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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS AND RAPID DETERMINATION OF ASSAY AND RELATED SUBSTANCES OF AMLODIPINE AND METOPROLOL IN PHARMACEUTICAL DOSAGE FORM

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Abstract : An objective of this research is to develop a novel and common reverse phase liquid chromatographic method for the simultaneous determination of Assay and impurities in Amlodipine and Metoprolol from Pharmaceutical dosage form. The chromatographic separation of Amlodipine, Metoprolol and their impurity peaks was achieved using YMC-Triart PFP, C18 Column (250 mm x 4.6 mm, 5 µm particle size). Mobile phase composed of Buffer solution 2 and acetonitrile (60: 40 v/v) was selected and a flow rate of 1.2 ml/minute is monitored with injection volume of 5 µl. Column oven temperature and autosampler temperature was maintained at 25ºC. Detection was carried out at 235 nm. The method was validated as per International Council on Harmonization (ICH) guidelines. The method was found to be linear and accurate as correlation coefficient and % recovery was within the acceptance criteria. System suitability criteria were also fulfilled. This method is specifically developed for rapid and simultaneous estimation of Amlodipine, Metoprolol as well as their impurities. All the impurities and drug substances elute before 25 minutes hence run time was set as 30 minutes for Assay and 40 minutes for Related Substances test. As all the impurities of Amlodipine elute within 25 minutes, developed reverse phase high-performance liquid chromatography (RP-HPLC) method is simple, fast and economical. The projected method can be utilized for routine analysis in the quality control department from pharmaceutical industry.

Keywords - Amlodipine, Metoprolol, RP-HPLC, Simultaneous estimation, Assay, Related Substances.

INTRODUCTION

Amlodipine besylate, chemically, (RS)-3-ethyl-5-methy-l-2-(2-amino ethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5- pyridinedicarboxylate benzene sulfonate, is a long acting calcium channel blocker, which is used as an antihypertensive agent [1]. Amlodipine is used to treat high blood pressure (hypertension) or chest pain (angina) and other conditions caused by coronary artery disease [2].

Metoprolol succinate is chemically (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol, a selective β_1-receptor blocker, which is also used as an antihypertensive agent [1]. Metoprolol succinate acts as a beta-adrenergic blocking agent, which reduces chest pain and lowers high blood pressure [3].

In the fixed dose combination of amlodipine (calcium channel blocker) and Metoprolol (cardio selective beta blocker); both the drugs have two different mechanisms and reduce blood pressure by acting on peripheral vascular resistance, stroke volume and heart rate. Advantages of this combination therapy are it effectively achieves target blood pressure, lower incidence of individual drug's side-effects, produces synergistic effects, increased patient compliance [4].

Literature reveals different Assay methods like HPTLC [5], synchronous fluorescence spectrofluorometric method [6], LC-MS/MS-method [7,10], UHPLC-MS/MS [7], liquid chromatography-tandem mass spectrometry method [8], HPLC [9], RP-UPLC Method [3] for simultaneous determination of assay of Amlodipine and Metoprolol. Moreover, RP-UPLC method [11] for simultaneous determination of related substances of Amlodipine and Metoprolol has also been reported. But no common method is reported so far for the simultaneous estimation of Assay of Amlodipine and Metoprolol as well as impurities in Amlodipine and Metoprolol. A successful attempt was made for quantitative determination of two drugs along with their impurities by RP-HPLC method. Furthermore, Metoprolol related compound D elutes at retention time of about 55 minutes as per related substances

method from USP monograph of Metoprolol Tablets. However, Metoprolol related compound D elutes at retention time of about 3.5 minutes with new developed RP-HPLC method. Therefore, the current work is worthwhile for development and validation of a novel and rapid RP-HPLC method for simultaneous estimation of Amlodipine and Metoprolol with their impurities in Pharmaceutical dosage form. Furthermore, the cost required for RP-HPLC method is very less as compared to that of RP-UPLC method.

I. MATERIALS AND METHOD

1. Method Development

Various mobile phase compositions and buffer systems were tried for achieving the optimum separation of Amlodipine, Metoprolol and their impurities. Mobile phase composed of buffer solution 2 and acetonitrile (60 : 40 v/v) gave the optimum resolution hence mobile phase composition of buffer solution 2 and acetonitrile (60 : 40 v/v) at the flow rate of 1.2 ml/minute was finalized. Different buffer systems were also tried in order to get suitable peak shapes and resolution. YMC-Triart PFP, C18 Column (250 mm x 4.6 mm, 5 µm particle size) was selected. To analyse both drugs along with their impurities, detection was tried at various wavelengths but 235 nm was selected as the detection wavelength as both the drugs showed maximum absorption. The retention time was found to be 9.21 and 21.07 minutes for Amlodipine and Metoprolol respectively. The optimized chromatographic conditions and system suitability parameters were tabulated in Table 1.

Instrumentation

The high performance liquid chromatography (HPLC) analysis was carried with Dionex Ultimate 3000 HPLC system using UV detector. Chromatographic data was acquired using Chromeleon 7.2 software.

Table 1 Optimized chromatographic conditions and system suitability parameters

Chromatographic Conditions		
Column	YMC-Triart PFP, C18 Column (250 mm x 4.6 mm, 5 µm particle size)	
Flow rate	1.2 ml/minute	
Injection volume	5 µL	
Wavelength	235 nm	
Column oven temperature	25ºC	
Autosampler temperature	25ºC	
Run time	30 minutes for Assay and 40 minutes for Related Substances	
Retention time	21.07 minutes – Amlodipine 9.20 minutes – Metoprolol 16.75 minutes - Amlodipine related compound A 7.91 minutes - Metoprolol related compound A 4.93 minutes - Metoprolol related compound B 6.94 minutes - Metoprolol related compound C 3.57 minutes - Metoprolol related compound D	
System suitability parameters		
	Amlodipine	Metoprolol
Tailing factor (Limit: NMT 2.0)	1.13	1.15
Theoretical plates (Limit: NLT 2000)	12839	11992
% Relative standard deviation (RSD)	0.6	0.6

Preparation of buffer solution 1

7.0 g of Sodium dihydrogen phosphate monohydrate was weighed and dissolved in 1 Litre of water. pH was adjusted to 3.0 ± 0.05 using dilute Orthophosphoric acid solution. This solution was then filtered through 0.45 µ Nylon membrane filter and mixed well.

Preparation of buffer solution 2

1.0 g of 1-decane sulfonic acid was weighed and dissolved in 1 Litre of water. pH was adjusted to 3.0 ± 0.05 using dilute Orthophosphoric acid solution. This solution was then filtered through 0.45 µ Nylon membrane filter and mixed well.

Mobile phase preparation

Buffer solution 2 and acetonitrile were mixed in the ratio of 60:40 v/v. The mobile phase was then mixed well and degassed.

Diluent

Buffer solution 1 and acetonitrile were mixed in the ratio of 50:50 v/v and mixed well.

Preparation of reference solution

Preparation of Reference solution for Assay:

27.7 mg of Amlodipine Besylate Working standard and 190.5 mg Metoprolol Succinate working standard were weighed and transferred into a 50 ml volumetric flask. 30 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent. Then the solution was mixed well and injected. (Concentration: 554.8 ppm of Amlodipine Besylate equivalent to 400 ppm of Amlodipine and 3810 ppm of Metoprolol Succinate equivalent to 4000 ppm of Metoprolol tartrate).

