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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ALISKIREN, AMLODIPINE AND HYDROCHLORTHIAZIDE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, and precise RP-High Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Aliskiren (ALS), Amlodipine (AML) and Hydrochlorothiazide (HCTZ) combined dosage form has been developed and validated. The components were well separated using Hypersil BDS, 250 x 4.6 mm, 5µ column using Acetonitrile:1ml TEA in 1000 potassium phosphate buffer 0.01M (40:60% v/v) as mobile phase at a flow rate of 1.0 mL/min. The eluents were detected at 228 nm using UV detector. The retention time of HCTZ 3.3 min, ALS was found to be 5.9 min and that of AML was 8.0 min. The linearity was observed between 6.25-37.5µg/mL, 75-450µg/mL and 2.5-15µg/mL for HCTZ, ALS and AML respectively. The marketed dosage form was analysed by using the developed method. The mean recoveries were 100±2% for three compounds. The method was validated for system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. The developed method was used for the stability studies (short, long and auto sampler) and forced degradation studies (acidic, alkaline, oxidative and photolytic). This validated method can be used for the routine quality control testing of HCTZ, ALS and AML combined dosage form.

Keywords: Aliskiren, Amlodipine and Hydrochlorothiazide, HPLC, Tablet formulation

INTRODUCTION

Aliskiren Hemifumarate (ALN), (2S, 4S, 5S, 7S) N-(2-carbamoyl-2-methylpropyl)-5-amino-4hydroxy-2, 7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate is a Rennin inhibitor. Anti- hypertensive Agent**.** Various analytical methods have been reported for the assay of ALN alone or in combination with other anti - hypertensive agents in pharmaceutical formulations. They include UV spectroscopy[2,4] , high performance liquid chromatography[1,2], high performance thin layer chromatography[3,4], LC MS[5] and LC - MS/ MS[6].

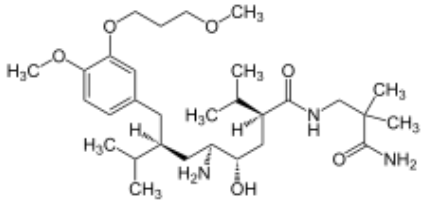

Fig. 1: The Chemical Structures of Aliskiren Hemifumarate

Amlodipine besylate (AMB), 2-[(2- amino ethoxy) - methyl] - 4 - (2 – chloro phenyl) -1, 4 -dihydro 6- methyl- 3, 5- pyridine dicarboxylic acid 3 ethyl- 5- methyl ester, benzene sulfonate, is a potent dihydro calcium channel blocker. Various analytical methods have been reported for the assay of AMB alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV spectroscopy2-4, high performance liquid chromatography [5,8], high performance thin layer chromatography[3,4] LC MS[5] and LC - MS/ MS[6].

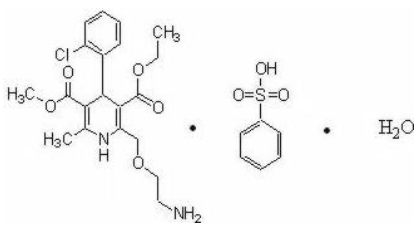

Fig. 2: Amlodipine Besylate

Hydrochlorothiazide (HCT), 6 - chloro - 3, 4 dihydro - 7 - sulfamoyl - 2H - 1, 2, 4 - benzothia diazine - 1, 1 – dioxide, is a thiazide diuretic. It increases sodium and chloride excretion in distilled convoluted tubule. Many analytical methods for HCT alone and in combination with other drugs by stability indicating method, RPHPLC methods, spectro photometric methods and in plasma.

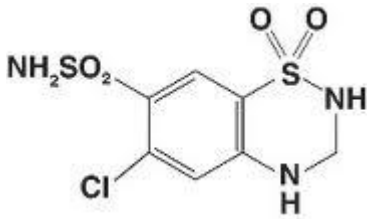

Fig. 3: Hydrochlorothiazide

All the three drugs are official in USP[7]. Amlodipine besylate and Hydrochlorothiazide are official in IP[8] and BP[9]. Literature survey revealed that there are several methods were reported for the estimation of ALN, AMB and HCT individually as well as in combination with some other drugs. As no method is available for their simultaneous determination , however, it is essential to develop a suitable analytical method for simultaneous estimation of ALN, AMB and HCT in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, precise, accurate and specific RP-HPLC method for the simultaneous determination of ALN, AMB and HCT in bulk and in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference in Harmonization (ICH)[10] guidelines, by assessing its selectivity, linearity, accuracy, and precision, limit of detection and limit of quantification.

