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RESEARCH

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Development and validation of stability indicating UPLC method for the simultaneous estimation of triamterene and hydrochlorothiazide in combined dosage forms using quality by design approach

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Abstract

Background: According to the information gathered from the literature, no technique for UPLC of triamterene and hydrochlorothiazide employing QbD in the formulations has been published. The technique development by incorporating QbD and validating for accuracy, linearity, precision, LOQ, LOD, ruggedness and selectivity as per ICH is part of the work's modernity.

Results: Screening investigations led to the selection of cmps. Peak tailing was evaluated as a metric of technique robustness based on these important analytical attributes, namely retention time. With a 0.1 percent OPN: methanol (40:60) mobile phase, a flow rate of 0.3 ml/min, a wave length of 224 nm, an injection volume of 41, and a run time of 6 min, the best chromatographic separation was attained.

Conclusions: The method was verified using ICH criteria, which ensure a high level of linearity, accuracy, precision, specificity and robustness. As a result, the suggested approach is regarded as a quick and accurate method for estimating triamterene and hydrochlorothiazide at the same time.

Keywords: QbD, UPLC, OPA, ICH guidelines, Triamterene, Hydrochlorothiazide, Degradation studies

Introduction

Triamterene a pteridine derivative (2,4,7 triamino-6-phenyl pteridine). Triamterene is potassium-sparing diuretic. Its chemical formula is $C_{12}H_{11}N_7$, and its molecular weight is 253.26 g/mol. It appears as a crystalline solid with an odorless yellow powder, 316 °C is the melting point. Triamterene is soluble in formic acid, ethanol and dilute hydroxides but not in benzene, chloroform, ether or dilute hydroxides. Edema induced by congestive heart failure and liver cirrhosis is treated with triamterene. Triamterene in combination with hydrochlorothiazide is suggested for the treatment of hypertension. Triamterene inhibits the sodium–potassium exchange pump in the luminal membrane of major cells of the distal tubule, cortical collecting tubule and collecting duct of the kidney [1–6] (Fig. 1).

A benzothiadiazine derivative is hydrochlorothiazide (6-chloro3, 4 dihydro -2H-1,2,4-benzothiadiazine 1,1-dioxide 7-sulfonamide). $C_7H_9C_{12}N_3O_4S_2$ is its chemical formula and its molecular weight is 297.7 g/mol. It is white in color, odorless and crystalline solid. Melting point is 274 °C. Hydrochlorothiazide is water soluble, freely soluble in NaOH, *n*-butyl amine,

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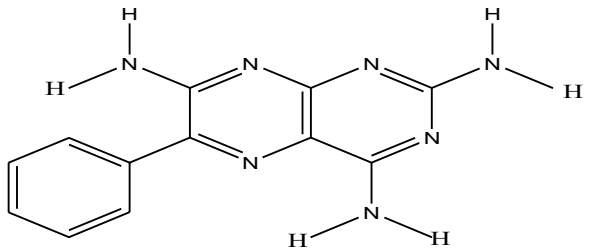

Fig. 1 Chemical structure of triamterene

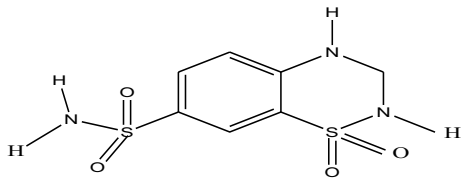

Fig. 2 Chemical structure of hydrochlorothiazide

dimethyl formamide, sparingly soluble in methanol and insoluble in ether, chloroform and dilutes mineral acids. Hydrochlorothiazide can be used alone or in combination with treat edema caused by congestive heart failure, hepatic cirrhosis, nephritic syndrome, acute glomerular nephritis and chronic renal failure. It is a drug that is used to treat hypertension. Hydrochlorothiazide prevents sodium and water from being reabsorption in the distal convoluted tubule, allowing for more water to be eliminated in the urine. Kidneys excrete the substance [7] (Fig. 2).

In New Drug Applications, the Food and Drug Administration [FDA] has allowed the use of the "Quality by Design" [QbD] approach to analytical processes [NDA]. The main goal of QbD is to carry out experimental procedures in order to better understand the process, product qualities and design control and tests that are carried out with in scientific boundaries with a minimum understanding of the development phase attained throughout the products life cycle. The QbD analytical testing technique is similar to that of suitable analysis at the appropriate periods. It is based on risk assessment and research, and it aids in the development of a robust and tenacious approach that adheres to ICH principles. Pharmaceutical companies are adopting the QbD idea for variety of reasons. In QbD, the variables impacting robustness are taken into account while developing analytical methods [8–11].

Literature review has been proved that the RPHPLC method available for the same combination of drugs to estimate simultaneously but no method is available on QbD with UPLC. So, an attempt has been done to estimate triamterene and hydrochlorothiazide by QbD with UPLC [12–14].

Experimental

Standard drugs

Triamterene and Hydrochlorothiazide pure drugs are procured from Hetero pharmaceutical laboratory, Hyderabad, as a gift sample with purity of 98–100% and 95–100%, respectively.

Reagents

HPLC grade acetonitrile from MOLYCHEM, HPLC grade methanol and water from LICHROSOLV [MERCK], and ortho-phosphoric acid from MERCK in the investigation.

Methodology

The aim of this method is split-plot design used to optimize an ultra-high-resolution liquid chromatography technique. For estimating triamterene and hydrochlorothiazide at the same time. The parameters such as mobile phase ratio, buffer pH and QbD to build and validate the UPLC, as well as to gain, a thorough knowledge of the ideas via optimal technique performance. The method developed is based on newer analytical techniques which are based on QbD approach [3]. Simultaneous estimation of triamterene and hydrochlorothiazide was developed by HPLC. There was No UPLC with QbD approach for this combination so want to develop this method [4].

Instrumentations and chromatographic conditions

For method development, a water UPLC aquity model with auto sampler and PDA detector (PDA -2996) was employed. At ambient temperature (25 °C), chromatographic separation was done in isocratic mode using a Dikmaleapsil C18 column with diameter of 4.6 × 50 mm and particle size of 2.7 m with a phase. The column was equilibrated with mobile phase before chromatographic analysis to ensure that the stationary phase was saturate. A UV/VIS spectrophotometer (LABINDIAUV3000 + pHmeter) ADWA-AD1020, weighing machine (AtcosetER-200A), pipettes, and burettes were used as well the experimental design was created during the design expert R version 12.0 program.

Preparation of solvent and solution

Preparation of 0.1% OPA

In a 1000-ml volumetric flask, 1 ml orthophosphoric acid was added and HPLC grade water was utilized to make up the volume. The solution was degassed in an ultrasonic water bath for 10 min before being vacuum filtered through a 0.45 filter, giving 0.1% orthophosphoric acid.

Preparation of mobile phase

400 ml 0.1% OPA buffer and 600 ml methanol were mixed and degassed for 10 min in an ultrasonic water bath before being filtered through 0.45 filters under vacuum.

Preparation of diluent

The mobile phase was used as the diluents.

Preparation of the triamterene and HCTZ standard and sample solution

Stock solution preparation

Accurately weight and transfer 75 mg of TRIAMTERENE and 50 mg of HCTZ working standard to a 100-ml clean dry volumetric flask, then add around 70 ml of diluents, sonicate dissolve fully, and then top up with the same solvent.

Standard solution preparation

Pipette 0.3 ml of the stock solution into a 10 ml volumetric flask and dilute to the desired concentration with diluent. Further pipette 0.3 ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. (22.5ppm of Traimterene and 15ppm of HCTZ).

Sample solution preparation

Weigh and transfer 75 mg of TRAIMTERENE and 50 mg of HCTZ into a 100-ml clean dry volumetric flask (Tablet powder = 285 mg). Add around 70 ml of diluents and sonicate until completely dissolved. A 0.45 micron filter should be used to filter it. Pipette 0.3 ml of the stock solution into a 10-ml volumetric flask and diluents to the desired concentration with diluents. A standard sample of 10 ml should be put into the system.

Preparation of working standard solution

Preparation of 50% solution

Accurately weigh and transfer 37.5 mg of triamterene and 25 mg of HCTZ working standard to a 100-ml volumetric flask. Add around 70 ml of diluents and sonicate until completely dissolved. Using the same solvent, increase the volume to the desired level. (This is an off-the-shelf option). Pipette 0.3 ml of the stock solutions into a 10-ml volumetric flask and diluents to the desired concentration with diluents.

Preparation of 100% solution

Accurately weigh and transfer 75 mg of TRAIMTERENE and 25 mg of HCTZ working standard to a 100-ml volumetric flask. Add around 70 ml of diluents and sonicate until completely dissolved using the same solvent, increase the volume to the desired level. (This is an off-the-shelf option). Pipette 0.3 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluent.

Preparation of 150% solution

In a 100-ml volumetric flask weigh 112.5 mg TRAIMTERENE and 75 mg HCTZ standard, add around 70 ml of diluents sonicate it and make up to the volume using solvent.(stock solution).Pipette 0.3 ml of the stock solutions into a 10-ml volumetric flask and dilute with diluents to the desired concentration.

Preparation of 0.01 ug/ml solution

Accurately weigh and transfer 75 mg of TRAIMTERENE working standard to a 100-ml clean dry volumetric flask, then add around 70 ml of diluents and sonicate to completely dissolve it, then make up the volume with the same solvent. (This is a stock solution).

Pipette 0.3 ml of the aforementioned stock solutions into a 10-ml volumetric flask and dilute with diluents to the desired concentration.

Pipette 1 ml of the aforementioned stock solution into a 10-ml volumetric flask and dilute with diluents to the desired concentration.

Pipette 0.05 ml of the aforementioned stock solution into a 10-ml volumetric flask and dilute with diluents to the desired concentration.

Preparation of 0.04 ug/ml solution

To a 100-ml clean dry volumetric flask, accurately weigh and transfer 75 mg of triamterene working standard, then add roughly 70 ml of diluents, sonicate to dissolve completely, and to pup with the same solvent. (Stock response).

Pipette 0.3 ml of the stock solutions into a 10-ml volumetric flask and dilute to the desired concentration with diluents.

Pipette 1 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluents.

Pipette 0.18 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration using diluents.

Preparation of 0.01 ug/ml solution

In a 100-ml clean dry volumetric flask, accurately weigh and transfer 50 mg of HCTZ working standard, then add roughly 70 ml of diluents, sonicate to dissolve completely and to pup with the same solvent. (Stock response).

Pipette 0.3 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluents.
Pipette 1 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentrations with diluents.
Pipette 0.05 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluent.

Preparation of 0.03 ug/ml solution

In a 100-ml clean dry volumetric flask, accurately weigh and transfer 50 mg of HCTZ working standard, then add around 70 ml of diluent, sonicate to dissolve completely, and to pup with the same solvent. (Stock response).

Pipette 0.3 ml of the stock solutions into a 10-ml volumetric flask and dilute to the desired concentration with diluent.
Pipette 1 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluents.
Pipette 0.2 ml of the stock solution into a 10-ml volumetric flask and dilute to the desired concentration with diluents.

