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AN ASSAY METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND BENAZEPRIL IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A short selective, precise, accurate and sensitive for the estimation of Benazepril and Amlodipine was done by RP-HPLC. The assay of Benazepril and Amlodipine was performed with tablets and the % assay was found to be 100.08 and 100.66 which shows that the method is useful for routine analysis. The linearity of Benazepril and Amlodipine was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.9 for Benazepril and Amlodipine which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.5 and 0.7 for Benazepril and Amlodipine which shows that the method is repeatable when performed in different days also.

KEYWORDS: Benazepril and Amlodipine, Validation, stability indicating method, degradation products.

INTRODUCTION

Benazepril,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 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.

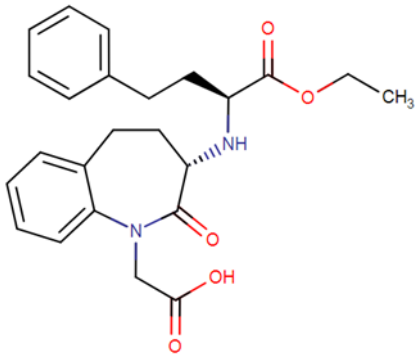

Fig. 1: Benazepril, 2-[(3S)-3-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin -1-yl]acetic acid

Amlodipine, 3-ethyl 5-methyl 2-[(2-aminoethoxy) me thyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine -3,5-dicarboxylate 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.

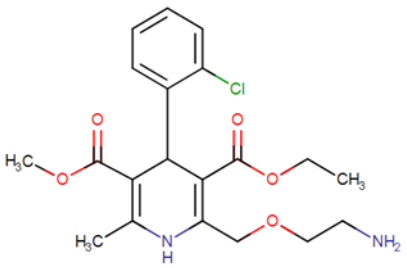

Fig. 2 Amlodipine, 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

Experimental

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Inertsil ODSC18 (4.6×150mm 5µm)
Buffer	:	Phosphate buffer pH 4.8
pH	:	3.0
Mobile phase	:	Buffer: Acetonitrile (35:65)
Flow rate	:	0.8 ml per min
Wavelength	:	242 nm
Injection volume	:	20 µl
Run time	:	10 min.

Preparation of buffer and mobile phase

Preparation of phosphate buffer pH 4.8

Weigh accurately 6.8gms of potassium di hydrogen orthophosphate dissolved in 1000 ml of HPLC water Ph was adjusted up to 3.0. Final solution was filtered through 0.44 µm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 350 ml (35%) of above buffer and 650 ml of Acetonitrile HPLC (65%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of the benazepril & amlodipine standard & sample solution

Standard Solution Preparation

Accurately weigh and transfer 40 mg of Benazepril and 20 mg of Amlodipine 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 diluent.

Sample Solution Preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 40 mg of Benazepril and 20 mg Amlodipine sample into a 100 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 15 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.45 micron Injection filter. (Stock solution).

Further pipette 1.5ml of Benazepril and Amlodipine from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Benazepril and Amlodipine peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSION

