Research Article

Ecofriendly Stability Indicating RP-UHPLC Method for Simultaneous Estimation of Amiloride and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form using AGREE and GAPI Tools

Neetu Sabarwal[1*], Suman Jain[1], DD Agarwal[2]

[1]School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior, Madhya Pradesh, India.
[2]School of Studies in Chemistry, Jiwaji University, Gwalior, Madhya Pradesh, India.

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ABSTRACT

An easy, quick and reliable RP-UHPLC technique for the quantification of amiloride (AML) and hydrochlorothiazide (HCTZ) is established as well as validated in the present article using the green analytical chemistry (GAC) tools. The mobile phase consisted of a mixture of 0.1% formic acid with ethanol in a 55:45% v/v ratio delivered at a flow rate of 0.5 mL/min through a 120 × 4.6 mm Hypersil gold ODS end-capped reverse phase column having a particle size of 3 microns. AML and HCTZ were detected at 265 nm with respective retention periods of 2.982 and 5.654 minutes. This methodology was rigorously validated in alignment with ICH guidelines. The calibration plots exhibited linearity over the concentration ranges of 2.5 to 25 μg/mL for AML and 25 to 150 μg/mL for HCTZ. The detection limits for AML and HCTZ were 0.69 and 0.61 μg/mL whereas quantification limits were 2.10 and 1.84 μg/mL. At three different levels of standard addition, the percentage recovery was determined to be between 99.37 and 100.7%. The precision of the method for both intra as well as inter-day fell under the acceptable range (RSD < 2). Both the bulk medication as well as its formulation were subjected to a forced degradation trial and the results indicate that the technique is stability-indicating and can differentiate between the peaks of the active analytes and the degradants. The evaluation of the method's environmental impact utilized green analytical chemistry tools such as GAPI and AGREE revealing a greenness rate exceeding 75%, which is excellent. Hence, the established chromatographic approach was straightforward, highly sensitive, quick, precise, cost-effective as well and eco-friendly than the earlier reported methods making it suitable for routine quality control testing in the industrial sector.

INTRODUCTION

Amiloride hydrochloride (AML), chemically designated as 3,5-diamino-N-(diaminomethylene)-6-chloropyrazinecarboxamide monohydrochloride dihydrate, serves as an oral antihypertensive and diuretic agent. It is notable for its ability to conserve potassium yet demonstrate minimal natriuretic and diuretic effects.[1] Fig. 1 illustrates the chemical structure of amiloride. Hydrochlorothiazide (HCTZ) a diuretic from the benzothiadiazide class, is frequently employed in antihypertensive therapy, either alone or in combination with other drugs, by inhibiting the reabsorption of Na^+ and Cl^- in the DCT. Its chemical designation is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide and Fig. 2 depicts its chemical configuration. Additionally, this combination lowers blood pressure in a synergistic way.[2]

Few analytical methodologies exist for concurrently analyzing AML and HCTZ according to the literature studies. The methodologies include spectrophotometry[3-5] and HPLC techniques.[6-10] Some liquid chromatography-tandem mass spectrometric[11-14] methods have been

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*Corresponding Author: Ms. Neetu Sabarwal

Address: School of Studies in Pharmaceutical Sciences, Jiwaji University, Gwalior, Madhya Pradesh, India.

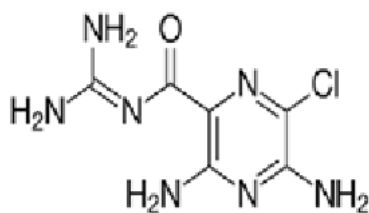**Email ✉:** neetubhalla1979@gmail.com

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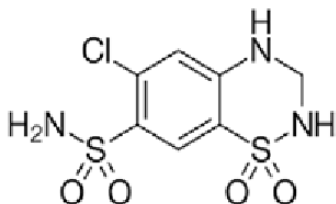
Fig. 1: Structure of amiloride

Fig. 2: structure of hydrochlorothiazide

documented in scientific sources for measuring trace amounts of these pharmaceuticals, especially in biological matrices. Additionally, a Polarography[15] method has been recorded. Numerous experimental methodologies have been recorded for the distinct measurement of AML[16] and HCTZ[17-20] in bulk, individual dosage forms, or human plasma. These approaches present ecological hazards linked with the utilization of toxic solvents, while the tenets of sustainable analytical practices in chemistry remain unutilized.