MATERIALS AND METHODS

Reagents and chemicals

Aliskarin, Amlodipine and Hydrochlorothiazide combined tablets were procured from the market. HPLC grade acetonitrile (S.D. Fine

Chemicals, Ahmedabad, India) methanol and water (Finar chemicals Ltd., Ahmedabad, India), ortho phosphoric acid (Spectrochem Pvt Ltd., Mumbai, India) and nylon filter (Millipore Pvt., Ltd, Bangalore, India) were used for study.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a Waters HPLC instrument PDA detector, Stationary Phase used was Hypersil BDS, 250 x 4.6 mm, 5µ columnparticle size and mobile phase consisting of Buffer, 1ml TEA-acetonitrile (60: 40, v/v; pH adjusted to 3.1 ± 0.02 with ortho phosphoric acid) was used. The flow rate was 1.0 ml/min and the effluents were monitored at 228 nm. The mobile phase was filtered through nylon 0.45 µm membrane filter (Millipore Pvt., Ltd, Bangalore, India). Injection volume was 10 µL. All weighing were done on analytical balance.

Preparation of mobile phase

The mobile phase was prepared withAccurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000mlof Volumetric flask added about 900ml of milli-Q water and degas to sonicated and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.1 with dil. Orthophosphoric acid solution. Buffer and Acetonitrile taken in the ratio 60:40.The mobile phase was degassed for 15 minutes before use.

Preparation of Standard Solutions

Accurately Weighed and transferred 25mg,5mg&12.5mg of Aliskiren and Amlodipine and Hydrochlorothiazide working Standards into a 10 ml and 25ml and 25ml clean dry volumetric flask, add 7ml and 15ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents..From the above stock solution,1.2 ml ,0.5ml &0.5ml was Pippeted out in to a 10ml Volumetric flask and made up to the final volumewith diluent to obtain a working standard solution of 300µg/ml Aliskiren& 10µg/ml Amlodipine&25µg/ml HCTZ .

Preparation of Sample Solutions

5 tablets were weigh and calculated the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 500 mL volumetric flask, 260mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was Pippeted out into a 10 ml volumetric flask and made up to 10ml with diluent. The solution was filtered through 0.45µm-47mm membrane filter.

RESULTS AND DISCUSSION

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

To evaluate the linearity of the method, six different dilutions were made from the standard stock solutions in the working range of 6.25-37.5µg/mL,75-450µg/mL and 2.5-15µg/mL for Hydrochlorothiazide, Aliskiren and Amlodipine respectively.

In order to determine the accuracy of the method, three different concentrations (50%, 100% and 150%) of tablet formulation were used and their recovery was calculated. Regarding the determination of the precision (repeatability) five replicate injections of the working standard Hydrochlorothiazide, Aliskiren and Amlodipine were injected and the relative standard deviation (RSD) of the peak areas were calculated for the replicate injections. To determine the LOD and LOQ, serial dilutions of the combination were made from the standard stock solution the signal from the samples was compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio (S/N) of 3:1 and 10:1 respectively.

Linearity and Range

The calibration curve was plotted over the concentration range of 6.25-37.5µg/mL,75-450µg/mL and 2.5-15µg/mL for Hydrochlorothiazide, Aliskiren and Amlodipine respectively. Dilutions and final concentration were shown in table 1, 2&3. Each of this drug solution (10 µL) was injected under the operating chromatographic conditions as described above. The goodness-of-fit (R^2) was found to be 0.9999 indicating functional linear relationship between.

