Original Article

STABILITY INDICATING UPLC METHOD FOR ESTIMATION OF BENAZEPRIL AND HYDROCHLOROTHIAZIDE IN BULK AND COMBINED DOSAGE FORM

ABSTRACT

Objective: The main objective was to develop stability indicating UPLC technique for simultaneous estimation of Benazepril and Hydrochlorothiazide in bulk and formulation.

Methods: 0.1% Triethylamine phosphate: Methanol (25:75v/v) was used as the mobile phase. Benazepril linearity was found to be 4-20 µg/ml and Hydrochlorothiazide linearity was found to be 5-25 g/ml. The detection wavelength was 236 nm, and the retention period of Benazepril was 3.4 min and Hydrochlorothiazide was 5.4 min with a flow rate of 1.0 ml/min. According to the ICH guidlines, the proposed method was validated and stress studies revealed that the drugs are prone to alkali and peroxide stress conditions.

Results: The calibration curve was plotted, and the regression equations for Benazepril were y = 2,01,491.67x+60,532.30 with a correlation coefficient (r^2) of 0.9997 and Hydrochlorothiazide were y = 64,635.86x-74,607.10 with a correlation coefficient (r^2) of 0.9994. According to the accuracy research, the percent recovery of Benazepril is 99.09-100.69 % and that of Hydrochlorothiazide is 98.27-101.88%, both of which are within the ICH recommendations. Benazepril has a limit of detection of 0.08 g/ml-0.24 g/ml and Hydrochlorothiazide has a limit of quantitation of 0.03 g/ml-0.10 g/ml. The procedure was found to be straightforward, linear, rapid, exact, repeatable, and robust. It was determined that the % RSD was within ICH norms. Stress degradation tests showed the drug's vulnerability to oxidative, thermal, photolytic, acid, basic, and neutral hydrolysis stress conditions. Under the circumstances of alkali and peroxide stress, it was discovered that the drug degraded most quickly.

Conclusion: The developed chromatographic technique under consideration was suitable for the accurate, precise, and quick simultaneous measurement of hydrochlorothiazide and benazepril in both their bulk and medicinal dose forms.

Keywords: Benazepril, Hydrochlorothiazide, UPLC method, ICH Guidelines, Stability testing

INTRODUCTION

A prodrug called benazepril (BZP) is used to treat hypertension and heart failure, to lessen proteinuria and renal disease in nephropathies patients, and to prevent stroke, myocardial infarction, and cardiac mortality in high-risk individuals when hydrolyzed by esterases. Benazepril and benazeprilat inhibit the angiotensin-converting enzyme (ACE) in both humans and animals [1]. Angiotensin I is converted to the vasoconstrictor chemical angiotensin II by the angiotensin converting enzyme (ACE). Aldosterone is also stimulated by angiotensin II to be produced in the adrenal cortex. BZP's chemical structure is seen in fig. 1.

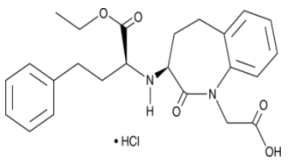

Fig. 1: Structure of benazepril

Fig. 1 shows the chemical structure of BZP. Chemically, BZP is 2-[(3S)-3-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl]acetic acid [2, 3]

Hydrochlorothiazide (HTZ) is a thiazide diuretic used to treat edoema caused by a variety of diseases, as well as hypertension. The most commonly given thiazide diuretic is hydrochlorothiazide. Both high blood pressure and edoema are treated with it. Although hydrochlorothiazide is still frequently used, angiotensin-converting enzyme drugs are replacing it. There are several possible combinations of angiotensin converting enzyme inhibitors or angiotensin II receptor blockers with hydrochlorothiazide [4]. Chemical structure of HZT is shown in fig. 2.

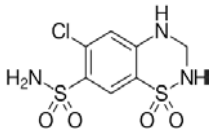

Fig. 2: Structure of hydrochlorothiazide

Fig. 2 shows structure of HTZ. Chemically, HTZ is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1lambda6,2,4-benzothiadiazine-7-sulfonamide.
Hydrochlorothiazide and benazepril are prescribed to patients with high blood pressure. Hydrochlorothiazide and benazepril are prescribed to patients with high blood pressure. By lowering certain molecules that harden blood vessels, it increases blood flow. Few UV [5-9], HPTLC [10-12], and HPLC [13-25] procedures for BZP and HTZ in bulk and formulations with other medications have been described, according to a study of the literature. In order to estimate BZP and HTZ in bulk and formulations, a stability-indicating chromatographic approach has been developed. The suggested approach may be used to routinely assess BZP and HTZ in pharmaceutical dosage forms and bulk samples since it is quick, easy, accurate, and reproducible. The formulation sample recoveries were in good agreement with their label claims, and no formulation excipients interfered with the estimate. As a result, this procedure is straightforward and convenient for testing bulk pharmaceuticals in pure form and mixes on a regular basis.