Currently, there exists just one stability-indicating RP-HPLC technique[21] documented for the concurrent measurement of AML and HCTZ in pharmaceutical formulations. At present, there is an absence of a documented stability-indicating approach for the estimation of AML and HCTZ that integrates RP-UHPLC with environmentally sustainable analytical principles. In the method reported the moving phase was a 50:50 mixture of water and acetonitrile according to volume. Since, ethanol is an environmentally friendly solvent, it was substituted for acetonitrile in the present procedure. The primary objective of employing this UHPLC instrument in the research endeavor was to achieve enhanced peak symmetry, elevated plate count, superior resolution (separation factor), reduced noise levels, minimal solvent consumption and reduced waste generation per analysis, as the developed methodology necessitates an environmentally conscious approach. Hence, there exists an opportunity to create a novel, straightforward, efficient, and environmentally friendly UHPLC method. This study focused on establishing a straightforward, sensitive, fast, dependable, environmentally friendly, and specific RP-UHPLC stability-indicating approach for the concurrent measurement of AML alongside HCTZ in pure and marketed preparations. The ICH Q2 (R1) standards are followed during the method validation.

Pharmaceutical industries are proposing the adoption of an environmentally sustainable analytical method for drug development and analysis. The use of miniaturized techniques like RP-UHPLC complies with 11 of the 12 green analytical criteria,[22-24] with the exception of solvent-free analysis. Stability studies of pharmaceutical products and their related substances represent essential factors in the development of innovative medications and formulations. The prediction of shelf life is pivotal in the formulation of all dosage forms, influencing specific storage conditions and guiding labeling recommendations. Such studies are imperative for the acceptance and approval of any pharmaceutical product, ensuring that overall quality, safety, and effectiveness are preserved over its entire shelf life.

Materials And Methods

Chemicals and Reagents

Amiloride hydrochloride and hydrochlorothiazide, each exhibiting a purity greater than 99%, were obtained from Anphar Laboratories Pvt. Ltd. in Jammu and Hetero Drugs Pvt. Ltd. in Hyderabad, respectively. Multicomponent medication tablets (Biduret) were obtained from a local pharmacy. Honeywell Research Chemicals in Mumbai supplied LC-MS grade 0.1% formic acid, whereas SD Fine Chemical in Mumbai, India, provided HPLC grade ethanol. Millipore provided the Milli-Q water utilized in this investigation. Rankem Limited, India, supplied analytical-grade sodium hydroxide, hydrochloric acid, and hydrogen peroxide.

Instrumentation

Thermo Fischer Dionex UHPLC UltiMate 3000 series paired with a PDA detector (DAD-3000) was used for the experiment. A 150×4.6 mm Hypersil gold ODS end-capped reverse phase column with 3 μ particle size was used for the separation procedure. Other instruments used in the experiment were a pH meter (Analab Scientific Private Ltd), sonicator (PCI Analytics), analytical balance (Citizen CX 265), and UV-visible spectrophotometer (Shimadzu UV 1700 & 1800), hot air oven (LabHosp Instrument) and photostability chamber (LabHosp Instrument).

Preparation of Solutions

Preparation of mobile phase and diluent

In 0.1% formic acid to ethanol in a 55:45 v/v ratio makes up the optimized mobile phase. Ethanol and water were combined in a volume/volume ratio of 50:50 to create the diluent.

Preparation of standard stock solutions

In 50 mg of pure hydrochlorothiazide and 10 mg of amiloride should be weighed precisely before being put into 100 and 200 mL clean, dry volumetric flasks,

respectively. After adding 50 mL of diluent and sonicating it for five minutes, diluents should be added to reach the final volume.

Preparation of working standard solution

To produce a 5 µg/mL amiloride and 50 µg/mL hydrochlorothiazide concentration, 1-mL of each of the two stock solutions was moved to a 10 mL volumetric flask and diluted to 10 mL.

Preparation of calibration solutions

By gradually diluting the stock solution, calibration standard solutions were created, yielding concentration ranges of 2.5 to 25 µg/mL for amiloride and 50 to 150 µg/mL for hydrochlorothiazide.

