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Novel RP-HPLC Method Development and Validation for Estimation of Moexipril In Bulk Dosage Form

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ABSTRACT:

A simple, fast, precise reverse phase and isocratic HPLC method was developed for the determination of Moexipril dosage forms. Chromatographic separation was achieved on a Zorbax SB C18(150mm×.4.6mm & 5.0µm)using mobile phase consisting of a mixture of Sodium Phosphate Buffer (pH 3.0): Acetonitrile (70:30) with detection of 240 nm and The flow rate was 1.0mL/min. The injection volume was 20µL,The retention time of Moexipril was 1.326 mins and total run time of Moexpril was 10 mins. Linearity was observed in the range 50-150 µg /ml for Moexipril (r^2 =0.9926) for drugs estimated by the proposed methods was in good agreement with the label claim. Moexipril was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies.

Key words: Moexipril, chromatographic separation, high resolution, shorter retention, bioequivalence

1. Introduction:

Chemically moexipril at is described as (3S) -2-[(2S)-2-{[(2S)-1-ethoxy-1- oxo- 4- phenylbutan2-yl]amino}propanoyl]-6,7-dimethoxy-1,2,3,4-tetra hydroisoquinoline-3-carboxylic acid (Figure 1). Moexipril is a non-sulfhydryl group drug substance that inhibits the angiotensin-converting enzyme (ACE). It is applied to the management of hypertension. It causes the blood vessels to relax and widen. Reduced blood pressure can lessen the risk of kidney damage, heart attacks, and strokes. Moexipril reduces blood pressure by the inhibition of ACE activity. The transformation of inert angiotensin decapeptide into the vasoconstrictor angiotensin II is catalyzed by the peptidyl dipeptidase ACE. Strong peripheral vasoconstrictor angiotensin II causes the adrenal cortex to secrete more aldosterone, which in turn suppresses the release of renin. ACE and kininase II, an endothelium-dependent vasodilator, are comparable. Kininase II is the enzyme that breaks down bradykinin.are inhibited by moexipril about 1000-fold. The literature search revealed that a few methods like spectroscopic, potentiometric, TLC, HPLC and LC-MS methods for the measurement of moexipril were reported. In this study, we report a rapid, sensitive, accurate, and precise HPLC method for moexipril in bulk and tablet samples.

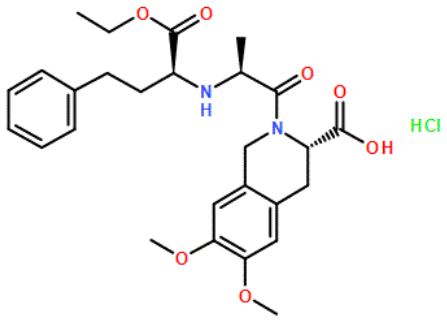

Figure.1.Structure of Moexipril

2. Materials and Methods:

2.1. Standard:

Moexipril

2.2. Product:

Moexipril Hydrochloride (15mg) tab

2.3. Chemicals and Reagents:

HPLC grade chemicals like Ammonium acetate ,Orthophosphoric acid, are purchased from Rankem, AR grade solvents and reagents like Methanol, Acetonitrile , Sodium Phosphate monobasic, Sodium dihydrogen phosphate, Disodium hydrogen phosphate and also Ultrapure water are purchased from Merck.

2.4. Instruments Used:

Electronic balance make Shimadzu, Syringe made Hamilton, Ultra sonicator make by Citizen, Digital Ultrasonic Cleaner, pH meter make by Global digital, UV-Visible Spectrophotometer made by Nicolet evolution 100 with operating software Vision Pro, HPLC made by Shimadzu(LC 20 AT VP) with operating software Spin chrome (LC SOLUTIONS) ,HPLC column Zorbax SB C18(150mm×.4.6mm & 5.0µm).

2.5. Preparations for Experiments:

2.5.1. Buffer Preparation:

Accurately weighed and transferred 11.9g Sodium Phosphate monobasic in to 1000mL of volumetric flask and sonicated to dissolved adjusted pH 3.0 with diluted ortho phosphoric acid. Filtered through 0.45µm membrane filter.

2.5.2. Mobile Phase Preparation:

Mixed 700mL of Buffer and 300mL of Acetonitrile, degassed by sonication.

