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ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS IN THE METOPROLOL SUCCINATE ER TABLETS

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ABSTRACT

A novel gradient reverse phase Ultra performance liquid chromatographic (UPLC) method was developed for the quantification of Metoprolol impurities and degradation products in the Metoprolol succinate ER tablets. The method was developed using Aquity BEH C18, 100 X 2.1 mm, 1.7 µm column with mobile phase containing a gradient mixture of solvent A and B. 0.13% SLS in 0.1% phosphoric acid and Acetonitrile in 60:40 v/v ratio was used as solvent A , 0.13% SLS solution and Acetonitrile in 20:80 v/v ratio was used as solvent B. The gradient program (T/%B) was set as 0/0, 8/0, 12/40, 16/40 16.5/90, 22/90, 22.5/0 and 28/0 with a flow rate of 0.28 mL/minute. The eluted compounds were monitored at 223 nm and the very good separation achieved for the USP listed Impurities and degradation product Diol impurity. The run time was 28 min with column temperature 25 ºC. Metoprolol succinate ER tablets were subjected to the stress conditions of oxidation, acid, base, hydrolytic, thermal and light degradation. Metoprolol peak did not show any flag in the purity table, thus proved the stability-indicating nature of the method. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness.

KEYWORDS

Degradation product, Method development, Method validation, and UPLC.

1. INTRODUCTION

Metoprolol succinate is a beta blocker [1-3], and chemically it is a (±) 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate. Metoprolol succinate extended-release tablets [4, 5] are indicated for the treatment of stable, symptomatic heart failure of ischemic, hypertensive, or cardiomyopathic origin. The tablets comprise a multiple unit pellet systems and each pellet acts as a separate drug delivery unit and is designed to deliver Metoprolol continuously over the dosage interval.

Metoprolol succinate Extended release tablets are official in the United States pharmacopoeia [6] and there was no impurities and degradation products quantification method in the Tablets USP monograph. Related substances method was given in drug substance monograph and it is not capable to quantify degradation product Diol impurity and the run time for each injection is about 60 minutes. So far to our present knowledge, no UPLC [7] method was available in the literature for the quantification of Metoprolol impurities and degradation products.

Attempts were made to develop a simple, rugged, robust and cost effective method [8] with shorter run time. This paper also deals with the validation [9] of the developed UPLC method for the accurate quantification of impurities and degradation products in the Metoprolol succinate ER tablets.

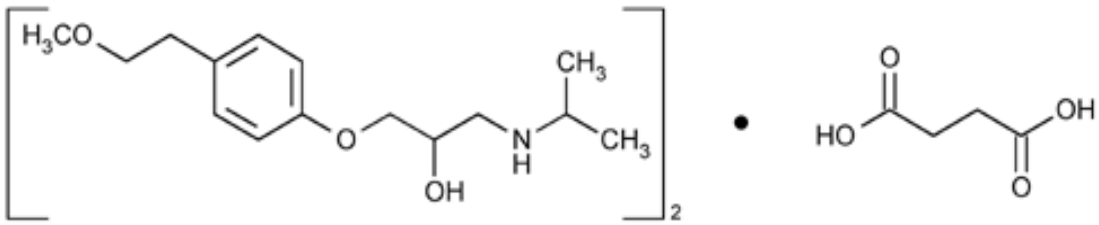

Fig.1. Metoprolol succinate

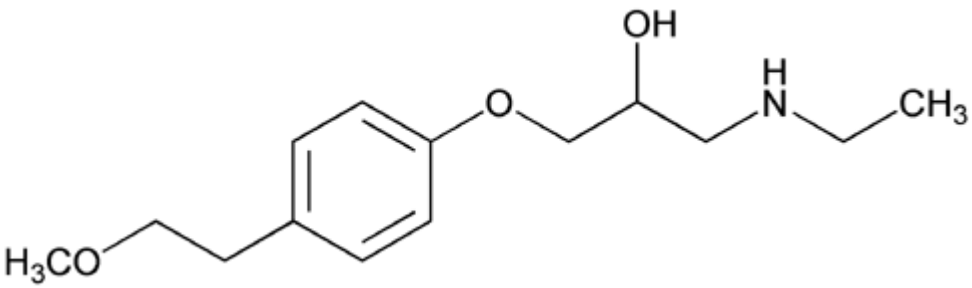

Fig.2. USP Related compound A (Impurity A)