Preparation of working sample solution

About 20 biduret tablets, each containing 5 mg of AML and 50 mg of HCTZ, were weighed, pulverized to a fine powder, and then poured into a dry sterile 100 mL volumetric flask in an amount equal to one tablet. The solution was prepared by dissolving it in 30 mL of diluent, followed by sonication for a duration of ten minutes at ambient temperature, after which it was adjusted to the designated mark. The solution was then subjected to filtration through a 0.45 µm filter and calibrated to the designated volume. In 1-mL of the aforementioned filtrate was transferred into a 10 mL volumetric flask and diluted to a total volume of 10 mL with diluent, yielding a concentration of 5 µg/mL of amiloride and 50 µg/mL of hydrochlorothiazide.

RP-UHPLC Method Development and Optimization

The liquid chromatographic technique was meticulously established taking into account the physico-chemical properties of particular pharmaceuticals. The most efficient method was reversed-phase liquid chromatography because the chosen drugs were fully soluble in polar and moderately polar solvents including formic acid, ethanol, and water. In this method, a polar moving phase was used in conjunction with a non-polar stationary phase. The ideal chromatographic conditions were determined *via* a systematic series of experiments that entailed adjusting specific variables, such as the composition of the mobile phase in isocratic mode, the flow rate, detection wavelength, injection volume and column temperature while maintaining constancy in other variables. This approach ensured reasonable resolution, peak form, retention period and performance. A 55:45 ratio of 0.1% formic acid to ethanol was selected for optimum results, accompanied by a 0.5 mL/min flow rate in isocratic elution mode. In order to achieve the requisite separation, 10 µL of the standard solution was introduced into a 150 x 4.6 mm Hypersil Gold ODS end-capped reverse phase column equipped with a PDA detector maintained at ambient temperature. The detection took place at 265 nm.

Method Validation [25]

The optimized chromatographic technique underwent validation against various recommended criteria, adhering to the ICH Q2(R1) guidelines. The solution's stability and the chromatogram's peak purity were examined by comparing the purity angle and purity threshold values obtained from the data acquisition software Chromeleon.

Analysis of BIDURET Tablet (Marketed Formulation of AML and HCTZ)

The analytical method that was devised was employed to assay the concentration of amiloride and hydrochlorothiazide in biduret-5/50 mg tablets (Glaxo Smithkline). The response of the combined dosage form at 265 nm was measured using RP-UHPLC to quantify AML and HCTZ. The regression equations for AML and HCTZ in the technique were employed to determine the concentrations of these substances in the sample solution. The chromatogram was examined for extraneous peaks that resulted from excipients in the formulation at the ideal retention time in order to verify the specificity of the procedure.

Forced Degradation Studies [26,27]

Investigations into forced degradation were conducted in alignment with ICH recommendations. Q1A (R2).[25,26] Both the individual medications, their laboratory-prepared mixture, and their commercial formulations underwent a series of rigorous stress conditions, including acid, alkali, oxidative, heat and light environments, to evaluate the effects of degrading impurities. Forced degradation was conducted under specified conditions: acid (2N HCl for 6 hours at 60°C), alkali (2N NaOH for 6 hours at 60°C), oxidation (30% H_2O_2 for 6 hours at 60°C), thermal (48 hours in a hot air oven at 105°C), and photolytic (exposed to UV light in a photolytic chamber for 7 days). Likewise, blank solutions were subjected to stress conditions to determine any impact on the retention times of AML and HCTZ. Stressed samples were evaluated in contrast to the standard using the suggested RP-UHPLC method. Each stressed sample's percentage deterioration findings were calculated and compared to the unstressed sample.

Greenness Profile Determination

In order to determine the environmental sustainability of an analytical methodology, it is essential to examine several variables like the quantity and toxicity of the compounds used, waste produced, energy consumption, number of procedural steps, miniaturization, and automation. Two green metrics were incorporated into our analysis: the Analytical GREEnness calculator (AGREE) and the green analytical process index (GAPI). The GAPI [28,29] employs a color coding system where green, yellow, and red represent low, medium, and high impacts on the environment, respectively. The method employs five

pentagrams to assess and measure the ecological impact throughout each phase of the analytical process. Other factors like safety and health are also taken into account by the GAPI tool. AGREE is another quantitative method that offers a score showing the degree of conformance with the 12 fundamental tenets of sustainable analytical chemistry. It can be accessed with readily available software. [30]