Preparation of Reference solution for unspecified impurities:

2.0 ml of Reference solution for Assay was diluted to 100 ml with diluent. 1.0 ml of above solution was further diluted to 10 ml with diluent. Solution was mixed well and injected. (Concentration: 0.8 ppm of Amlodipine and 8 ppm of Metoprolol tartrate)

Preparation of Reference stock solution for Amlodipine Related Compound A:

2.5 mg of Amlodipine Related Compound A Impurity standard was weighed and transferred into a 50 ml volumetric flask. 30 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent and solution was mixed well. (Concentration: 50 ppm of Amlodipine Related Compound A)

Preparation of Reference stock solution for Metoprolol Related Compound A:

5.0 mg of Metoprolol Related Compound A Impurity standard was weighed and transferred into a 25 ml volumetric flask. 15 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent and solution was mixed well. (Concentration: 50 ppm of Metoprolol Related Compound A)

Preparation of Reference stock solution for Metoprolol Related Compound B:

5.0 mg of Metoprolol Related Compound B Impurity standard was weighed and transferred into a 25 ml volumetric flask. 15 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent and solution was mixed well. (Concentration: 50 ppm of Metoprolol Related Compound B)

Preparation of Reference stock solution for Metoprolol Related Compound C:

5.0 mg of Metoprolol Related Compound C Impurity standard was weighed and transferred into a 25 ml volumetric flask. 15 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent and solution was mixed well. (Concentration: 50 ppm of Metoprolol Related Compound C)

Preparation of Reference stock solution for Metoprolol Related Compound D:

5.0 mg of Metoprolol Related Compound D Impurity standard was weighed and transferred into a 25 ml volumetric flask. 15 ml of diluent was added and the resulting solution was sonicated until dissolved. Volume was made up to the mark with diluent and solution was mixed well. (Concentration: 50 ppm of Metoprolol Related Compound D)

Preparation of Impurity Reference solution for Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D:

1.0 ml of Reference stock solutions for Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D (50 ppm each) were further diluted to 25 ml with diluent. Solution was mixed well and injected. (Conc.: 2 ppm each of Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D).

Preparation of Test solution

20 tablets were weighed and average weight was calculated. These tablets were then crushed to a fine powder by suitable means and transferred the crushed powder equivalent to the 20 mg of Amlodipine / 200 mg of Metoprolol into a 50 ml volumetric flask. 70 ml of diluent was added and the solution was sonicated for 15 minutes with intermittent shaking. Solution was cooled and diluted up to the mark with diluent. Then solution was mixed well and injected. (Conc.: 554.8 ppm of Amlodipine Besylate equivalent to 400 ppm of Amlodipine and 3810 ppm of Metoprolol Succinate equivalent to 4000 ppm of Metoprolol tartrate).

2. Method Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines [12]. The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Specificity

Spectral purities of chromatographic peaks of Amlodipine, Metoprolol & their impurities were evaluated for the interference of the diluent, placebo. Chromatographic peaks should be well separated & no co-elution of impurities with main peak as well as other peaks. Peak purity should not be less than 990 for impurity peaks. For specificity, chromatograms of blank, reference solution, test solution, placebo solution, test solution spiked with known impurities and individual impurities solution ware compared.

Limit of Quantification (LOQ)

It is the lowest amount of the analyte in the sample that can be determined with acceptable precision & accuracy. LOQ solution of Amlodipine, Metoprolol and their impurities were prepared based on S/N ratio ≥ 10:1. Signal to noise ratio & % RSD of the area obtained was calculated.

Limit of Detection (LOD)

It is the lowest amount of the analyte in the sample that can be detected but not necessarily quantitated. LOD solution of Amlodipine, Metoprolol and their impurities were prepared based on S/N ratio ≥ 3:1. Signal to noise ratio was calculated.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. The correlation coefficient, percentage y-intercept, slope of the regression line & residual sum of squares of the areas of each level was calculated.

Linearity for Assay method of Amlodipine and Metoprolol was established by analysing serial dilutions of a stock solution of the working standard. Five concentrations in the rage of 50% to 150% of the target concentration were prepared for Assay method. Refer table 2 for linearity solution preparation.

For specified and unspecified impurities in related substances test, linearity was performed in the rage of LOQ to 120% of the impurity limit concentration level. Refer table 3 for linearity concentration levels preparation for unspecified impurities and table 4 for linearity concentration levels preparation for specified impurities.

Table 2 Linearity concentration levels preparation of Assay of Amlodipine and Metoprolol

% level	Volume of stock solution of Amlodipine (ml) (1600 ppm)	Volume of stock solution of Metoprolol (ml) (16000 ppm)	Diluted to (ml)	Final concentration of Amlodipine in ppm	Final concentration of Metoprolol in ppm
50%	2.5	2.5	20	200.0	2000.0
80%	4.0	4.0	20	320.0	3200.0
100%	5.0	5.0	20	400.0	4000.0
120%	6.0	6.0	20	480.0	4800.0
150%	7.5	7.5	20	600.0	6000.0

Table 3 Linearity concentration levels preparation of Amlodipine and Metoprolol (for unspecified impurities)

% level	Volume of stock solution of Amlodipine (ml) (8 ppm)	Volume of stock solution of Metoprolol (ml) (80 ppm)	Diluted to (ml)	Final concentration of Amlodipine in ppm	Final concentration of Metoprolol in ppm
LOQ 40%	1.0	1.0	25	0.32	3.20
50%	1.25	1.25	25	0.40	4.00
80%	2.0	2.0	25	0.64	6.40
100%	2.5	2.5	25	0.80	8.00
120%	3.0	3.0	25	0.96	9.60

Table 4 Linearity concentration levels preparation of Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D

% level	Volume of stock solution of Amlodipine Related Compound A (ml) (50 ppm)	Volume of stock solution of Metoprolol Related Compound A, B, C, D (ml) (200 ppm)	Diluted to (ml)	Concentration of Amlodipine Related Compound A in ppm	Concentration of Metoprolol Related Compound A, B, C, D in ppm
LOQ 40%	0.4	0.4	25	0.80	3.20
50%	0.5	0.5	25	1.00	4.00
80%	0.8	0.8	25	1.60	6.40
100%	1.0	1.0	25	2.00	8.00
120%	1.2	1.2	25	2.40	9.60

Accuracy

The accuracy of the Assay / Related Substances method was determined by recovery experiments of known concentrations of actives / impurities. It is carried out by spiking known amount of actives / impurities in the placebo.

The accuracy was evaluated by 9 determinations of the recovery rate for Assay & 12 determinations of the recovery rate for Related Substances. Data from triplicate determinations was collected at 3 concentration levels from 50%, 100%, 150% of the target concentration for Assay method and 4 concentration levels from LOQ, 80% 100%, 120% for Related Substances method. The recovery and mean recovery were calculated. Refer Tables 5, 6 and 7 for accuracy solution preparation.

Method Precision (Repeatability)

The repeatability of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. It was evaluated at 100 % w.r.t. test concentration from 6 determinations of the same homogenous samples of finished dosage forms.

For Related Substances method, the sample was spiked with impurities of the impurity limit concentration level.

Standard deviation & relative standard deviation from the results obtained was calculated.