Method validation

Linearity

The approach was verified for linearity in accordance with ICH recommendations. Prepare 5 different concentrations from stock solution. In a volumetric flask, 0.1 ml of stock solutions collected and volume is increased to 10 ml, yielding 7.5 g/ml solution. Similarly, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml stock solutions pipette out and the volume is increased to 10 ml to prepare 15 g/ml, 22.5 g/ml, 30 g/ml, and 37.5 g/ml for levels II, III, IV, and V, respectively. The peak area of each level is assessed after injection into the chromatographic apparatus. A graph with concentration on the x-axis and peak area on the y-axis issued to compute the correlation.

Precision

The degree of reproducibility or repetition of test findings for analytical technique is measured by precision. Six individual sample solutions were made to determine the methods' precision, following the protocol outlined in the assay by weighting individual tablet powder. The triamterene and hydrochlorthiazide percent assays were determined.

Accuracy

The correctness of the conventional addition procedure was determined before it was used. The sample solutions were made in three distinct concentrations, 50%, 100%, and 150%, as described in production of solutions. The standard stock solution was made in the same ways as the assays. Each concentration level was examined in three duplicates. The percent recovery and mean recovery numbers were calculated using the quantity contributed and amount found data.

Detection and quantification limits

The LOD and LOQ are the lowest analyze concentrations that can be reliably detected and quantified (LOQ). For LOD and LOQ determinations, 0.01 g/ml and 0.03 g/ml hydrochlorothiazide solutions were produced and injected into the UPLC. The technique was duplicated three times and each injection was replaced six times. The percent RSD value was calculated.

Degradation studies

The international conference harmonization issued recommendations titled "stability testing of novel drugs substance and products" to high light the intrinsic stability properties of active chemicals, which were followed to conduct stressed gradation experiments on triamterene and hydrochlorothiazide. Both samples were subjected to hydrolytic degradation tests in acidic and alkaline settings, temperature include degradation tests, oxidative degradation tests and UV degradation tests. The investigations were carried out using the stock solutions that had been developed before.

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of the above solution into a 10-ml volumetric flask, and then add 3 ml of 0.1 N HCL. After that, the volumetric flask was kept at 60 °C for 24 h before being neutralized with 0.1 N NaOH and diluents were added to bring it up to 10 ml. After filtering the solution using 0.44 micro-syringe filters, pour it into vials.

Hydrolytic degradation under alkaline condition

Pipette 0.3 ml of the above solution into a 10-ml volumetric flask, followed by 3 ml of 0.1 N NaOH. After that, the volumetric flask was kept at 60 °C for 24 h before being neutralized with 0.1 N HCL and diluents were added to bring it up to 10 ml. After filtering the solution using 0.44 micro-syringe filters, pour it into vials.

Thermal-induced degradation

Triamterene and HCTZ samples were put in a Petri dish and baked for three hours at 110 °C in a hot air oven. After that, the sample was collected, diluted with diluents and then injected into HPLC for analysis.

Oxidative degradation

10-ml volumetric flask was filled with 1 ml of 30 percent w/v hydrogen peroxide, and the volume was brought up to the mark using diluents. After that, the sample solutions using 0.45 micro-syringe filters pour into vials.

Photodegradation

Pipette out 0.3 ml of the above stock solutions into a 10-ml volumetric flask, expose to sunlight for 24 h, and the dilute to desired concentration. After filtering the solution using 0.45 micro-syringe filters, pour it into vitals.

Results

Screening design for suitable chromatographic conditions

To choose an appropriate solvents system or mobile phase, A QbD tested a number of solvents, including methanol: orthrophosphoric acid buffer and methanol: phosphate buffer and methanol: acetonitrile: methanol with various pH combination and amounts. Finally, in isocratic mode of separation, the condition optimized was 0.1 percent orthophosphoric acid: methanol in a C18 column with dimensions of 4.6 × 50 mm and particle size of 2.7 m, injection volume of 4 and flow rate of 0.3 ml per min in a C 18 column with dimension of 4.6 × 50 mm and particle size of 2.7 m, injection volume if 41, and flow rate of 0.3 ml per min. These ideal parameters were determined using the Shapiro–Wilk test.

Design expert 12 software was used to examine the data collected.

The program recommended a quadratic model, and the general equation for this model is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_1 X_1^2 + \beta_2 X_2^2 + \beta_3 X_3^2.$$

where 0 represents the arithmetic average of all quantitative results from all experimental runs; 1, 2, 3 represent the coefficient calculated from the observed experimental values of Y; and X_1, X_2 and X_3 represent the code level of variables. Variables X_1, X_2 and X_3 have quantifiable influence on each other of the Y_1, Y_2 and Y_3 solutions, as shown ninth equation. For each dependent variable, ANOVA was used to create mathematical models, which were then translated into 3D surface graph and equations.

The impact to find dependent factors (X_1, X_2 and X_3) and their interactions terms (X_1X_2, X_1X_3 and X_2X_3) on each dependent variable was assessed. Nonlinearity is detected by looking at exponential terms.

Details of chromatographic conditions are mentioned in Table 1

Table 1 Optimized chromatographic conditions

Instruments	WATER UPLC Acquit model with auto sampler and PDA detector
Temperature	25 °C
Mode of separation	Isocratic mode
Column	Dismal leapsil C 18 column (4.6 × 50 mm, 2.7 µm)
Mobile phase	0.1% orthophosphoric acid: methanol (40:60)
Flow rate	0.3 ml/min
Wavelength	224 nm
Injection volume	4 Micron liter
Run time	6 min

Table 2 Linearity for triamterene

S. nos.	Concentration(µg/ml)	Area
1	7.5	428,475
2	15	666,276
3	22.5	884,564
4	30	1,122,752
5	37.5	1,380,840
Correlation coefficient	0.999	

Method validation

The adjusted chromatographic setting allowed for excellent drug separation and no drug degradation throughout the analysis. For the parameters listed below, the UPLC technique was verified.

Linearity

In the range of concentration of 7.5–37.5 µg/ml for triamterene and 5–25 µg/ml for hydrochlorothiazide, the study of linearity was conducted. For triamterene, the regression equation was $Y = 31{,}483X + 188{,}220$ with $R^2 = 0.9992$, while for hydrochlorothiazide it was $Y = 31{,}591$X-8299 with $R^2 = 0.9998$. Linear reaction for both medications across concentration range studied. For triamterene and hydrochlorothiazide linearity tablets are mentioned in Tables 2 and 3 and linearity graphs are mentioned in Figs. 3 and 4.

Table 3 Linearity for hydrochlorothiazide

S. nos.	Concentration (µg/ml)	Area
1	5	145,856
2	10	310,711
3	15	467,567
4	20	625,427
5	25	778,275
Correlation coefficient		0.999

Acceptance criteria: correlation coefficient should be not less than 0.99

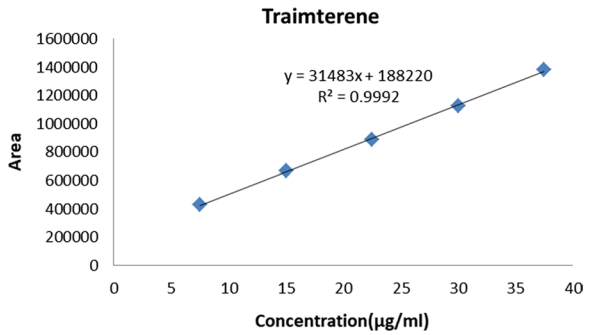

Fig. 3 Linearity graph of triamterene

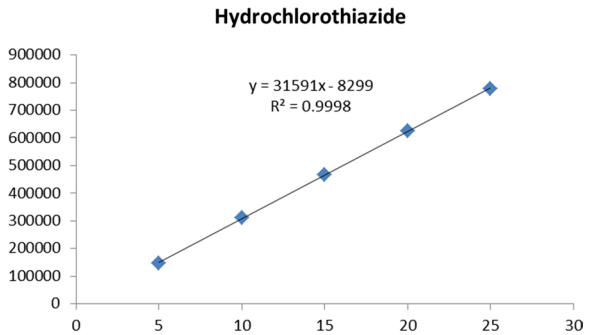

Fig. 4 Linearity graph of hydrochlorothiazide

Precision

Standard solution injected six times into UPLC to determine accuracy, and the area for all six injections was calculated for triamterene and hydrochlorothiazide, the percent RSD was determined to be 0.5 and 1.2, respectively. The percent RSD that was obtained was determined to be with in the required limits. (NMT 2%). The method's intermediate precision/ruggedness was determined in the same way as the precision, but on separate days. There region of six duplicate injections percent RSD must determine in prescribed limits (Table 4).

Table 4 Precision results for triamterene and hydrochlorothiazide

Injection	Triamterene area	HCTZ area
1	838,408	245,103
2	835,617	246,003
3	832,556	240,386
4	837,966	246,088
5	830,038	249,300
6	839,801	245,551
Ave	835,731.0	245,405.2
SD	3774.4	2874.0
Percent RSD	0.5	1.2

Acceptance: The percent relative standard deviation should not be more than 2%

Accuracy

For 50%, 100% and 150% concentrations, accuracy data revealed a percent recovery of 101.05, 100.73 and 100.45, respectively, with a mean recovery of 100.75 ($n=3$). For 50%, 100% and 150% concentrations, hydrochlorothiazide had a percent recovery of 99.89, 101.82 and 107.86, respectively, with a mean recovery of 103.19 ($n=3$). Data on accuracy indicated that both medications had a high percent recovery rate. Accuracy results of both drugs are mentioned in Tables 5 and 6.

Detection limit

Percentage recovery of 50%, 100% and 150% concentrations was 101.05, 100.73 and 100.45, respectively, with a mean recovery of 100.75 ($n=3$).

Hydrochlorothiazide had a percent recovery of 99.89, 101.82 and 107.86 at 50, 100 and 150% concentrations, respectively, with a mean recovery of 103.19 ($n=3$). Both drugs had a high % recovery rate, according to data on accuracy (Figs. 5, 6, 7, 8, 9, 10).

The S/N values obtained are given in Table 7.

Degradation studies

Stress degradation investigations such as hydrolytic degradation in acidic settings, hydrolytic degradation in basic conditions, heat-induced deterioration, oxidative degradation and photodegradation are carried out according to the ICH recommendations. During the analysis, there was no evidence of drug degradation. Acid, base, thermal, peroxide and photo-sample degradation peak results are mentioned in Tables 8, 9, 10, 11 and 12 (Figs. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24).