MATERIALS AND METHODS

Instruments

An Agilent Technologies UPLC system, a variable wavelength programmable UV identifier, and a Rheodyne injector with a 20l fixed circle were all used throughout the chromatographic process. The opposing stage was a Cosmosil C18 (250 mm x 4.6ID, 5 micron). For Spectrophotometric assessments and gauging, the PGB 100 electronic equilibrium balance from Wenser High Precision Balance Model was employed.

Reagents and chemicals

Benazepril and Hydrochlorothiazide was procured from Pharma Tech Solutions as a gift sample. The tablet Benazepril+HCTZ 20/25 mg tablet of Sandoz PVT LTD purchased from local market. HPLC quality Methanol and water were purchased from Merck Specialities Private Limited, Mumbai.

Chromatographic conditions

Cosmosil C18 (250 mm x 4.6ID, particle size: 5 micron) was employed in the chromatographic procedure at a wavelength of 236 nm. 0.1% triethylamine buffer pH 4.8: Methanol (25:75 v/v) was used as the mobile phase for elution, and the same solvent was used to prepare the standard and sample solutions. By injecting the 20 µl and increasing the flow rate to 1.0 ml/min, the elution was evaluated.

Preparation of standard stock solutions

Accurately Benazepril and hydrochlorothiazide working standards were weighed and placed into a 100 ml clean, dry volumetric flask. A 3/4 volume of diluent was added, and the mixture was sonicated for 5 min before being made up to the final volume using diluents. Benazepril's final concentration is 40 µg/ml, whereas Hydrochlorothiazide's ultimate concentration is 50 µg/ml. These medications' working standard solutions were created by properly diluting the corresponding stock solution with mobile phase.

Selection of mobile phase

Benazepril (40 µg/ml) and Hydrochlorothiazide (50 µg/ml) standard solutions were introduced into the RP-HPLC equipment and ran in different solvent systems. Initially, different mobile phase solutions, such as phosphate buffer and methanol were explored in the isocratic mode to discover the optimal conditions.

HPLC method development

Optimisation of the RP-HPLC method

The HPLC technique was developed for the simultaneous measurement of Benazepril and Hydrochlorothiazide. Different mobile phases were investigated for process optimisation, but appropriate retention periods, hypothetical plates, and high resolution were seen with 0.1% Triethylamine Buffer pH 4.8: Methanol (25:75v/v) using Cosmosil C18 (250 mm x 4.6 mm ID, Particle size: 5 m) via Isocratic method. Results are shown in table 1.

Table 1: Optimized chromatographic conditions

Mobile phase	0.1 % Triethylamine Buffer pH 4.8: Methanol (25:75v/v)
Selection of column	Cosmosil C18 (250 mm x 4.6 mm ID, Particle size: 5 µm)
Injection volume	20 µl
Flow rate	1.0 ml/min
Column temperature	Room temperature
Detection wavelength	236 nm
Run Time	7.5 min
Retention time	Benazepril (3.4 min) and Hydrochlorothiazide (5.4 min)

Validation of RP-HPLC method

The optimised RP-HPLC technique was validated in compliance with the ICH Q2 (R) requirements [26].

Linearity

The chromatograms were obtained after each injection of a different test solution concentration. In order to create a series of Benazepril and Hydrochlorothiazide test preparations, 1–5 ml of the stock solution containing the appropriate amounts of Benazepril (40 µg/ml) and Hydrochlorothiazide (50 µg/ml) were taken and placed in five 10 ml volumetric flasks before being topped up with mobile phase. Three injections of a 20µ l volume of each concentration into the HPLC were made under optimal chromatographic conditions.

Accuracy

Samples are typically produced to cover 50%, 100% and 150% of the nominal sample preparation concentration. These samples are analysed, and the recoveries for each are computed [27].