Table 5 Accuracy concentration levels preparation of Assay of Amlodipine and Metoprolol

% level	Weight of placebo (in mg)	Amount of Amlodipine added (in mg)	Amount of Metoprolol added (in mg)	Diluted to (ml)	Final concentration of Amlodipine in ppm	Final concentration of Metoprolol in ppm
50%	1590.0	10.0	100.0	50	200.0	2000.0
100%	1590.0	20.0	200.0	50	400.0	4000.0
150%	1590.0	30.0	300.0	50	600.0	6000.0

Table 6 Accuracy concentration levels preparation of Amlodipine and Metoprolol (for unspecified impurities of Amlodipine and Metoprolol)

% level	Weight of placebo (in mg)	Volume of stock solution of Amlodipine (ml) (8 ppm)	Volume of stock solution of Metoprolol (ml) (80 ppm)	Diluted to (ml)	Final concentration of Amlodipine in ppm	Final concentration of Metoprolol in ppm
LOQ 40%	1590.0	1.0	1.0	50	0.32	3.2
80%	1590.0	4.0	4.0	50	0.64	6.4
100%	1590.0	5.0	5.0	50	0.80	8.0
120%	1590.0	6.0	6.0	50	0.96	9.6

Table 7 Accuracy concentration levels preparation of Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D

% level	Weight of placebo (in mg)	Volume of stock solution of Amlodipine Related Compound A (ml) (50 ppm)	Volume of stock solution of Metoprolol Related Compound A, B, C, D (ml) (200 ppm)	Diluted to (ml)	Final concentration of Amlodipine Related Compound A in ppm	Final concentration of Metoprolol Related Compound A, B, C, D in ppm
LOQ 40%	1590.0	0.8	0.4	50	0.8	3.2
80%	1590.0	1.6	0.8	50	1.6	6.4
100%	1590.0	2.0	1.0	50	2.0	8.0
120%	1590.0	2.4	1.2	50	2.4	9.6

Intermediate Precision

The intermediate precision of the assay & Related Substances method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst on different instrument as well. Standard deviation & relative standard deviation from the results obtained was calculated. The result of Intermediate Precision is compared with results of the first day analysis.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The influence of slightly changed parameters of the chromatographic conditions was tested to demonstrate sufficient robustness of the method. In detail, effects of the change in column oven temperature & flow rate were studied.

Robustness test was carried out by analysing reference solution, test solution as such (for Assay) and spiked test solution (For Related Substances) for % content to access the impact of each changed parameter.

II. RESULTS AND DISCUSSION

The proposed method for simultaneous determination of Assay and Related Substances in Amlodipine and Metoprolol was developed and validated for the parameters specificity, repeatability, intermediate precision, linearity, accuracy & robustness as per the ICH guidelines.

Specificity

By comparing the chromatograms of blank, reference solutions, test solution as such, spiked test solution and placebo solution; it was observed that there was no interference of any peak at the retention time of Amlodipine, Metoprolol and impurities. Chromatographic peaks were well separated & no co-elution of impurities with main peak as well as other peaks. Refer fig. 1 to 6 for the representative chromatograms of reference solutions and test solutions.

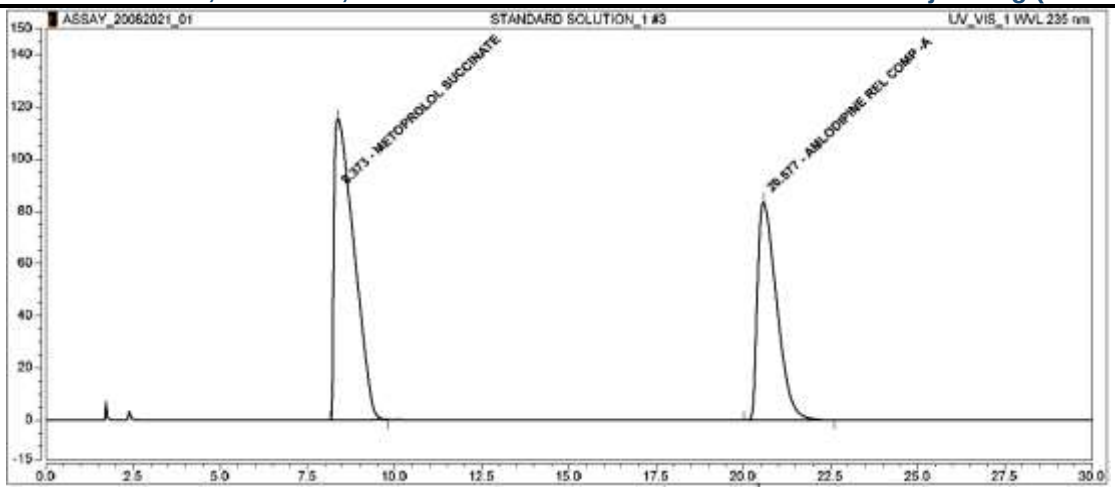

Figure 1 Representative chromatogram of reference solution for Assay

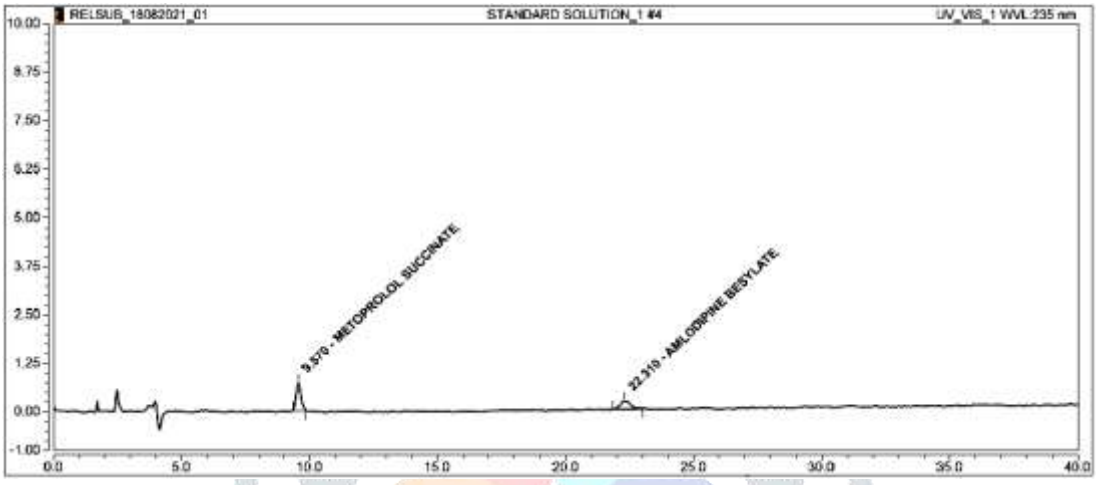

Figure 2 Representative chromatogram of reference solution for Unspecified impurities