Table 5 The accuracy results for triamterene

Percentage concentration	Area*[n]	Amount added in mg	Amount found in mg	Percentage recovery	Mean recovery
50	422,827	37.5	37.89	1.1.05	100.75
100	842,991	75	75.55	100.73	
150	1,261,001	112.5	113.01	100.45	

n = 3 determinations

Table 6 Hydrochlorothiazide accuracy results

%Concentration	Area	Amount added in mg	Amount found in mg	Percentage recovery	Mean recovery
50%	236,219	25	24.97	99.89	103.19
100%	481,571	50	50.91	101.82	
150%	765,167	75	80.89	107.86	

Acceptance criteria: the percentage recovery between 98.0 and 105.0%

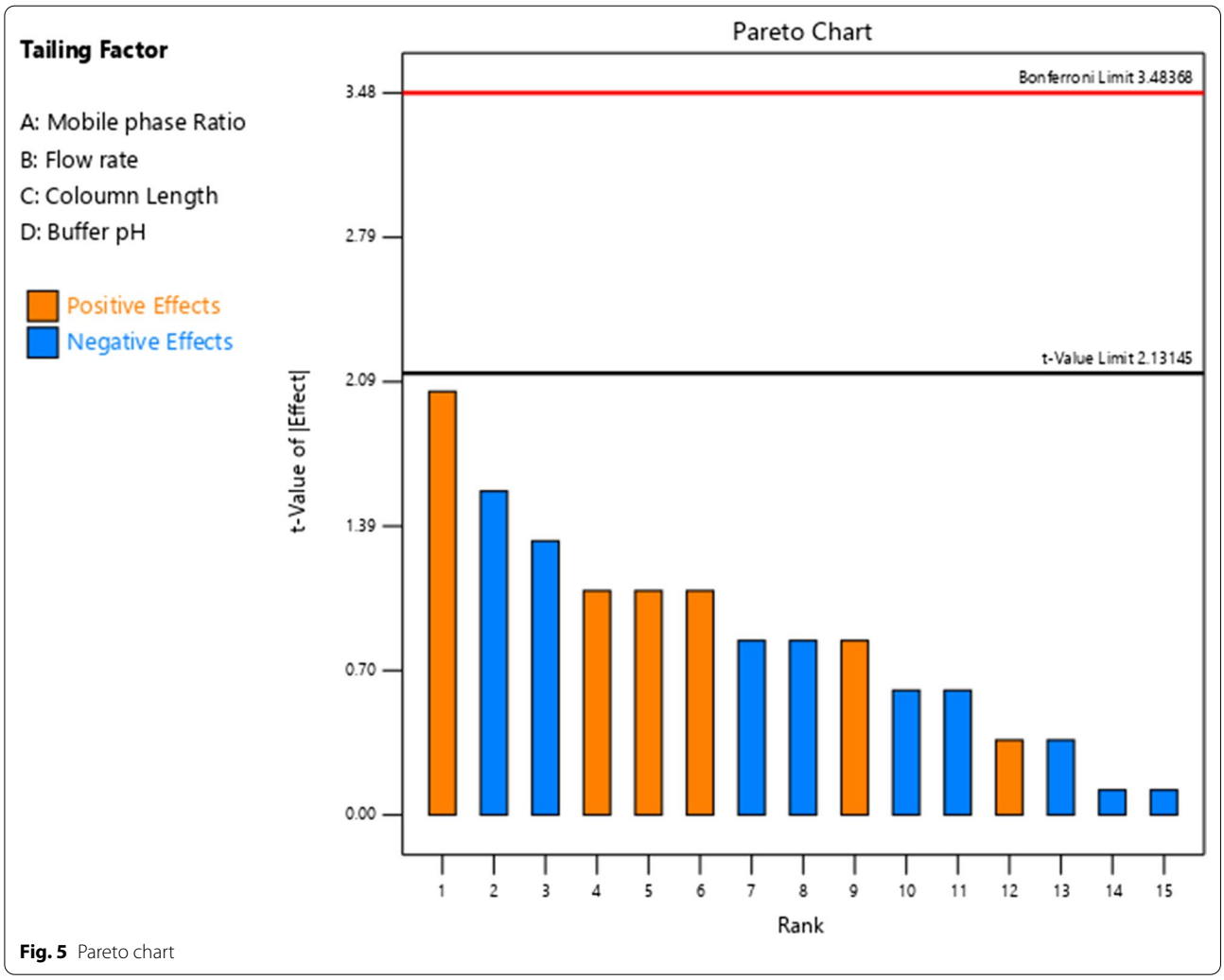

Fig. 5 Pareto chart

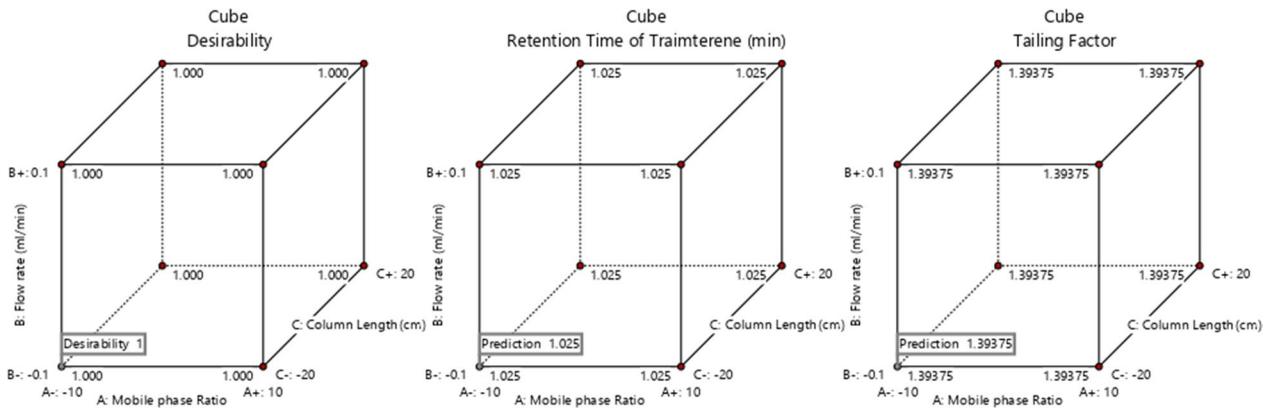

Fig. 6 Cube desirability, flow rate, mobile phase

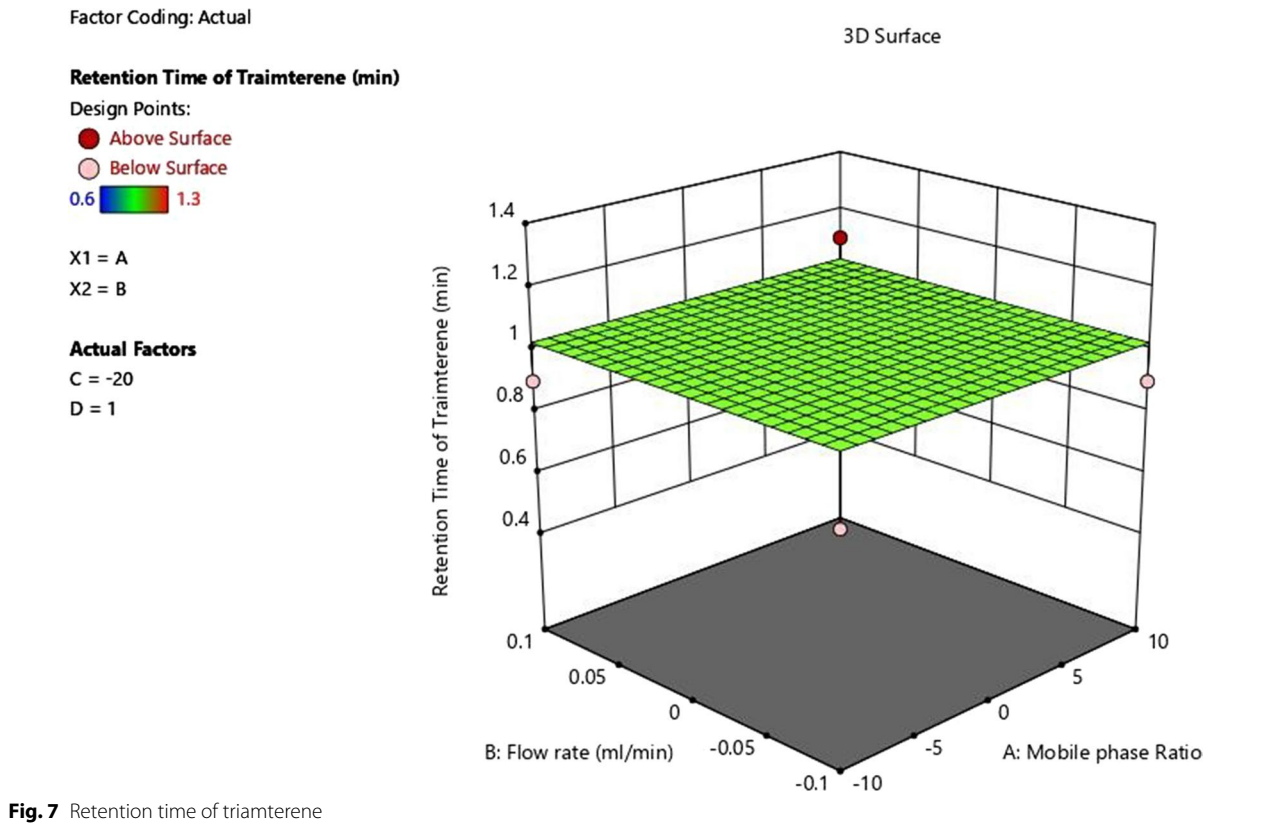

Fig. 7 Retention time of triamterene

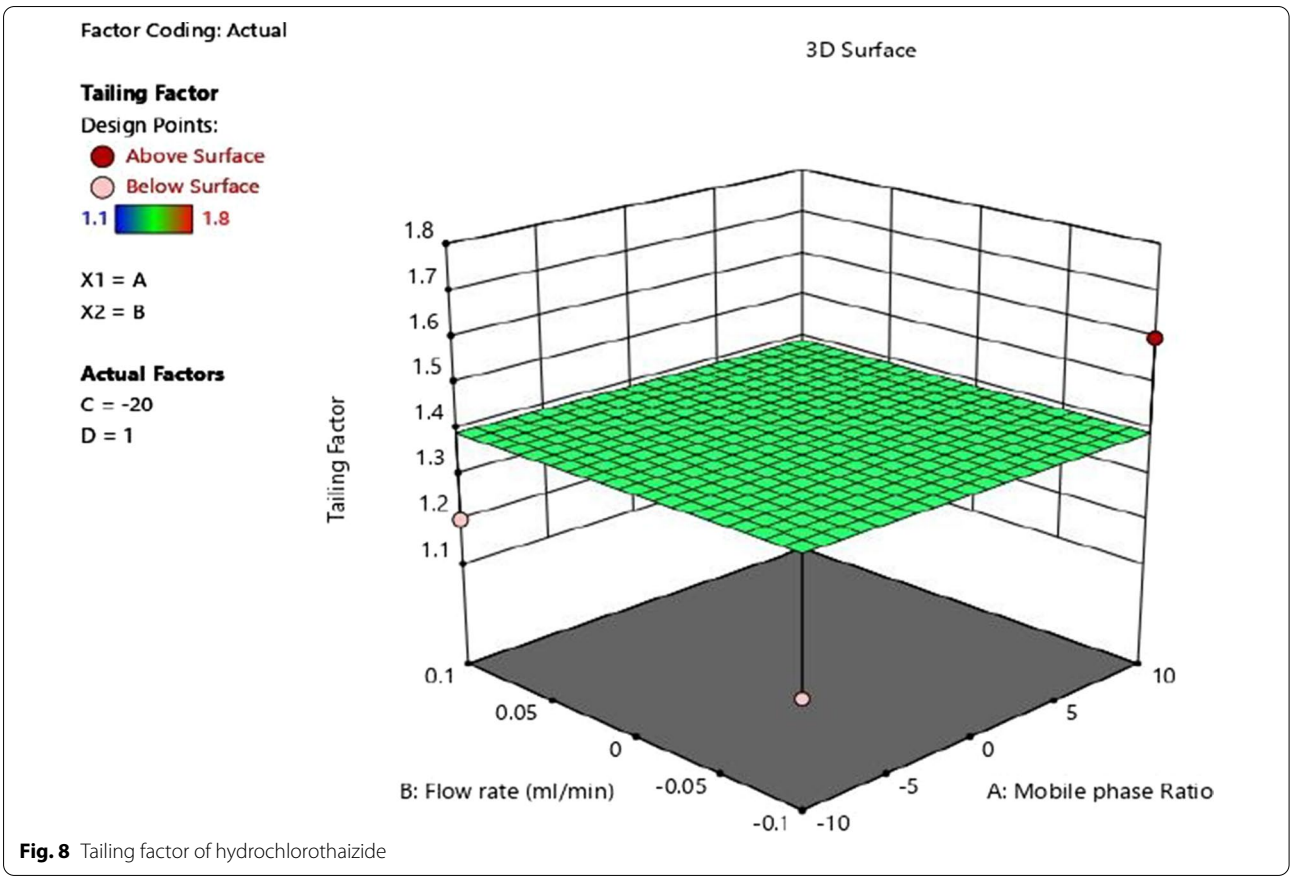

Fig. 8 Tailing factor of hydrochlorothaizide

Table 7 Detection limit [LOD, LOQ]