Precision

The intraday precision research was carried out by creating a test solution of the same concentration and analysing it three times during the day. To determine interday precision, the identical process was used on two distinct days. The outcome was given as % RSD [28, 29].

Limit of quantitation (LOQ) and limit of detection (LOD)

The LOD and LOQ were computed using the slope(s) of the calibration curve and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was assessed by altering the chromatographic circumstances, such as the composition of the mobile phase, the detection wavelength, the flow rate, etc. The % RSD should be given. Small adjustments were allowed under ideal circumstances, and the method's resilience was established. We tried individual variations of detecting wavelength of±2 nm and flow rate of±0.1 ml/min. Solutions of 100 % test concentration with the required modifications were injected into the system in triplicate under ideal circumstances.

Ruggedness

Ruggedness is the investigation of the influence of external factors on the approach. To assess the ruggedness of the suggested approach, factors were purposefully altered. These factors included system variation, various analysts, and atmospheric fluctuations. Three concentrations of the test solution were injected into the HPLC system at a flow rate of 1.0 ml/min by two separate analyzers after preparing it in accordance with the test method.

Assay of marketed formulation

20 tablets of Sandoz PVT LTD's commercialised Benazepril with HCTZ Weighed and crushed into a fine powder, 20/25 mg tablets were used. The average weight of the tablet sample was measured transferred to a volumetric flask measuring 100 ml, and the capacity was then filled with diluent. Sonicate while intermittently rotating for ten minutes. Using a 0.45m membrane filter, the aforementioned solution was filtered to produce a stock solution that contained 200 ug/ml of BZP and 250 ug/ml of HTZ. To be used for analysis, 0.3 ml of the solution was taken out, diluted to 10 ml, and then injected into the system.

System suitability

The system suitability properties were examined at in order to validate the approach, column performance, and system. The system was injected with a standard solution of BZP and HTZ six times, and system suitability traits were evaluated.

Force degradation studies

The BZP and HTZ drugs combinations were subjected to forced degradation experiments [30]. The principle stress conditions were ^{1}N HCl (1 h at 60 °C), ^{1}N NaOH (1 h at 60 °C), 3% H_2O_2 (24 h), dry heat (24 h), and UV radiation (24 h) [31]. The stress conditions applied are shown in table 2.

Table 2: Forced degradation conditions according to ICH guidelines

Test condition	Acidic degradation	Alkaline degradation	Oxidative degradation	Thermal degradation	Photolytic degradation
Benazepril and Hydrochlorothiazide	[1]N HCl, 1 h at 60 °c	[1]N NaOH, 1 h at 60 °c	3%H_2O_2, 24 h	Thermal stress for 24 h	Photolytic stress for 24 h

RESULTS AND DISCUSSION

Linearity

Linearity was defined as an analytical method's capacity to provide test results that are exactly proportionate to the analyte concentration within a certain range. Plotting the peak area of the HPLC chromatograph against the pertinent concentrations resulted in the calibration graph. HTZ was shown to be linear in the concentration range of 1–5 µg/ml, while BZP was found to be linear in the 5–25 µg/ml range. Fig. 3 and 4 illustrate the graphs.

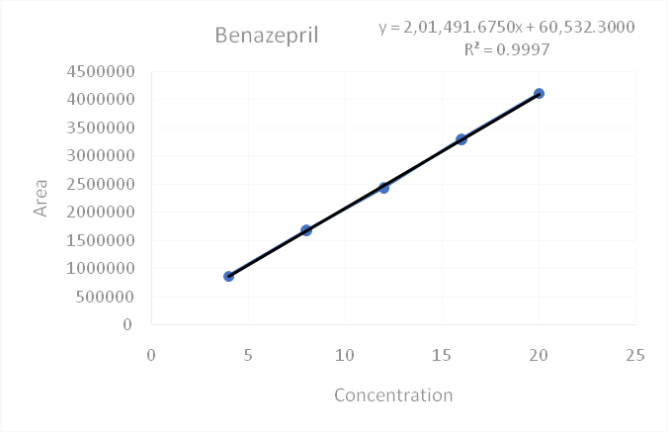

Fig. 3: Calibration curve for benazepril, fig. 3 shows calibration curve of BZP and regression equation of Benazepril was found to be y = 2,01,491.67x+60,532.30 with correlation coefficient (r^2) of 0.9997