2.5.3. Preparation of standard stock solution

Weighed accurately 100mg of Moexipril in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase. Working standard solution 100µg/ml of Moexipril was prepared by diluting 5ml to 50ml with mobile phase from above stock solution respectively.

2.5.4. Preparation of sample solution:

10 tablets (each tablet contains 15mg of Moexipril) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weighed crushed powder equivalent to 50 mg of Moexipril in 50 ml of volumetric flask and dissolve in 20ml of mobile phase by 10min of sonication and make up the volume with mobile phase. Prepared 100µg/mL sample solution by further diluted 5mL above sample stock solution to 50mL with mobile phase and mixed well.

2.5.5. Preparation of Placebo solution:

Weighed Placebo powder equivalent to 100 mg of Moexipril in 100 ml of volumetric flask and dissolve in 50ml of mobile phase by 30min of sonication and make up the volume with mobile phase. Centrifuged sample at 5000rpm for 10min.Prepared 100µg/mL sample solution by further diluted 5mL above sample stock solution to 50mL with mobile phase and mixed well.

3. Method Development

3.1. Solubility Studies:

10mg of MOEXIPRIL was weighed and transferred in to 100 ml volumetric flasks and dissolved in different solvents and then make up to the mark with respective solvents and prepare 10 µg /ml of solution by diluting 1ml to 10ml with same solvent. These studies are carried out at 25 ^{0}C.

Table.1. Solubility studies.

Solvent Name	**Moexipril**
Water	Soluble
Methanol	Soluble
Ethanol	Soluble
Triethylamine	Soluble

Observation: Methanol is selected for initial standard stock solution preparation

3.2. Determination of Working Wavelength (λ_{max})

Preparation of Standard solution: 10mg of MOEXIPRIL was weighed and transferred in to100 ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

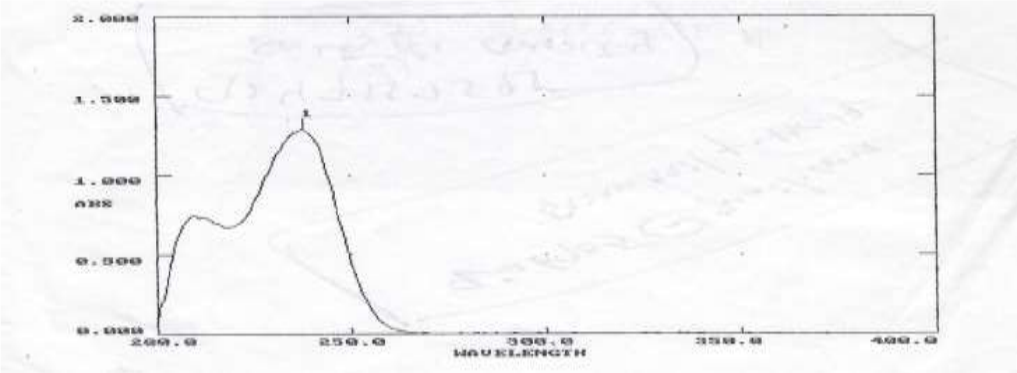

Figure.2. UV-VIS Spectrum of moexipril (240nm)

Observation: The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 230nm for **Moexipril**(Figure.2), 240nm was selected as detector wavelength for the HPLC chromatographic method.

3.3. Optimized Chromatographic conditions:

After several trails with mobile phase the method was optimized with chromatographic parameters Zorbax SB C_{18} (150mm×.4.6mm & 5.0µm) analytical column using mobile phase consisting of a mixture of Sodium Phosphate Buffer (pH 3.0): Acetonitrile (70:30) with detection of 240 nm and the flow rate was 1.0mL/min. The injection volume was 20µL,the retention time of Moexipril was 1.326 mins and total run time of Moexpril was 10 mins. olumn

4. Results and Discussion:

Method validation: Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness study. It was validated according to ICH Q2 (R1) guideline.

4.1. System Suitability:

The system suitability parameters were determined by preparing standard solution of Moexipril. The prepared solution was injected six times and the parameters like peak tailing, resolution and plate count were determined.