S/N Sample	LOD (accepted *S/N* ratio = 3)	LOQ (accepted *S/N* ratio = 10)
Triamterene	3.07	10.09
Hydrochlorothiazide	2.95	9.93

Discussion

Analytical quality by design (QbD) with UPLC method for estimation of triamterene and hydrochlorothiazide combined dosage forms. Target profile was retention time, plate count, resolution and tailing factor. Mobile phase ratio, pH buffer solution, length of column and temperature were identified as critical quality attributes. Quality by design was applied for two-level factorial design by design expert 12 software was used. The quality by design approach was successful. (15–16). Different method validation parameters like linearity Co-relations should not less than 0.999. The Precision for percentage recovery is between 98.0% and 105.0%. LOD for triamterene and hydrochlorothiazide is 3.07 and 2.95. LOQ for triamterene and hydrochlorothiazide is 10.09 and 9.93 stress degradation studies have done for triamterene and hydrochlorothiazide for acid degradation were 0.925 and 1.7442 for base 0.984 and 1.744 for thermal peroxide 0.918 and 1.736 for peroxide effect 0.918 and 1.736 and for photo-sample 0.945 and 1.518 the developed method was validated according to ICH guidelines [15, 16].

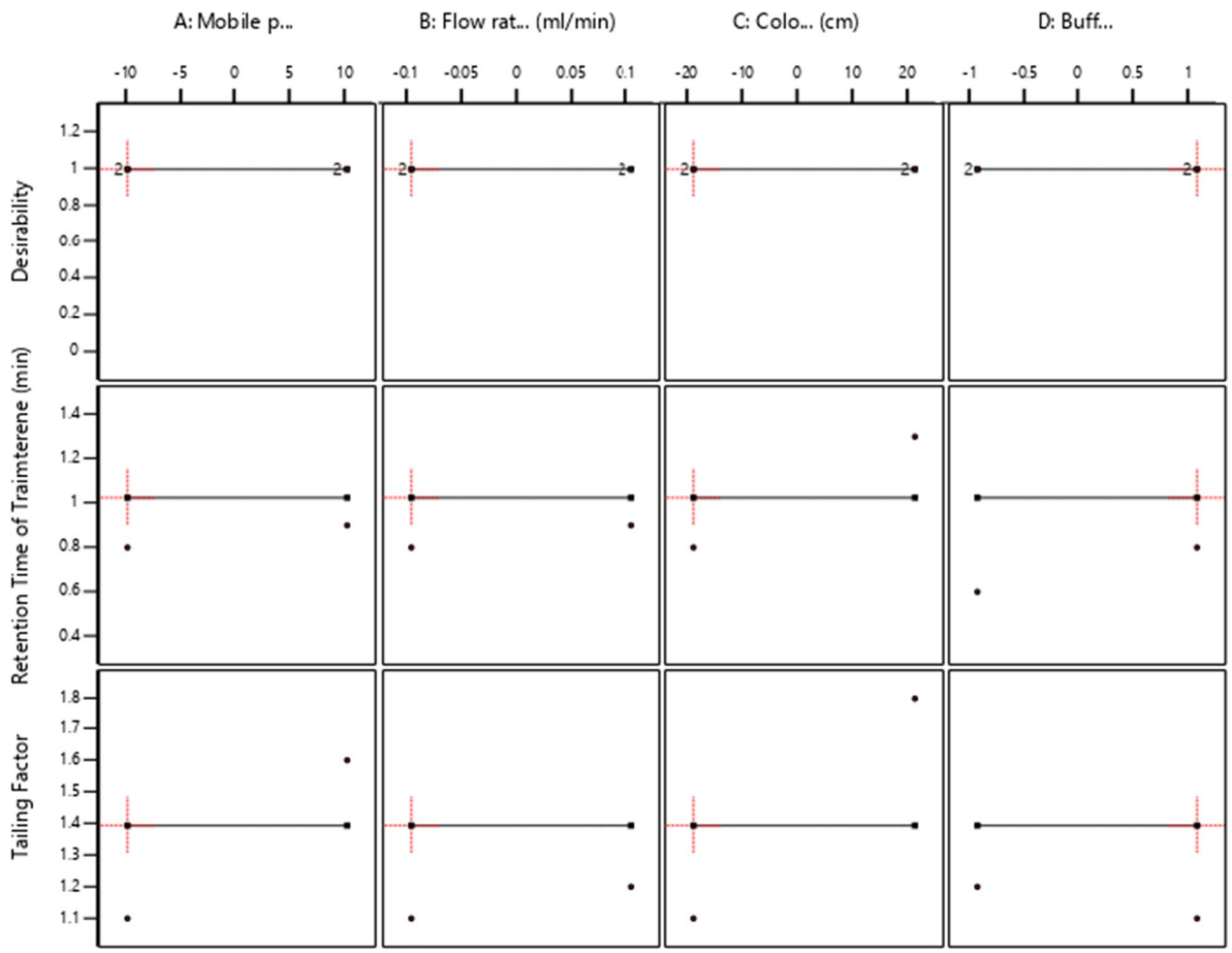

Fig. 9 Factors responses

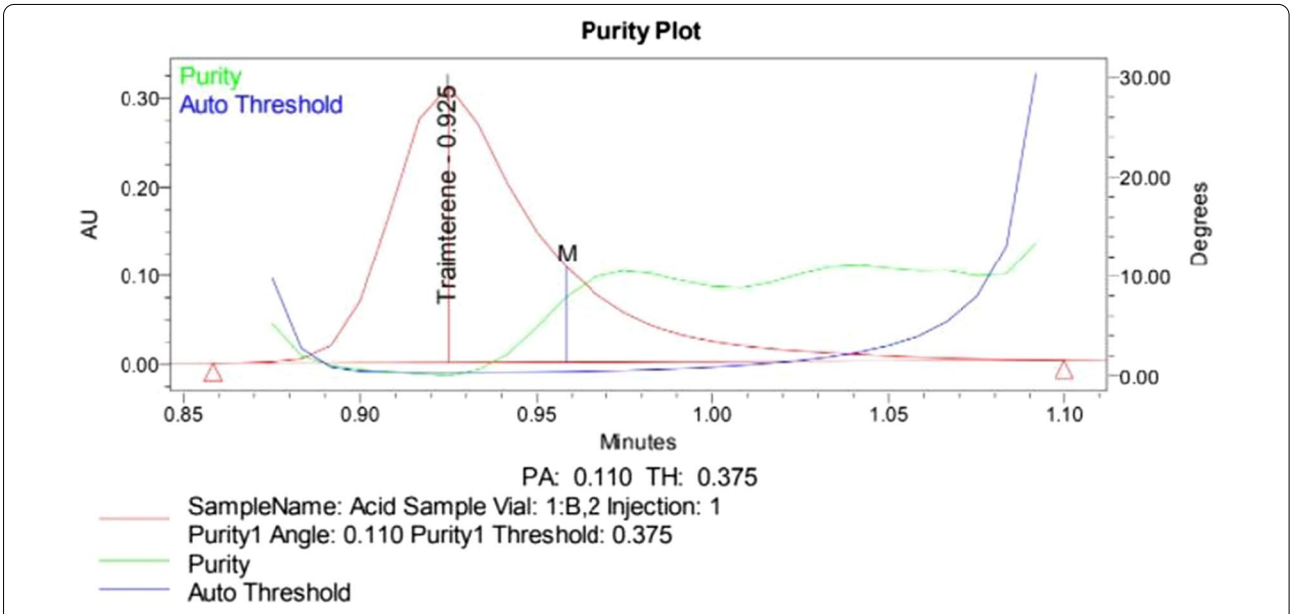

Fig. 10 Purity plot of triamterene of acid degradation

Table 8 Acid degradation peak results

	Sample	Retention time	Area	Height	USP	USP plate	Purity1 angle	Purity1 threshold
01	Peak1	0.067	284	278	0.91	77.75		
02	Triamterene	0.925	822,994	302,607	1.72	2402.80	0.110	0.375
03	Peak1	1.285	17,228	6303	1.66	5408.99	2.203	4.363
04	Peak1	1.548	28,793	7051		4588.52	26.586	4.024
05	HCTZ	1.742	931,060	233,776	1.29	4582.89	0.262	0.348
06	Peak1	2.516	1305	442	0.60	32,440.89		
07	Peak1	4.134	152	228	1.60	347,087.42		
08	Peak1	5.817	113	225	1.00	649,614.07		

Table 9 Base degradation peak results

	Sample	Retention time	Area	Height	USP tailing	USP plate	Purity angle	Purity threshold
01		0.067						
02	Peak1	0.921	998,471	396,915		292.97		
03	Triamterene	0.981	810,006	307,869		1445.16	12.801	3.904
04	Peak3	1.285						
05		1.393	9952	3440	1.26	4686.18	2.959	3.398
06	Peak4	1.549	14,828	4598	1.12	4742.60	1.990	2.620
07	HCTZ	1.744	937,058	238,214	1.34	4367.71	0.391	0.286
08		2.083	125	208	1.11	71,046.96		
09		2.225	90	180	1.00	216,122.28		
10	Peak6	2.516						
11		2.760	202	255	1.21	119,996.94		
12		2.809	152	282	1.00	222,599.98		
13		3.507	459	426	0.60	106,744.19		
14	Peak7	4.134						
15		4.533	113	226	1.00	939,602.49		
16		5.010	177	249	1.30	421,643.03		
17	Peak8	5.709	99	181	1.09	877,705.41		

Conclusion

On design expert software version 12 a quality by design methodology was used to build a UPLC technique foe triamterene and hydrochlorothiazide. Mobile phase, flow rate, column, length, and buffer pH were all employed as independent variables. According to ICH criteria, the approach was validated. Based on the results from method development and degradation experiments, the developed UPLC method demonstrated high linearity, precision, accuracy, LOD, LOQ and ruggedness for determination. The method may also be employed in the build an ultra-performance liquid chromatography for simultaneous quantification of triamterene, hydrochlorothiazide in tablet and bulk dosage form.

