between the peak area of MPS and concentration of MPS in the range of 10-50 µg/ml. The high R^2 value was indicative of good linearity.

The limit of detection (LOD) and the limit of quantitation (LOQ), which represents the sensitivity of the method, for MPS were 0.0189 µg/ml and 0.0630 µg/ml, respectively.

Precision

System precision and method precision were assessed by repeated analysis (n=5) of standard MPS solution and MPS tablet sample solution, respectively at a concentration of 30 µg/ml MPS. The results are represented in Table 1. The proposed method gave satisfactory results for the system and method precision as the %RSD values did not exceed 0.137 and 0.089%, respectively.

Table 1: System and method precision

System precision		**Method precision**	
Concentration of MPS (µg/ml)	**Peak area**	**Concentration of MPS (µg/ml)**	**% Recovery**
30	379331	30	99.9
30	380281	30	100.0
30	379830	30	99.8
30	380134	30	100.0
30	380912	30	99.8
Mean peak area – 380097.6		**Mean recovery** - 99.90	
% RSD – 0.137		**%RSD** – 0.089	

Accuracy

Accuracy of the proposed method was established by the recovery study of known amount of MPS standard added to a placebo matrix for tablets at three different concentration levels (80 %, 100 % and 120 % of target concentration). The samples were analyzed (3 replicates were injected) by the proposed method and the added amounts were calculated. The recovery was presented as percentage. The recovery

values ranged from 99.81 to 100.56 (± 0.109- 0.374%), Table 2. These results indicated the adequate accuracy of the method.

Table 2: Accuracy results for metoprolol succinate

Spiked level (%)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	RSD (%)
80	8	7.985	99.81	0.374
100	10	10.056	100.56	0.261
120	12	12.042	100.35	0.109

Robustness and Ruggedness

The method robustness and ruggedness was established at a concentration of 30 µg/ml MPS. In order to measure the method robustness, the HPLC parameters were deliberately varied and in parallel the chromatographic profile was observed and recorded. The studied parameters were: column temperature (±5°C), flow rate (±10%), pH of the buffer (±0.2). The system suitability parameters were measured to demonstrate the robustness of the method. The results (Table 3) indicated that the small change in the conditions did not significantly affect the system suitability. Therefore, the method is robust.

Table 3: Results of method robustness

Parameter	Condition	System suitability parameters		
		Peak area	Tailing factor	Theoretical plates
Flow rate by ± 10% (ml)	1.0	389331	1.2	5658
	0.9	370281	1.4	4155
	1.1	379830	1.3	3770
Column temperature by ± 5 (°C)	27	388624	1.2	5625
	22	376235	1.4	3528
	32	375624	1.4	3460
pH of Buffer solution by ± 0.2	3.8	386321	1.2	5479
	3.6	374521	1.3	4169
	4.0	379962	1.3	3484

Table 4: Results of ruggedness

Amount of MPS (µg/ml)	Analyst 1/ Instrument 1	Analyst 2/ Instrument 2
	% Recovery	% Recovery
30	99.95	99.65
30	100.03	100.31
30	99.89	99.49
30	100.11	100.52
30	99.85	99.49
Average	99.966	99.892
%RSD	0.094	0.436

Method ruggedness was done to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness of the method was determined by analyzing MPS standard solution under optimized chromatographic conditions with two different analysts and instruments. There was no significant change in the retention time of MPS was observed and the %RSD was <0.5% (Table 4) indicating the ruggedness of the proposed method.

Tablet sample and standard solution stability

The stability of MPS in standard and tablet sample solutions during analysis was determined by repeated analysis of both the samples during the course of experimentation on the same day and also after storage of the MPS solution (30 µg/ml) for 0, 24 and 30 hr under controlled room temperature (25±1°C) and under refrigeration (8±1°C). The solutions are considered stable, if the difference in percentage assay results from 0 to 24 hr and 30 hr is not more than 2%. The results are summarized in Table 5. The results suggesting that the MPS standard and tablet sample solutions can be stored without degradation over the time period studied.

Table 5: Results of MPS stability in standard and tablet sample solutions

Sample	Time (hr)	Sample stored at 25±1°C		Sample stored at 8±1°C	
		Recovery (%)	Difference (%)	Recovery (%)	Difference (%)
Standard	0	99.8	-	99.7	-
	24	100.4	0.6	100.2	0.5
	30	100.7	0.9	100.4	0.7
Tablet	0	102.1	-	101.1	-
	24	102.8	0.7	101.7	0.6
	30	102.2	0.1	102.2	0.5

Applicability of the proposed HPLC method

From the results obtained after method validation, it is evident that the proposed method gave satisfactory results with the analysis of MPS in bulk. Therefore, commercial tablets containing MPS (12.5 mg/tablet) were subjected to the analysis by the proposed method. The percentage recovery was 99.95 ± 0.215%. This satisfactory value indicated the applicability of the developed method for the routine quality control of MPS tablets without interference from the excipients found in the tablet dosage form.

Conclusion

In the present work, a sensitive HPLC method with UV detection has been developed and validated for determination of metoprolol succinate in bulk and in its tablet dosage forms. From the results of validation parameters, the proposed method was found to be sensitive, accurate, precise, robust, rugged and specific. Stability data shows that the MPS was stable for atleast 30 hr. The proposed method, hence, can be applied for routine quality control analysis of metoprolol succinate in bulk and tablet dosage forms.

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