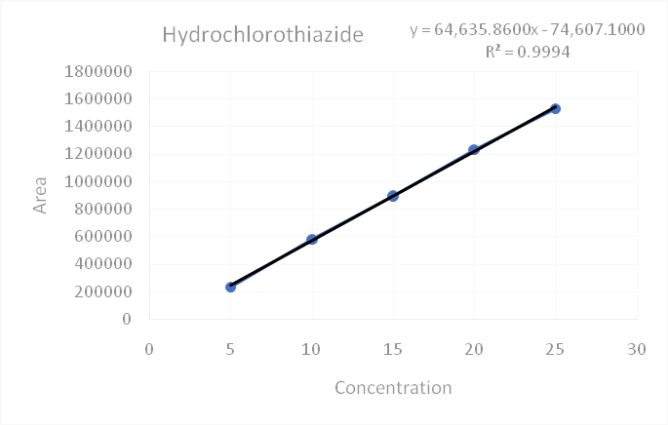

Fig. 4: Calibration curve for hydrochlorothiazide, fig. 4 shows calibration curve of HZT and regression equation of Hydrochlorothiazide was found to be y = 64,635.86x-74,607.10 with correlation coefficient (r^2) of 0.9994

Accuracy

The method's accuracy specifies how near the method's findings are to the real value. The results of the accuracy tests indicated that the procedure is accurate within acceptable limits. The percentage RSD for BZP and HTZ is determined, and all findings are within acceptable bounds. With a maximum RSD of 2.0%, the allowable accuracy was within the range. Table 3 displays the results.

Table 3: Recovery studies

Level of addition	% Mean recovery		Amount found ([µg/ml]), mean±SD [n=3]		% RSD	
	BZP	HTZ	BZP	HTZ	BZP	HTZ
50%	100.19	100.19	38086.33±0.45	31825.00±1.07	0.45	1.06
100%	99.86	99.84	84553.33±0.30	79160.67±1.85	0.30	1.85
150%	99.76	99.33	210825.33±0.72	153930.00±0.43	0.72	0.43

Data are expressed as mean±SD, n=3

Precision

The repeatability of test results is ensured by intraday and interday precision. Both BZP and HTZ exhibited % RSD values less than two. Table 4 displays the results.

Robustness

Robustness was examined using various deliberate alterations in chromatographic settings, such as variation in flow Rate and wavelength. According to the robustness investigation, the RSD for the BZP and HTZ is less than 2%. Hence it is robust and complies per ICH guidelines. Table 5 displays the results.

Ruggedness

Ruggedness was studied by different analysts. From the robustness study % RSD was found to be within the limit of 2 % for the BZP and HTZ. Hence it is complying as per ICH guidelines. Table 6 presents the outcomes.

Table 4: Precision studies (Intra-day and Inter-day)

Drug	Conc. [µg/ml]	Intra-day amount found [µg/ml]		Inter-day amount found [µg/ml]	
		mean±SD [n= 3]	% RSD	mean±SD [n= 3]	% RSD
BZP	4	88025.23±6485.06	0.74	88590.33±2924.65	1.23
	12	244653.33±9883.02	0.40	244513.33±9662.60	1.07
	20	410825.33±13332.38	0.32	410496.33±9353.15	0.61
HTZ	4	23825.00±9737.71	1.10	23571.00±949.10	0.40
	12	89910.67±4762.32	0.19	89607.67±639.50	0.07
	20	153190.00±5096.90	0.12	153980.33±4994.10	0.32

Data are expressed as mean±SD, n=3

Table 5: Data for robustness study of benazepril and Hydrochlorothiazide

S. No.	Parameter	Condition	Benazepril, [n=3]				Hydrochlorothiazide [n=3]			
			Area	Mean	SD	% RSD	Area	Mean	SD	%RSD
1	Variation in	0.9	2441534	2451361	9988	0.41	896145	895817	1523	0.17
2	Flow Rate	1	2451048				897150			
3	(ml/min)	1.1	2461503				894157			
1	Change in	234	2458791	2445190	14133	0.58	897421	895259	1948	0.22
2	Wavelength	236	2446202				893640			
3	(nm)	238	2430578				894715			

Table 6: Data for ruggedness study of benazepril and hydrochlorothiazide

S. No.	Analyst	Benazepril				Hydrochlorothiazide			
		Area	Mean area*	SD	% RSD	Area	Mean area*	SD	% RSD
1	Analyst-I	2451623	2456199	7711	0.31	896457	896160	394	0.04
		2451872				895712			
		2465102				896312			
2	Analyst-II	2441524	2446808	4629	0.19	897421	894423	3730	0.42
		2450154				895602			
		2448745				890245			

*Data are expressed as mean±SD, n=3

Specificity

Analysing reference medications and sample drugs helped to determine the specificity of the technique. Excipients and contaminants had no effect on conventional medications. Hence the method is specific as shown in fig. 5.