Table 10 Thermal degradation peak results

	Sample	Retention time	Area	Height	Tailing	Plate count	Purity1 angle	Purity1 threshold
01	Peak1	0.067						
02	Triamterene	0.918	802,817	305,133	1.61	2408.53	0.212	0.379
03		1.158	1266	637	2.61	7046.27		
04	Peak3	1.285						
05		1.385	25,485	7798	1.15	3842.44	1.298	1.706
06	Peak4	1.542	30,086	8623		3901.05		
07		1.598	6812	3162		1494.43		
08	HCTZ	1.736	922,762	231,601	1.32	4218.36	0.176	0.278
09	Peak6	2.516						
10		3.342	159	317	1.00	305,940.06		
11	Peak7	4.033	105	209	1.00	419,284.37		
12	Peak8	5.817						

Table 11 Peroxide peak results

	Sample	Retention time	Area	Height	Tailing	Plate count	Purity1 angle	Purity1 threshold
01	Peak1	0.067						
02		0.534	187	260	1.32	4940.33		
03	Triamterene	0.918	811,590	305,421	1.72	2381.52	0.292	0.391
04	Peak3	1.285						
05		1.385	25,714	7887	1.16	3802.83	1.274	1.777
06	Peak4	1.543	36,867	8718		3957.11	15.224	1.501
07	HCTZ	1.736	917,267	230,369	1.32	4198.72		
08	Peak6	2.516						
09		3.700	87	174	1.00	386,435.81		
10	Peak7	4.134						
11	Peak8	5.817						

Table 12 Photo-sample peak results

	Sample	Retention time	Area	Height	USP	USP plate	Purity1 angle	Purity1 threshold
01		0.058	59	118	1.00	186.13		
02	Peak1	0.067						
03	Triamterene	0.945	79,948	303,779	1.44	1892.67	0.097	0.346
04	Peak3	1.285						
05	Peak4	1.394	41,876	11,566		1281.92		
06	HCTZ	1.518	91,547					
5	229,882	1.29	3293.06	0.286	0.289			
07	Peak6	2.516						
08		2.708	97	193	1.00	89,503.44		
09		3.298	256	296	1.05	158,840.70		
10		3.925	341	259	0.74	175,710.08		
11	Peak7	3.958	69	137	1.00	716.09		
12	Peak8	5.850	90	181	1.00	598,183.92		

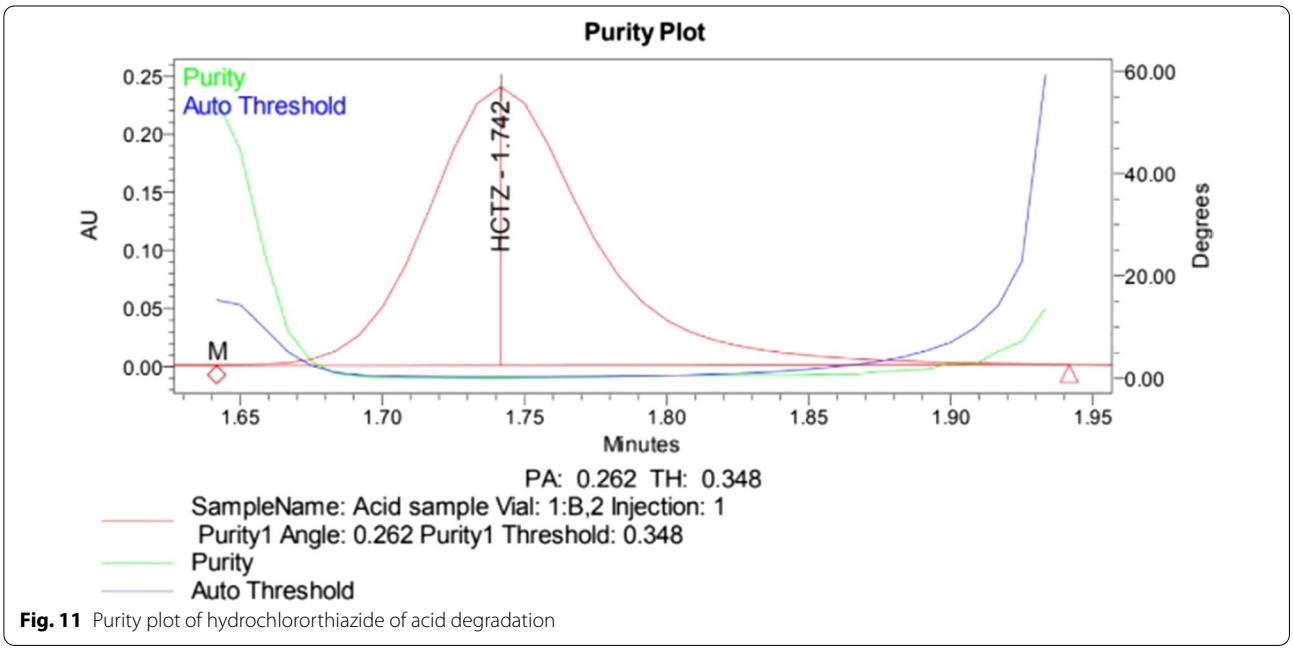

Fig. 11 Purity plot of hydrochlorothiazide of acid degradation

SAMPLE INFORMATION

Sample Name:	Acid Sample	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Traim_HCTZ Validation
Vial:	1:B,2	Acq. Method Set:	Traim_HCTZ UPLC_1
Injection #:	1	Processing Method:	Traim_HCTZ Stability 1
Injection Volume:	4.00 ul	Channel Name:	ACQ- 2996 224 nm -1.2
Run Time:	6.0 Minutes		