RESULTS AND DISCUSSION

Method Development and Optimization

Amiloride and hydrochlorothiazide were analyzed in pure and commercial formulations using an appropriate green LC method established by optimizing the RP-UHPLC methodology. A Hypersil gold ODS end-capped reverse phase column (150 x 4.6 mm, 3 µ particle size) was used with a moving phase that consisted of 0.1% formic acid and ethanol (55:45 v/v) at a wavelength of 265 nm. This configuration yielded a retention period of 2.98 minutes for AML and 5.65 minutes for HCTZ, with tailing factors of 1.05 and 1.12 for AML and HCTZ, respectively, along with satisfactory theoretical plates and resolution. Fig. 3 demonstrates the blank chromatogram whereas Fig. 4 represents the optimized chromatogram of pure AML and HCTZ samples.

Method Validation

Specificity

The specificity of the method was evaluated through the analysis of chromatograms from the mobile phase, standard solution, and sample solution to determine the potential interference of excipients in the sample. The HPLC chromatograms obtained for the drug exhibited no interference from peaks within the retention time ranges. The peak purity angles for AML and HCTZ are 0.215 and 0.261, respectively, while the purity thresholds for AML and HCTZ are 0.433 and 0.488, respectively.

System suitability testing

By looking at features like theoretical plates, asymmetry or tailing factor, and resolution, the viability of the well-established chromatographic method for AML and HCTZ was evaluated. System suitability assessment involved
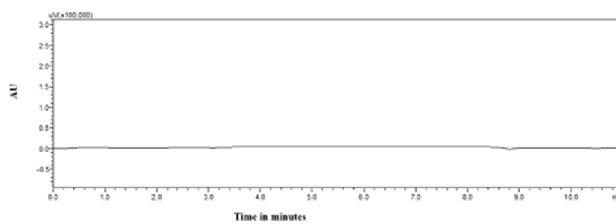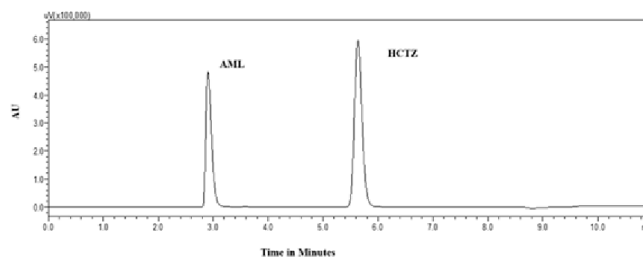

Fig. 3: Blank chromatogram

Fig. 4: Optimized chromatogram of standard amiloride and hydrochlorothiazide at 265 nm

the evaluation of the tailing factor and plate count values by introducing six replicates of a working standard solution comprising 5 µg/mL of AML and 50 µg/mL of HCTZ following the chromatographic conditions outlined in the proposed methodology. Multiple criteria, such as plate count, retention time, and peak tailing, were found to comply with USP guidelines. The %RSD values for tailing factor and plate count from the six duplicate injections of AML and HCTZ were within the prescribed range demonstrating outstanding peak symmetry. Table 1 has the tabulated values.

Linearity

The linearity of the technique was evaluated at multiple concentration ranges, specifically between 2.5 and 25 µg/mL for AML and between 25 and 150 µg/mL for HCTZ. To confirm the linearity of the detector's response across various drug concentrations, an aliquot of 10µl from each concentration range was introduced into the RP-UHPLC instrument six times, and the peak regions of the obtained chromatograms were documented. The proposed method demonstrated linearity for both

Table 1: System suitability results

System suitability parameter	Acceptance criteria	Drug name	
		Amiloride	Hydrochlorothiazide
Retention time	-	2.98	5.65
USP plate count	*NLT 2000	2241	2661
USP tailing	**NMT 2.0	1.05	1.12
USP resolution	*NLT 2.0	-	6.675
%RSD	**NMT 2.0	0.425	0.726

n = 6. * Not Less Than. ** Not More Than

Table 2: Linearity studies results

S. No.	AML Conc. (µg/mL)	AML Area (µAU)	HCTZ Conc. (µg/mL)	HCTZ Area (µAU)
1	2.5	76285	25	154322
2	5	159255	50	310567
3	10	332377	75	447760
4	15	467610	100	599436
5	20	623243	125	744165
6	25	786257	150	894876
Correl.coeff	0.9991		0.9996	
Slope	31206		5873.2	
Intercept	4433.4		12182	

pharmaceuticals. The calibration curve was created by graphically illustrating the correlation among the peak regions as well as individual concentrations of AML and HTCZ. The calibration curves were analyzed to determine the correlation coefficient, y-intercept, and slope. With a correlation coefficient (r^2) of more than 0.999 for both AML and HCTZ, the calibration curve clearly indicates a robust linear relationship. Table 2 displays the calibration data for AML and HCTZ, whereas Figs 5 and 6 depict the linearity graphs for each drug, respectively.

