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SIMULTANEOUS ESTIMATION AND FORCED DEGRADATION STUDIES OF AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN A PHARMACEUTICAL DOSAGE FORM USING RP-HPLC METHOD

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ABSTRACT

The present study describes the stability indicating RP-HPLC method for simultaneous estimation of Amiloride hydrochloride and Hydrochlorothiazide in pharmaceutical dosage forms. The proposed RP-HPLC method was developed by using Shimadzu prominence-i LC-2030 HPLC system equipped with UV detector and chromatographic separation was carried on Shim-pack GIST C18 (250 × 4.6 mm, 5 µ) column at a flow rate of 1mL/min and the run time was 5 min. The mobile phase consisted of water and acetonitrile in the ratio of 50:50% v/v and eluents were scanned using UV detector at 285 nm. The retention time of Amiloride hydrochloride and Hydrochlorothiazide was found to be 2.06 and 3.12 min, respectively. A linearity response was observed in the concentration range of 12–28 µg/mL for Amiloride hydrochloride and 120–280 µg/mL for Hydrochlorothiazide, respectively. Limit of detection and limit of quantification for Amiloride hydrochloride were 0.52µg/mL and 1.57µg/mL and for Hydrochlorothiazide are 0.708µg/mL and 2.14µg/mL, respectively. The stability indicating method was developed by subjecting the drugs to stress conditions such as acid and base hydrolysis, oxidation, humidity and photo- and thermal degradation and the degraded products formed were resolved successfully from the samples.

KEYWORDS

Amiloride hydrochloride, Hydrochlorothiazide, RP-HPLC, Degradation and Validation.

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INTRODUCTION

Amiloride hydrochloride, an antikaliuretic-diuretic agent, is a pyrazine-carbonyl guanidine that is unrelated chemically to other known antikaliuretic or diuretic agents[1,2,12]. Chemically, Amiloride hydrochloride is 3, 5 - diamino - 6 -chloro- N - (diaminomethylene) pyrazine carboxamide mono hydrochloride, dihydrate[3,5] (Figure No.1). It is a pyrazine compound inhibiting sodium reabsorption

through sodium channels in renal epithelial cells. This inhibition creates a negative potential in the luminal membranes of principal cells, located in the distal convoluted tubule and collecting duct. Negative potential reduces secretion of potassium and hydrogen ions. Amiloride is used in conjunction with diuretics to spare potassium loss.

Hydrochlorothiazide is diuretic agent. Chemically Hydrochlorothiazide is 6-chloro-3, 4-dihydro-2*H*-1, 2, 4- benzothiadiazine-7-sulphonamide 1, 1 -dioxide and its structural formula as in[3,5] (Figure No.2). It is a thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipid us, and hypoparathyroidism[11,13].

Through literature survey reveals that few analytical methods such as RP-HPLC[2,8,9] and UV methods[4] are reported for simultaneous estimation of Amiloride hydrochloride and Hydrochlorothiazide in pharmaceutical dosage forms. However, so far there is no stability indicating method reported. Therefore, the present investigation was carried out to develop new, simple, precise, rapid, and cost-effective stability indicating RP-HPLC method for the simultaneous estimation of Amiloride hydrochloride and Hydrochlorothiazide in pharmaceutical dosage form. The proposed method was used successfully to separate the degraded products from the samples.

MATERIAL AND METHODS

Reagents and chemicals

Amiloride hydrochloride and Hydrochlorothiazide standards were provided by Alkem Laboratories, Navi Mumbai, Maharashtra, India and Torrent Pharmaceuticals ltd., Sikkim, Maharashtra, India and commercial tablet dosage form BIDURET was purchased from local market. The HPLC grade acetonitrile and water were purchased from Thomas Baker. Analytical grade orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine Chemicals.

HPLC Instrument

The chromatographic separation was carried out by Shimadzu prominence-i LC-2030 HPLC system equipped with UV detector and auto sampler. The Lab Solution software was used for signal monitoring and processing. UV chamber has been used for photolytic degradation and hot air oven was employed for thermal degradation.

Chromatographic Conditions

The chromatographic separation of analytes was carried out using Shimadzu RP-HPLC system with Shim-pack GIST C18 (250 × 4.6 mm, 5 µ) column. The mobile phase consists of water and acetonitrile in the ratio of 50:50% v/v and column temperature was maintained at 25°C. The analytes were detected at 285 nm using UV detector. The run time was set at 5 min at a flow rate of 1mL/min.