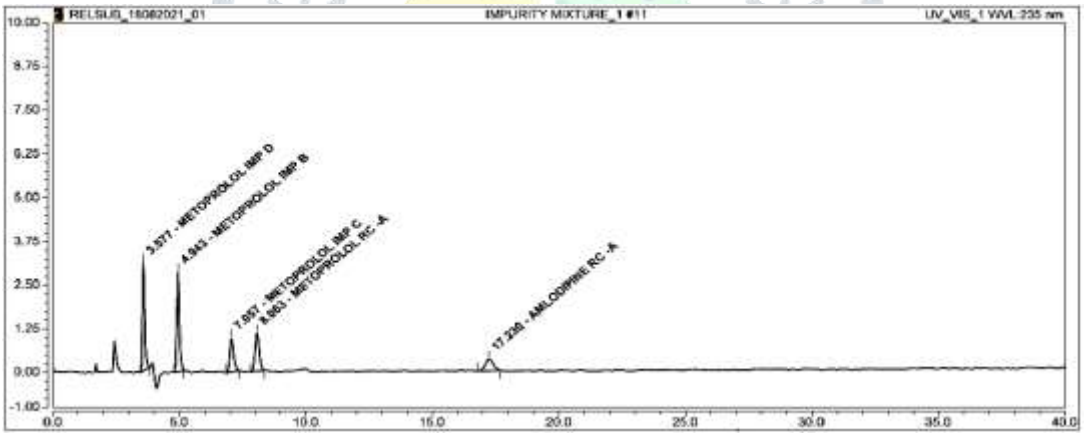

Figure 3 Representative chromatogram of Impurity Reference solution

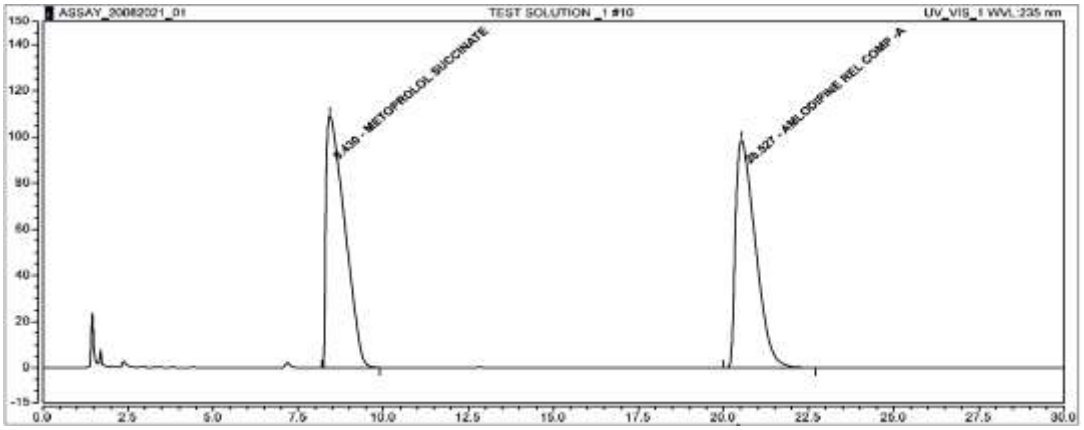

Figure 4 Representative chromatogram of test solution For Assay

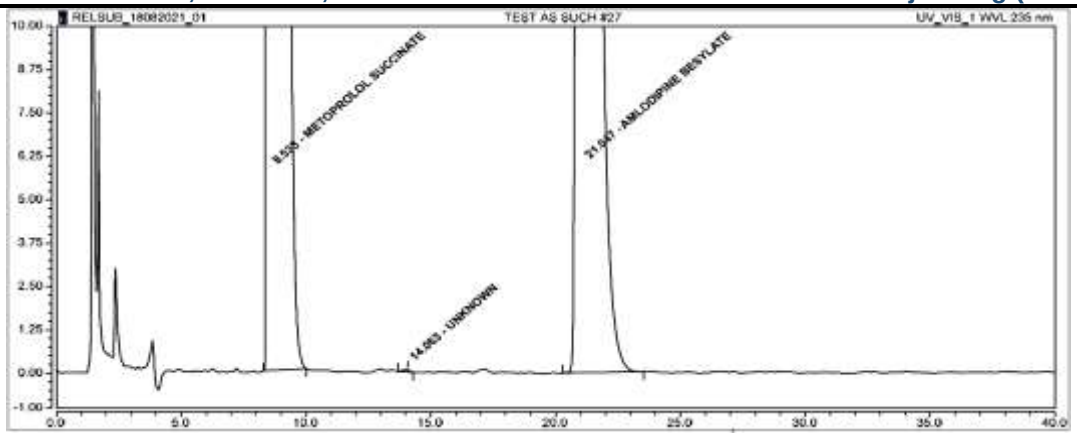

Figure 5 Representative chromatogram of test solution as such for Related Substances

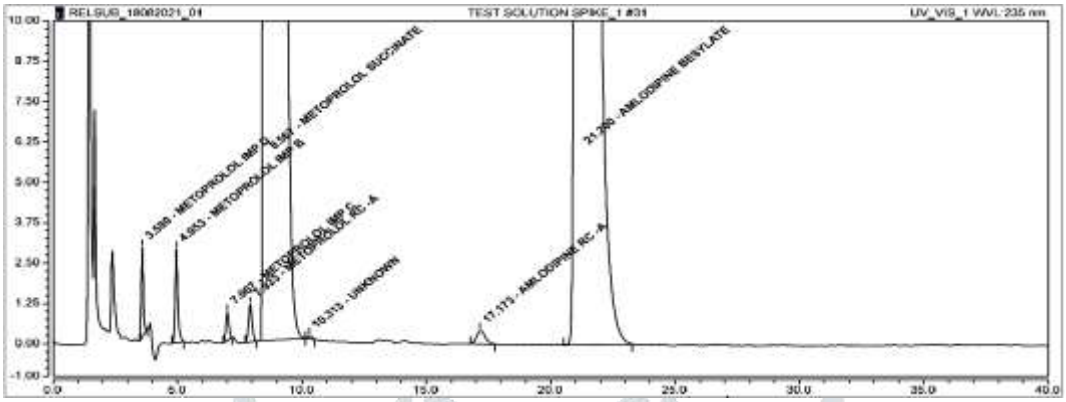

Figure 6 Representative chromatogram of test solution spiked with impurities for Related Substances for Related Substances

Limit of Quantification (LOQ)

LOQ concentration obtained for Metoprolol Related Compound A, B, C, D and unspecified impurities is 0.08% w.r.t. (with respect to) test concentration. LOQ concentration obtained for Amlodipine Related compound A is 0.2% w.r.t. test concentration. Amlodipine, Amlodipine Related Compound A, Metoprolol and Metoprolol Related Compound A, B, C, D peaks from LOQ chromatograms were clearly visible with signal to noise ratio more than 10:1 and % RSD less than 10.0 %.

Limit of Detection (LOD)

LOQ concentration obtained for Metoprolol Related Compound A, B, C, D and unspecified impurities is 0.026% w.r.t. test concentration. LOQ concentration obtained for Amlodipine Related compound A is 0.066% w.r.t. test concentration. Amlodipine, Amlodipine Related Compound A, Metoprolol and Metoprolol Related Compound A, B, C, D peaks from LOQ chromatograms were clearly visible with signal to noise ratio more than 3:1.

Linearity & Range

Linearity study for Assay method was carried out by using five concentrations ranging from 50% to 150% for Amlodipine and Metoprolol. Furthermore linearity study for specified and unspecified impurities in Related Substances method was carried out by using five concentrations ranging from LOQ to 120%.