Precision

This method entails the preparation of six replicated samples with identical concentrations of HCTZ (50 µg/mL) and AML (5 µg/mL) and their injection into an RP-UHPLC machine. The peak area values that were observed served as the basis for calculating the mean, standard deviation, and percent relative standard deviation (RSD).

Fig. 5: Standard calibration plot of AML at 265 nm

Fig. 6: Standard calibration plot of HCTZ at 265 nm

Very high precision or closeness between findings is indicated by a score of less than 2% RSD. The intra and inter-day precision results for AML and HCTZ fell under the permissible ranges. The precision was acceptable, as evidenced by the percentage RSD value that was obtained.
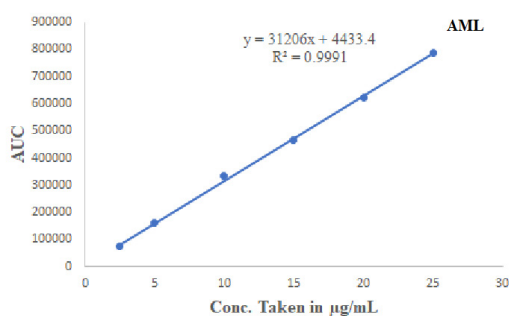
Intraday precision results

Six duplicate samples comprising AML (5 µg/mL) and HCTZ (50 µg/mL) were introduced into an RP-UHPLC machine and assessed on the same day. The %RSD values recorded for AML was 0.933 and for HCTZ was 0.818, both of which are below the maximum recommended threshold of 2%. Additionally, the percentage assay values approached 100%, indicating that the proposed method yielded precise results. Table 3 presents the intraday precision results for AML and HCTZ.
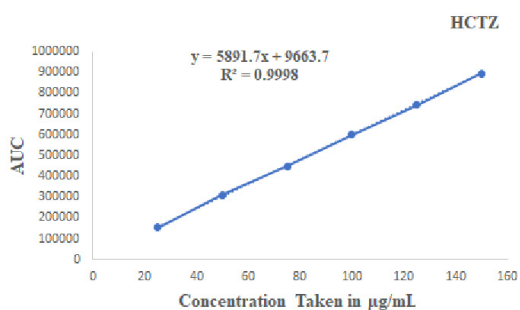
Inter-day precision results

Six duplicate samples comprising AML (5 µg/mL) and HCTZ (50 µg/mL) were introduced into an RP-UHPLC machine and analyzed on a separate day. Peak regions were used to calculate the mean, standard deviation, and %RSD. The proposed method exhibited precision, yielding %RSD values of 0.407 for AML and 0.306 for HCTZ, both under 2%. The percentage assay values for AML and HCTZ approached 100%. The findings are displayed in Table 4.

Accuracy

The suggested RP-UHPLC technique's accuracy was assessed through percentage recovery analysis. The standard addition procedure was used in the accuracy method, which involved adding AML and HCTZ at three different concentrations: 50, 100, and 150%, conducted in triplicate as per the design protocol. The approach's accuracy was evaluated through the percentage recovery of AML and HCTZ. The %recovery values were determined to be 100.3 to 100.7% for AML and 99.37 to 100.27% for HCTZ, meeting the acceptable standards. The %RSD values were significantly below the established threshold of 2%, demonstrating the high accuracy of the established method. Table 5 summarises the results of accuracy.