Preparation of Standard Stock Solution

Standard stock solutions of Amiloride hydrochloride and Hydrochlorothiazide were prepared separately by dissolving 100 mg of Amiloride hydrochloride and 100 mg of Hydrochlorothiazide in 100mL volumetric flasks with water : acetonitrile (50 : 50% v/v) as diluent and sonicated for 10 min. From the above solution 0.2mL of Amiloride hydrochloride and 2mL of Hydrochlorothiazide were transferred separately to 10mL volumetric flasks and 1 ml 10% OPA was added as a supporter and sonicated for 5 min, made up the volume with diluent to get 20µg/mL of Amiloride hydrochloride and 200µg/mL of Hydrochlorothiazide standard stock solution.

PREPARATION OF SAMPLE SOLUTION

Ten tablets (BIDURET tablets: 5 mg Amiloride hydrochloride and 50 mg Hydrochlorothiazide) were weighed and the average weight of each tablet was calculated; then the weight equivalent to 10 tablets was transferred into a 100 mL volumetric flask; 30 mL of diluent was added and sonicated for 25 min; further the volume was made up with diluent and filtered. From the filtered solution, 2 mL was pipetted out into a 10 mL volumetric flask and

1 ml 10% of OPA was added as a supporter and sonicated for 5 min, and volume made up to 10 mL with diluent.

FORCED DEGRADATION STUDIES

Forced degradation studies of the drug formulation were carried out by treating the drug samples under stress induced conditions like acid and base hydrolysis, oxidation, humidity and photo- and thermal degradation and interference of the degraded products was investigated. These studies help to know the inherent stability characteristic of the active molecules in drug product and the possible degradation products[1].

Acid Degradation

Acidic degradation was carried out by adding 5 ml of 1N Hcl and after 45 minutes neutralizing the mixture by adding 5ml of 1N NaOH.

Alkali Degradation

Alkali degradation was carried out by adding 5 ml of 1N Na OH and after 45 minutes
Neutralizing the mixture by adding 5ml of 1N Hcl.

Oxidative Degradation

Oxidative degradation was performed by exposing the drug to 5 ml of 3% (v/v) H_2O_2 for 45 minutes.

Photolytic Degradation

Photolytic degradation was carried out by exposing the drug content to UV light inside a UV chamber for 7 days.

Thermal Degradation

Thermal degradation was performed by placing the drug in an oven at 105°C for 6 h to study dry heat degradation.

Humidity Degradation

Humidity degradation was performed by placing the drugin desiccator at 25°C, 95%RH for 120 hours.

RESULTS AND DISCUSSION

Method Development

A series of trials was conducted with different columns like Inertsil ODS and SHIM-PACK C-18 column with different mobile phases to develop a suitable RP-HPLC method for estimation of Amiloride hydrochloride and Hydrochlorothiazide in tablet dosage form, and finally a typical chromatogram was obtained with water and acetonitrile in the ration of 50: 50% v/v. The chromatographic separation was performed on SHIM-PACK C-18 (250 × 4.6 mm, 5 µ) column by injecting 20µL and analytes were detected with UV detector at285 nm. The retention time of Amiloride hydrochloride and Hydrochlorothiazide was found to be 2.06 and 3.12 min, respectively. Forced degradation studies were also carried using the developed method and the degraded compounds were effectively resolved from the Amiloride hydrochloride and Hydrochlorothiazide in tablet dosage form. The optimized conditions were given in Table No.1.

Method Validation

The validation was performed with above developed RP-HPLC method for simultaneous estimation of Amiloride hydrochloride and Hydrochlorothiazide according to ICH guidelines. Various parameters were evaluated such as system suitability, precision, accuracy, linearity, robustness, LOD, and LOQ[6,7,9].

System Suitability

System suitability was performed to verify the acceptability of the resolution and repeatability of the system. System suitability was performed by injecting six replicate injections of the standard solution (100%) and parameters such as peak area, USP tailing, theoretical plates, retention time, and peak asymmetry were evaluated. The % RSD was determined and reported within the limits. The results were shown in Table No.2.

Accuracy

The accuracy of the proposed method was evaluated by calculating the recovery studies of the test drug at three different concentration levels (80%, 100%, and 120%) by standard addition method. A known amount of Amiloride hydrochloride and Hydrochlorothiazide was added to pre quantified sample solution and three replicates of each concentration were injected in developed chromatographic conditions. The mean percentage recovery of Amiloride Hydrochloride and Hydrochlorothiazide was varied between 99.9 and 101.7% indicating that the developed method was

found to be accurate. The percentage recovery results are shown in Table No.3.

Precision

The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The method precision and system precision studies were carried out by injecting six replicates of both standard and test solutions with the same concentration. The % RSD was calculated from the chromatograms and results obtained were within the limits of 2% and proposed method was found to be precise. The precision data was given in Table No.4.

Linearity

The linearity of the method was determined at different concentration levels ranging from 12 to 28µg/mL of Amiloride hydrochloride and from 120 to 280µg/mL of Hydrochlorothiazide. All the concentrations were prepared and injected into the system. The linearity curve was constructed by plotting peak area versus concentration of the analyte. From the results obtained the proposed method was found to be linear. The regression coefficient (r^2) was found to be 0.9997 and 0.9996 for Amiloride Hydrochloride and Hydrochlorothiazide respectively, was shown in (Figure No.5).