Table 1: Linearity values of Hydrochlorothiazide by RP- HPLC method

Concentration (µg/ml)	Peak area
0	0
6.25	151468
12.5	312157
18.75	472499
25	625272
31.25	785236
37.5	948502

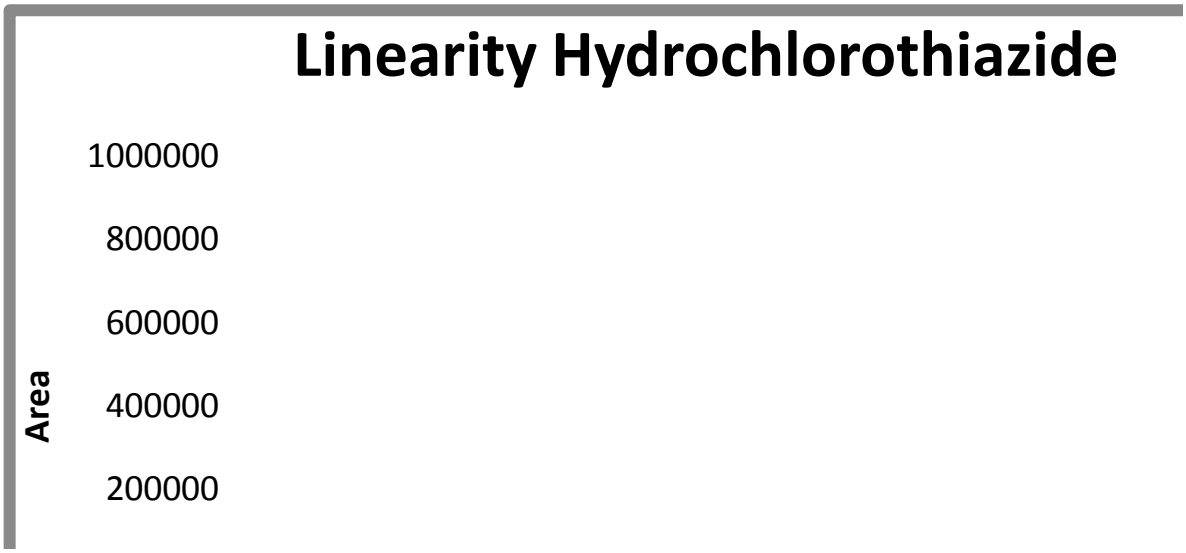

Fig. 3: Linearity Curve of Hydrochlorothiazide

Table 2: Linearity values of Amlodipine by RP-HPLC method

Concentration (µg/ml)	**Peak area**
0	0
2.5	67403
5	131048
7.5	201909
10	265406
12.5	332902
15	406942
Concentration (µg/ml)	**Peak area**
0	0
75	210216
150	433783
225	632681
300	858034
375	1063917
450	1288703

