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Review Paper

A Comprehensive Review on RP-HPLC Method Development and Validation for Simultaneous Estimation of Metoprolol Succinate and Azelnidipine

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ABSTRACT

The Present study focuses on the systematical method development and validation of quantitative determination by using analytical methods for Metoprolol Succinate and Azelnidipine. The study is simple, sensitive and highly accurate High-Performance Liquid Chromatography method has been studies as well as validated for determination of Metoprolol succinate and Azelnidipine in bulk and pharmaceutical dosage form. Both the drugs are anti-hypertensive medications. Metoprolol is cardio-selective β1 adrenergic receptor blockers and Azelnidipine a calcium channel blocker. The present analytical method was developed on Shimadzu HPLC LC-2010. The HPLC chromatography was performed using a technique like Reversed Phase C18 column with mobile phase based are dependent on the polarity of the molecules. The overall study outlines the RP-HPLC determination of metoprolol succinate and azelnidipine. The calibration curves demonstrate the excellent result of linearity about the selected concentration ranges, with correlation coefficient values. Accuracy studies showed satisfactory percentage recovery within acceptable limits as well as precision study involving low Relative-Standard Deviation values, that confirm the quantification of the method. RP-High Performance Liquid Chromatography method is successfully applied for the quantitative estimation of Metoprolol Succinate and Azelnidipine in pharmaceutical dosage forms

INTRODUCTION

Metoprolol succinate belongs to the class of selective β_1-adrenergic receptor blocker antagonist that is commonly prescribed for treatment of

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hypertension, angina pectoris, heart failure, and other cardiovascular disorders and Azelnidipine is Calcium Channel Blocker as well use in the treatment of hypertension. Due to its extensive use in pharmaceutical formulations, accurate and reliable analytical methods are essential to ensure drug quality, safety, and efficacy. The development and rigorous validation of analytical procedures are essential for the accurate quantitative determination of Metoprolol succinate and Azelnidipine in bulk drugs substance and finished dosage forms. Validated methods, following ICH guidelines, ensure precision, accuracy, reproducibility, and regulatory compliance in routine quality control analysis.

Chromatographic techniques are commonly employed due to their sensitivity, simplicity, and cost effectiveness. Therefore, developing and validating robust analytical methods for Metoprolol succinate and Azelnidipine.

1.2 Method Development-

Method validation is carried out to ensure that a laboratory or analytical method is for its required purpose and consistently produces reliable, accurate, and reproducible results. The objective is to provide evidence of documents that the method performs effectively under specified conditions and meets the defined requirements for its required use, whether in research, clinical, or industrial setting.

Components of method validation include:

- Accuracy
- Precision
- Specificities
- Linearity Range
- Limit of the Detections (LOD)
- Limit of Quantification (LOQ)

1.2.1 VALIDATION OF ANALYTICAL METHOD:

Validating a quantitative method is the process by which it is established, by laboratory knowledge gaining, that performance characteristics of the technique meet the requirements for the intended analytical application. Typical analytical performance characteristic for Analytical method validation are listed below

a. Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value.

b. Precision

Precision describes the degree of agreement or the spread among a series of results obtained by repeated measurement of the same homogeneous sample under specified conditions. Precision is evaluated at three levels: repeatability, intermediate precision, and reproducibility.

c. Specificity

It is defined as the ability of a method to measure accurately and unambiguously the analyte of interest in the presence of other components that may be present in the sample matrix, such as impurities, degradation products, or excipients.

d. Intermediate precision

Intermediate precision captures the variability observed within a single laboratory when measurements are made on different days, by different analysts, or using different equipment.

e. Linearity

The linearity is quantitative study of capacity to generate test results that are directly proportional within a defined range to the concentration of substances in the sample.

f. Robustness

Robustness reflects the intrinsic resilience of a method — its ability to remain unaffected by small, deliberate changes in critical method

parameters. A robust method provides confidence in its reliability and suitability for routine usable under normal conditions.

g. Ruggedness

Ruggedness is a calculation of how consistently an analytical method performs when deliberately subjected to variations in operating conditions — for instance, different laboratories, different analysts, different instruments, different reagent batches, different assay timings, or different temperatures.

1.3 CHROMATOGRAPHY

Chromatography encompasses a collection of analytical techniques that are used for separation, identification, and quantitative determination of chemical constituents present in complex mixtures.

In simple terms, chromatography may be defined as follows:

"It is the technique in which drug components of a mixtures are separated based upon the rates at which they are carried or moved through a stationary phase (column) by a gaseous or liquid mobile phase". Chromatography is mainly classified into two categories based on the type of mobile phase:

A. Liquid Chromatography

B. Gas Chromatography

Separation of components occurs due to factors such as differences in migration rates, capillary action, and adsorption.

