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R E S E A E C H A R T I C L E

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Amlodipine and Benazepril In Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A simple, Accurate, rapid and precise method was developed for the simultaneous estimation of the Amlodipine and Benazepril in Tablet dosage form. The chromatogram was run through Inertsil ODS (4.6 x 100mm, 5µm). Mobile phase containing0.1%TEA: Methanol: Acetonitrile is taken in the ratio 40:30: 30 was pumped through the column at a flow rate of 1 ml/min. The buffer used in this method was 0.1%TEA buffer. The temperature was maintained at 25°C. Optimized wavelength selected was 235 nm. The retention time of Amlodipine and Benazepril were found to 2.41 min and 3.31. % RSD of Amlodipine and Benazepril were found to be 0.3 and 0.7 respectively. % Recovery was obtained at 100.6% and 99.9% for Amlodipine and Benazepril respectively. LOD, LOQ values obtained from regression equations of Amlodipine and Benazepril were 3.30, 10.02 and 2.98, 9.98 respectively. The accuracy and reliability of the method were assessed by evaluation of linearity, precision (intra-day and inter-day % RSD >2 for Amlodipine and Benazepril), accuracy and specificity, in accordance with ICH guidelines. This method has been successively applied to the pharmaceutical formulation and was validated according to ICH guidelines.

Keywords: Amlodipine, Benazepril, RP-HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Amlodipine is chemically known as 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. This drug used in the hypertension.

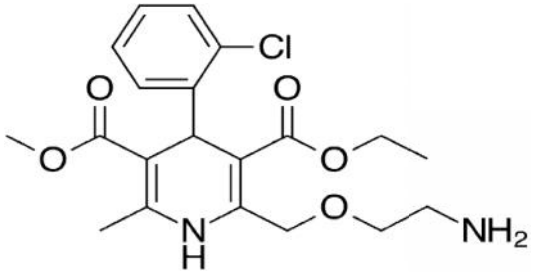

Fig 1: Structure of Amlodipine

Benazepril is chemically known as 2-[(3S)-3-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl]acetic acid. indicated for the treatment of Hypertension.

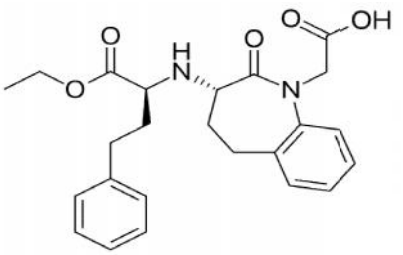

Fig 2: Structure of Benazepril

The literature review shows very few methods for Amlodipine and Benazepril by simultaneous estimation by RP-HPLC. Hence, it was felt that, there is a need of new, precise and much efficient analytical method development for the simultaneous estimation of Amlodipine and Benazepril in pharmaceutical dosage form[5].

Present work is aimed to develop a new, simple, fast, rapid, accurate, and reproducible RP-HPLC method for the simultaneous analysis of Amlodipine and Benazepril. The developed method will be validated according to ICH guidelines. Degradation studies were also performed[6-10].

2. Materials and Methods

Instruments Used:

Table 1: Instrument specification

Instrument	Model
HPLC	WATERS, software: Empower, 2695 separation module, uv detector.
UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
pH meter	Adwa – AD 1020
Weighing machine	Afcoset ER-200A
Pipettes and Burettes	Borosil
Beakers	Borosil

Chemicals Used:

Table 2: Chemicals specification

Chemical	Company Name
Amlodipine	PHARMATRAIN
Benazepril	PHARMATRAIN
KH_2PO_4	FINER chemical LTD
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	MOLYCHEM
Ortho phosphoric Acid	MERCK

Wave length selection:

UV spectrum of 10 µg/ml Amlodipine and 10 µg/ml Benazepril in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 235 nm. At this wavelength both the drugs show good absorbance.