Table.2.System suitability results

Name of the Standard	Moexipril	Tailing factor	Plate count
Standard-01	2670447	1.3	3333
Standard-02	2675047	1.3	3353
Standard-03	2660261	1.3	3331
Standard-04	2681945	1.3	3326
Standard-05	2673126	1.3	3315
Average	2672165	1.3	3332
%RSD	0.3	0.3	0.4

Acceptance Limits: %RSD should not exceed 0.85, USP tail factor should not be greater than 2.0.Column efficiency, and More than 2000 theoretical plates are required on top of the column.

Observation: The %RSD of Moexipril was 0.3, USP tailing was 1.3 and plate count of Moexipril was observed as 3332.

Conclusion: System suitability results were met with acceptance criteria, hence system is suitable.

4.2. Specificity:

Specificity of the method was determined by injecting blank and placebo to check whether peaks in the blank and placebo are eluting with drugs peaks. So this method was considered to be specific.

Table.3.Specificity Results

S. No.	Solution details	Area of Moexipril
1	Blank	Not Detected
2	Placebo solution	Not Detected
3	Standard	2670555
4	Test solution	2670829

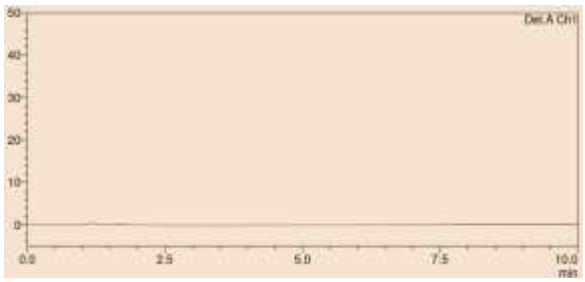

Figure.3. Chromatogram of Blank

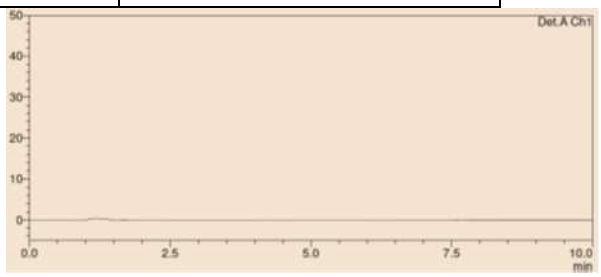

Figure.4.Chromatogram of Placebo

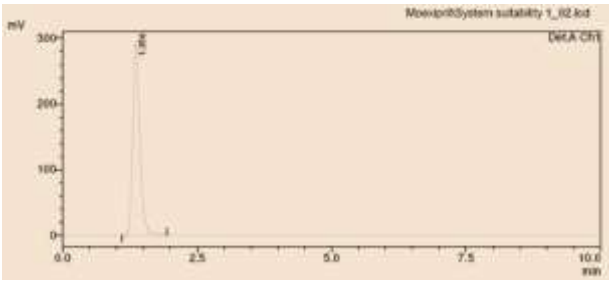

Figure.5. Chromatogram of Moexipril Standard

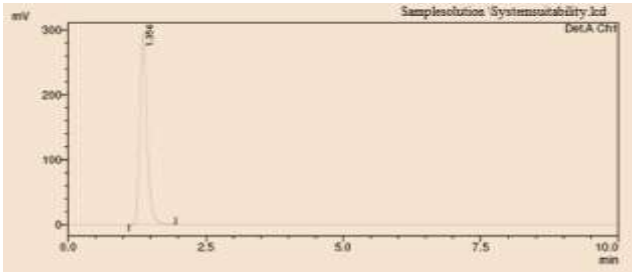

Figure.6. Chromatogram of Sample solution

Acceptance Limits: Each peak needs to be properly resolved. Do not interfere with the Moexipril peak of other peaks. NLT 0.99 is the recommended maximum purity for Moexipril.

Observation: There was no interference observed at the retention time of Moexipril due to blank and Placebo.

Conclusion: Due to no interference with retention time of moexipril peak. This method is considered as specific to moexipril.

4.3 Precision:

Precision of the method refers to the reproducibility of value on repeated measurements.

4.3.1 Method Precision:

The prepared solution was injected six times on different intervals of same day and %RSD of the values is estimated to understand the precision of the method.