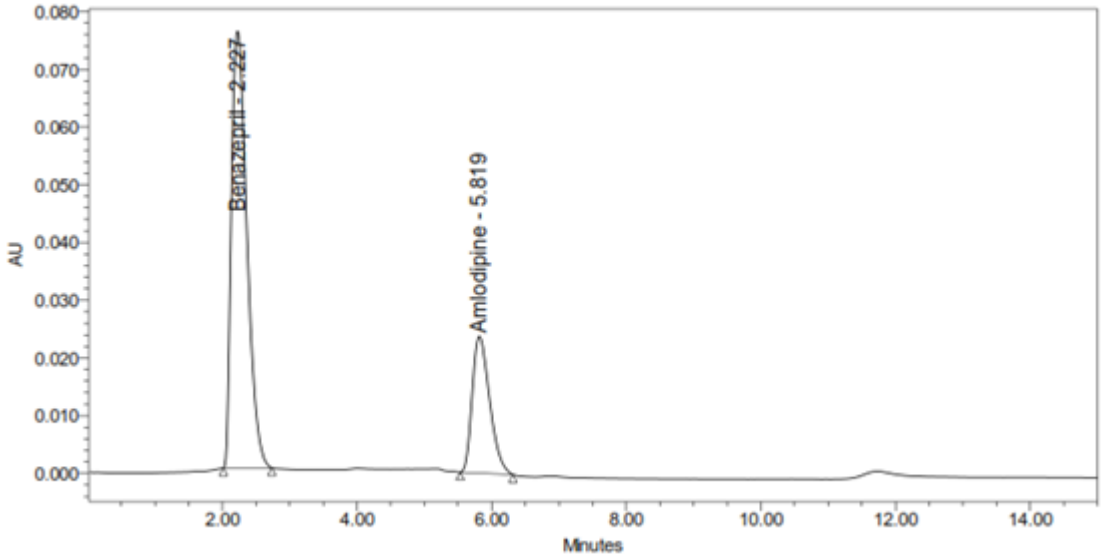

Fig. 3: Standard Chromatogram of Benazepril and Amlodipine.

Table 1: Results of system suitability parameters.

S. No.	Name	RT(min)	Area (µVsec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Benazepril	2.227	121623	75723		1.54	3765.98
2	Amlodipine	5.819	42389	23721	7.98	1.38	5742.70

Table 2: Results of Linearity of Benazepril and Amlodipine.

S. No.	Benazepril		Amlodipine	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	20	42486	10	14430
2	40	82149	20	29766
3	60	124321	30	43753
4	80	161418	40	57246
5	100	201924	50	72471

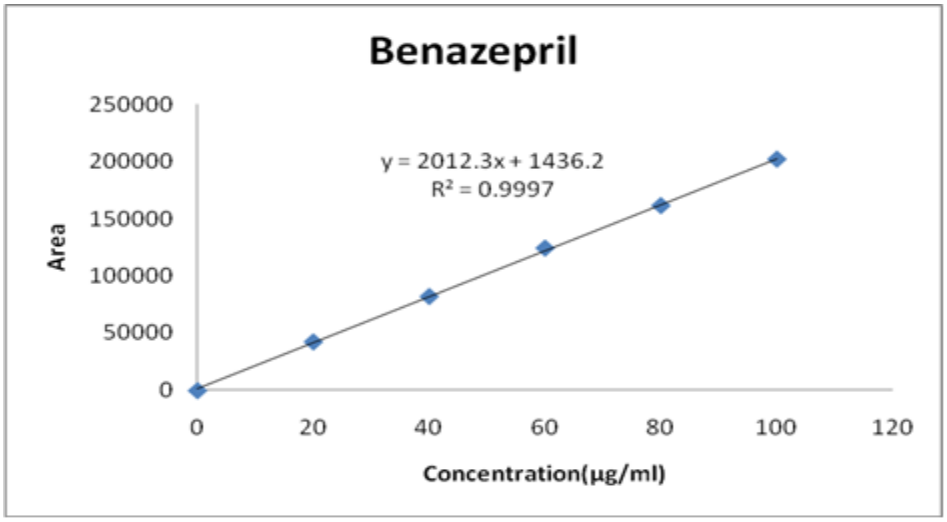

Figure 4: Calibration graph for Benazepril.

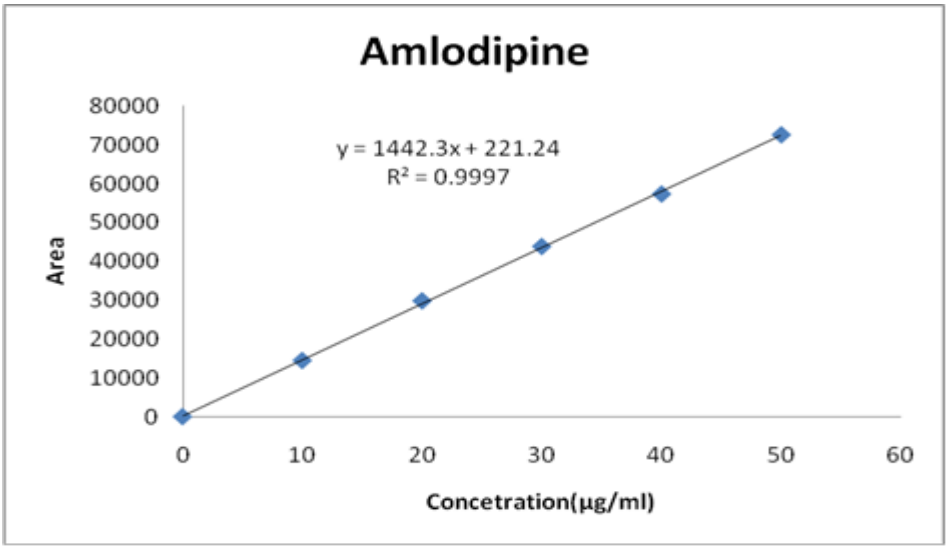

Figure 5: Calibration graph for Amlodipine.