LOD and LOQ

In the present study the LOD and LOQ of Amiloride hydrochloride and Hydrochlorothiazide were evaluated based on the standard calibration curve method. Limit of detection is performed to know the lowest concentration level of the analytes that gives measurable response. LOD and LOQ for Amiloride hydrochloride are 0.52µg/mL and 1.57µg/mL and for Hydrochlorothiazide are 0.708µg/mL and 2.14µg/mL respectively.

Robustness

Robustness of the proposed method has been evaluated by small deliberate changes in the system parameters such as flow rate, wavelength and temperature. It was found that none of the above parameters caused alteration in the peak area, retention time, and USP tailing by small changes like ±0.2 mL change in flow rate, ±2nm wavelength, and ±2°C change in temperature. The % RSD was found to be within the limits and the method was found to be robust. The robustness results were shown in Table No.5.

Assay of Marketed Formulation

Analysis of marketed formulation (BIDURET tablets: 5 mg Amiloride hydrochloride and 50 mg Hydrochlorothiazide) was purchased from local market. Ten tablets were weighed and average weight was calculated; weight equivalent to 10tablets was transferred into a 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 25 min, and further the volume was made up with diluent and filtered. From the filtered solution 2 mL was pipetted out into a 10 mL volumetric flask and 1 mL 10% of OPA is added as supporter made up to 10 mL with diluent. From the resulting solution 20µL was injected into HPLC system and peak areas were recorded. The % assay of the marketed formulation was found to be 99.85% for Amiloride hydrochloride and 99.99% for Hydrochlorothiazide was shown in Table No.6.

Forced Degradation Studies

ICH degradation was attempted to various stress conditions such as acid hydrolysis (using 1N Hydrochloride), base hydrolysis (using 1N Na OH), oxidative hydrolysis (using 3% H2O2), thermal degradation (heated at 105°C for 6hours), humidity degradation (using 25°C, 95%RH) and photolytic degradation (using UV light inside a UV chamber for 7 days), to evaluate the ability of the proposed method to separate Amiloride Hydrochloride and Hydrochlorothiazide from its degradation products. It was found that Hydrochlorothiazide was degraded in acid, base and humidity condition. The results of stress studies were shown in Table No.7.

Table No.1: Optimized Chromatographic Condition

S.No	Parameter	Optimized condition
1	Column	SHIM-PACK C-18 (250 × 4.6 mm, 5 µ) column
2	Mobile phase	Water and acetonitrile in the ration of 50 : 50% v/v
3	Flow rate	1 mL/min
4	Flow rate	UV detector at 285 nm
5	Injection volume	20µL
6	Temperature	25°C
7	Retention time	Amiloride hydrochloride 2.06 min and Hydrochlorothiazide 3.12 min

Table No.2: System Suitability Parameters

S.No	Parameters	Amiloride hydrochloride	Hydrochlorothiazide
1	Retention time (min)	2.06	3.12
2	USP plate count	3747	5939
3	USP tailing	0.990	1.181

Table No.3: Percentage Recovery Results of Amiloride hydrochloride and Hydrochlorothiazide

S.No	Spiked	Percentage Recovery		Mean Percentage Recovery	% RSD	
		Amiloride hydrochloride	Hydrochlorothiazide		Amiloride hydrochloride	Hydrochlorothiazide
1	80%	100.3	101.7	101.1	0.68	0.39
		101.3	101.2			
		101.6	100.9			
2	100%	100	100	100.1	0.36	0.06
		100.2	99.9			
		100.7	100			
3	120%	100.4	100.1	100.9	0.20	0.80
		101.2	101.4			
		101	101.6			

Table No.4: Results of Method Precision for Amiloride hydrochloride and Hydrochlorothiazide

S.No	Sample No	Amiloride hydrochloride	Hydrochlorothiazide
		% Assay	% Assay
1	Injection 1	100.5	101.6
2	Injection 2	99.9	100.1
3	Injection 3	101.2	101.2
4	Injection 4	100.9	100.7
5	Injection 5	100.2	99.9
6	Injection 6	100	100.4
7	Average	100.4	100.7
8	Sd	0.51	0.65
9	%Rsd	0.51	0.65

Table No.5: Results of Robustness

S.No	Parameter	Amiloride hydrochloride			Hydrochlorothiazide		
		RT	NTP	TF	RT	NTP	TF
1	Flow rate 0.8 mL	2.567	4627	0.975	3.889	7076	1.18
	Flow rate 1.2 mL	1.729	2830	1.00	2.620	4970	1.19
2	Temperature 23°C	2.069	3781	0.998	3.138	5962	1.18
	Temperature 27°C	2.068	3759	0.988	3.123	5983	1.18
3	Wavelength283nm	2.068	3661	0.983	3.131	5888	1.18
	Wavelength287nm	2.063	3763	0.984	3.124	6014	1.18