Table 3: Precision results of intraday study

	Amiloride			Hydrochlorothiazide		
S.No.	Conc. (μg/mL)	Area (μAU)	%Assay	Conc. (μg/mL)	Area (μAU)	%Assay
1		159295	99.2		310876	101.7
2		158954	99		310256	101.5
3	5	161325	100.5	50	306787	100.3
4		160256	101		306152	100.1
5		160089	99.7		305758	99.8
6		162436	101.2		310096	101.4
SD	0.934			0.825		
Mean	100.1			100.8		
%RSD	0.933			0.818		

Table 4: Precision results of interday study

	Amiloride			Hydrochlorothiazide		
S.No.	Conc. (μg/mL)	Area (μAU)	%Assay	Conc. (μg/mL)	Area (μAU)	%Assay
1		159285	99.2		306254	100.1
2		160804	100.2		304675	99.6
3	5	160725	100.1	50	306653	100.2
4		160456	99.9		306186	100.1
5		160889	100.2		304886	99.7
6		161036	100.3		307123	100.4
SD	0.407			0.306		
Mean	99.983			100.017		
%RSD	0.407			0.306		

Table 5: Accuracy results

Recovery level	Amount added (μg/mL)	Peak area	Amount recovered (μg/mL)	%Mean Recovery±SD	%RSD
Amiloride					
50%	7.5	239882	7.52	100.3±0.78	0.775
1000%	10	318420	10.06	100.6±0.2	0.199
150%	12.5	397440	12.59	100.7±0.4	0.397
Hydrochlorothiazide					
50%	75	450083	74.55	99.37±0.38	0.381
100%	100	601370	100.31	100.27±0.23	0.230
150%	125	748343	125.34	100.11±0.17	0.181

Robustness

The robustness of an analytical technique indicates its reliability under standard operating conditions, as it assesses the technique's capacity to endure minor yet deliberate variations in method parameters. By varying the flow rate (±0.1 mL/min), changing the mobile phase composition (change in solvent ratio), and modulating the wavelength (±2 nm), the method's robustness was evaluated. Analytical results were unaffected by minor adjustments to chromatographic variables such as flow rate, mobile phase composition and wavelength, demonstrating sufficient robustness for the suggested method. The results met the established acceptance criteria and indicate that the proposed RP-UHPLC approach is robust for the analysis of amiloride and hydrochlorothiazide. Table 6 presents the results of the robustness analysis.

Table 6: Robustness results

| Parameters | Condition | Area (µAU) n = 3 | | Area (µAU) n = 3 | |
| | | Amiloride | | Hydrochlorothiazide | |
		Mean peak area ± SD	%RSD	Mean peak area ± SD	%RSD
Flow rate (±0.1 mL/min)	0.4 (mL/min)	158772 ± 432.4	0.272	311301 ± 850.6	0.273
	0.6 (mL/min)	159244 ± 347.63	0.218	311585 ± 216.7	0.070
Mobile phase composition	45:55	158679 ± 251.10	0.158	311919 ± 185.4	0.059
	50:50	159578 ± 160.41	0.101	311955 ± 79.0	0.025
Wavelength (±2 nm)	263 nm	159461 ± 294.36	0.185	311721 ± 150.9	0.048
	267 nm	159301 ± 157.47	0.099	311871 ± 99.7	0.032

LoD and LoQ

Using the standard deviation and the slope of the calibration curve, the following formulas were used to determine the limits of detection (LoD) and quantification (LoQ) for amiloride and hydrochlorothiazide.

$$LoD = 3.3\ \sigma/s$$
$$LoQ = 10\ \sigma/s$$

where 'σ' stands for the standard deviation of the response and 's' for the slope of the curve.

The detection limit (LoD) for AML was 0.69 µg/mL and for HCTZ it was 0.61 µg/mL, suggesting that even trace levels of both substances can be identified. The limit of quantification was determined to be 2.10 µg/mL for AML and 1.84 µg/mL for HCTZ, demonstrating that even minimal concentrations of AML and HCTZ can be accurately measured.

Stability

The stability of AML and HCTZ was evaluated by maintaining the reference solution at ambient room temperature and within the temperature range of 2 to 8°C. The %RSD was obtained by evaluating the results of previously stored standard solutions against freshly prepared solutions. Measurements of AML and HCTZ were conducted at 6, 12, 18, and 24 hours. No significant deviation (<2%) was observed, affirming that the solutions were stable for a duration of 24 hours. The storage conditions do not influence the efficacy of amiloride and hydrochlorothiazide.

Analysis of Marketed Formulation

Six samples of the AML and HCTZ tablets (Biduret) were assayed in order to determine the assay value. The standard deviation was measured at 0.584, accompanied by a relative standard deviation percentage of 0.58. The average values recorded for AML and HCTZ were 5.10 mg (102%) and 50.55 mg (101.1%), respectively. In comparison to the amount stated in the BIDURET tablets, the assay results were within the permitted range. Excellent assay findings indicated that a validated method should be developed.