Table 3: Results of Precision for Benazepril.

Injection	Area
Injection-1	123578
Injection-2	122050
Injection-3	123931
Injection-4	121272
Injection-5	123137
Injection-6	122702
Average	122778.3
Standard Deviation	990.2
%RSD	0.8

Table 4: Results of Precision for Amlodipine.

Injection	Area
Injection-1	43604
Injection-2	43572
Injection-3	43902
Injection-4	43587
Injection-5	44286
Injection-6	44447
Average	43899.7
Standard Deviation	385.1
%RSD	0.9

Table 5: Accuracy (recovery) data for Benazepril.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	60984.7	20	20.04	100.21	100.42
100%	122049.7	40	40.11	100.28	
150%	184002.3	60	60.47	100.78	

*Average of three determinations

Table 6: Accuracy (recovery) data for Amlodipine.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	21510	10	10.07	100.69	100.64
100%	43071.7	20	20.16	100.81	
150%	64352.7	30	30.12	100.41	

Table 7: Results of LOD.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Benazepril	54	164	3.04
Amlodipine	54	162	3.00

Table 8: Results of LOQ.

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Benazepril	54	541	10.02
Amlodipine	54	543	10.06

Degradation Studies

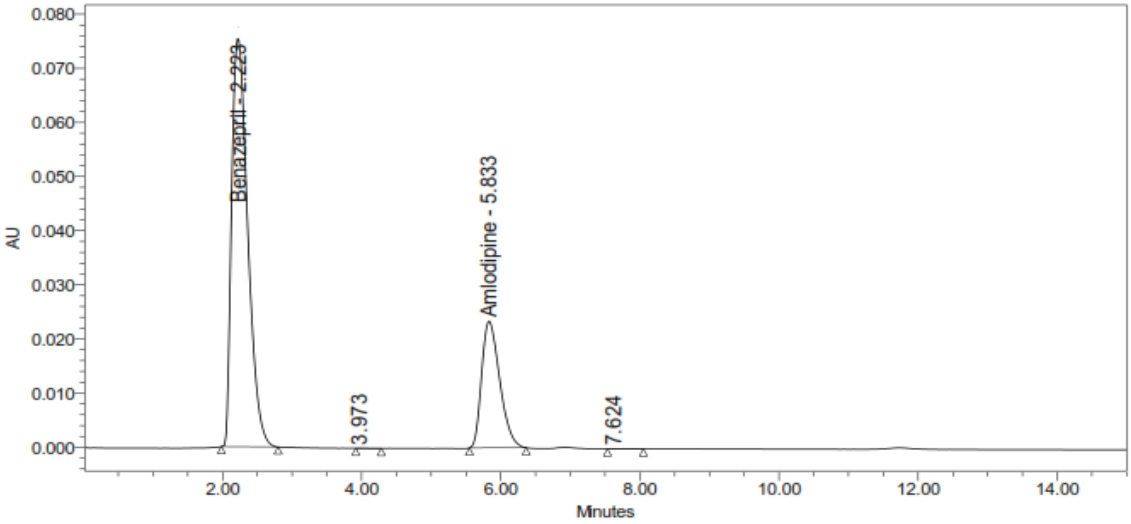

Figure 6: Chromatogram showing Acid degradation.