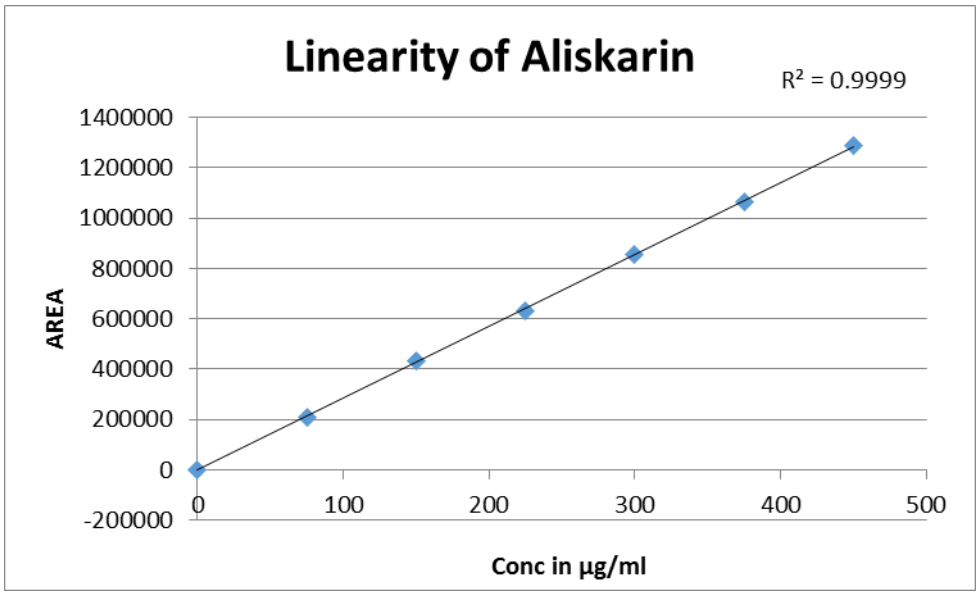

Fig. 5: Linearity Curve of Aliskiren

Accuracy

Accuracy was determined by recovery studies of Aliskiren, Amlodipine and HCTZ, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 4, 5 & 6. The study was done at three different concentration levels.

Precision

The precision (repeatability) of an analytical method refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the %RSD. The precision study (**Table 7**) showed that method has a good reproducibility which was approved by the analysis of five replicate injections of the working standard solution.

Table 4: Accuracy of Drug Product Data for Hydrochlorothiazide

Conc. %	**Conc. HCT (mg/ml)**	**% Accuracy (Recovery)**	**Average**
50%			
50%	12.55	100.4	101
50%	12.69	101.6	
50%	12.64	101.1	
100%			
100%	24.95	99.8	100.3
100%	25.35	101.4	
100%	24.95	99.8	
150%			
150%	37.58	100.2	99.6
150%	37.22	99.3	
150%	37.25	99.3	

Table 5: Accuracy of Drug Product Data for Amlodipine

Conc. %	Conc. HCT (mg/ml)	% Accuracy (Recovery)	Average
50%			
50%	5.04	100.9	101.2
50%	5.06	101.3	
50%	5.07	101.4	
100%			
100%	10.02	100.2	99.8
100%	9.93	99.3	
100%	9.99	99.9	
150%			
150%	15.07	100.5	100.4
150%	15.12	100.8	
150%	15	100	

Table 6: Accuracy of Drug Product Data for Aliskiren

Conc. %	Conc. HCT (mg/ml)	% Accuracy (Recovery)	Average
50%			
50%	149.94	99.96	100.4
50%	149.22	99.48	
50%	152.53	101.68	
100%			
100%	302.09	100.70	99.9
100%	299.59	99.86	
100%	297.02	99.01	
150%			
150%	454.06	100.90	99.8
150%	446.63	99.25	
150%	446.25	99.17	

Detection and quantification limit (LOD &LOQ)

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be taken as LOQ of the. Our method showed the (LOD) for Aliskiren, Amlodipine and HCTZ were found to be 0.255µg/ml, 0.003µg/ml and 0.075µg/ml, respectively and The LOQ values for Aliskiren, Amlodipine and HCTZ were found 0.772 µg/ml, 0.012µg/ml and 0.224 µg/ml respectively.

System suitability testing

System suitability is used to verify that the system is adequate for the analysis to be performed. Our method shows all the values for the system suitability parameters are within limits .The column efficiency is about 7090, 6611 and 6807 theoretical plates for Aliskiren, Amlodipine and HCTZ, respectively. The tailing factors are about 1.4, 1.1 and 1.2 for Aliskiren, Amlodipine and HCTZ respectively.

Robustness

The robustness of the proposed method was evaluated by slight modification in the organic composition and pH values of aqueous phase of the mobile phase and flow rate. During these studies it was found that there was not much change retention time, area and symmetry of peak. The developed method was used for the assay of commercially available tablets and six replicate determinations were performed. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from excipients.

Specificity and Stability indicating studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Intentional degradation was carried out by exposing of samples to stability condition 1 N HCl, at 60 ^{0}C, 1 N NaOH, at 60 ^{0}C ,20% H_2O_2 at 60 ^{0}C , water at 60°C for 30min and UV. They were then analysed against control.