A graphs of concentration taken in µg/mL (x-value) verses response (area observed) (y-axis) are shown in Figures 7 to 8 for Assay test and 11 to 17 for Related Substances test. Plots of Residuals against concentration for Amlodipine and Metoprolol are shown in Figures B. 9 and B. 10 respectively. Refer tables 8, 9 and 10 for the correlation coefficient, percentage y-intercept, slope of the regression line & residual sum of squares results.

The Assay method is found to be linear in the range of 201.375 µg/ml (50%) to 604.125 µg/ml (150%) of the target concentration for Amlodipine and 1997.095 µg/ml (50%) to 5991.286 µg/ml (150%) of the target concentration for Metoprolol.

Related substances method is found to be linear in the range of LOQ – 0.3219 µg/ml (40%) to 0.9658 µg/ml (120%) for unspecified impurities of Amlodipine, LOQ – 3.1995 µg/ml (40%) to 9.5985 µg/ml (120%) for unspecified impurities of Metoprolol, LOQ – 0.8358 µg/ml (40%) to 2.5075 µg/ml (120%) for Amlodipine Related Compound A, LOQ – 3.2845 µg/ml (40%) to 9.8534 µg/ml (120%) for Metoprolol Related Compound A, LOQ – 3.5373 µg/ml (40%) to 10.6118 µg/ml (120%) for Metoprolol Related Compound B, LOQ – 3.3299 µg/ml (40%) to 9.9898 µg/ml (120%) for Metoprolol Related Compound C and LOQ – 3.4496 µg/ml (40%) to 10.3488 µg/ml (120%) for Metoprolol Related Compound D.

Table 8 Observation for linearity of Amlodipine and Metoprolol (For Assay)

Parameter	Values		Acceptance Criteria
	Amlodipine	Metoprolol	
Correlation coefficient R	1.000	0.999	> 0.999
%Y – axis intercept	2.53	2.71	≤ ± 3 %
Slope of regression line	7.9	1.0	To be reported
Residual sum of squares	6783.9	10420.1	To be reported

Table 9 Observation for linearity of Amlodipine and Metoprolol (for unspecified impurities of Amlodipine and Metoprolol)

Parameter	Values		Acceptance Criteria
	Amlodipine	Metoprolol	
Correlation coefficient R	1.000	0.998	> 0.999
%Y – axis intercept	- 0.002	4.04	≤ ± 3 %
Slope of regression line	16.42	1.14	To be reported
Residual sum of squares	0.035	0.25	To be reported

Table 10 Observation for linearity of Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D

Parameter	Values					Acceptance Criteria
	Amlodipine Related Compound A	Metoprolol Related Compound A	Metoprolol Related Compound B	Metoprolol Related Compound C	Metoprolol Related Compound D	
Correlation coefficient R	0.990	0.995	0.997	0.995	0.997	> 0.999
%Y – axis intercept	0.80	4.41	5.46	- 2.96	- 0.01	≤ ± 3 %
Slope of regression line	4.16	1.53	2.29	1.07	1.98	To be reported
Residual sum of squares	1.07	1.07	0.04	0.56	0.56	To be reported

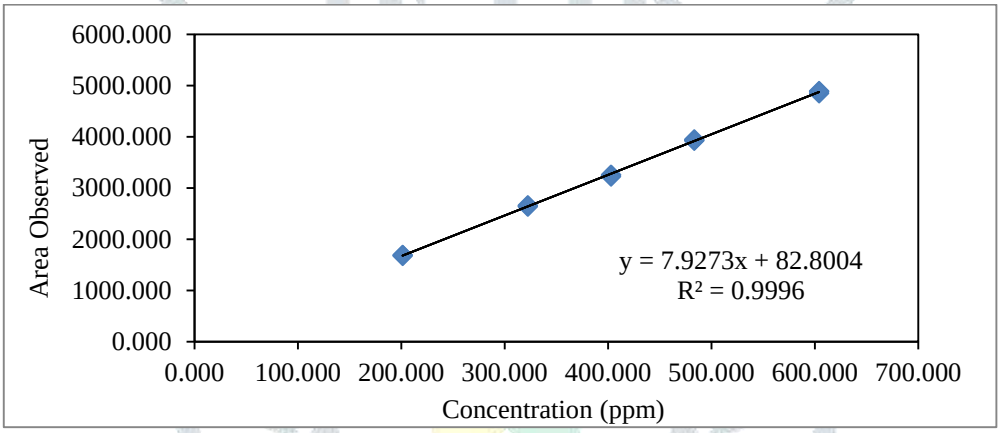

Figure 7 Linearity plot of Amlodipine (For assay)

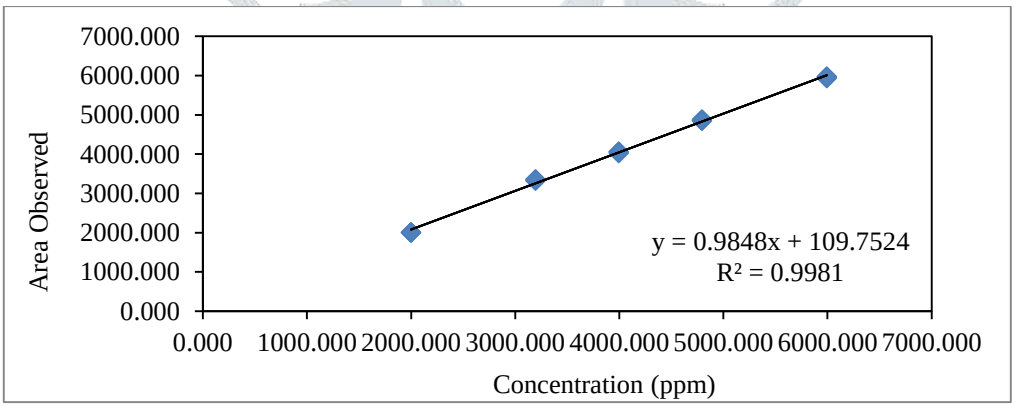

Figure 8 Linearity plot of Metoprolol (For assay)

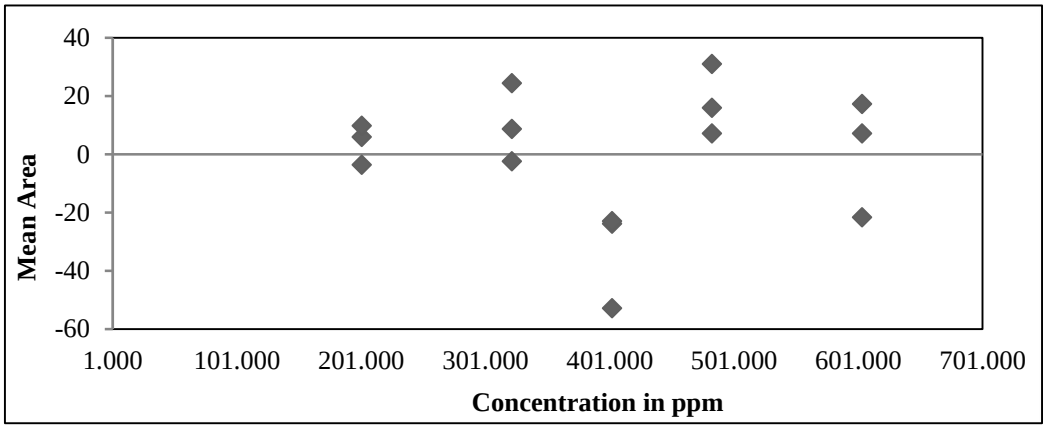

Figure 9 Plot of Residuals against concentration for Amlodipine (For assay)