Optimized Chromatographic Conditions:

Instrument used	:Waters HPLC with auto sampler and UV detector
Temperature	:Ambient (25° C)
Mode of separation	: Isocratic mode
Column	:Inertsil ODS (4.6 x 100mm, 5µm)
Buffer	:0.1% TEA
Mobile phase	:0.1% TEA: Methanol: Acetonitrile (40:30:30)
Flow rate	:1 ml/min
Wavelength	:235 nm
Injection volume	:20 µl
Run time	:10 min

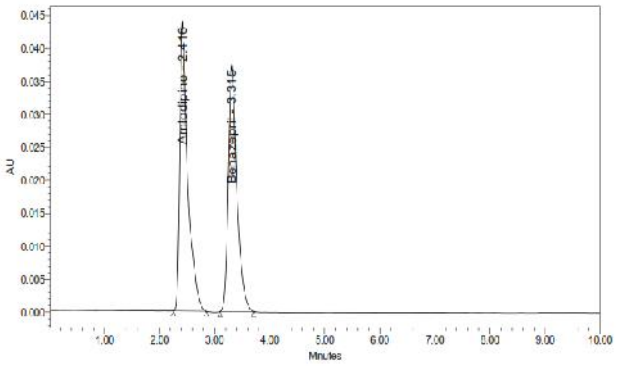

Fig 3: Optimized Chromatogram of Amlodipine and Benazepril

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Amlodipine and 40 mg of Benazepril working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample Solution Preparation:

Accurately weigh and transfer equalent to 20 mg of Amlodipine and 40 mg of Benazepril sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 0.1% TEA buffer:
Take 1ml of TEA in 1000ml volumetric flask make up with HPLC grade water, final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.

Mobile Phase Optimization:
Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer, Acetonitrile: methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to 0.1% Tryethyl amine buffer: Methanol: Acetonitrile in proportion 40: 30: 30 v/v respectively.

Diluents Preparation:
The Mobile phase was used as the diluents.

3. Results and Discussions

Method Development

RP- HPLC method was developed by considering the system suitability parameters i.e. resolution between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Amlodipine at 1.53 min and Benazepil at 1.40 min. The total run time is 10 minutes with all system suitability parameters as ideal for the mixture of standard solutions.

Tailing factor for the peaks due to Amlodipine and Benazepril in Standard solution should not be more than 2.0. Theoretical plates for the Amlodipine and Benazepril peaks in Standard solution should not be less than 2000. Resolution for the Amlodipine and Benazepril peaks in standard solution should not be less than 2.

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The RP-HPLC method developed was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The method was validated for the parameters in terms of system suitability, selectivity, linearity, accuracy, precision, ruggedness, and robustness, limit of detection (LOD) and limit of quantization (LOQ).

Specificity:
For Specificity Blank and Standard are injected into system. There is no any interference of any peak in blank with the retention time of the analytical peaks.