Table.4. Method Precision Results

S. No.	Solution details	%Assay of Moexipril
1	Test solution preparation-1	99.5
2	Test solution preparation-2	100.0
3	Test solution preparation-3	100.1
4	Test solution preparation-4	100.3
5	Test solution preparation-5	100.7
6	Test solution preparation-6	100.7
Average		99.2
Std Dev		0.34
%RSD		0.3

Table.5. Intermediate Precision Results

S. No.	Solution details	%Assay of Moexipril
1	Test solution preparation-1	100.4
2	Test solution preparation-2	100.0
3	Test solution preparation-3	100.4
4	Test solution preparation-4	100.2
5	Test solution preparation-5	100.2
6	Test solution preparation-6	100.3
Average		100.3
Std Dev		0.13
%RSD		0.1

4.3.2. Intermediate Precision:

The prepared solution was injected six times on different days and different intervals of same day and %RSD of the values is estimated to understand the precision of the method.

Acceptance criteria: The % RSD should not be more than 2%

Observation: The % RSD of moexipril for method precision and intermediate precision was found to be 0.3 and 0.1 respectively.

Conclusion: Precision data at Q point time was within acceptance criteria of NLT 80% and %RSD below 2.0.

4.4. Linearity and Range:

Injected each level in to the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration (on x-axis concentration and on y-axis peak area) and calculated the R^2.The linearity was determined by injecting the LOQ, 50%, 80%, 100%, 120%,150% of spiked solutions.

Table.6. Preparation of the Standard Stock

Volume Taken(mL)	Volume diluted to	Concentration(µg/mL)
1	20	50
1.5	20	75
2	20	100
2.5	20	125
3	20	150

Table.7. Linearity Results of Moexipril

S. No	Name of the Solution	Area of Moexipri
1	Linearity solution, Level-1 (50%)	1009147
2	Linearity solution, Level-2 (80%)	1953325
3	Linearity solution, Level-3 (100%)	2667662
4	Linearity solution, Level-4 (120%)	3312980
5	Linearity solution, Level-5 (150%)	4105525
Slope		33117.1
Intercept		661701.3
Correlation coefficient		0.9926

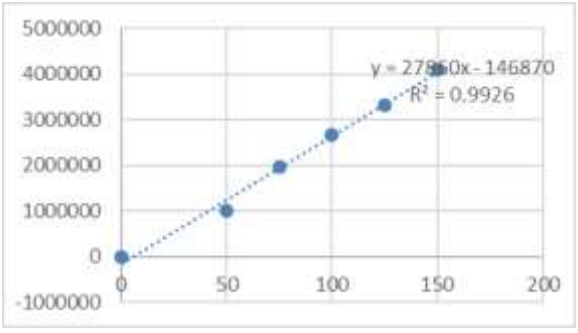

Figure.7. Linearity Graph

Acceptance criteria: The correlation coefficient value should not be less than 0.999 for Moexipril

Observation: The correlation coefficient value obtained 0.9926 for Moexipril.

Conclusion: Correlation coefficient between Moexipril concentration and peak area was calculated by linear regression and was found to be within the acceptance criteria.

4.5. Accuracy and Recovery:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100% & 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%.

Table.8. Results for Recovery of Moexipril

Recovery level	**Accuracy of Moexipril**			**Average % Recovery**
	Area	**Average area**	**%Recovery**	
50%	1351906	1345438.7	100.7	100.8
	1340602			
	1343808			
100%	2676139	2683344.3	100.4	
	2669772			
	2704122			
150%	4004102	4058614.0	101.2	
	4080919			
	4090821			

Acceptance criteria: The mean % recovery of the standard and sample at each level should be not less than 98% and not more than 102%.

Observation: The % Recovery of Moexipril was found to be 100.8%

Conclusion: The recovery results indicate that the test method has an acceptable level of accuracy. The results were found to be within the limits.

4.6. Limit of Detection (Lod):

LOD is determined by the analysis of samples with a known concentration of analyte and by establishing that minimum level at which the analyte can reliably detect, but not necessarily quantitated as precise value, under the stated experimental conditions.

$$\mathrm{LOD} = \frac{3.3\sigma}{\mathrm{S}}$$

Where, σ = the standard deviation of the response and S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation: LOD of Moexipril was found to be 5.74 µg/mL.