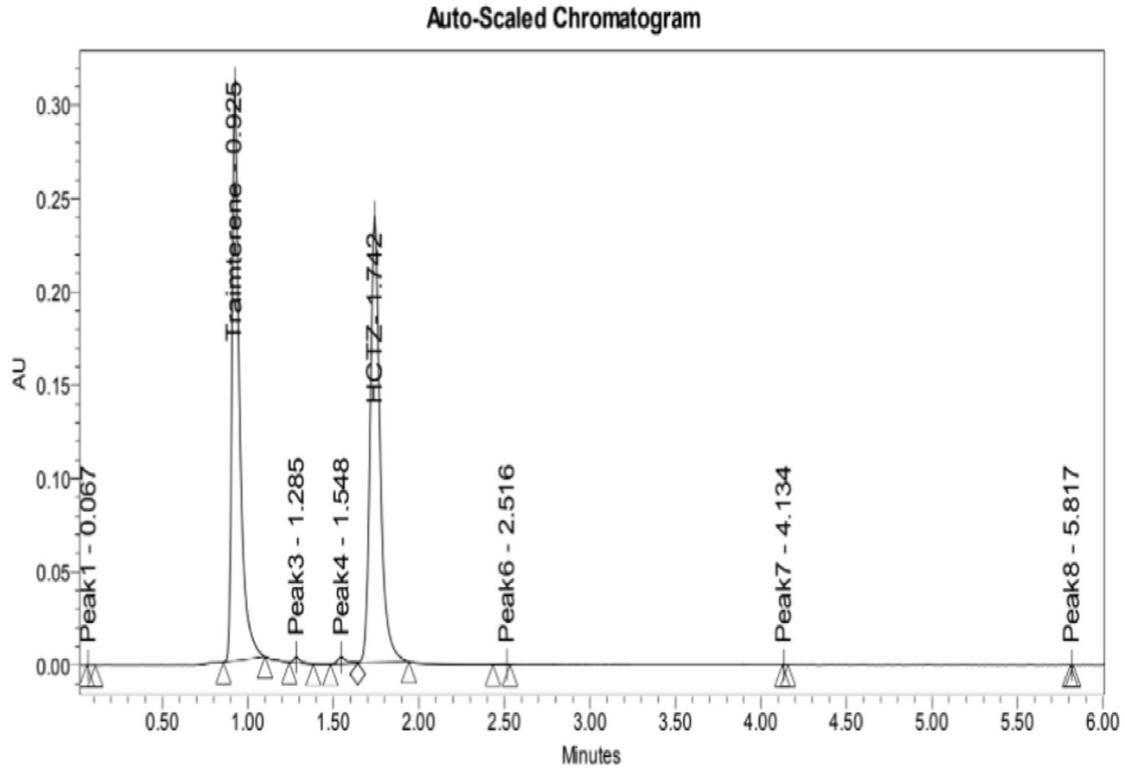

Fig. 12 Acid degradation chromatogram

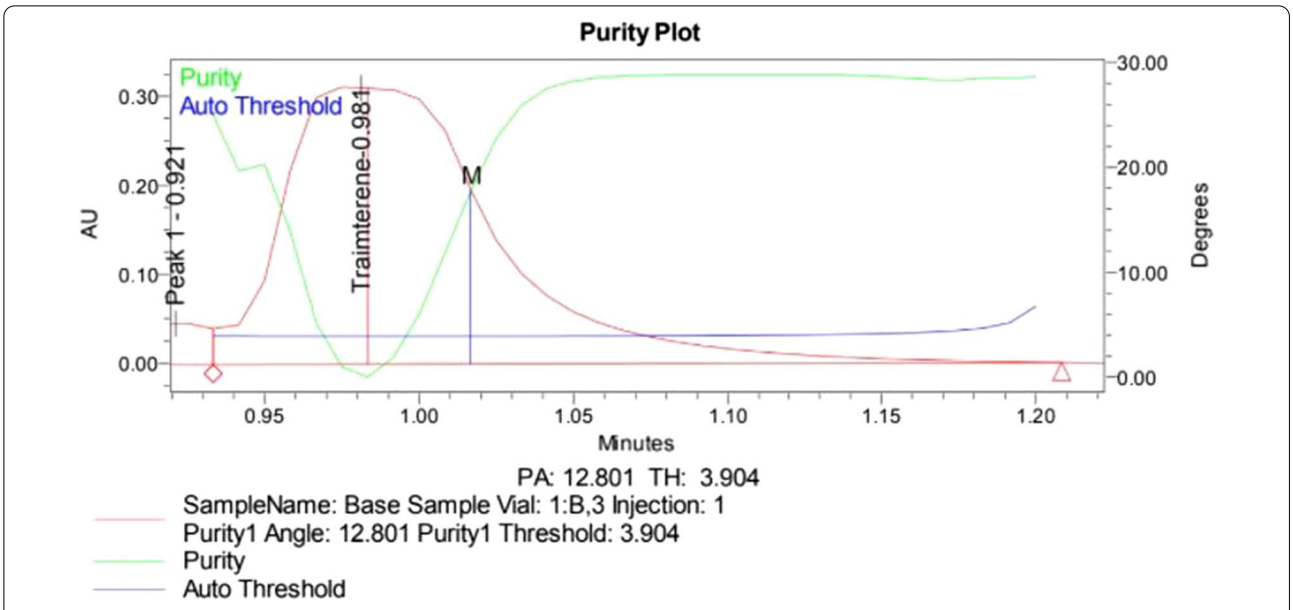

Fig. 13 Purity plot of triamterene of base degradation

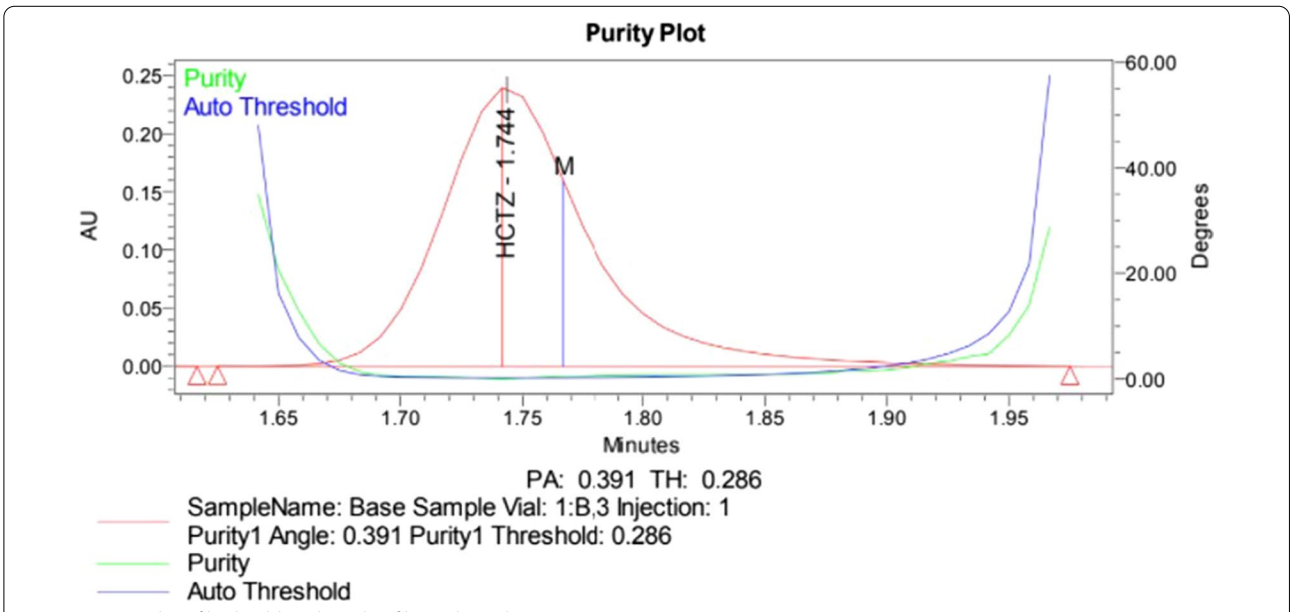

Fig. 14 Purity plot of hydrochlorothiazide of base degradation

SAMPLE INFORMATION

Sample Name:	Base Sample	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Traim_HCTZ Validation
Vial:	1:B,3	Acq. Method Set:	Traim_HCTZ UPLC_1
Injection #:	1	Processing Method:	Traim_HCTZ Stability 1
Injection Volume:	6.00 ul	Channel Name:	ACQ- 2996 224 nm -1.2
Run Time:	6.0 Minutes		