Forced Degradation Studies

According to the results of the degradation study, degradation peaks generated during stress studies were not interfering with the AML and HCTZ peaks. There was a difference between the percentage assay results obtained for AML and HCTZ from samples treated with different stress conditions and those obtained from samples that were untreated. Table 7 presents a summary of the findings from the stress studies conducted. The forced degradation results of AML and HCTZ are reported in Figs 7 to 10.
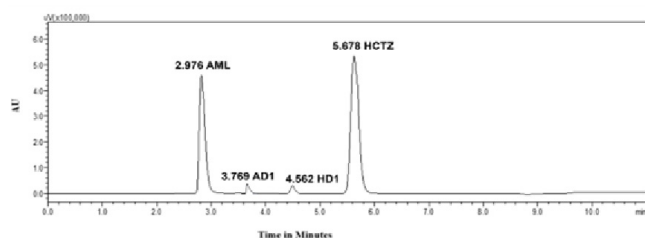
Acid degradation

For acidic degradation, 10 mL of stock solution in a round bottom flask was treated for 6 hours in a thermostat controlled at 60°C with 10 mL of 1N HCl. After cooling, the stressed sample underwent neutralization using 10 mL of 2N NaOH, then subsequently diluted with the diluent according to the test requirements. A 10 µL of

Table 7: Forced degradation results of AML and HCTZ

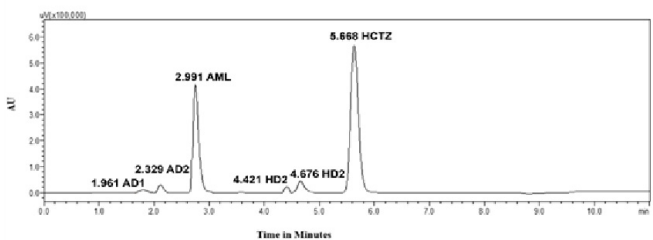| Stress conditions | Amiloride | | Hydrochlorothiazide | |
	%Assay	%Degradation	%Assay	%Degradation
Acid degradation	89.1	10.9	96.5	3.5
Alkali degradation	91	9	87	13
Oxidative degradation	94	6	92.5	7.5
Thermal degradation	99.9	-	96	4
Photolytic degradation	99.9	-	99.9	-
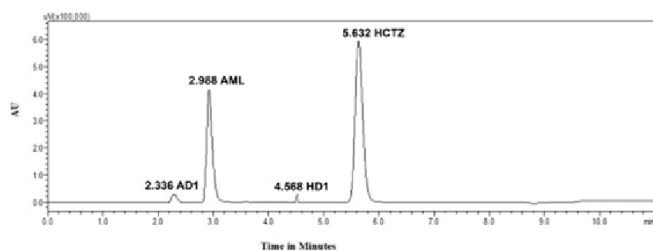
Fig. 7: UHPLC chromatogram of acid stressed sample

Fig. 8: UHPLC chromatogram of alkali stressed sample

Fig. 9: UHPLC chromatogram of oxidative stressed sample
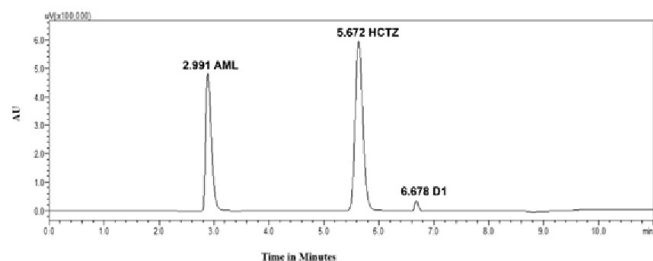
Fig. 10: UHPLC chromatogram of theramal stressed sample

acid-degraded sample was introduced into the RP-UHPLC machine and measured for peak area. 10.9 and 3.5% degradation of AML and HCTZ, respectively with 1-1 extra degradant peaks were observed in this study. The same procedure is repeated for sample (formulation) solution.