Fig. 5 exhibits a well-defined peak of BZP and HTZ at the determined retention times of 3.4 min for BZP and 5.4 min for HTZ.

Assay of marketed formulation

The % Assay of Benazepril+HCTZ 20/25 mg tablet of Sandoz PVT LTD was calculated. The percentage assay was discovered to be BZP (99.77%) and HTZ (98.03 %), which was in good accord with the label claim.

System suitability parameters

System appropriateness criteria were examined to validate the system, approach, and column performance. The system was given six injections of a conventional mixture of hydrochlorothiazide and benazepril, and its suitability was assessed. Table 7 presents the results of the study.

Degradation studies

The stability and specificity of the analytical approach, as well as the predicted degradation products, may all be determined with the help of stress testing the drugs material. Tests for degradation were run on solutions containing 15 µg/ml each of BZP and HTZ. Results are displayed in table 8.

Table 7: System suitability parameter

Parameter	BZP	HTZ
Retention time (min)	3.4	5.4
Theoretical plates	9052	8214
Asymmetry factor	0.50	0.37
Tailing factor	1.08	1.21

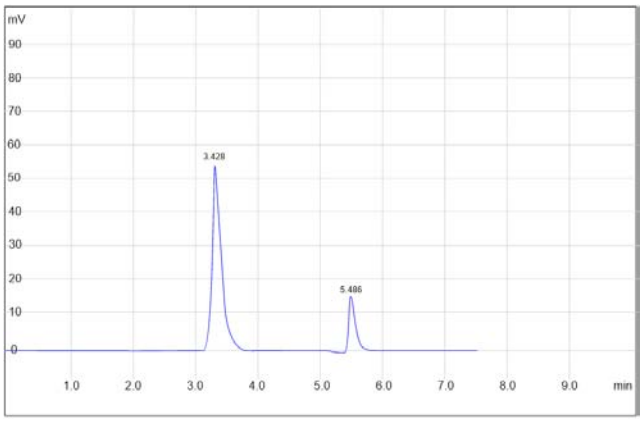

Fig. 5: Chromatograph of benazepril and hydrochlorothiazide

Table 8: Results of forced degradation studies

S. No.	Condition	Drugs	Area of sample	Area of standard	% Drug recovered	% Degradation
1	Acid stress	BZP	2346264	2440154	96.15	3.85
		HTZ	869641	896145	97.04	2.96
2	Alkali Stress	BZP	2266574	2440154	92.89	7.11
		HTZ	795440	896145	88.76	11.24
3	Oxidative Stress	BZP	1782448	2440154	73.05	26.95
		HTZ	584048	896145	65.17	34.83
4	Thermal Stress	BZP	2388035	2440154	97.86	2.14
		HTZ	870236	896145	97.11	2.89
5	Photolytic Stress	BZP	2405410	2440154	98.58	1.42
		HTZ	888993	896145	99.20	0.80