Table 7: Precision of Standard Injection Data:

Injection No.	Nominal Concentration		
	HCTZ	**AMLODIPINE**	**ALISKIREN**
I	649713	276501	746692
II	653945	279801	747660
III	643719	273497	734936
IV	652388	275416	735813
V	645243	277154	741465
VI	652868	274666	743190
Average	649646	276172.5	741626
SD	4264	2202	5350
RSD	0.70%	0.80%	0.70%

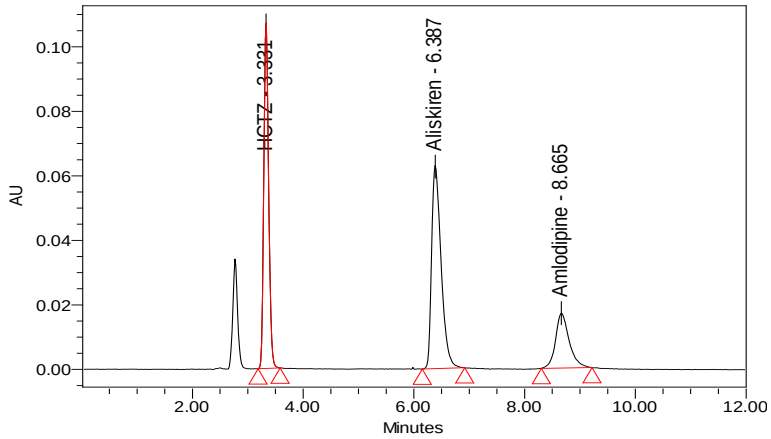

Fig. 6: Standard chromatogram for HCTZ, Aliskarin and Amlodipine Combined Dosage form

Table 8: Specificity and stability indicating study

Stress Condition	Aliskarin			Amlodipine			HCTZ		
	% Degradation	Purity angle	Purity Threshold	% Found	Purity angle	Purity Threshold	% Degradation	Purity angle	Purity Threshold
0.1 N HCL at 60°C for 30 Min	11.4	0.718	2.57	11.9	2.418	2.758	10.4	0.477	1.462
0.1 N NaOH at 60°C for 30 Min	6.5	0.28	0.464	7.9	1.123	1.649	7.7	0.418	0.649
3% H2O2 at 60°C for 30 Min	6.4	0.299	0.498	7.7	0.596	0.889	6.4	0.186	0.395
Heat at 60°C	4.6	0.305	0.49	5.7	0.737	0.888	9.6	0.437	0.485
Water at 60°C for 30 Min	2.4	0.28	0.474	1.3	0.795	0.993	2.2	0.447	1.125
Photolysis	2.2	0.234	0.436	3.3	0.886	1.102	2.5	0.25	0.422