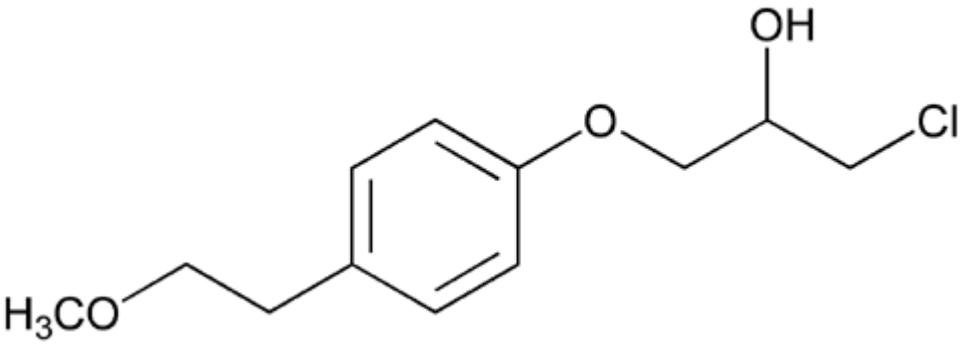

Fig.3. USP Related compound B (Impurity B)

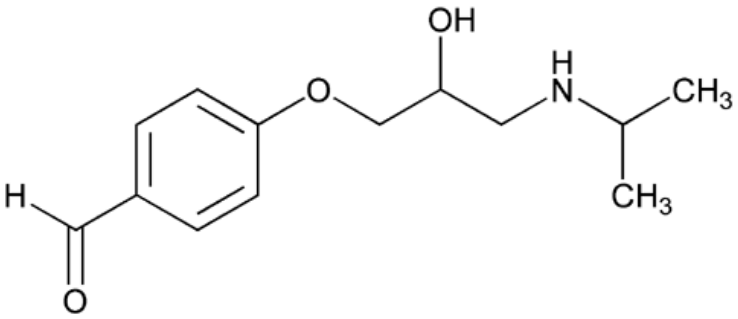

Fig.4. USP Related compound C (Impurity C)

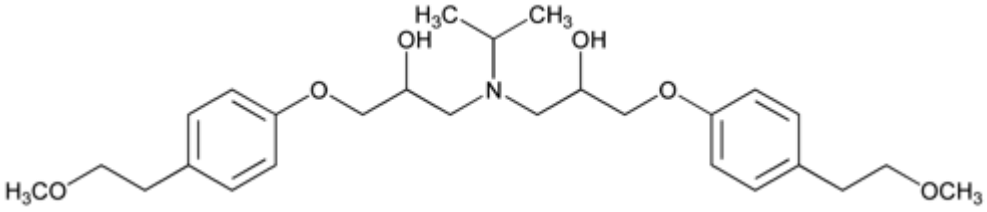

Fig.5. USP Related compound D (Impurity D)

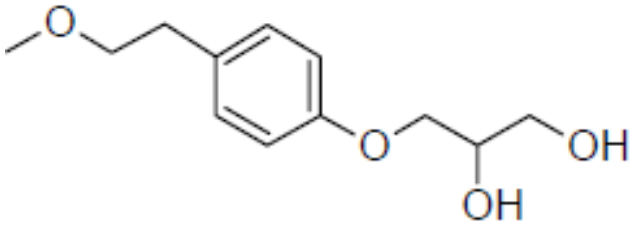

Fig.6. Metoprolol Diol impurity

2. EXPERIMENTAL

2.1. Chemicals and reagents

The Liquid chromatographic gradient grade acetonitrile, Sodium lauryl sulphate and orthophasphoric acid were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore MilliQ Plus water purification system.

2.2. Equipment

Waters UPLC PDA system was used and the output signal was monitored and processed using empowers software.

2.3. Chromatographic Conditions

The method was developed using Aquity BEH C18, 100 X 2.1 mm, 1.7 µm column with mobile phase containing a gradient mixture of solvent A and B. 0.13% Aqueous Sodium lauryl sulphate solution in 0.1% phosphoric acid was used in Mobile phase. 0.13% SLS solution and Acetonitrile in 60:40 v/v ratio was used as solvent A, 0.13% SLS solution and Acetonitrile in 20:80 v/v ratio was used as solvent B. The gradient program (T/%B) was set as 0/0, 8/0, 12/40, 16/40 16.5/90, 22/90, 22.5/0 and 28/0 with a flow rate of 0.28 mL/minute. The run time was 28 min with column temperature 25 ^{o}C and injection volume of 1 µL. Solvent A was used as a diluent for the preparation of samples.