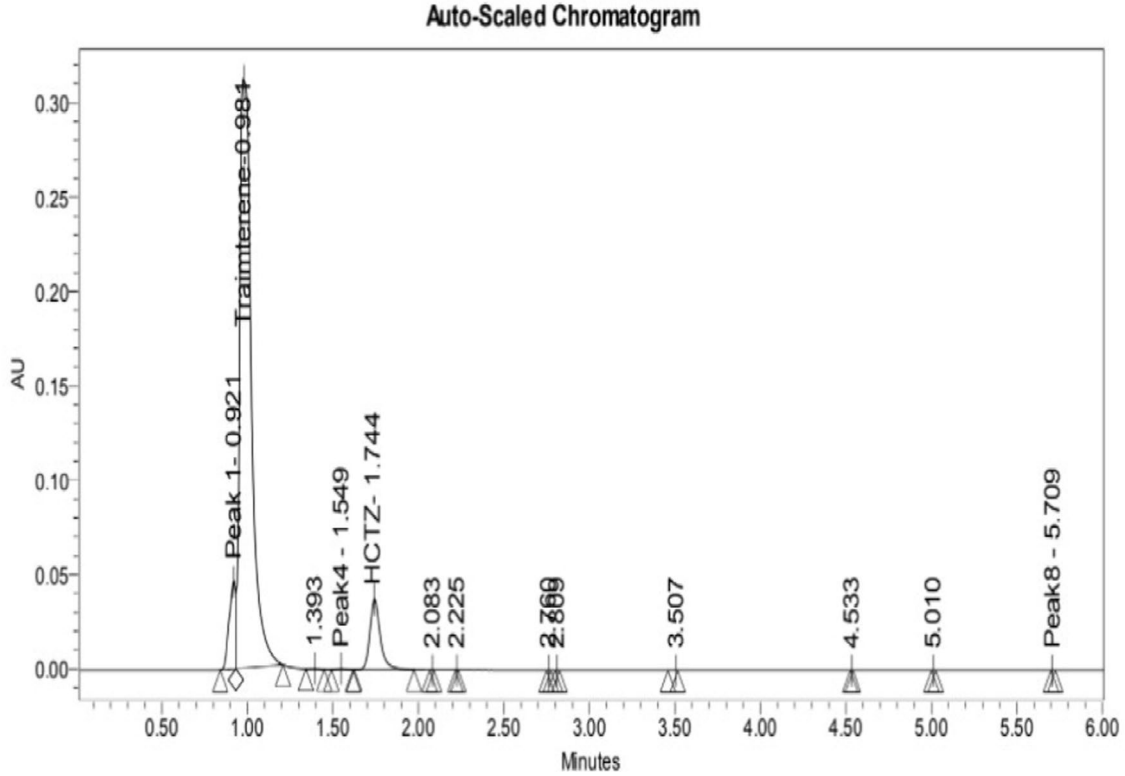

Fig. 15 Base degradation chromatogram

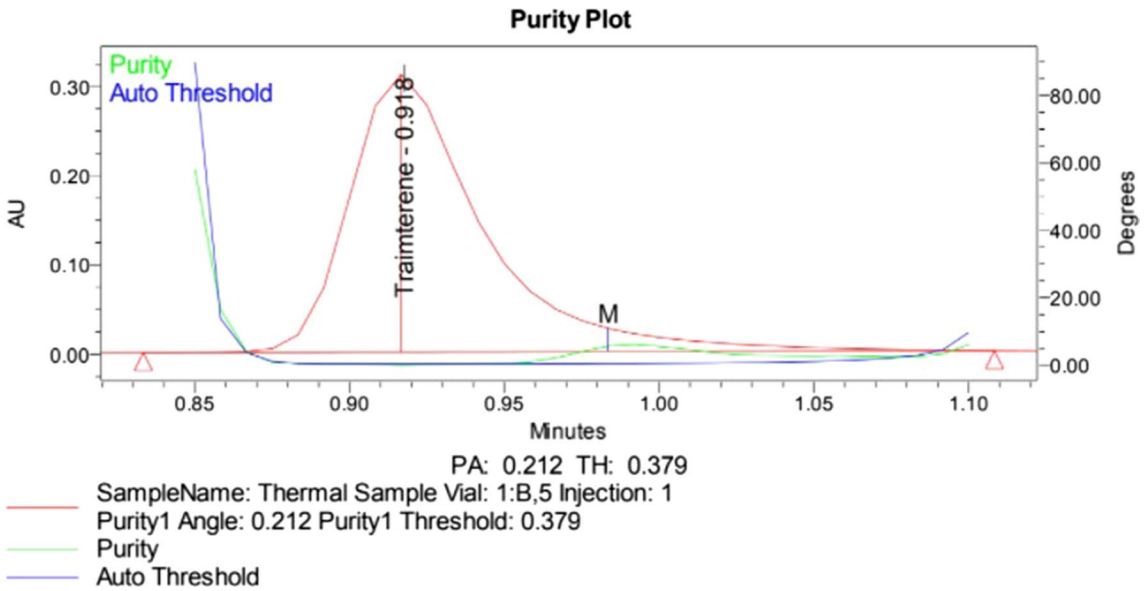

Fig. 16 Purity plot of triamterene of thermal degradation

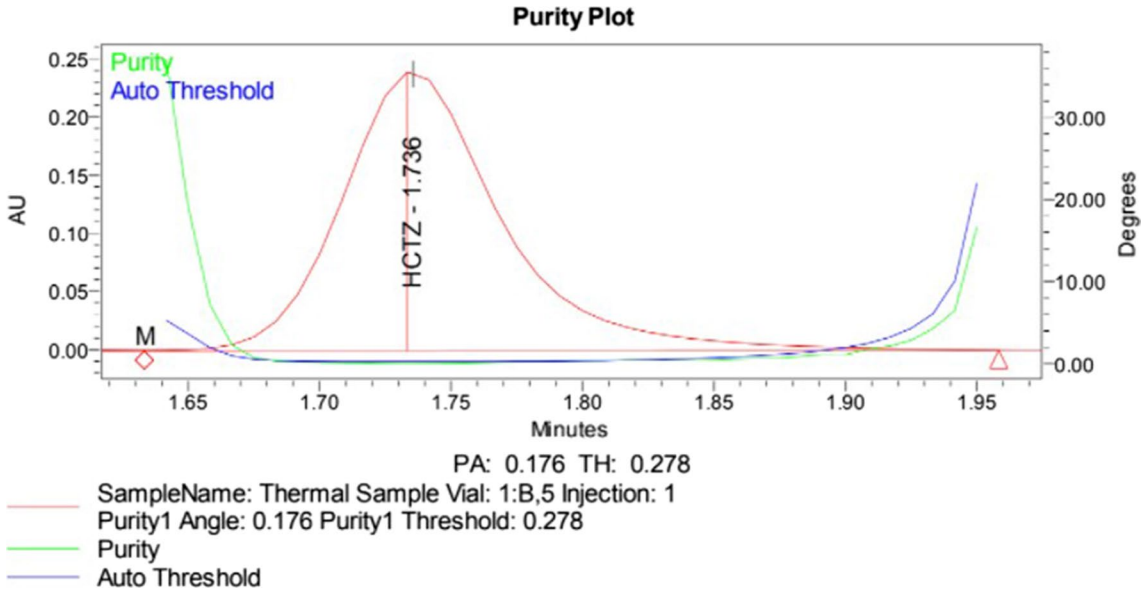

Fig. 17 Purity plot of hydrochlorothiazide of thermal degradation

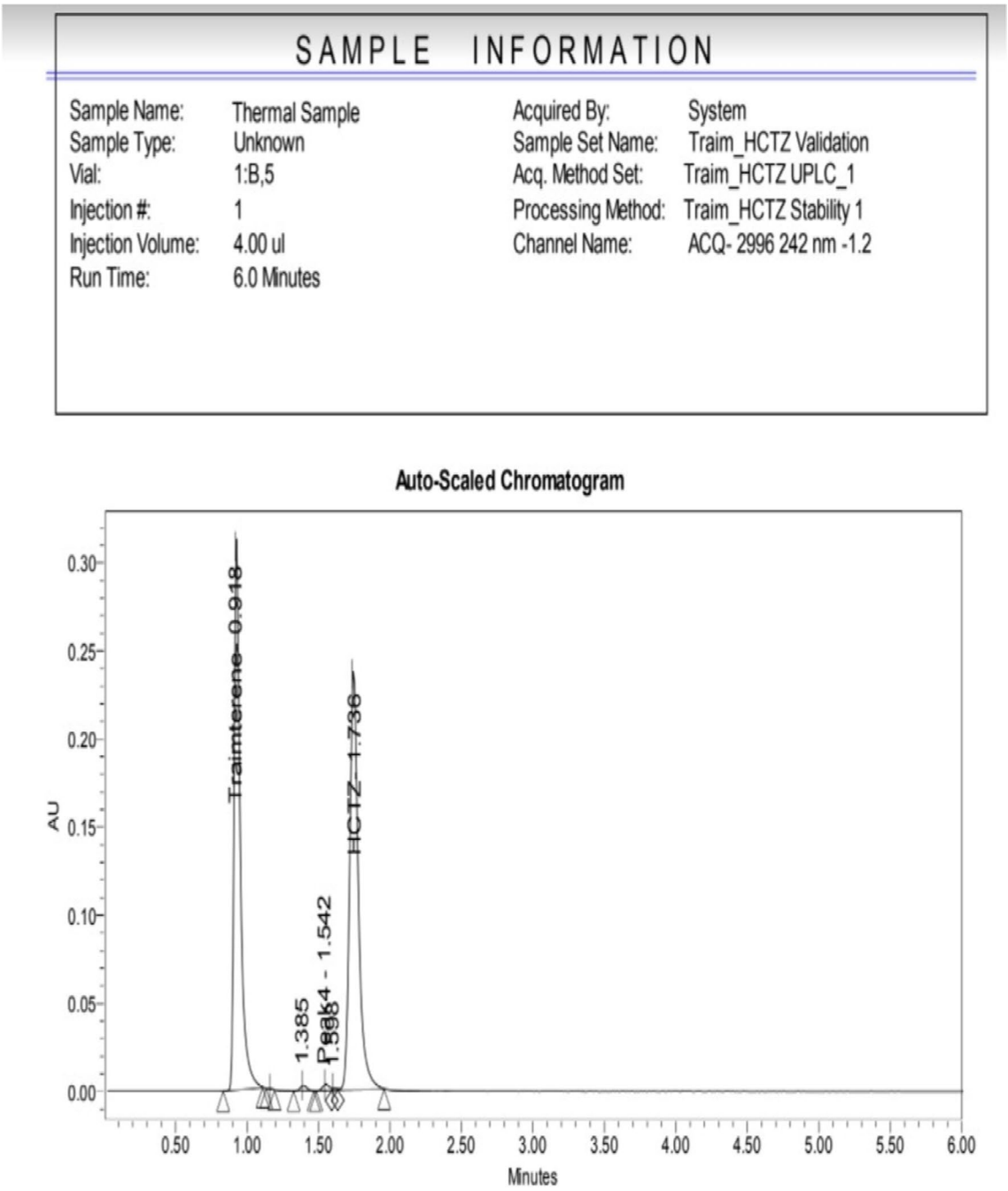

Fig. 18 Thermal degradation of chromatogram

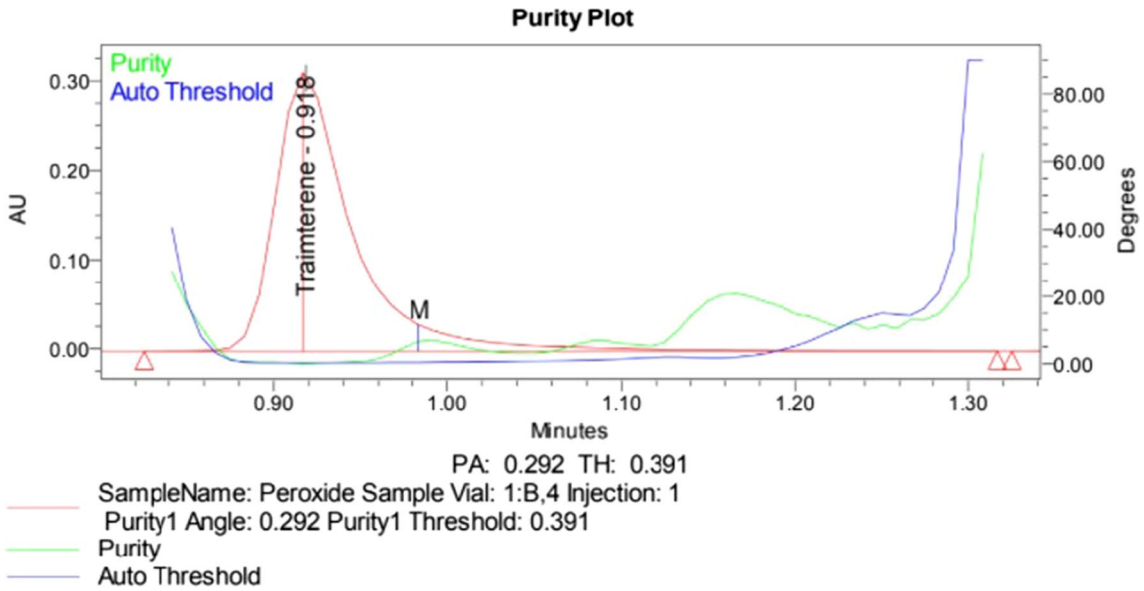

Fig. 19 Purity plot of triamterene of peroxide

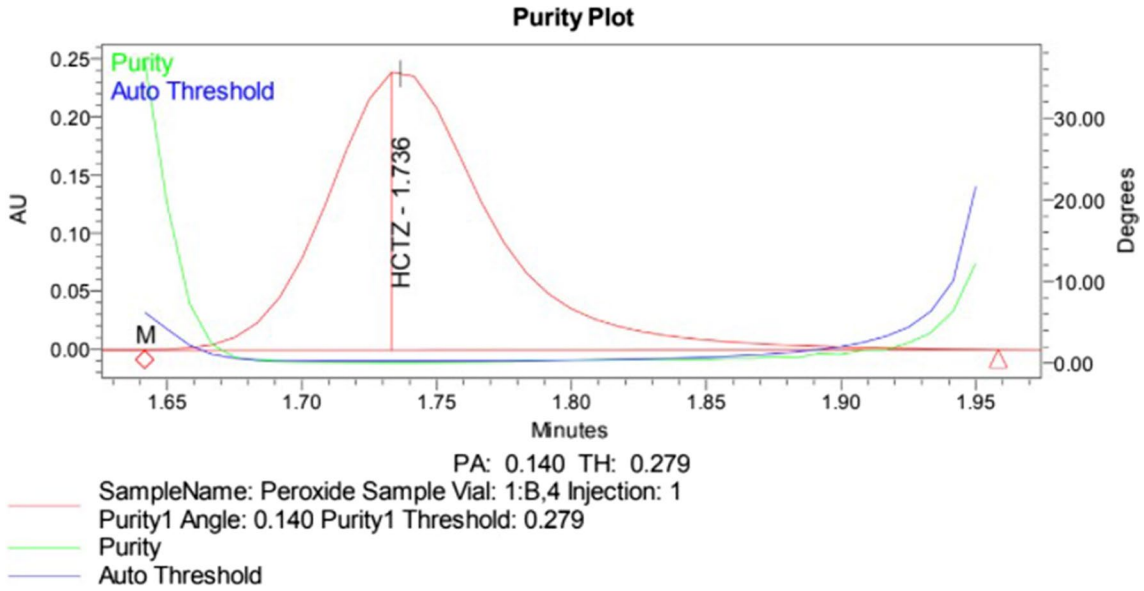

Fig. 20 Purity plot of hydrochlorothaizide of peroxide

SAMPLE INFORMATION

Sample Name:	Peroxide Sample	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Traim_HCTZ Validation
Vial:	1:B,4	Acq. Method Set:	Traim_HCTZ UPLC_1
Injection #:	1	Processing Method:	Traim_HCTZ Stability 1
Injection Volume:	4.00 ul	Channel Name:	ACQ- 2996 242 nm -1.2
Run Time:	6.0 Minutes		