4.7. Limit of Quantification (LOQ):

LOQ is the least concentration of drugs in a sample which is estimated with appropriate precision and accuracy under the affirmed experimental conditions.

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{S}}$$

Where, σ = the standard deviation of the response and S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation: LOQ of Moexipril was found to be 18.96 µg/mL.

4.8. Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.5ml/min), Flow plus (0.8ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected.

Table.9.Robustness results for Moexipril:

Name of the Parameter	%RSD	Theoretical Plates	Tailing factor
Low Column Oven Temperature(25°C)	0.9	3945	1.43
High Column Oven Temperature(35°C)	0.7	3976	1.42
Lower Wavelength(251nm)	0.3	3964	1.43
Higher Wavelength(261nm)	0.4	3956	1.45

Acceptance Criteria: The % amount found should be between 98% to 102%. % relative standard deviation should not be more than 2.0%

Observation: System suitability met the acceptance criteria in Robustness parameters hence method is Robust.

Conclusion: The test method was found to be robust against changes in flow rate, buffer pH, column temperature, and organic ratio.

4.9. Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Table.10.Ruggedness results for Moexipril

MOEXIPRIL	%Assay	MOEXIPRIL	%Assay
Analyst 01	99.90%	**Analyst 01**	98.62%
Analyst 02	98.34%	**Analyst 02**	99.58%
%RSD	0.11	**%RSD**	0.26

Acceptance criteria: The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Observation: The obtained % RSD for Moexipril was 0.11 for analyst 1 and 0.26 for analyst 2 respectively.

Conclusion: From the above results % Assay and %RSD obtained is within the acceptance criteria 2%, so method is rugged.

5. Forced degradation studies:

The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is “studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline “Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods”.

1. Thermal Degradation: Stress testing is likely to be carried out on single batch of the drug substance (API). Thermolytic degradation may lead to hydrolysis / dehydration / isomerization / epimerization / decarboxylation / rearrangements and some kinds of polymerization reactions. ICH guidelines suggest that thermolytic degradation study should be carried out at temperatures (in 10 increments e.g. 50ºC, 60ºC, etc.) above that for accelerated testing and withdraw the sample at different time intervals during reaction condition. If reasonable degradation (i.e. 5-20%) has seen, testing can be stopped at this point.

2. Photolytic Degradation: The photochemical stability of the drug was studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 24hours. For HPLC study, the resultant solution was injected into the system and the chromatogram were recorded to assess the stability of sample.

3. Acidic Degradation: Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCl then kept in oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

4. Base Degradation: Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in oven at 60°C for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml with mobile. Phase, then place the sample in the vial and measure the chromatogram

5. Peroxide Degradation: Sample solution of Moexipril (100µg/ml) and 1 ml of 20% hydrogen peroxide (H_2O_2) was mixed. For HPLC study, 100µg/ml was injected into the system and the chromatogram was recorded to assess the stability of sample.

Table.11. Data of Percentage of Degradation of Moexipril standard

Name of the Degradation	Condition	Peak Purity	Peak Purity Value	%Assay
Thermal Degradation	60°C/7Days	PASS	+	99.80
Photolytic Degradation	1.2mill/LUX Hours	PASS	+	99.73
Acid Degradation	5mL of 3N HCl/4Hrs at 80°C	PASS	+	99.88
Base Degradation	5mL of 3N NaOH /4Hrs at 80°C	PASS	+	99.81
Peroxide Degradation	5mL of 10% H_2O_2/4Hrs at Bench top	PASS	+	90.27

Acceptance Criteria: Peak purity should be Pass and Value should be in positive

Observation: Peak purity was obtained Pass, Purity value obtained in Positive, %degradation 5.8% obtained in Acid degradation, No interference was observed with treated blank

Conclusion: The proposed method gave good resolution of Mesalamine and its degradants. The method is confirmed to be stable indicating capability of distinguishing the active pharmaceutical ingredient (API) from any degradation (decomposition products) formed during the defined storage conditions during the stability testing period.

5. CONCLUSION

A new precise, accurate, rapid method has been developed for the estimation of Moexipril in bulk and its pharmaceutical dosage form by HPLC. From the above experimental results and parameters it was concluded that, this newly developed method for the estimation Moexipril was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future. From results the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of Educational institutions and Quality control laboratories.

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