2.4. Preparation of Stock Solutions

A stock solution of Metoprolol succinate (0.9 mg/mL) was prepared by dissolving appropriate amount of drug in Diluent. Working solution of

0.0045 mg/mL was prepared from the above stock solution for the impurities quantification.

2.5. Preparation of Sample Solution

Metoprolol succinate extended release tablets were taken in to the 500 ml volumetric flask, added about 200 mL of diluent and tablets were disintegrated with help of rotary shaking for about 30 minutes and sonication for 30 minutes. Sample solutions diluted to volume to get test concentration of 1140 ppm.

2.6. Method Validation

The proposed method was validated as per ICH guidelines.

2.6.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The stress conditions employed were; Acid, Base, Water, oxidation media, heat, moisture and light. To evaluate the ability of the proposed method to separate degradation products from Metoprolol peak, Peak purity was carried out by using PDA detector.

2.6.2. Precision

The precision of the related substances method was verified by injecting six individual preparations of Metoprolol succinate ER tablets test preparation spiked with impurities and degradation product Diol impurity.

The intermediate precision of the method was also evaluated using different analyst and different instruments.

2.6.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values were determined at a signal-to-noise ratio of about 3:1 and 10:1 respectively, by injecting a series of dilute impurity solutions. Precision study was carried out at the LOQ level by injecting six individual preparations.

2.6.4. Linearity

Linearity test solutions for the related substance method were prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 300% of the 0.1% level. Correlation coefficient value, slope, Y-intercept and bias were calculated.

2.6.5. Accuracy

The accuracy study was carried out in triplicate at LOQ, to 300 % of the 0.1% concentration level. The percentages of recoveries for impurities were calculated.

2.6.6. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered. To study the effect of flow rate, flow was changed from 0.25 to 0.31 mL/min. The effect of the column temperature was studied at 20° and 30°C, effect of the percent organic strength was studied by varying acetonitrile composition, −10% and +10% with constant ratio of Buffer.

2.6.7. Solution Stability and Mobile Phase Stability

The solution stability of related substance method was carried out by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for two days. Mobile phase stability was carried out by injecting the freshly prepared sample solutions for two days.

3. RESULTS AND DISCUSSION

3.1. Method Development and Optimization

The objective of the Method development was to develop simple, Robust, Rugged stability indicating UPLC method with shortest possible run time. Related substances methods published in the literature have about 60-70 minutes run time for each injection by using HPLC systems. To reduce the run time attempts were made to develop highly sensitive method by using UPLC. Mobile phase composition and Gradient

programs optimized to achieve very good separation between impurities with lowest possible run time. Method is capable to quantify polar Degradation product, Diol impurity and non polar USP related compound-D within 28 minutes of run time.

