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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF AMLODIPINE AND BENAZEPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Amlodipine and Benazepril, in its pure form as well as in capsule dosage form. Chromatography was carried out on an Altima C_{18} (4.6 x 150 mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) as the mobile phase at a flow rate of 1.0 mL/min, the detection was carried out at 225 nm. The retention time of the Amlodipine and Benazepril was 2.102, 3.537 ±0.02 min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Amlodipine and 20-100 µg/ml of Benazepril. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Amlodipine, Benazepril, RP-HPLC, Validation.

INTRODUCTION

Amlodipine, initially approved by the FDA in 1987, is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure. The option for single daily dosing of amlodipine is an attractive feature of this drug. Amlodipine relieves the symptoms of chest pain associated with angina. In patients diagnosed with angina, daily administration of a single amlodipine dose increases total exercise time, the time to angina onset, and the time to 1 mm ST-segment depression on ECG studies, decreases anginal attack frequency, and decreases the requirement for nitroglycerin tablets. Amlodipine has a strong affinity for cell membranes, modulating calcium influx by inhibiting selected membrane calcium channels. This drug's unique binding properties allow for its long-acting action and less frequent dosing regimen. The IUPAC name of Amlodipine is (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chloro phenyl)-6-methyl-1, 4-dihydro pyridine-3, 5-dicarboxylate. The Chemical Structure of Amlodipine is shown in follows

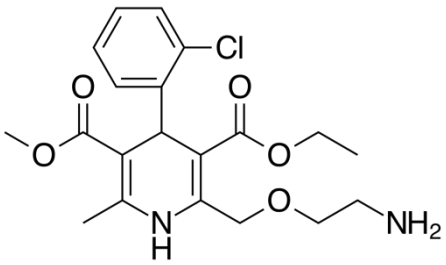

Fig-: Chemical Structure of Amlodipine.

Benazepril, brand name Lotensin, is a medication used to treat high blood pressure (hypertension), congestive heart failure, and chronic renal failure. Upon cleavage of its

ester group by the liver, benazepril is converted into its active form benazeprilat, a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. Benazepril, an angiotensin-converting enzyme (ACE) inhibitor, is a prodrug which, when hydrolyzed by esterases to its active Benazeprilat, is used to treat hypertension and heart failure, to reduce proteinuria and renal disease in patients with nephropathies, and to prevent stroke, myocardial infarction, and cardiac death in high-risk patients. Benazepril and Benazeprilat inhibit angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. Benazeprilat, the active metabolite of Benazepril, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin. Inhibition of ACE results in decreased plasma angiotensin. As angiotensin II is a vasoconstrictor and a negative-feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and stimulation of baroreceptor reflex mechanisms, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The IUPAC name of Benazepril is 2-[(3S)-3-[[(2S)-1-ethoxy-1-oxo-4-phenyl butan-2-yl] amino]-2-oxo-4, 5-dihydro-3H-1-benzazepin-1-yl] acetic acid. The Chemical Structure of Benazepril is shown in fig-1.

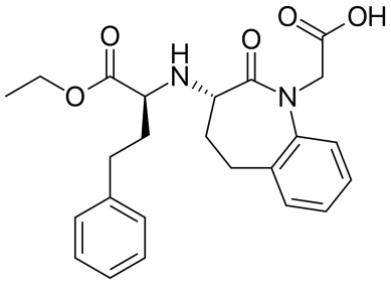

Fig-: Chemical Structure of Benazepril.

MATERIALS AND METHODS

Table 3: List of Instrument used.

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 4: List of Chemicals used.

S.No.	Chemical	Brand Names
1	Amlodipine	Sura labs
2	Benazepril	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Amlodipine and Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Amlodipine and 0.375ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Buffer and Mobile Phase

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Filter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of Mobile Phase
Accurately measured 500 ml (50%) of Methanol, 250 ml of Triethylamine buffer (25%) and 250 ml of Acetonitrile (25%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