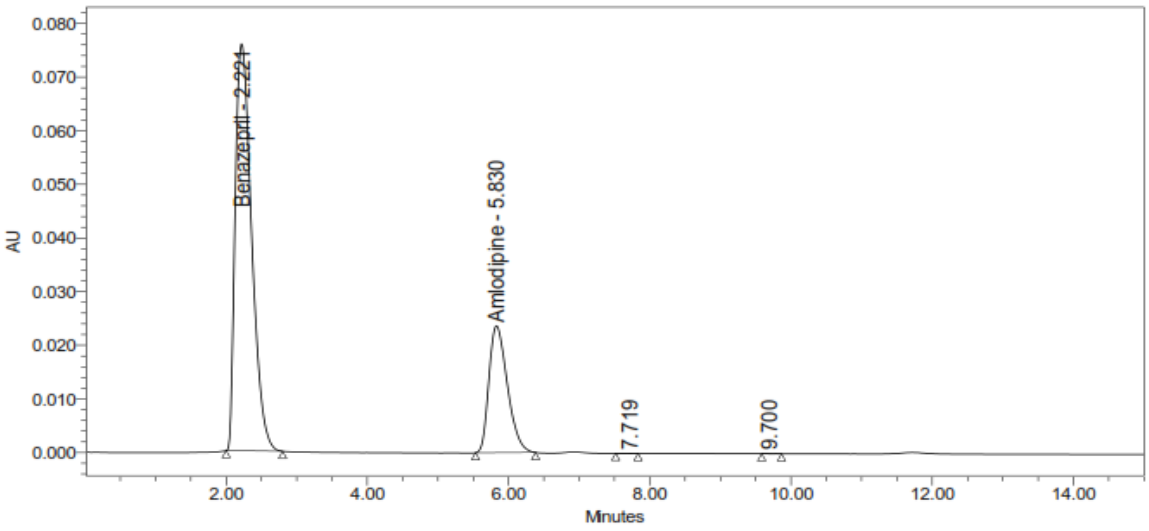

Figure 7: Chromatogram showing Base degradation.

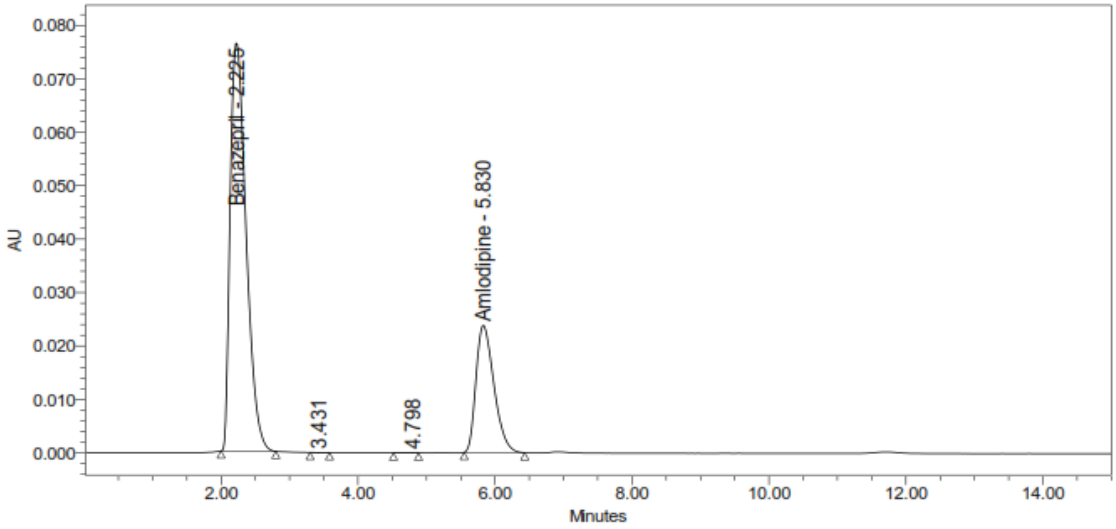

Figure 8: Chromatogram showing Peroxide degradation.