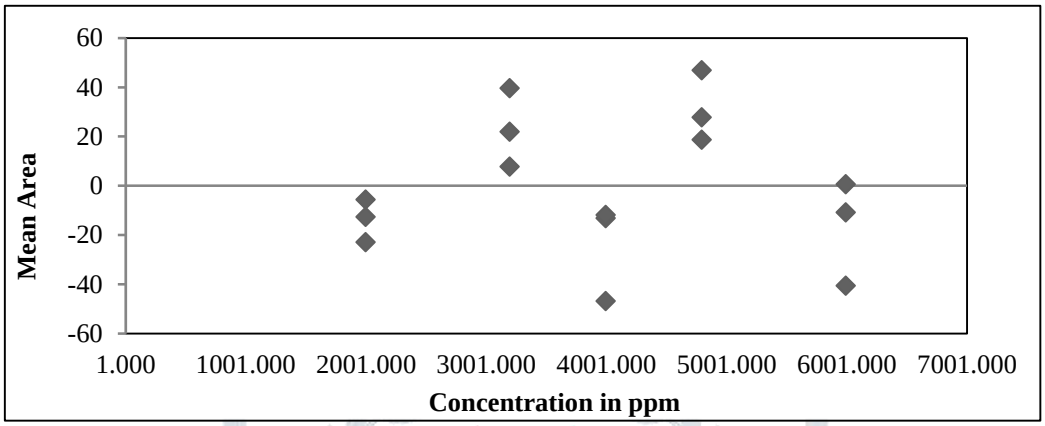

Figure 10 Plot of Residuals against concentration for Metoprolol (For assay)

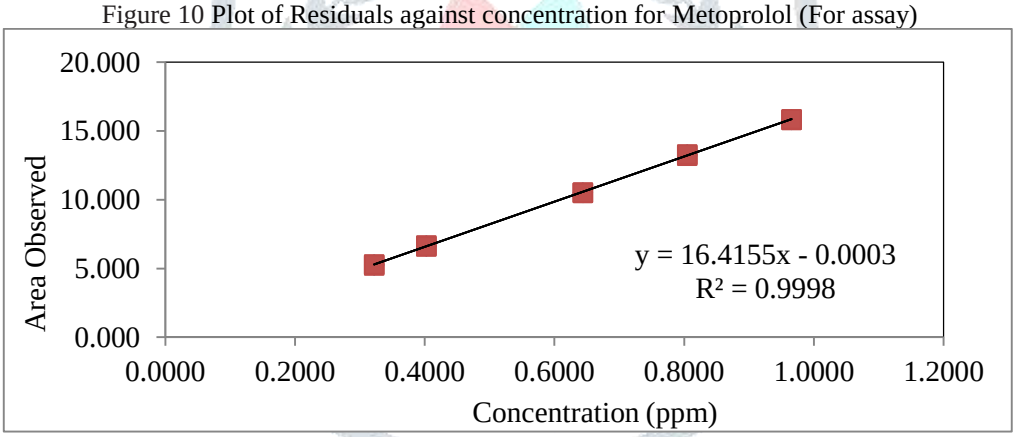

Figure 11 Linearity plot of Amlodipine (For Unspecified impurities in Related Substances)

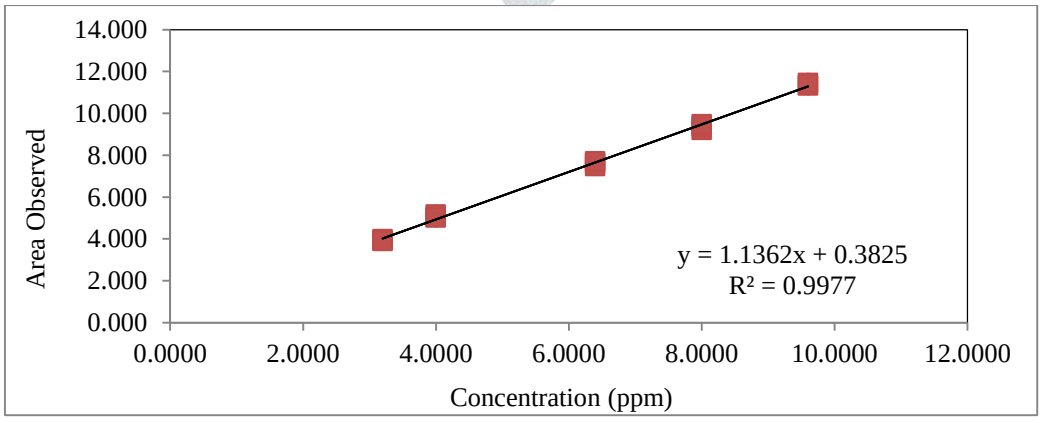

Figure 12 Linearity plot of Metoprolol (For Unspecified impurities in Related Substances)

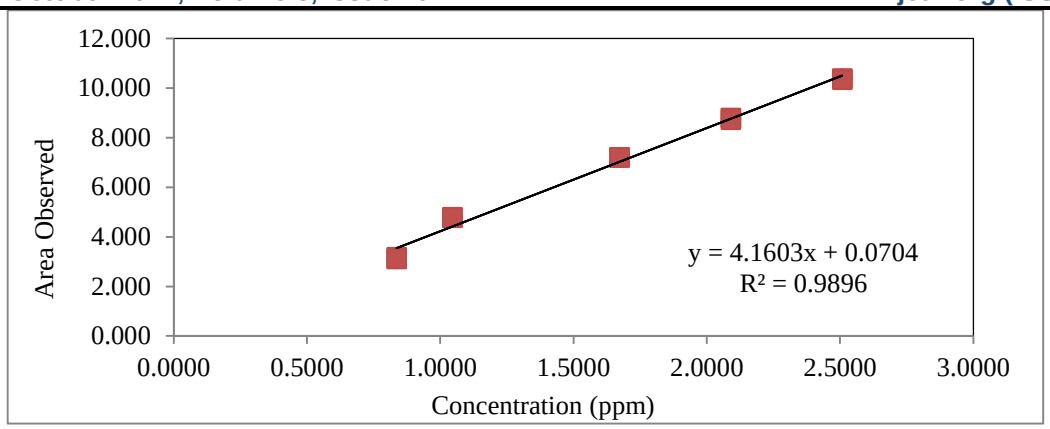

Figure 13 Linearity plot of Amlodipine Related Compound A (Related Substances)