Method Validation Parameters
System Suitability
Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15 ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug
Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15 ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution
Take average weight of one capsule and crush in a mortor by using pestle and weight 10 mg equivalent weight of Amlodipine and Benazepril sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.15 ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity
Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (5 ppm of Amlodipine & 20 ppm of Benazepril)
Pipette out 0.05ml of Amlodipine and 0.20 ml of Benazepril stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Amlodipine & 40 ppm of Benazepril)
Pipette out 0.1ml of Amlodipine and 0.40 ml of Benazepril stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 ppm of Amlodipine & 60 ppm of Benazepril)
Pipette out 0.15 ml of Amlodipine and 0.60 ml of Benazepril stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Amlodipine& 80 ppm of Benazepril)
Pipette out 0.2 ml of Amlodipine and 0.80 ml of Benazepril stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Amlodipine& 100 ppm of Benazepril)
Pipette out 0.25ml of Amlodipine and 1.0 ml of Benazepril stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure
Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision
Repeatability
Preparation of Amlodipine and Benazepril Product Solution for Precision
Accurately weigh and transfer 10 mg of Amlodipine and 10 mg of Benazepril working standard into a 10 ml of

clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15 ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy

For Preparation of 50% Standard Stock Solution

Accurately weigh and transfer 10 mg of Amlodipine and 10 mg of Benazepril working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.075ml of the above Amlodipine and 0.3 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For Preparation of 100% Standard Stock Solution

Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For Preparation of 150% Standard Stock Solution

Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml of Amlodipine and 1.2 ml of Benazepril from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Amlodipine and Benazepril and calculate the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard solution

Accurately weigh and transfer 10 mg of Amlodipine and 10 mg of Benazepril working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15 ml of the above Amlodipine and 0.60 ml of the Benazepril stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 40: 40:20, 60:10:30 instead (50:25:25), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimization of Analytical Method

Optimised Chromatographic Conditions

Mobile phase: Methanol: TEA Buffer pH-4.5: Acetonitrile (50:25:25% v/v/v)
Column: Altima C_{18} (4.6×150mm, 5.0 µm)
Flow rate: 1.0 ml/min
Wavelength: 225 nm
Column temp: 40ºC
Injection Volume: 10 µl
Run time: 7.0 minutes

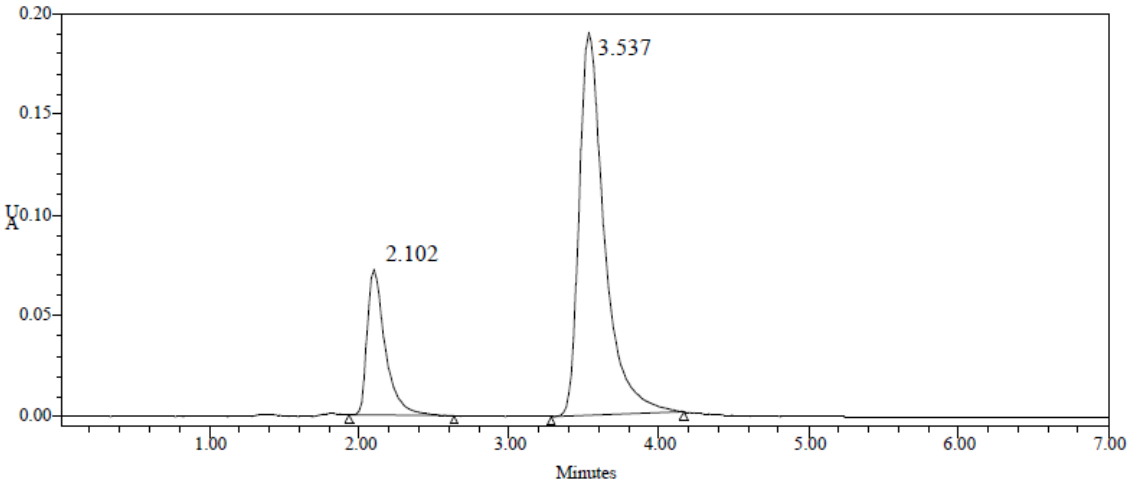

Fig-: Optimized Chromatographic Condition.

Analytical Method Validation

The developed method was validated for linearity and range, accuracy, precision, Limit of detection, Limit of quantitation and robustness as per ICH guidelines.

System Suitability

Table-: Results of System Suitability for Amlodipine.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Amlodipine	2.117	608452	71498	5643	1.9
2	Amlodipine	2.118	606820	126412	5432	1.6
3	Amlodipine	2.116	608452	126471	5123	1.6
4	Amlodipine	2.109	595267	129859	5207	1.7
5	Amlodipine	2.102	596608	124691	5481	1.6
Mean			**603119.8**			
Std. Dev			**6607.31**			
% RSD			**1.09**			

Table-: Results of System Suitability for Amlodipine.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Benazepril	3.547	2234724	188631	5043	1.2	2.07
2	Benazepril	3.539	2240080	2614821	5432	1.4	2.05
3	Benazepril	3.547	2234724	2321451	5987	1.5	2.0
4	Benazepril	3.565	2204466	2324710	5845	1.6	2.01
5	Benazepril	3.537	2209574	2531247	5371	1.6	2.01
Mean			**2224714**				
Std. Dev			**16399.05**				
% RSD			**0.73**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Amlodipine and Benazepril in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Amlodipine and Benazepril in pharmaceutical dosage form was found to be 99.6%, 99.8%.