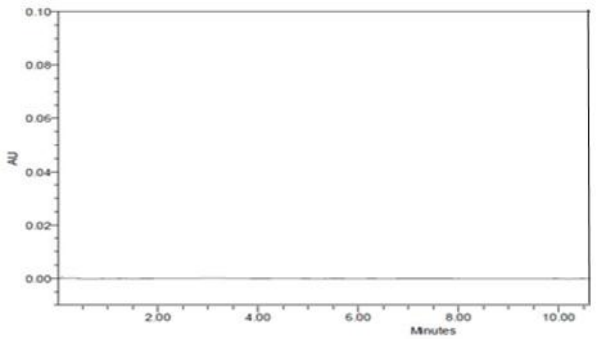

Fig 4: Chromatogram for Blank

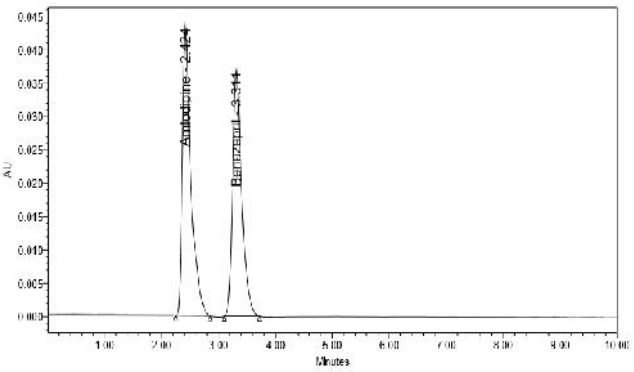

Fig 5: Chromatogram for Standard

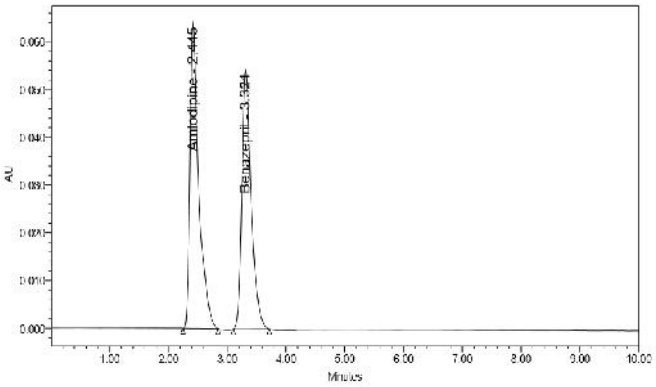

Fig 6: Chromatogram for Sample

The specificity test was performed for Amlodipine and Benazepril. It was found that there was no interference of impurities in retention time of analytical peak.

Accuracy:
The accuracy study was performed for 50%, 100% and 150 % for Amlodipine and Benazepril. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The results are tabulated in Table. No 4 &5.

Precision :

- Repeatability
- Intermediate Precision/ Ruggedness

Repeatability:
The precision study was performed for five injections of Amlodipine and Benazepril. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD. The results are tabulated in Table no 6.

Intermediate Precision/Ruggedness:
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 thespecified limits.The results are tabulated in Table no 7.

Linearity:
The linearity study was performed for the concentration of 20 ppm to 100 ppm Amlodipine and 12.5ppm to 62.5ppm Benazepril. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in table no-8& 9.

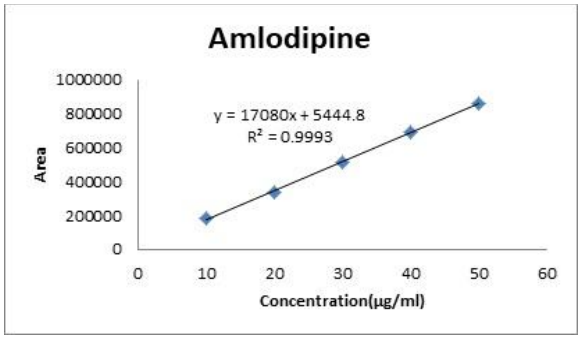

Fig 7: Linearity graph of Amlodipine

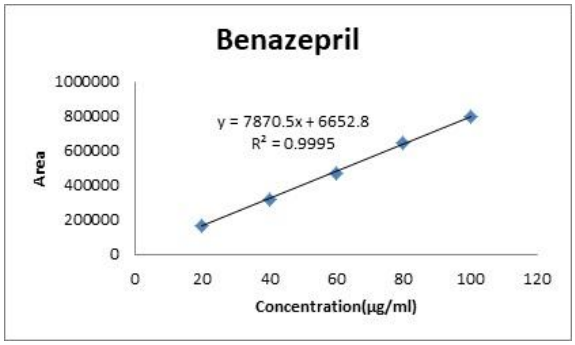

Fig 8: Linearity graph of Benazepril

The linearity study was performed for concentration range of 20µg -100µg Amlodipine and 12.5µg – 62.5 µg Benazepril and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

Limit of Detection:

The detection limit of Amlodipine was found to be 3.03

The detection limit of Benazepril was found to be 2.98

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution

Limit of Quantification: The quantification limit of Amlodipine was found to be 10.02

The quantification limit of Benazepril was found to be 9.98

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 30 ppm of Amlodipine& 60 ppm of Benazepril was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from ±10%.

Standard solution 30 ppm of Amlodipine & 60 ppm of Benazepril was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.On evaluation of the above results, it can be concluded that the variation in 10%.Organic composition in the mobile phase affected the method significantly. Hence it Indicates that the method is robust even by change in the Mobile phase ±10.

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Amlodipine and Benazepril using the proposed method.