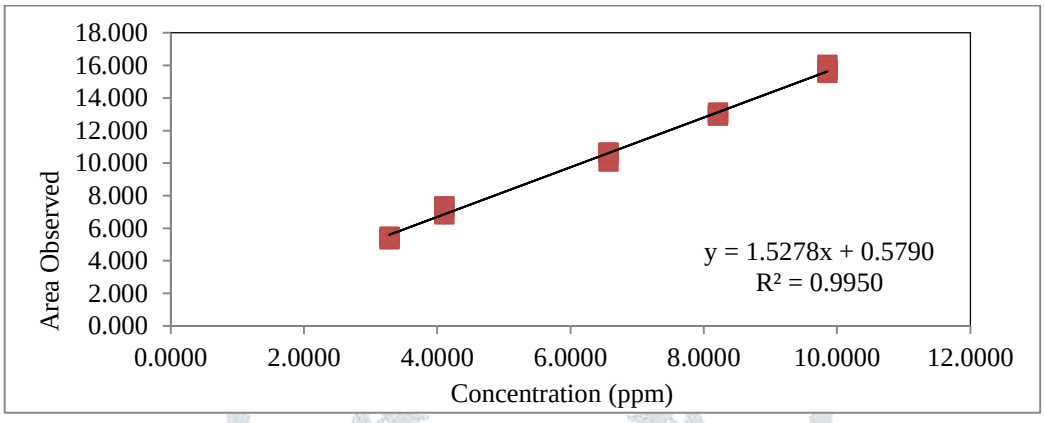

Figure 14 Linearity plot of Metoprolol Related Compound A (Related Substances)

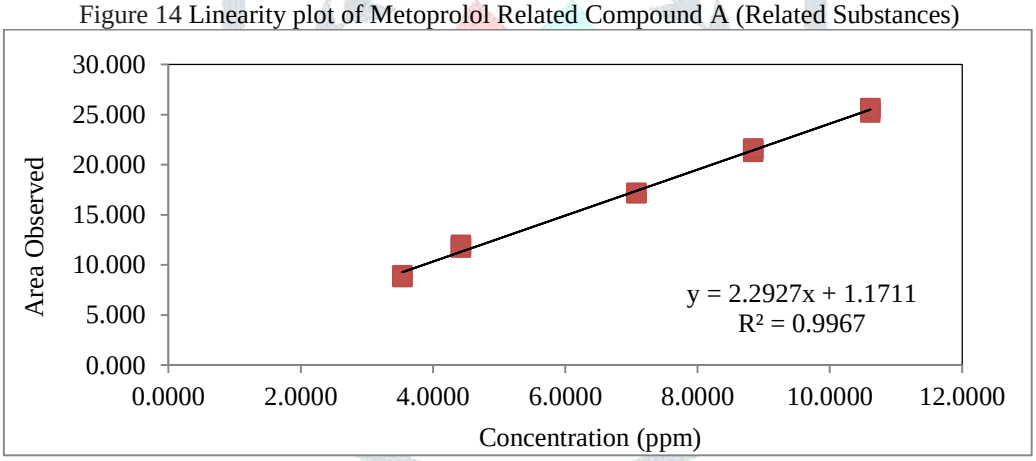

Figure 15 Linearity plot of Metoprolol Related Compound B (Related Substances)

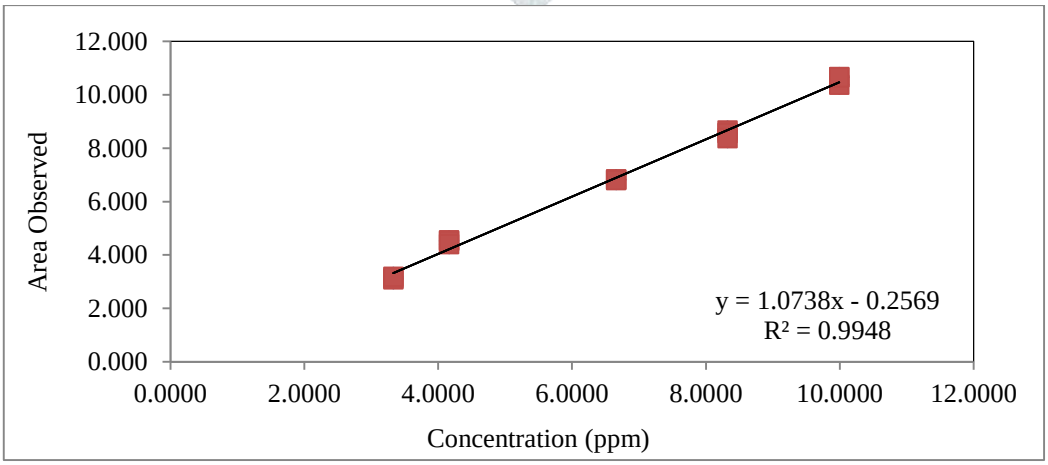

Figure 16 Linearity plot of Metoprolol Related Compound C (Related Substances)

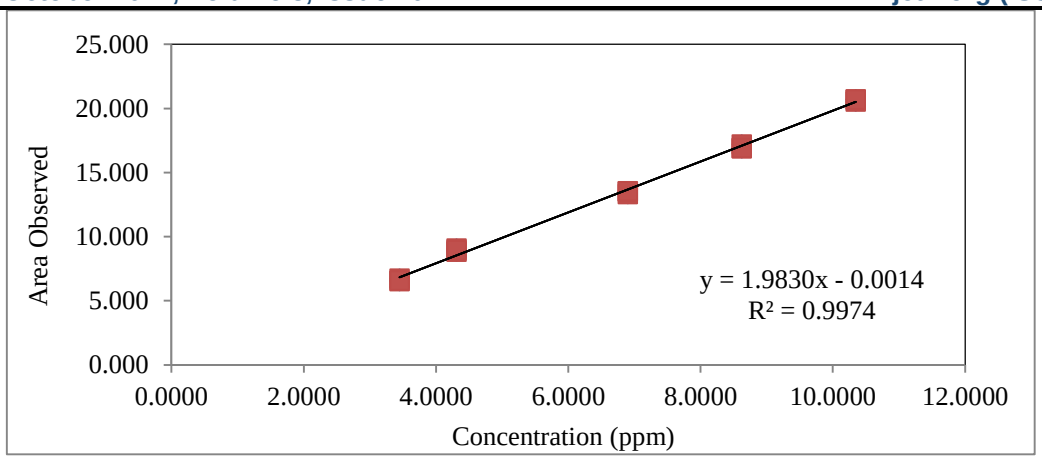

Figure 17 Linearity plot of Metoprolol Related Compound D (Related Substances)

Accuracy

The accuracy of Assay and Related Substances method was confirmed by evaluating the recoveries of Amlodipine, Metoprolol and impurities (Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D). The mean recovery values and individual recovery values were calculated for each drug substances and impurities.

Both the methods were found to be accurate because the % individual recovery and % mean recovery were within the acceptance criteria as given in the tables 11, 12 and 13. It showed that method is free from interference of excipients present in formulation.