Table 1. Various types of chromatographic techniques

Techniques	Stationary phase	Mobile phase
Column chromatography	Solid	Liquid
Partition chromatography	Liquid	Liquid
Paper chromatography	Liquid	Liquid
TLC	Liquid or Solid	Liquid
Gas liquid chromatography (GLC)	Liquid	Gas
Gas solid chromatography (GSC)	Solid	Gas
Ion exchange chromatography	Solid	Liquid

1.4 HPLC (High Performance Liquid Chromatography):

High performance liquid chromatography is the most widely used analytical technique utilizes the liquidified mobile phase separation of the components of mixture. These components are first dissolved in a solvent forced to flow through a chromatographic column under high pressure. In the column, the mixture is resolved into its components. From different choices of both solvent and stationary phases the interaction of solute with mobile and the stationary phase can be manipulated. HPLC is used either in the liquid-solid adsorption chromatography mode or the liquid-liquid partition chromatography mode, either normal or reversed phase. Both partition and adsorption chromatography operates on differences in solute polarity since polarity is important in determining both adsorption and solubility. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it could separate a wider variety of chemical mixtures.

Table 2. Scouting Parameters of HPLC

Parameters	Description of Parameters
Column	C8 C18
Mobile Phase	ACN: Buffur ACN :Water Methanol : Water Methanol: Buffur

2. EXPERIMENTAL WORK:

2.1 Materials and Reagents:

Following list of Reagents/Standards/Equipment was required during method validation. Name of standards Drugs metoprolol succinate, azelnidipine. Reagents Potassium dihydrogen orthophosphate, Orthophosphoric acid, Acetonitrile, Water. Instruments/ Equipments HPLC System Shimadzu-LC-2010CHT, Software-LC Solution, pH Meter-Labman, Sonicator-Life Care Instruments Pvt. Ltd., Analytical Balance-Lab Man, HPLC Column: C18, (4.6 mm x 15-cm), 5µm-Agilent.

Chromatographic Conditions: To validate the analytical method for % Assay of Metoprolol Succinate and Azelnidipine tablet and active pharmaceutical ingredient by High Performance Liquid Chromatography. The method was validated for certain Parameters like specificity and, precision, solution stability, accuracy, linearity based on approved protocol with predetermined acceptance criteria.

Mobile Phase Preparation:

Buffer Preparation:0.01M ammonium dihydrogen orthophosphate in 1000ml of water.

Mobile Phase Preparation: Mixture of 40ml Buffer and 60ml of Acetonitrile. With pH of 3.2.

Diluent: Methanol

To validate the analytical method for % Assay of Metoprolol Succinate and Azelnidipine tablet and active pharmaceutical ingredient by High Performance Liquid Chromatography. HPLC method mainly used for content determination assay of metoprolol succinate as well as azelnidipine tablet. The method was validated for certain Parameters like specificity and selectivity, precision, solution stability, accuracy, linearity and Range based on approved protocol with predetermined acceptance criteria.

Table 3. Typical Chromatographic Conditions

Equipment	Shimadzu (LC-2010C_{HT})
Column	HPLC Column: C18, (150 x 4.6mm),5µm
Rate of Flow	1.00 ml/min
Wavelength	275nm
Injection volume	20 µl

2.1.1 Preparation of solution:

1. Standard Solution:

Measure accurately 16mg Azelnidipine API and 25mg Metoprolol succinate API transfer it into a 25ml measuring flask and add 15ml of methanol and sonicate till dissolve and makeup volume with methanol. Take both 5ml of above solution in 50 ml of measuring flask and volume makeup by using methanol.

2. Sample Solution:

Take 10 tablets and measure its average weight (Tablet weight). Crush the tablets into fine powder. Take powder equivalent to average weight into 50ml of volumetric flask add 30 ml of

methanol sonicate for 10 mins. and makeup the volume with methanol. Filter above solution with whatmann filter paper No.41 and collect the filtrate. Take 5ml of above solution into 50 ml volumetric flask and makeup the volume with methanol.

3. System Suitability Parameters (Acceptance Criteria):

The relative standard deviation of metoprolol succinate and azelnidipine should not be more than 2.