Linearity: Linearity of the method was evaluated by constructing calibration curves at five concentration levels over a range of 5-25µg/ml. The calibration curve was linear and regression equation found to be y = 36199x + 13756 with correlation coefficient (r2) 0.9993 as shown in fig. 1.

Table-: Chromatographic Data for Linearity Study of Amlodipine.

Concentration Level (%)	Concentration g/ml	Average Peak Area
33.3	5	205035
66.6	10	381239
100	15	561128
133.3	20	740162
166.6	25	909922

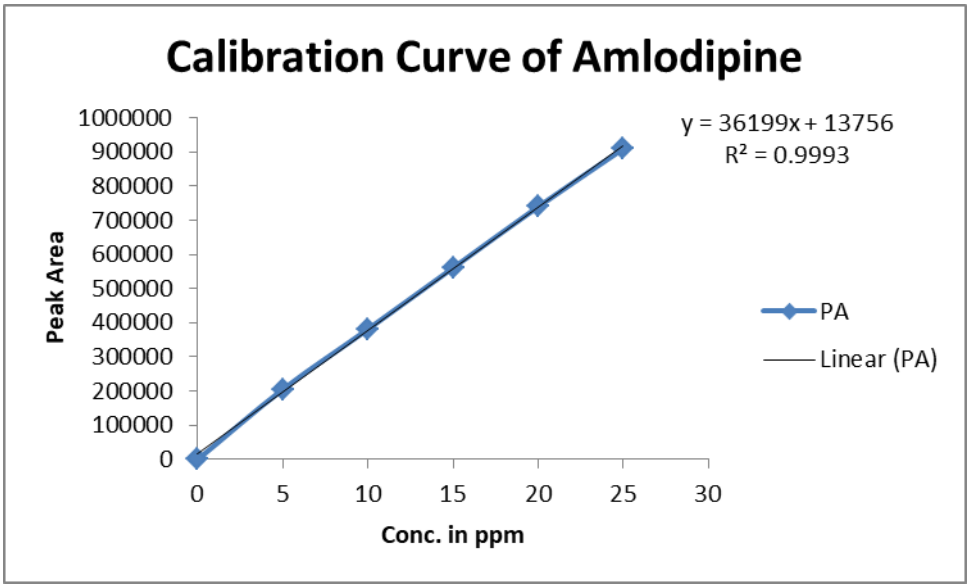

Fig-: Calibration Curve of Amlodipine.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Amlodipine is a straight line.

Y = mx + c

Slope (m) = 27760

Intercept (c) = 12827

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 12827. These values meet the validation criteria.

Benazepril

Linearity of the method was evaluated by constructing calibration curves at five concentration levels over a range of 20-100µg/ml. The calibration curve was linear and regression equation found to be y = 36199x + 13756 with correlation coefficient (r2) 0.9993 as shown in fig. 1.

Table-: Chromatographic Data for Linearity Study of Benazepril.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	20	757881
66	40	757881
100	60	1458941
133	80	2132457
166	100	2901811

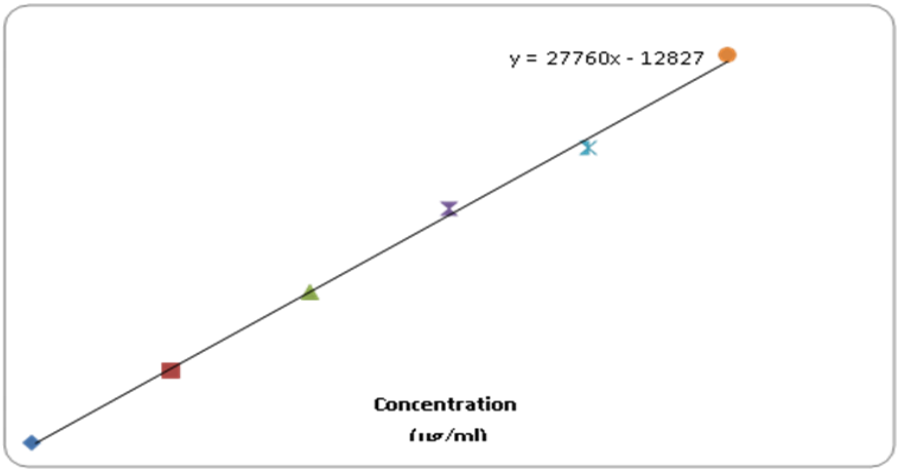

Fig-: Calibration Curve of Benazepril.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Benazepril is a straight line.