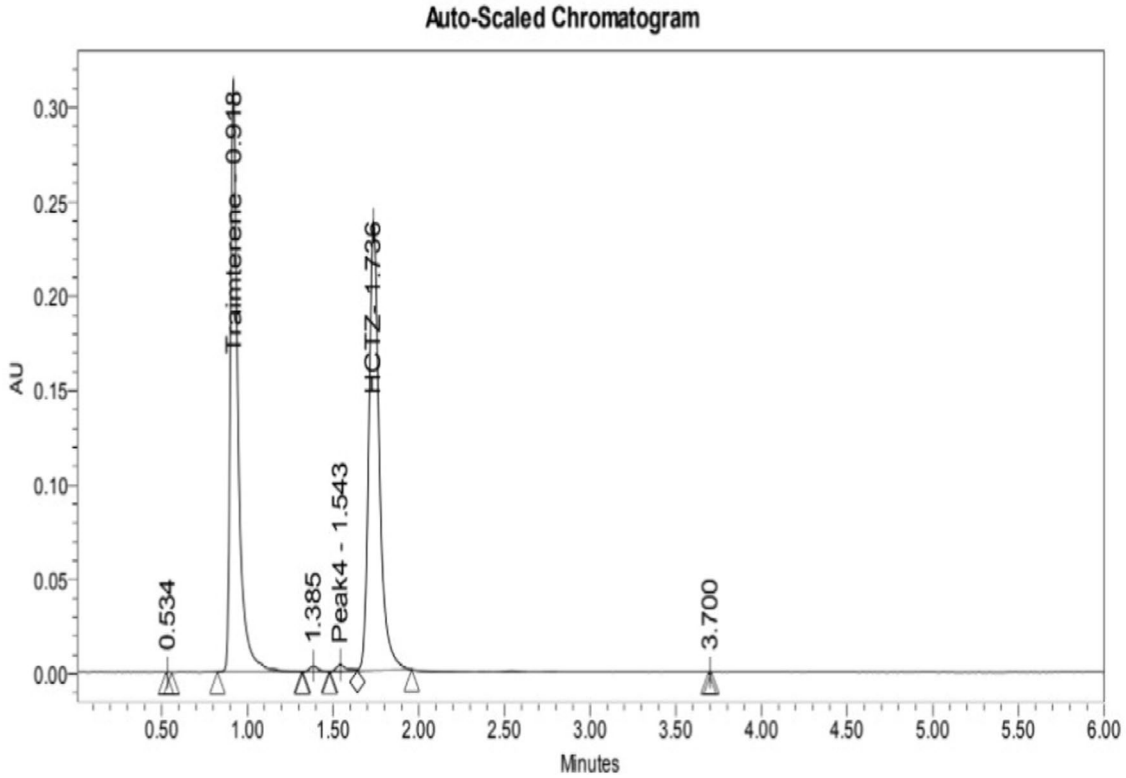

Fig. 21 Peroxide chromatogram

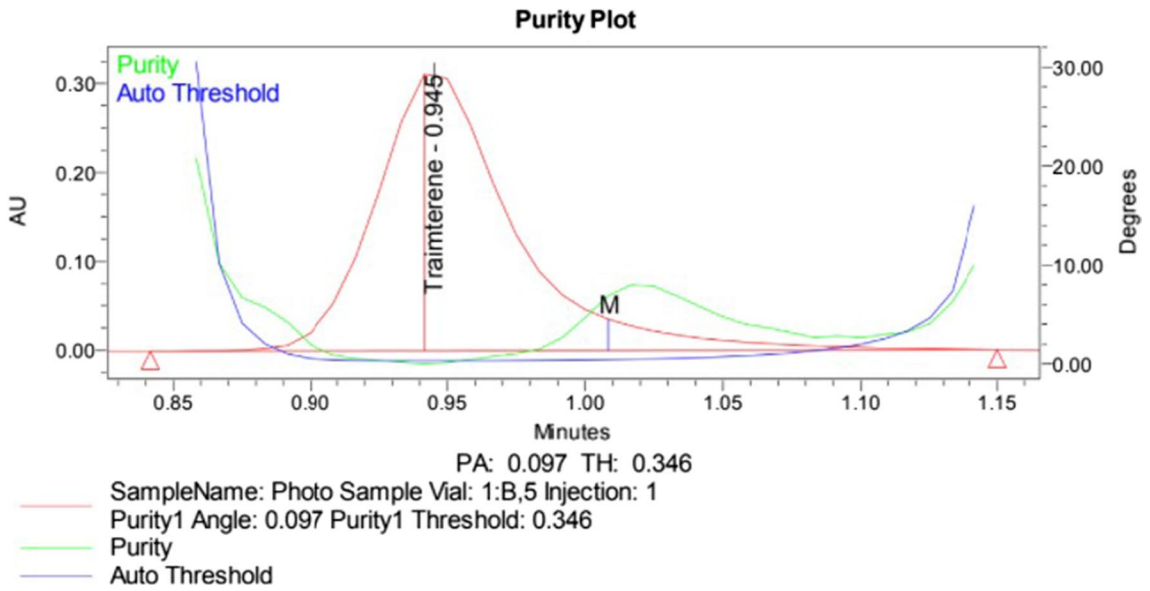

Fig. 22 Purity plot of triamterene of photo-sample

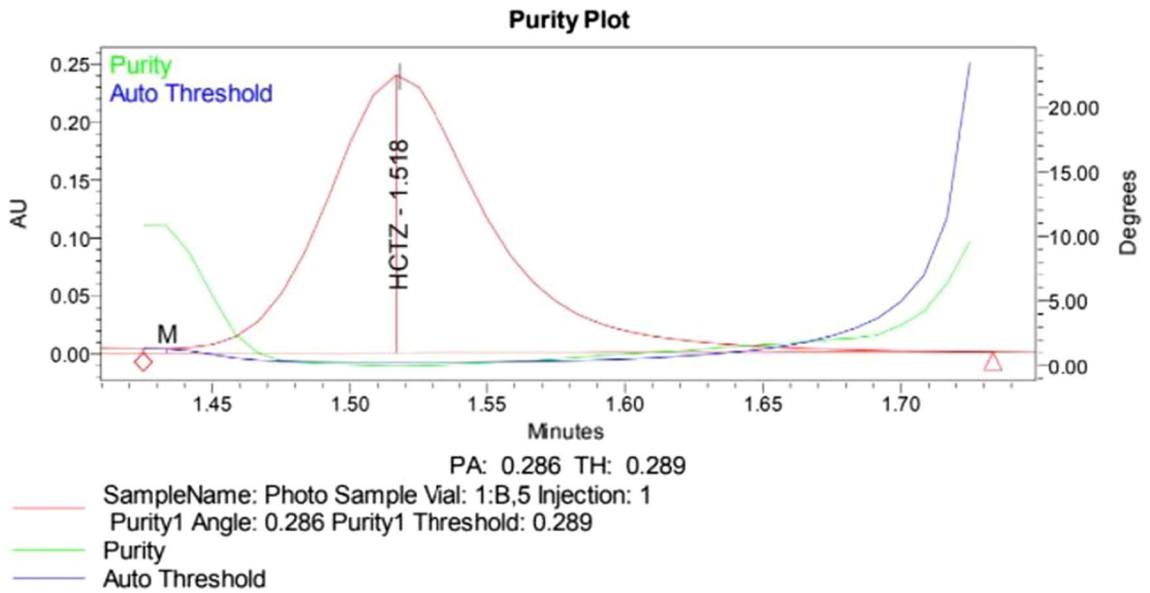

Fig. 23 Purity plot of hydrochlorothaizide of photo-sample

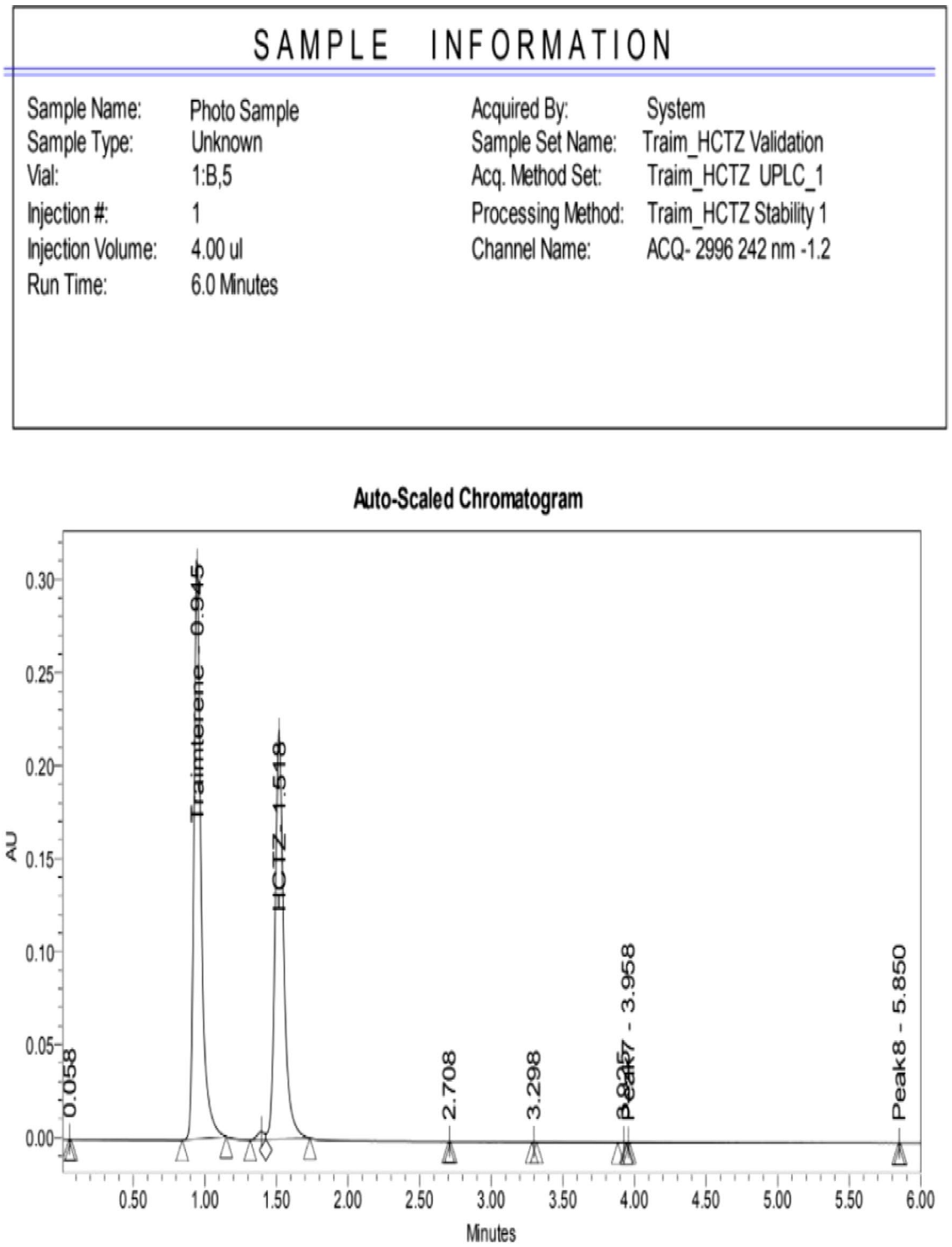

Fig. 24 Photo-sample chromatogram

Abbreviations
ICH: International Conference Harmonization; QbD: Quality by design; UPLC: Ultra-performance liquid chromatography; FDA: Food and drug administration; NDA: New drug applications; OPA: Orthophosphoric acid; LOD: Limit of detection; LOQ: Limit of quantification; HCTZ: Hydrochlorothiazide; NMT: Not more than; NLT: Not less than; RSD: Relative standard deviation; Avg: Average; g/ml: Gram per milliliter; HPLC: High-performance liquid chromatography; PDA: Photodiode array detection; Min: Minutes; RP-HPLC: Reverse phase high performance liquid chromatography; AQbD: Analytical quality by design; µg: Microgram; cmps: Critical method parameters.

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Author contributions
DAD, PGB contributed for the collection of triamterene and hydrochlorothiazide pure drug samples and the combined dosage forms, we performed the experiments and analyzed the data. We drafted the paper, all authors have reviewed and approved the manuscript. All authors read and approved the final manuscript.

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Declarations

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References

1. Santhosh SB, Sachin LD (2019) Analytical method development and optimization of Sofosbuvir drug: a QbD approach. IJPSR 10(1)
2. Peraman R, Bhadraya K, Padmanabha Reddy Y, Surayaprakash Reddy C, Lokesh T (2015) Analytical quality by design approach in RP-HPLC method development for the assay of etofenamatein dosage forms. Indian J Pharm Sci 77(6):751–757
3. Tome T, Zigart N, Casar Z (2019) Development and optimization of liquid chromotography analytical methods by using AQbD principles: overview and recent advances. Org Process Res 23:1784–1802
4. AbD El-Hay SS, ZHashem H (2016) High performance liquid chromatography for and simultaneous determination of performance liquid chromotography for simultaneous determination of xipimide, triamterene and hydrochlorothaizide in bulk drug samples and dosage forms. Acta Pharmaceutica 16(1):109
5. Chauhan VC, Shah VN, Shah DA, Parmar RR (2014) Development and Validation of RP-HPLC Method for simultaneous Estimation of Triamterene and Benzthiazide in Tablets. Pharma Tutor Mag 2:115
6. Antakli S, Nejem L, Kullah A (2018) Analytical spectrophotometric study for determining hydrochlorothiazide individually or with combination with triamterene in some pharmaceutical formulations. RJPT 11(6):115
7. Manisha C (2013) Significance of various chromotographic techniques in drug discovery and developmen. IJRPC 392:151
8. Taleuzzaman M (2016) Ultra performance liquid chromotography. AJAPC 2:1056
9. Priyadarshini R (2016) Chromotography the essence of bioanalysis. EJBPS 3:366–377
10. Sharath Chandra S (2013) Switch from HPLC to UPLC: a novel achivement in liquid chromotography techniques. IJPS 21:237–246
11. Rajneet K (2015) Analytical quality by design approach for development of a validate bioanalytical UPLC method of docetaxel trihhdrate. CPA 3:180
12. Pavan Kumar V (2015) Quality by design approach QBD for pharmaceuticals. IJDDR 17:0
13. Ramalingam P (2015) Analytical quality by design: a tool for regulatory flexibility and robust analytics. IJAC. https://doi.org/10.1155/2015/868727
14. Karmakar S (2011) Quality by design [QbD] based development of a stability indicating HPLC method for drug and impurities. JCS 49:439
15. Tharkor NS (2017) Implementing quality by design [QbD] in chromotography. AJAPC 4:1078
16. ICH Guidelines

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