Table 11 Recoveries of Amlodipine and Metoprolol (For Assay)

Level	% recovery of Amlodipine	% recovery of Metoprolol	Acceptance Criteria
50%	101	101	Individual Recovery = 97 % - 103 %
	101	102	
	101	101	
100%	102	99	
	101	100	
	101	100	
120%	102	99	
	102	100	
	101	99	
Mean	**101**	**100**	Mean Recovery 98 % – 102 %

Table 12 Recoveries of Amlodipine and Metoprolol (For Unspecified Impurities in Related Substances)

Level	% recovery of Amlodipine	% recovery of Metoprolol	Acceptance Criteria
LOQ	93	95	Individual Recovery = 80 % - 120 %
40%	91	94	
	94	95	
50%	91	101	
	91	101	
	91	100	
100%	97	97	
	97	99	
	95	98	
120%	97	100	
	97	101	
	96	100	
Mean	**94**	**98**	Mean Recovery 80 % - 120 %

Table 13 Recoveries of Amlodipine Related Compound A and Metoprolol Related Compound A, B, C, D (For Related Substances)

Level	% recovery of Amlodipine Related Compound A	% recovery of Metoprolol Related Compound A	% recovery of Metoprolol Related Compound B	% recovery of Metoprolol Related Compound C	% recovery of Metoprolol Related Compound D	Acceptance Criteria
LOQ	101	89	104	102	92	Individual Recovery 80% - 120%
40%	99	89	104	103	93	
	99	87	103	101	91	
	98	91	100	102	96	
50%	100	91	100	104	96	
	101	91	99	105	96	
	100	90	95	100	100	
100%	100	90	98	99	100	
	99	93	97	100	100	
	100	94	94	101	95	
120%	99	95	96	99	95	
	100	94	97	100	95	
Mean	**100**	**91**	**99**	**102**	**96**	Mean Recovery 80% - 120%

Method Precision (Repeatability)

The exactness of the method was defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations is well within the acceptance limit. Refer tables 14 and 15.

Intermediate Precision

The intermediate precision of the assay method is established by comparison of two independent repeatability experiments on 2 different days. Refer tables 16 and 17 for assay and Related Substances of Amlodipine and Metoprolol during intermediate precision and tables 18 and 19 for difference between two independent repeatability experiments on 2 different days.

Table 14 Repeatability of Amlodipine and Metoprolol (For Assay)

Sample No.	% Assay of Amlodipine	% Assay of Metoprolol
Sample 01	100.07	99.45
Sample 02	99.08	98.48
Sample 03	100.68	100.03
Sample 04	99.54	98.87
Sample 05	100.10	99.42
Sample 06	100.68	99.98
Mean	100.03	99.37
Standard Deviation **(STD Dev.)**	0.63	0.61
% RSD	**0.6**	**0.6**

Table 15 Repeatability of Amlodipine related compound A, Metoprolol related compound A, Metoprolol related compound B, Metoprolol related compound C, Metoprolol related compound D and unspecified impurities

Sample No.	% Recovery of Amlodipine related compound A	% Recovery of Metoprolol related compound A	% Recovery of Metoprolol related compound B	% Recovery of Metoprolol related compound C	% Recovery of Metoprolol related compound D	% Unspecified Impurity
01	99.20	93.50	105.32	87.44	86.20	BQL
02	104.92	93.50	105.32	87.44	90.99	BQL
03	106.83	98.42	105.32	87.44	90.99	BQL
04	108.74	98.42	105.32	87.44	86.20	BQL
05	108.74	98.42	105.32	92.30	90.99	BQL
06	103.01	98.42	100.74	87.44	90.99	BQL
Mean	105.24	96.78	104.56	88.25	89.40	BQL
STD Dev	3.702	2.541	1.869	1.983	2.473	NA

% RSD	3.52	2.63	1.79	2.25	2.77	**NA**

BQL: Below Quantification Limit

Table 16 Intermediate precision of Amlodipine and Metoprolol (For Assay)

Sample No.	**% Assay of Amlodipine**	**% Assay of Metoprolol**
Sample 01	99.64	99.90
Sample 02	99.33	99.66
Sample 03	99.52	99.89
Sample 04	99.26	99.53
Sample 05	99.52	99.84
Sample 06	99.17	99.62
Mean	99.41	99.74
STD Dev.	0.18	0.16
% RSD	**0.2**	**0.2**

Table 17 Intermediate precision of Amlodipine related compound A, Metoprolol related compound A, Metoprolol related compound B, Metoprolol related compound C, Metoprolol related compound D and unspecified impurities

Sample No.	**% Recovery of Amlodipine related compound A**	**% Recovery of Metoprolol related compound A**	**% Recovery of Metoprolol related compound B**	**% Recovery of Metoprolol related compound C**	**% Recovery of Metoprolol related compound D**	**% Unspecified Impurity**
01	101.11	93.50	100.74	87.44	90.99	BQL
02	103.01	93.50	96.16	92.30	90.99	BQL
03	104.92	93.50	96.16	87.44	90.99	BQL
04	108.74	93.50	105.32	87.44	95.78	BQL
05	108.74	93.50	100.74	82.58	90.99	BQL
06	104.92	93.50	100.74	87.44	90.99	BQL
Mean	105.24	93.50	99.98	87.44	91.79	BQL
STD Dev	3.056	0.000	3.447	3.072	1.955	NA
% RSD	2.90	0.00	3.45	3.51	2.13	**NA**

BQL: Below limit of quantification, NA: Not Applicable.

Table 18 Difference between two independent repeatability experiments for Assay of Amlodipine and Metoprolol

Parameter	**Amlodipine**		**Metoprolol**	
	1st day Repeatability	**2nd day Repeatability**	**1st day Repeatability**	**2nd day Repeatability**
Number of determinations	6	6	6	6
Mean (%) Assay	100.03	99.41	99.37	99.74
RSD (%)	0.6	0.2	0.6	0.2
Mean value difference (%)				
Acceptance Criteria: < 2.0 % absolute	**0.62**		**0.37**	

Table 19 Difference between two independent repeatability experiments for Related Substances

Name of Impurity	**1st day Repeatability**	**2nd day Repeatability**	**Difference (%)**	**Acceptance criteria**
	% Recovery			
Amlodipine Related Compound A	105.24	105.24	**0.00**	$\Delta \leq 10\%$ Absolute
Metoprolol Related Compound A	96.78	93.50	**3.28**	
Metoprolol Related Compound B	104.56	99.98	**4.58**	
Metoprolol Related Compound C	88.25	87.44	**0.81**	
Metoprolol Related Compound D	89.40	91.79	**2.39**	

% Impurities				
Unspecified impurity	BQL	BQL	**NA**	$\Delta \leq 0.05\%$

Robustness

Method was found to be robust as system suitability criteria is achieved for all the robustness parameters tested and the values obtained from % Assay, % recoveries of impurities, % Impurities are comparable to the values of method precision. Deliberate change in parameter did not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is robust. The results were tabulated below tables 20.

Table 20 Robustness observations

Name of Impurity	Method precision	Change in column oven temperature		Change in flow rate (in ml/minute)	
		20ºC	30 ºC	1.1	1.3
% Assay					
Amlodipine	100.03	100.03	99.41	101.14	99.96
Metoprolol	99.37	100.71	100.56	101.50	101.58
% Recovery of Impurities					
Amlodipine Related Compound A	105.24	105.78	104.42	104.42	104.69
Metoprolol Related Compound A	96.78	97.48	96.08	97.48	97.48
Metoprolol Related Compound B	104.56	105.21	105.86	105.21	105.86
Metoprolol Related Compound C	88.25	88.25	88.95	89.64	88.25
Metoprolol Related Compound D	89.40	90.08	89.40	90.76	89.40
% Impurities					
Unspecified impurity	BQL	BQL	BQL	BQL	BQL

III. CONCLUSIONS

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the simultaneous estimation of amlodipine and Metoprolol with their impurities in Pharmaceutical dosage form in accordance with the ICH guidelines. This developed method was novel and rapid giving good resolution among amlodipine and Metoprolol with their impurities. Thus, this method can be useful for the routine analysis of amlodipine and Metoprolol with their impurities in pharmaceutical tablet dosage form.

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