Table No.6: Percent Content of Marketed Formulation

S.No	Tablet	Drug	Amount taken	Amount found	%Assay
1	BIDURET(Amiloride Hydrochloride 5mg and Hydrochlorothiazide 50mg)	Amiloride hydrochloride	20 ppm	19.97 ppm	99.85
		Hydrochlorothiazide	200 ppm	199.98 ppm	99.99

Table No.7: Forced degradation studies of Amiloride hydrochloride and Hydrochlorothiazide

S.No	Stress Condition	Amiloride hydrochloride		Hydrochlorothiazide	
		%Assay	%Difference w.r.t control	%Assay	%Difference w.r.t control
1	Control	99.8	NA	99.5	NA
2	Acid degradation	99.4	0.4	72.2	27.3
3	Base degradation	99.4	0.4	72.2	27.3
4	Oxidative degradation	97.8	2	98.8	0.7
5	Photolytic degradation	99.5	0.3	99.2	0.3
6	Thermal degradation	99.7	0.1	99.3	0.2
7	Humidity degradation	99.7	0.1	89.6	9.9

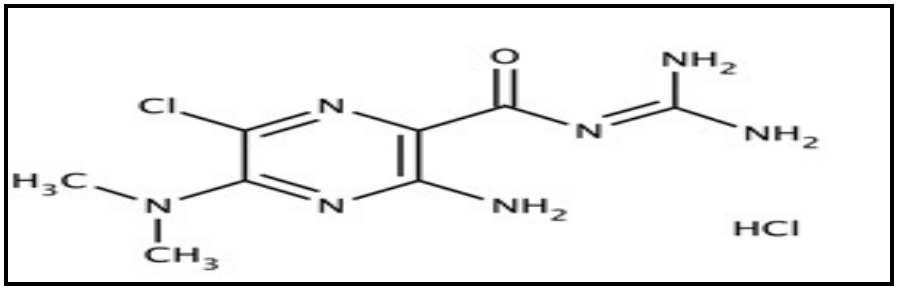

Figure No.1: Chemical Structure of Amiloride hydrochloride

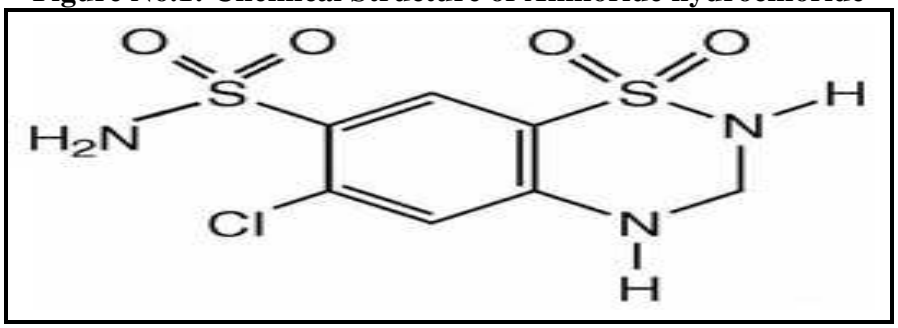

Figure No.2: Chemical Structure of Hydrochlorothiazide

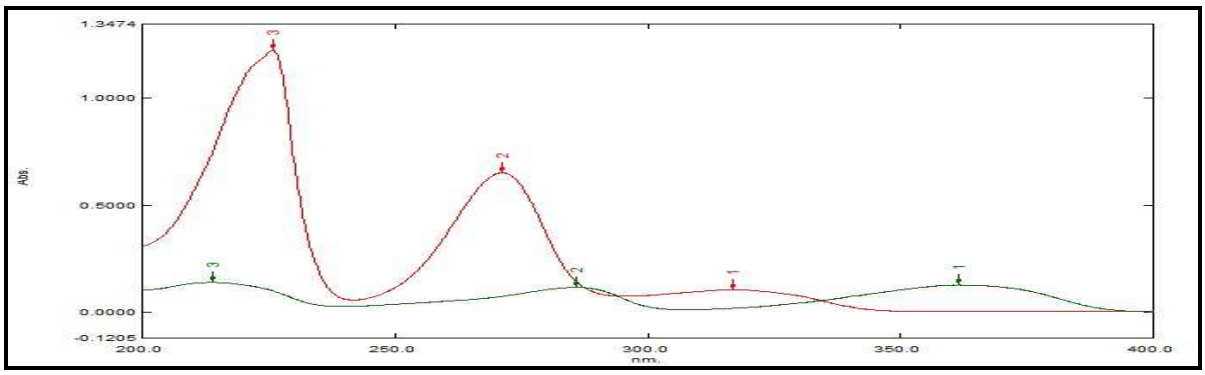

Figure No.3: Overlain Spectra of Amiloride hydrochloride and Hydrochlorothiazide

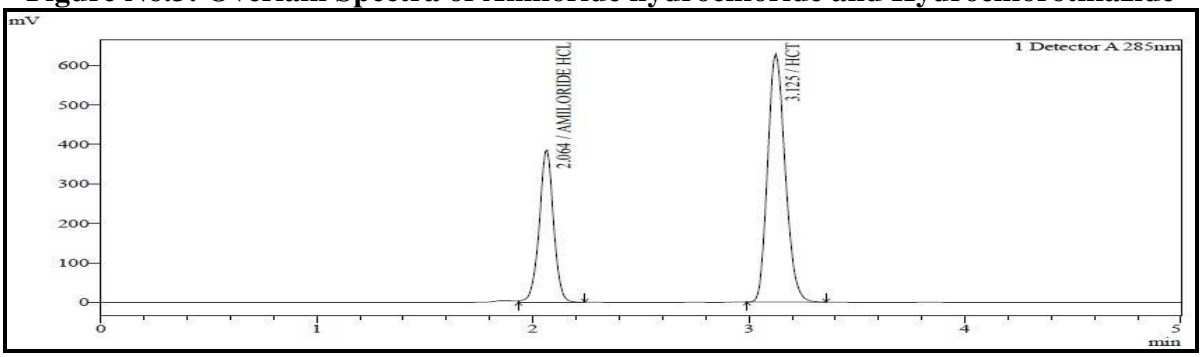

Figure No.4: Chromatogram of Standard Amiloride hydrochloride and Hydrochlorothiazide