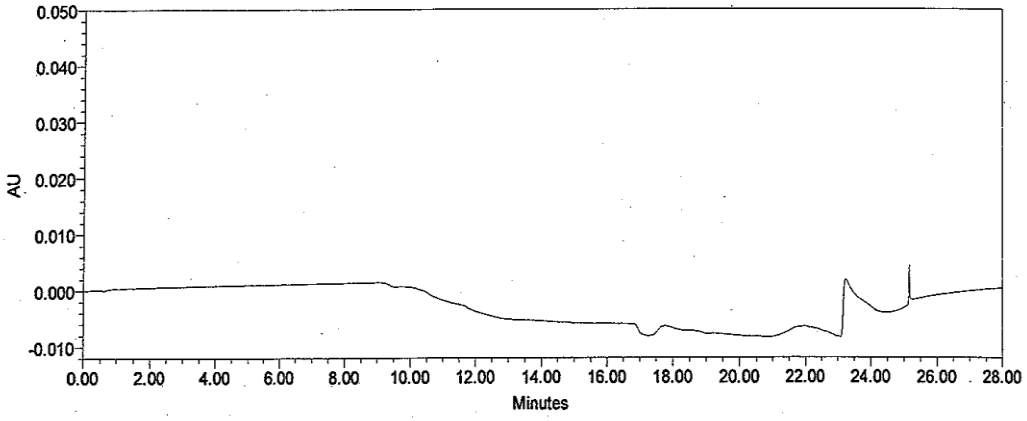

Fig.7: Blank chromatogram

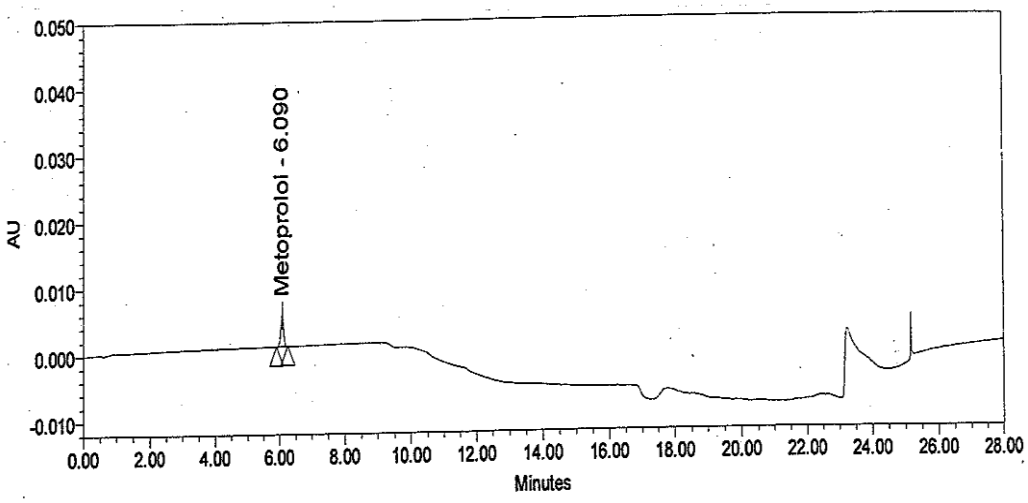

Fig.8: Standard chromatogram

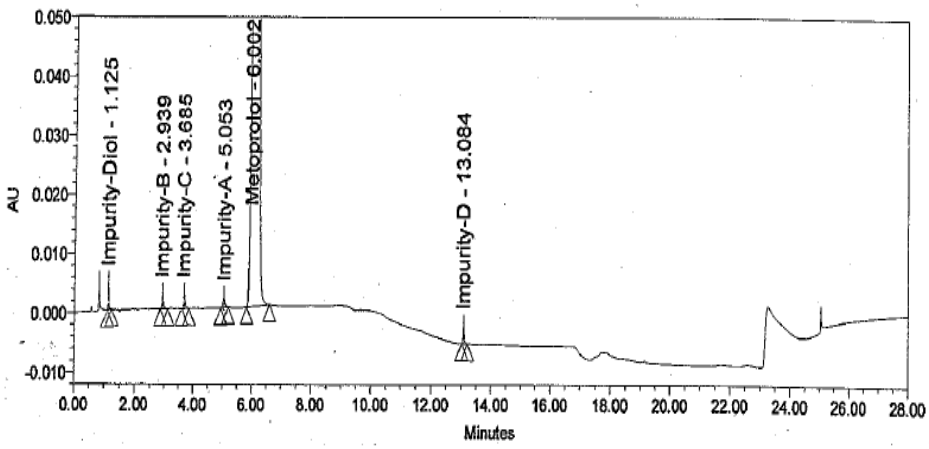

Fig.9: Test chromatogram spiked with Impurities

3.2. Validation of the Method

3.2.1. Specificity

All forced degradation samples were analyzed as mentioned in Section 2.6.1 using PDA detector to ensure the homogeneity and purity of Metoprolol. No purity flag was observed in any of the degradation conditions, which indicates the stability indicating nature of the method. % Degradation was summarized in **Table.1**.

Table 1: Forced degradation data.

Stress Condition	Drug Product		
	Peak Purity		
	Purity Angle	Purity Threshold	Purity flag
Stressed with 2N HCl solution for about 15 Hours at 60°C.	0.051	0.258	NO
Stressed with 2N NaOH solution for about 6 Hours at 60°C.	0.040	0.251	NO
Stressed with 6% Hydrogen peroxide (H_2O_2) for about 1 Hour at 60°C.	0.121	0.274	NO
Stressed with purified water for about 15 hours at 60ºC.	0.043	0.256	NO
Exposed to visible light for about 1.2 million Lux.	0.036	0.309	NO
Exposed to UV light both for about 200 watt hours/square meter.	0.037	0.309	NO
Dry heating done at 105°C for about 6 hrs.	0.067	0.258	NO
Exposed to humidity at 25°C, 90% RH for about 7 days	0.036	0.308	NO

3.2 2. Precision

The % R.S.D. for the of Metoprolol Related compounds was less than 2% conforming good precision of the method.