4. Parameters Evaluated for Validation Study:

- Specificity / Selectivity
- Precision
- Intermediate precision
- Accuracy
- Linearity & Range
- Solution Stability
- Robustness

3. RESULTS AND DISCCUSION:

Table 4. Optimization of RP-HPLC

Trial No.	Mobile Phase	Observation	Action
1	Water and Acetonitrile Ratio-(50:50)	No peak is observed	Add Buffer in mobile phase
2	Potassium Dihydrogen Orthophosphate and Acetonitrile Ratio- (60:40)	Only Peak is observed and peak shape is not clear	Change buffer to NaH2PO4
3	Sodium Dihydrogen Orthophosphate and Acetonitrile Ratio- (60:40)	Only one peak is observed	Change buffur to NH4H2PO4
4	Ammonium Dihydrogen Orthophosphate and Acetonitrile Ratio- (60:40)	Only one peak is observed	Change in ratio of buffur NH4H2PO4 and acetonitrile
5	Ammonium Dihydrogen Orthophosphate and Acetonitrile Ratio- (40:60)	Two peaks are observed but distance is too long	Add PH to mobile phase
6	Ammonium Dihydrogen Orthophosphate and Acetonitrile Ratio- (40:60)	Peak is Observed & Method optimize	

3.1 Metoprolol Succinate and Azelnidipine HPLC Method Development Parameters:

1) Specificity/Selectivity:

The specificity method was determined by analysing drugs and active pharmaceutical ingredients.

Table 5. System Suitability observed during Specificity

	Area	
Sample	Metoprolol Succinate	Azelnidipine
Blank		
Standard Solution	2498996	1116433
Sample Solution	2455773	1136053

2) Precision:

The Precision of an quantitative study determines closeness of agreement between a series of measurements obtained from multiple sampling of the same sample solutions as per standards. Precision Three Levels Consideration As, System Repeatability, Analysis Repeatability, Ruggedness.

3) System Suitability:

Precision under system repeatability conditions i.e. conditions where the system suitability was checked by injecting five replicate standard injections of pharmaceutical formulations like metoprolol succinate and azelnidipine.

Table 6. Observation of System Suitability

No. of Inj.	Metoprolol Succinate	Azelnidipine
1	2251564	1214499
2	2241480	1201298
3	2238857	1222729
Average	**2243967**	**1212842**
STDEV	6708.639	10811.160
RSD	**0.30**	**0.89**

4) Intermediate Precision

Table 6. Observation of Intermediate Precision

	Area	
No. of Inj.	**Metoprolol Succinate**	**Azelnidipine**
1	2334630	1130335
2	2330691	1122195
3	2343615	1134031

Precision under analysis repeatability conditions where test results are independent and obtained with similar methodology of same test items like same laboratory to laboratory using same operator and equipment's within short time interval.

Closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference sample. For establishing Accuracy of the method, prepared the sample solutions of different concentrations for Metoprolol Succinate and for Azelnidipine.

5) Accuracy:

Table 7. Observation of accuracy

Standard Solution		
	Area	
No. of Inj.	**Metoprolol Succinate**	**Azelnidipine**
1	**2387994**	**1306779**
2	**2373013**	**1302806**
3	**2363229**	**1314485**
Average	**2374745**	**1308023**
STDEV	**12473.053**	**5938.100**
RSD	**0.53**	**0.45**

6) **Limit of Detection:** The detection limit of individual testing procedure having less drug substance in the sample which is detected but not quantitated as per exact value.

Metoprolol Succinate-Azelnidipine-

$= \frac{3.3 \times Std.Deviation}{Slope} = \frac{3.3 \times 1552.040}{23188.0600}$

$\frac{3.3 \times Std.Deviation}{Slope} = \frac{3.3 \times 210611.74}{22178.2796}$

LOD = 0.2 ppm

LOD = 1.6 ppm

Limit of Quantification: The Quantification limit of individual testing procedure having less amount of drug substance in the sample which is quantitatively determined by using suitable precision.

7) **Linearity & range:**

Linearity Sample Stock Solution:

Weigh 20 mg Metoprolol Succinate and 20mg of Azelnidipine in 50ml calibrated flask sonicate, dissolve and dilute upto volume by using mobile phase.

Linearity of Metoprolol Succinate

Area	Conc in ppm	Conc. of Solution
0	0.0	
1810326	80.0	80
2084216	90.0	90
2309666	100.0	100
2445390	110.0	110
2789142	120.0	120

Linearity of Azelnidipine

Avg. Area	Conc. in ppm	Conc. of Solution
0	0.0	0
1,131,268	51.2	80
1,288,838	57.6	90
1,421,164	64.0	100
1,571,643	70.4	110
1,692,555	76.8	120

SUMMARY AND CONCLUSION

The Overall study determines the successful driven method development Reverse Phase -High Performance Liquid Chromatography methods and validation study for quantitative analysis of Azelnidipine and Metoprolol Succinate. Both methods were rigorously validated following ICH guidelines, are mainly simple, precise, accurate, linear, and robust for routine pharmaceutical analysis.

FUTURE SCOPE

The developed QbD-based UV spectroscopic and RP-HPLC methods provide a robust, accurate, and reliable approach for the simultaneous estimation of Metoprolol Succinate and Azelnidipine. Future work may involve application of these methods to stability studies, biological sample analysis, advanced chromatographic techniques, green analytical approaches, and routine industrial

quality control, thereby expanding their utility in pharmaceutical research and manufacturing.

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