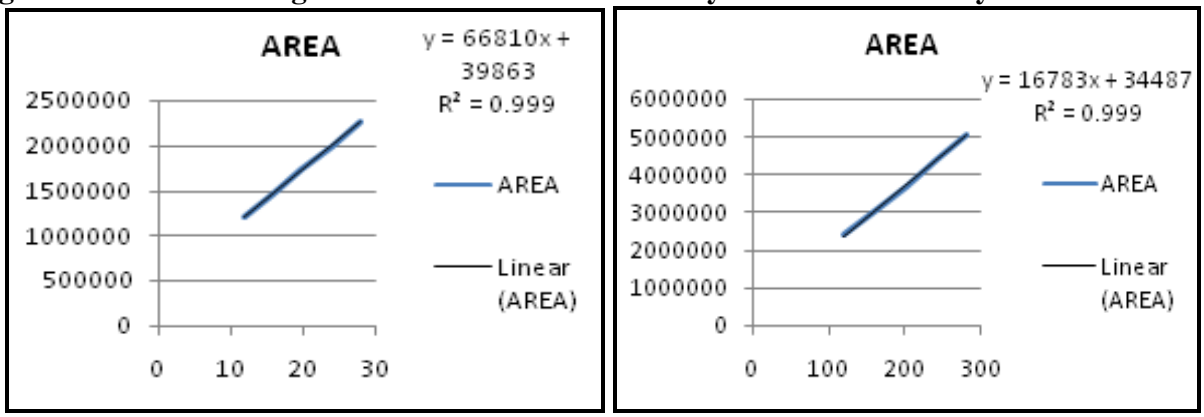

Figure No.5: Linearity Graph of Amiloride hydrochloride and Hydrochlorothiazide

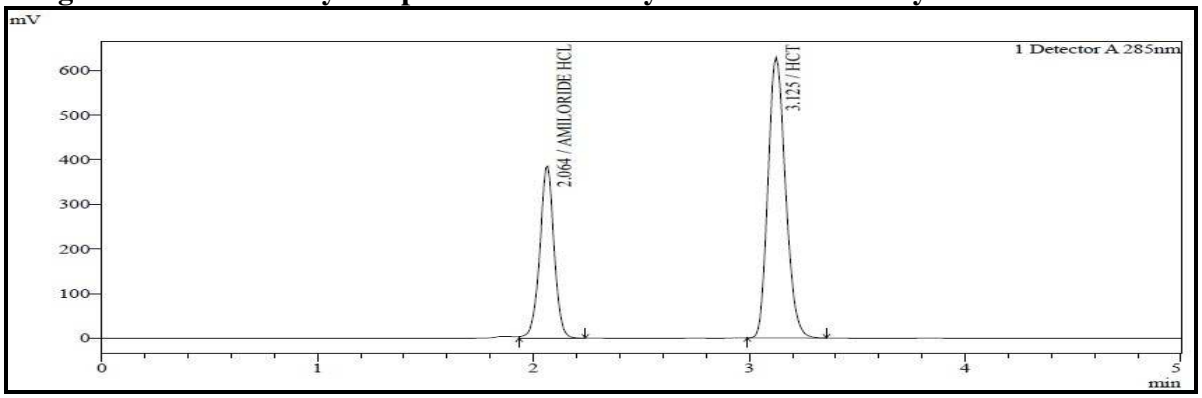

Figure No.6: Chromatograph of Untreated tablet

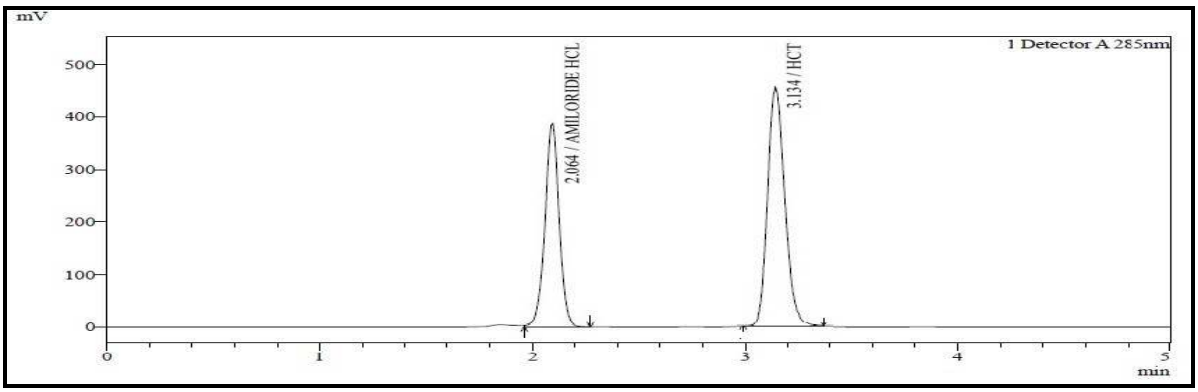

Figure No.7: Chromatograph of Acid degradation

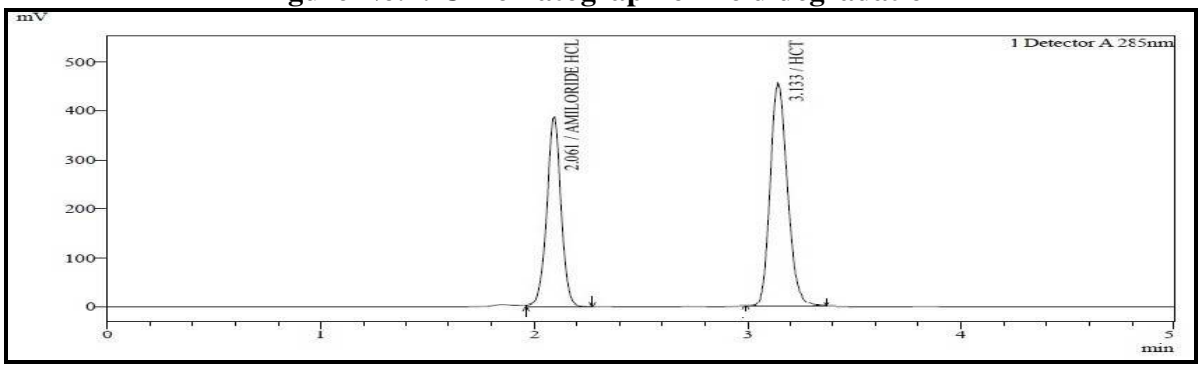

Figure No.8:Chromatograph ofBase degradation

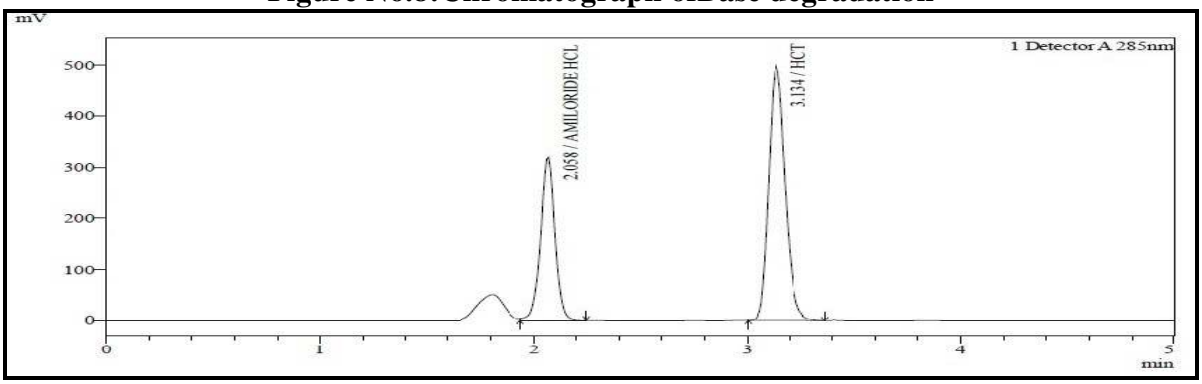

Figure No.9: Chromatograph of Oxidative degradation

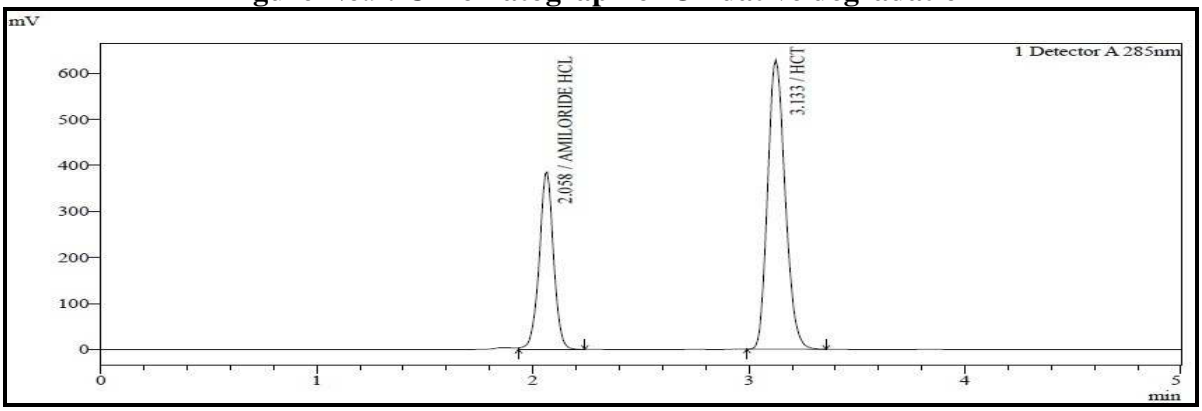

Figure No.10: Chromatograph of Photolytic degradation

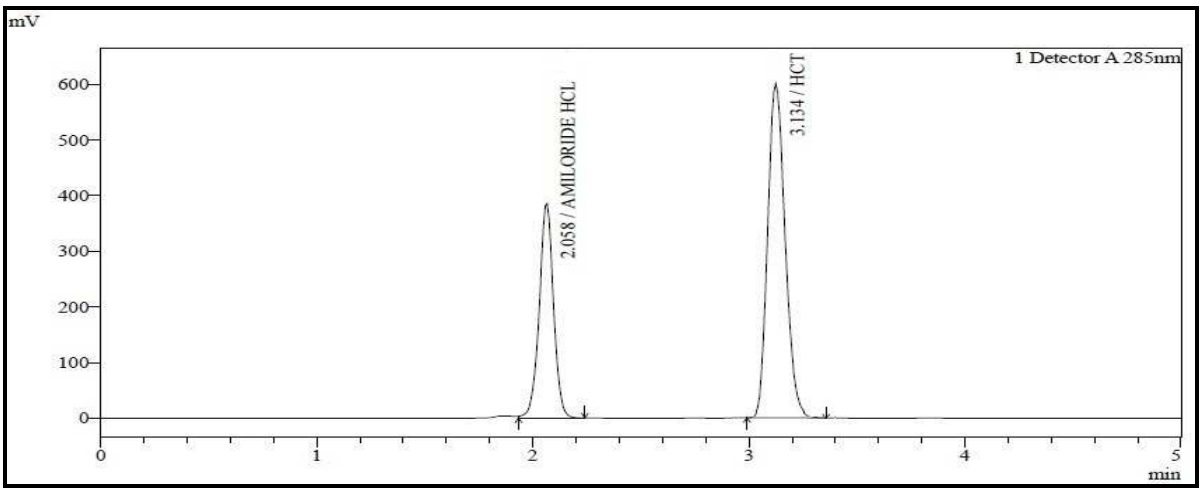

Figure No.11: Chromatograph of Thermal degradation

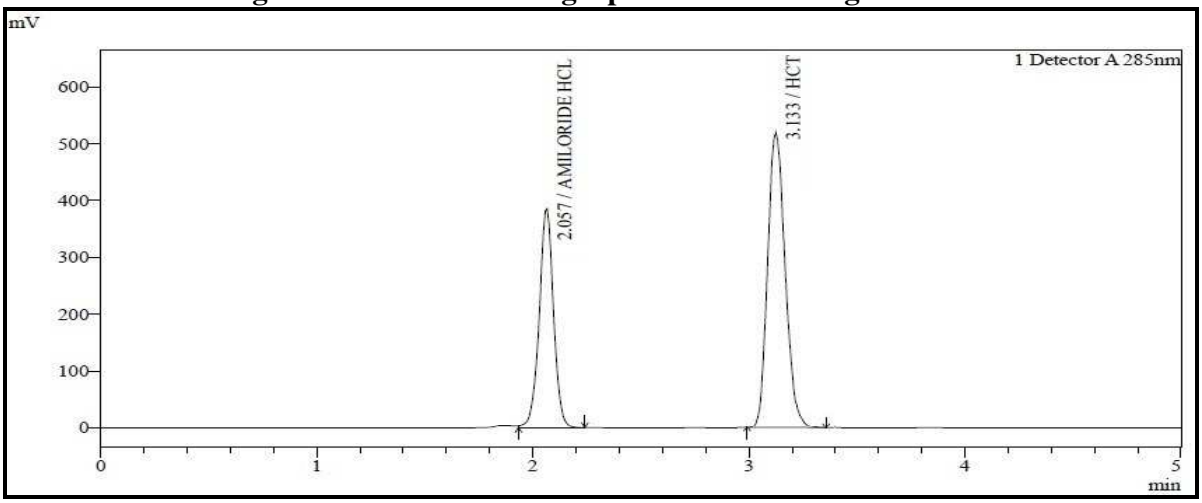

Figure No.12: Humidity degradation

CONCLUSION

In the present study, a stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of Amiloride hydrochloride and Hydrochlorothiazide in tablet dosage form. The validated method successfully used for stress testing analysis of Amiloride hydrochloride and Hydrochlorothiazide. The