Preparation of stock:

Accurately weigh and transfer 20 mg of Amlodipine and 40 mg of Benazepril working standard into a 10 ml clean dry volumetric flask 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)

Hydrolytic degradation under acidic condition:

Pipette 1.5 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluents. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition:

Pipette 1.5 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation:

Amlodipine and Benazeprile sample was taken in petridish and kept in Hot air oven at 110^0 C fo 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation:

Pipette 1.5 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 1.5 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Table 3: System Suitability Results for Amlodipine and Benazepril

S.No	Sample Name	Ret. Time	Area	Theoretical Plates	Tailing/Fronting Factor
1.	Amlodipine	2.416	505111	3459.86	1.56
2.	Benazepril	3.315	472665	5117.08	1.40

Table 4: Accuracy Results for Amlodipine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	253566.3	10	10	99.95	99.92
100%	510664	20	20.13	100.65	
150%	754661.7	30	29.75	99.16	

Table 5: Accuracy Results for Benazepril

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	237545	20	19.96	99.79	100.01
100%	476053	40	40	99.99	
150%	715902	60	60.15	100.25	

Table 6: Summarized Results for Amlodipine and Benazepril

Injection	Area for Amlodipine	Area for Benazepril
Injection-1	505591	470021
Injection-2	503459	473537
Injection-3	507867	476185
Injection-4	503326	470009
Injection-5	504599	475679
Injection-6	505716	479198
Average	505093.0	474104.8
Standard Deviation	1695.1	3647.5
%RSD	0.3	0.8

Table 7: Intermediate precision results for Amlodipine and Benazepril

Injection	Area for Amlodipine	Area for Benazepril
Injection-1	505591	470021
Injection-2	503459	473537
Injection-3	504693	477253
Injection-4	506230	476197
Injection-5	504599	479198
Injection-6	507803	474597
Average	505395.8	475133.8
Standard Deviation	1510.5	3197.4
%RSD	0.3	0.7

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

Table 8: Linearity Results for Amlodipine

S. No	Linearity Level	Concentration	Area
1	I	10	184810
2	II	20	339375
3	III	30	510488
4	IV	40	692083
5	V	50	862450
Correlation Coefficient			0.999

Table 9: Linearity Results: (for Benazepril)

S. No	Linearity Level	Concentration	Area
1	I	20	168693
2	II	40	318093
3	III	60	471391
4	IV	80	642911
5	V	100	793338
Correlation Coefficient			0.999

Table 10: Robustness results for Amlodipine and Benazepril

S. No	Flow Rate (ml/min)	System Suitability Results				USP Resolution
		Amlodipine		Benazepril		
		USP Tailing	USP Plate Count	USP Plate Count	USP Tailing	
1	0.9	1.52	3374.87	5100.55	1.44	3.01
2	1	1.53	3459.86	5117.08	1.40	3.14
3	1.1	1.57	3430.34	5150.94	1.37	3.04

Table 11: Robustness results for Amlodipine and Benazepril

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results				USP Resolution
		Amlodipine		Benazepril		
		USP Tailing	USP Plate Count	USP Plate Count	USP Tailing	
1	10% less	1.52	3422.22	5659.98	1.44	3.21
2	*Actual	1.53	3459.86	5117.08	1.40	3.14
3	10% more	1.57	3242.86	5365.28	1.37	2.66

* Results for actual Mobile phase composition (40:30:30) 0.1% Try ethyl amine buffer: Methanol: Acetonitrile has been considered from Accuracy stand.

Table 12: Degradation Results

Sample Name	Amlodipine		Benazepril	
	Area	% Degraded	Area	% Degraded
Standard	506355.0		475131	
Acid	487362	3.75	456373	3.95
Base	478637	5.47	446563	6.01
Peroxide	481627	4.88	451837	4.90
Thermal	472882	6.61	449377	5.42
Photo	479898	5.22	441345	7.11

4. Conclusion

The proposed RP-HPLC (Reverse phase High Performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the concentration range. The analytical method validation of Amlodipine & Benazepril by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Amlodipine & Benazepril. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness, therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

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