Table 2: Method precision data

Sample No.	Metoprolol Impurities									
	Diol Impurity		Related Compound–A		Related Compound–B		Related Compound–D		Related Compound–D	
	RRT	% Impurity	RRT	% Impurity	RRT	% Impurity	RRT	% Impurity	RRT	% Impurity
1	0.18	0.097	0.84	0.101	0.47	0.094	0.60	0.099	2.12	0.093
2	0.18	0.097	0.84	0.100	0.47	0.095	0.60	0.097	2.12	0.093
3	0.18	0.097	0.84	0.100	0.47	0.095	0.60	0.097	2.12	0.093
4	0.18	0.097	0.84	0.100	0.47	0.096	0.60	0.098	2.12	0.094
5	0.18	0.096	0.84	0.100	0.47	0.094	0.60	0.097	2.12	0.093
6	0.18	0.097	0.84	0.101	0.47	0.095	0.60	0.097	2.12	0.093
Average	NA	0.097	NA	0.100	NA	0.095	NA	0.098	NA	0.093
% RSD	NA	0.4	NA	0.5	NA	0.8	NA	0.9	NA	0.4

RRT; Relative retention time, NA: Not applicable

3.2.3. Limits of Detection and Quantification

Table 3: Limit of Detection and Limit of quantification

Name	% impurity	
	Limit of Detection	Limit of Quantification
Metoprolol	0.012	0.039
Diol Impurity	0.004	0.012
Impurity – A	0.010	0.040
Impurity – B	0.009	0.030
Impurity – C	0.007	0.024
Impurity – D	0.006	0.023

3.2.4. Linearity

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e. LOQ to 300 %. The correlation coefficient obtained was about 0.999 and the bias is less than 3%.

Table 4: Linearity

Name	Coefficient of correlation (r)	Intercept (C)	Slope (m)	% Bias
Metoprolol	0.999	-54.021	6448.857	0
Diol Impurity	0.999	233.184	9171.787	2
Related Compound – A	0.999	19.726	8228.573	0
Related Compound – B	0.999	-6.299	7852.968	0
Related Compound - C	0.999	48.238	9355.657	0
Related Compound – D	0.999	30.341	8319.888	0

3.2.5. Accuracy

The percentage recoveries of impurities are well within the limits of 85% to 115% at LOQ, and at 300% level. % Recovery values for impurities are presented in table.

Table 5: Accuracy

Name of the Impurity	Spike level	Mean % Recovery
Diol Impurity	At LOQ	94.9
	At 300%	92.0
Related Compound – A	At LOQ	97.7
	At 300%	95.5
Related Compound – B	At LOQ	91.1
	At 300%	90.8
Related Compound – C	At LOQ	96.8
	At 300%	95.1
Related Compound – D	At LOQ	91.2
	At 300%	91.3

3.2.6. Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), the relative retention times are same as that of regular method conditions. This Data illustrates the robustness of the method.

Table 6: Flow rate variation

Metoprolol Succinate Related Substances			
Impurity Name	**Relative Retention time**		
	0.25 mL/min	0.28 mL/min	0.31 mL/min
Related Compound – A	0.84	0.84	0.84
Related Compound – B	0.49	0.49	0.48
Related Compound – C	0.62	0.61	0.61
Related Compound – D	2.02	2.18	2.33
Diol Impurity	0.19	0.19	0.19

Table 7: Temperature variation

Metoprolol Succinate Related Substances			
Impurity Name	**Relative Retention time**		
	20°C	25°C	30°C
Related Compound – A	0.84	0.84	0.84
Related Compound – B	0.48	0.48	0.48
Related Compound – C	0.61	0.61	0.61
Related Compound – D	2.10	2.14	2.19
Diol Impurity	0.18	0.19	0.19

Table 8: Organic composition variation

Metoprolol Succinate Related Substances			
Impurity Name	**Relative Retention time**		
	90%	100%	110%
Related Compound – A	0.84	0.84	0.84
Related Compound – B	0.48	0.48	0.48
Related Compound – C	0.61	0.61	0.61
Related Compound – D	2.17	2.14	2.13
Diol Impurity	0.19	0.19	0.19

3.2.7. Solution Stability and Mobile Phase stability

No significant changes were observed in the content of impurities during solution stability and mobile phase stability experiments. Sample solutions and mobile phases used during the related substance determination were stable for 2 days.

4. CONCLUSIONS

The gradient UPLC method developed for the quantification of Metoprolol impurities and degradation products is precise, accurate, linear,

robust, rugged and specific. Satisfactory results were obtained from validation of the method. The method is stability-indicating and can be used for routine analysis of production samples and Stability samples.

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