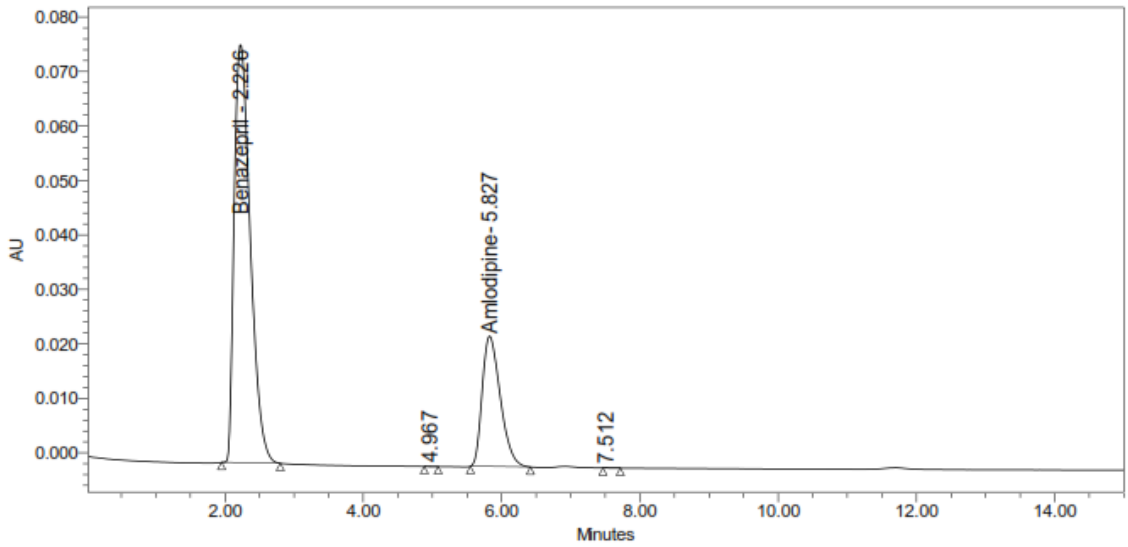

Figure 9: Chromatogram showing Thermal degradation.

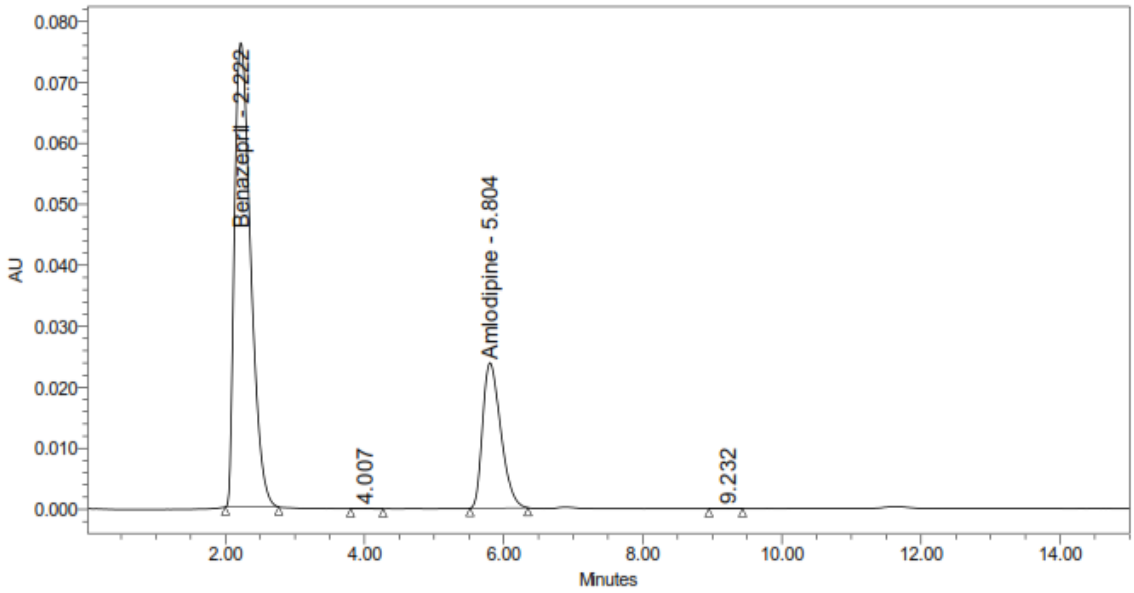

Figure 10: Chromatogram showing Photo degradation.

Table 9: Results for Stability of Benazepril and Amlodipine.

Sample Name	Benazepril		Amlodipine	
	Area	% Degraded	Area	% Degraded
Standard	121403		42673	
Acid	119736	1.37	41662	2.37
Base	117533	3.19	41776	2.10
Peroxide	113863	6.21	40623	4.80
Thermal	116563	3.99	40572	4.92
Photo	115552	4.82	39772	6.80

Table 10: Results of Assay for Benazepril and Amlodipine.

	Label Claim (mg)	% Assay
Benazepril	20	100.08
Amlodipine	10	100.66

SUMMARY AND CONCLUSION

The estimation of Benazepril and Amlodipine was done by RP-HPLC.

The assay of Benazepril and Amlodipine was performed with tablets and the % assay was found to be 100.08 and 100.66 which shows that the method is useful for routine analysis.

The linearity of Benazepril and Amlodipine was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.9 for Benazepril and Amlodipine which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.5 and 0.7 for Benazepril and Amlodipine which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.42% and 100.64% for Benazepril and Amlodipine. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ are 3.00 and 10.00. The LOD and LOQ for Benazepril was found to be 3.04 and 10.02 and LOD and LOQ for Amlodipine was found to be 3.00 and 10.06.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits. Which shows that the method is having good system suitability and precision under given set of conditions.

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