Alkali degradation

As part of the alkali degradation process, 10 mL of stock solution in a round bottom flask was treated with 10 mL of 2N NaOH for six hours under laboratory conditions at 60°C. In order to obtain the necessary dilution, the stressed sample underwent cooling, was neutralized with 10 mL of 2N HCl and then subsequently diluted with the appropriate

diluent. In 10 µL of the sample were introduced into the RP-UHPLC machine, the peak regions of which were determined. About 9% and 13% degradation of AML and HCTZ, respectively with 1-1 extra degradant peaks were observed.in this study.

Oxidative degradation

A volume of 10 mL from the stock solution was carefully transferred into a 10 mL round-bottom flask. After that, 10 mL of an oxidative agent (30% H_2O_2) was added to the mixture. The reaction mixture was shaken occasionally while being kept at reflux at 60°C for at least six hours. After cooling the solution was filtered and 10 µL was introduced into the RP-UHPLC machine. In this study, a degradation of 6% for AML and 7.5% for HCTZ was observed, along with the presence of 2 -2 additional degradant peaks.

Thermal degradation

To examine thermal deterioration, 10 mL of the stock solution was moved to a 10 mL volumetric flask and heated to 105°C for 6 hours in a hot air oven. After diluting the resultant solution to the desired concentration, 10 µL was added to the system for the RP-UHPLC analysis, and the chromatograms were then recorded. In the heat degradation study, AML exhibited no degradation, but HCTZ had a 4% degradation.

Photolytic degradation

In the photostability chamber, 10 mL of the stock solution was placed into a petri dish and subjected to ultraviolet light (1.2 mL ux hours and 200 watts hours per sq. mts of UV-visible) for 48 hours. After the resulting solution was diluted to get the final concentration for the RP-UHPLC investigation, 10 µL was introduced into the UHPLC machine and chromatograms were captured. The study did not reveal any degradation as a result of the drug's exceptional stability.

Green Analytical metric assessment:

Until a method has been evaluated using appropriate assessment tools, it cannot be claimed to be eco-friendly. This study employed two assessment tools to examine the method, namely the GAPI and the AGREE. Twelve green analytical principles are incorporated into AGREE software. In order to assess the level of compliance with sustainability criteria, every principle receives a score that varies from 0.1 to 1.0. The result is a circular design resembling a clock, featuring a total AGREE score of 0.74 and a color image at its center as depicted in Fig. 11 (b). There are 15 pictograms and five pentagrams displayed by the GAPI tool. A key feature of the pictograms used is that the pictogram representing the number 1 is colored red, representing the type of sample treatment used. On the other hand, yellow indicates pictograms that pertain to sample preparation and sample handling. A green pictogram indicates the reagents and instruments that
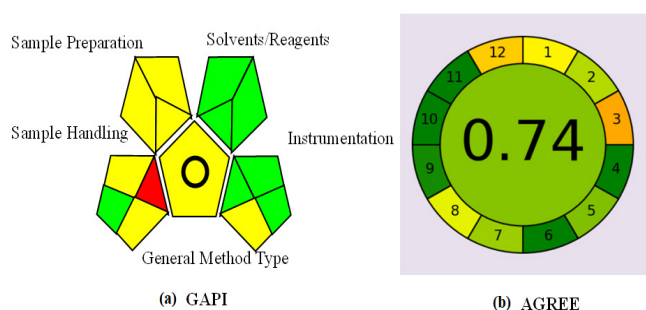
Fig. 11: Representative pictograms of (a) GAPI and (b) AGREE

were used in this study. The outcomes derived from the GAPI are illustrated in Fig. 11 (a)

CONCLUSION

The proposed RP-UHPLC method provides an effective means for examining the shelf life and storage conditions of the pharmaceuticals. The established and verified stability-indicating method is user-friendly, precise, rapid, and environmentally sustainable for the quantitative determination of AML and HCTZ. The proposed method's effectiveness has been confirmed to meet ICH Q2 guidelines. Using the suggested RP-UHPLC approach, the degradation peaks produced during forced degradation investigations further demonstrate improved resolution and separation of AML and HCTZ from the degradation products. Using GAP metric tools like AGREE and GAPI, the optimized analytical approach was evaluated and found to be an excellent green method that adheres to GAP principles. As a result, this approach has no negative environmental effects. This approach is characterized by satisfactory precision and accuracy, cost-effectiveness, rapid analysis, and environmental safety. It is readily adaptable for application in industrial and quality control environments. This study's future implications may compel the scientific community to adopt and refine eco-friendly procedures for testing diverse chemical compounds with green solvents.

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