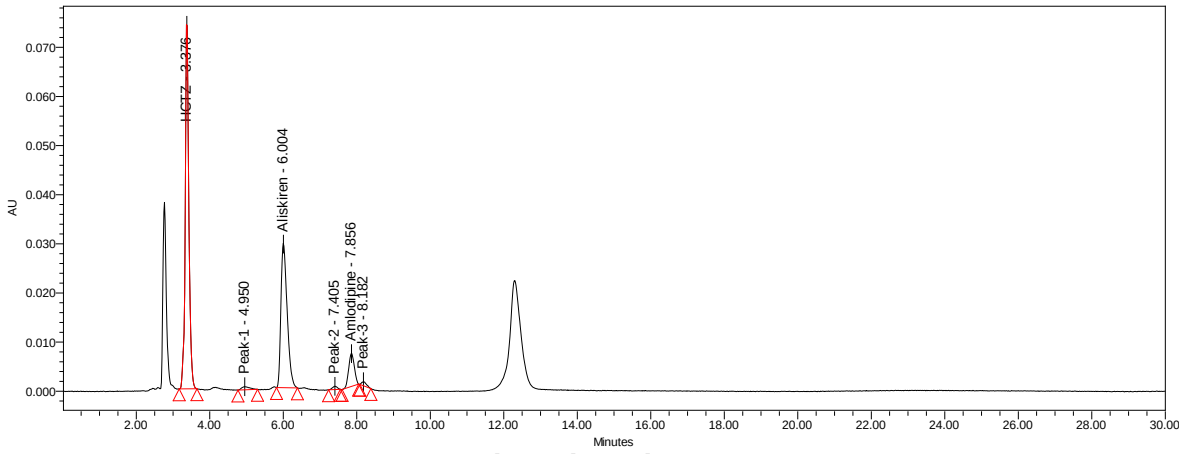

Fig. 7: Acid Degradation Chromatogram

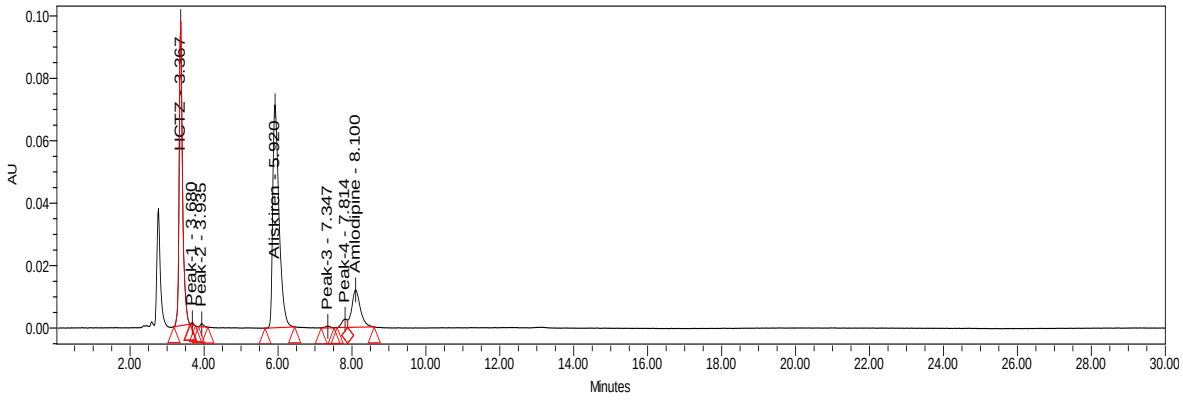

Fig. 8: Base Degradation Chromatogram

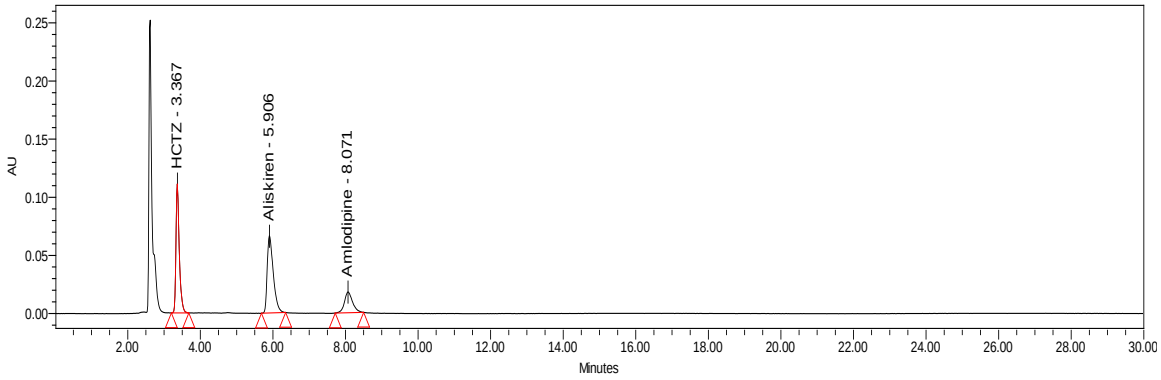

Fig. 9: Peroxide Degradation Chromatogram

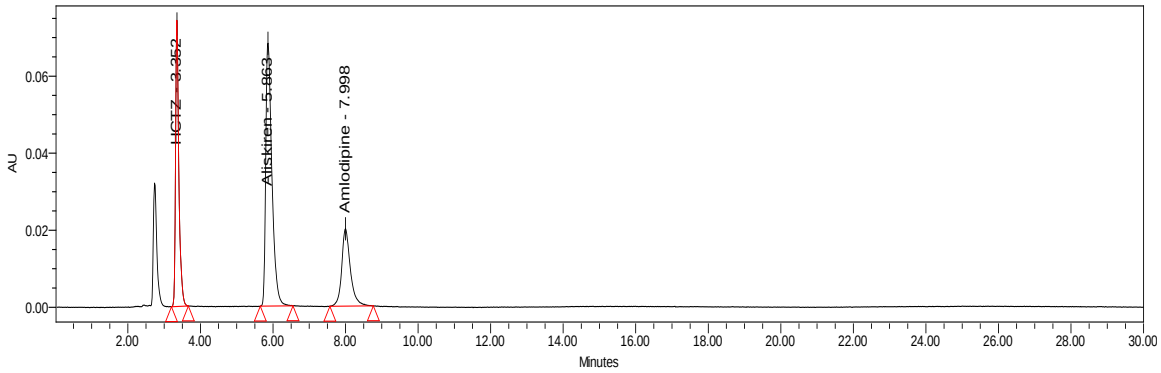

Fig. 10: Thermal Degradation Chromatogram

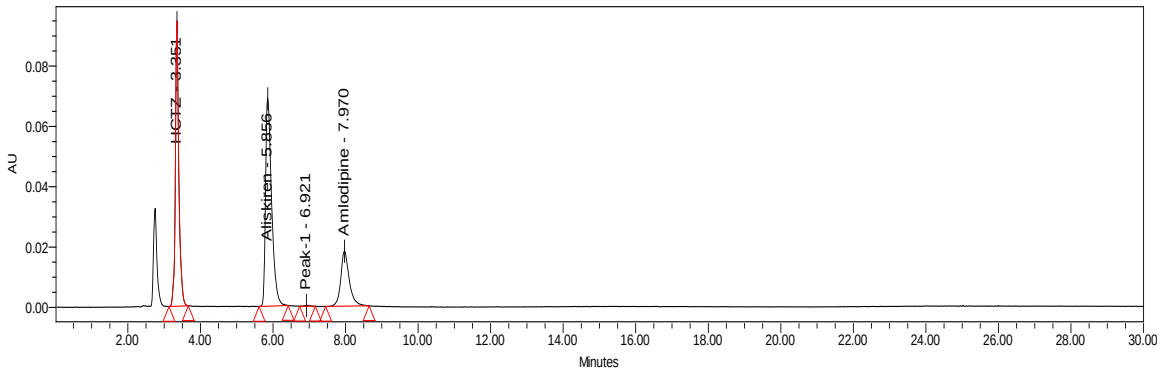

Fig. 11: Water Degradation Chromatogram

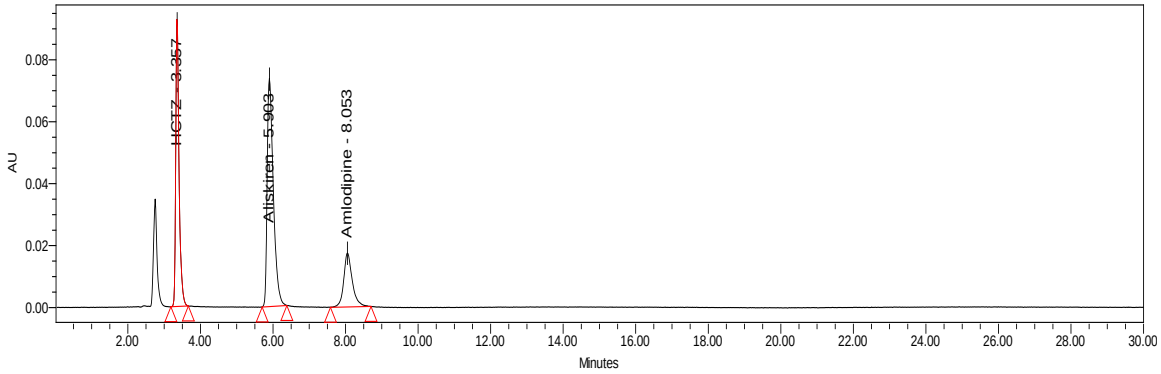

Fig. 12: Photolytic Degradation Chromatogram

CONCLUSION

The stability indicating HPLC method was successfully developed and it showed several advantages over other known methods for the analysis of these agents since it is an economical, well resolved peaks in single method that can be used for assay of the three active ingredients. The method was validated in accordance to the ICH guidelines shown linearity, accuracy, precision, selectivity, stability and system suitability. The method can also be used for purity and degradation evaluation.

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