stress testing studies revealed that the method was successfully employed to resolve the degraded products from the sample. The proposed method was proved selective, accurate, precise, and rapid and it can be used for the routine analysis of the Amiloride hydrochloride and Hydrochlorothiazide in the formulation.

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CONFLICT OF INTEREST

The authors confirm that this paper has no conflict of interests.

REFERENCES

1. Sebaey Mahgoub. Novel Stability-Indicating RP-HPLC Method for Determination of Hydrochlorothiazide, Amiloride Hydrochloride and Timolol Maleate in Tablet Dosage Form, *Columbia International Publishing American Journal of Modern Chromatography,* 3(1), 2016, 23-32.
2. Kopuri. Gayathri, Anusha K, Srinivasa Rao D. Development of a new HPLC method for simultaneous estimation of Amiloride and Hydrochlorothiazide, *JCPS,* 8(4), 2015, 661-663.
3. Lakshmi Narasimham *et al,* Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms, *J. Chem. Metrol,* 4(1), 2010, 1-20.
4. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, Monographs no. 73, 885, Merck and Co., 15th Edition, 2015.
5. Indian pharmacopoeia published by, The Indian pharmacopoeia commission Ghaziabad, 2, 6th Edition, 2010, 795-797, 1451-1452.
6. Abd El-Hay S S *et al,* High performance liquid chromatography method for simultaneous determination of xipamide, triamterene and hydrochlorothiazide in bulk drug samples and dosage forms, *Acta Pharm,* 66, 2016, 109-118.
7. Madhavi A, Sathishkumar Shetty A. Simultaneous Determination and Validation of Ramipril And Hydrochlorothiazide by RP-HPLC Method, *International Journal of Chem Tech Research,* 10(3), 2017, 321-325.
8. Jaivik V. Shah *et al,* Simultaneous quantification of Amiloride and hydrochlorothiazide in human plasma by liquid chromatography–tandem mass spectrometry, *Journal of Pharmaceutical Analysis,* 7(5), 2017, 288-296.
9. Nidhal S. Mohammed and Ahmed J. Mohammed. Development and Validation of RP-HPLC Method for the Determination of Hydrochlorothiazide in Bulk Drug and Pharmaceutical Dosage Form, *Chromatography Research International,* Article ID 1693024, 2016, 1-7.
10. Khaleda H. Al-Saidi, Suhad Abdlaziz and Sahar Semer. Simultaneous Determination of Amiloride Hydrochloride And Hydrochlorothiazide In Pharmaceuticals By Derivative Spectrophotometry, *Journal of Al-Nahrain University,* 13(4), 2010, 52-61.
11. Nagori B P and Renu Solanki. RP-HPLC Method for Simultaneous Estimation of Furosemide and Amiloride Hydrochloride in Tablet Formulation, *Indian J Pharm Sci*, 72(3), 2010, 384-387.
12. Anand kumar R *et al,* RP-HPLC Method for Simultaneous Estimation of Hydrochlorothiazide, Ramipril and Telmisartan in Tablet Dosage Form, *Journal of Pharmacy Research,* 5(3), 2012, 1290-1293.
13. Tengli A R and Gurupadayya B M. Method Development and Validation of Tablet Dosage form Containing Losartan, Atenolol and Hydrochlorothiazide Using Internal Standard by RPHPLC, *J Chromat. Separation Techniq,* 4(5), 2013, 1-5.

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