Y = mx + c

Slope (m) = 27760

Intercept (c) = 12827

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 12827. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-: Results of Repeatability for Amlodipine.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Amlodipine	2.108	602223	128898	2586	1.6
2	Amlodipine	2.105	607748	129233	2947	1.4
3	Amlodipine	2.113	607302	127409	2468	1.6
4	Amlodipine	2.109	608674	127047	2146	1.9
5	Amlodipine	2.109	607376	129859	2307	1.7
Mean			**606665**			
Std. Dev			**2542.3**			
% RSD			**0.42**			

Table-: Results of Method Precision for Benazepril.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Benazepril	3.552	2220333	2231111	1.6	2371
2	Benazepril	3.550	2221573	2674210	1.6	2841
3	Benazepril	3.564	2215483	2231261	1.5	2816
4	Benazepril	3.564	2217379	2421301	1.5	2872
5	Benazepril	3.565	2211255	2324710	1.6	2845
Mean			**2217205**		**1.6**	**2841**
Std. Dev			**4100.8**			
% RSD			**0.18**			

Intermediate Precision

Day 1

Table-: Results of Intermediate Precision for Amlodipine.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Amlodipine	2.108	596608	128898	2547	1.6
2	Amlodipine	2.105	598959	129233	2944	1.4
3	Amlodipine	2.113	595728	127409	2361	1.6
4	Amlodipine	2.109	594485	127047	2546	1.9
5	Amlodipine	2.109	595267	129859	2207	1.7
6	Amlodipine	2.102	596608	124691	2481	1.6
Mean			**596209**			
Std. Dev			**1718.7**			
% RSD			**0.29**			

Table-: Results of Intermediate Precision for Benazepril.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Benazepril	3.552	2207732	2231134	2371	1.5	2.04
2	Benazepril	3.550	2202266	2674210	2841	1.6	2.03
3	Benazepril	3.564	2209375	2247461	2816	1.6	2.01
4	Benazepril	3.564	2204037	2454301	2872	1.6	2.05
5	Benazepril	3.565	2204466	2324710	2845	1.6	2.02
6	Benazepril	3.537	2209574	2531247	2371	1.6	2.03
Mean			**2205575**				
Std. Dev			**2899.8**				
% RSD			**0.13**				

Day 2

Table-: Results of Intermediate Precision Day 2 for Amlodipine.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Amlodipine	2.102	602155	127998	5586	1.5
2	Amlodipine	2.105	603662	134844	5636	1.6
3	Amlodipine	2.112	603931	161103	5432	1.6
4	Amlodipine	2.113	607302	127409	5468	1.6
5	Amlodipine	2.109	608674	127047	5146	1.9
6	Amlodipine	2.109	607376	129859	5307	1.7
Mean			**605516.7**			
Std. Dev			**2602.622**			
% RSD			**0.42**			

Table-: Results of Intermediate Precision for Benazepril.

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Benazepril	3.537	2241579	2263528	2371	1.6	1.98
2	Benazepril	3.552	2236409	2224418	2414	1.6	
3	Benazepril	3.560	2239093	2233725	2384	1.6	8.97
4	Benazepril	3.564	2215483	2231261	2816	1.5	
5	Benazepril	3.564	2217379	2421301	2872	1.5	
6	Benazepril	3.565	2211255	2324710	2845	1.6	
Mean			**2226866**				
Std. Dev			**13567.02**				
% RSD			**0.60**				

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table-: The Accuracy Results for Amlodipine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table-: The Accuracy Results for Amlodipine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Amlodipine

= 3.3 × 3188.4/36199

= 0.2µg/ml

Benazepril

= 3.3 × 39656.07/56304

= 2.3µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Amlodipine

= 10×3188.481242/36199

= 0.8µg/ml

Benazepril

= 10 × 39656.07/56304

= 7.04µg/ml

Robustness

Table-: Results for Robustness of Amlodipine.

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

Table-: Results for Robustness of Benazepril.

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

SUMMARY AND CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Amlodipine and Benazepril in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Amlodipine and Benazepril was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was

found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Amlodipine and Benazepril in bulk drug and in Pharmaceutical dosage forms.

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