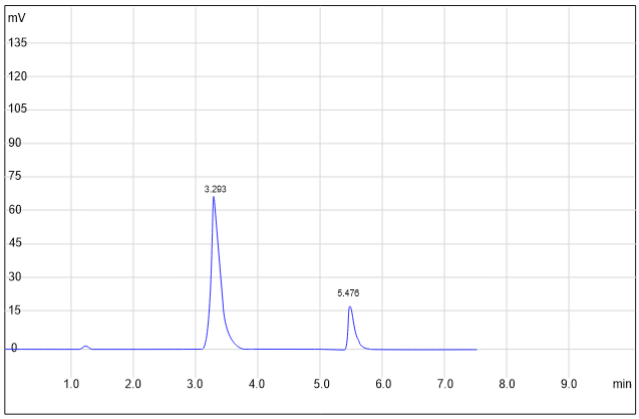

Fig. 6: Acid stressed chromatogram

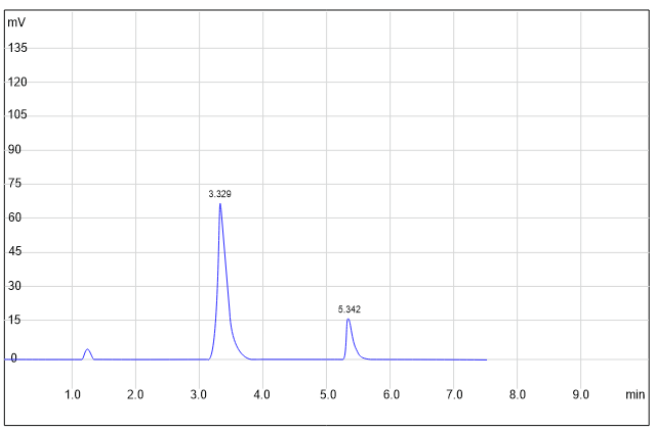

Fig. 7: Alkali stressed chromatogram

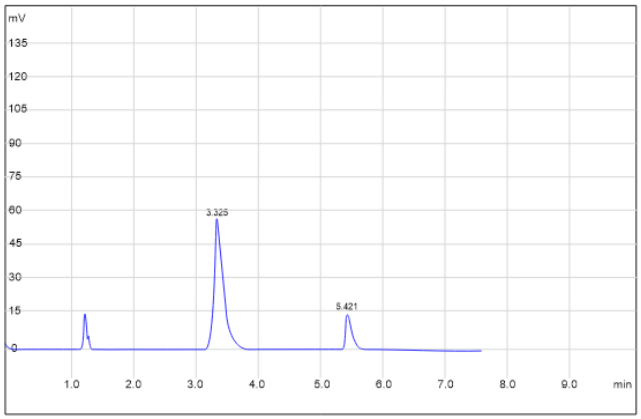

Fig. 8: Peroxide-stressed chromatogram

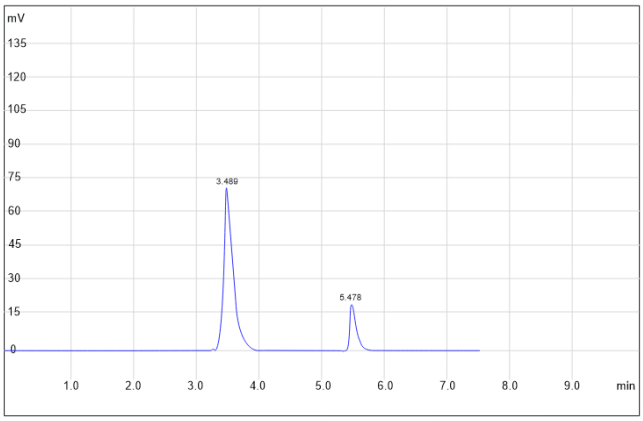

Fig. 9: Thermal stressed chromatogram

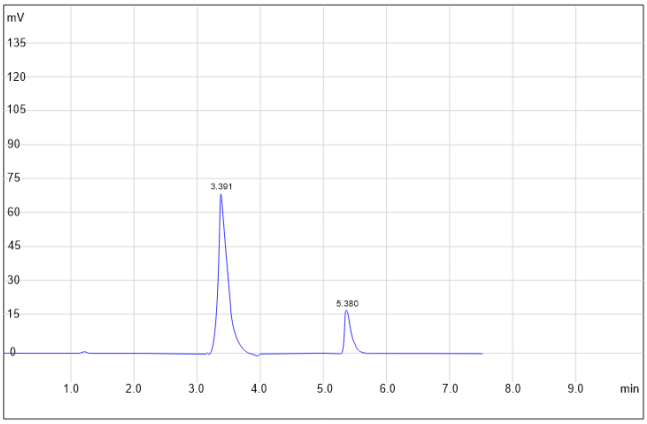

Fig. 10: Photolytic stressed chromatogram

Fig. 6, 7, 8, 9 and 10 shows some additional peaks at various Rt values. The drugs were found prone to acidic, alkali and oxidation condition.

DISCUSSION

The UPLC technique for simultaneous measurement of Benazepril and Hydrochlorothiazide was developed and validated. 0.1% Triethylamine phosphate: Methanol (25:75v/v) was used as the mobile phase. Benazepril linearity was found to be 4-20 µg/ml and Hydrochlorothiazide linearity was found to be 5-25 µg/ml. The calibration curve was plotted, and the regression equations for Benazepril were y = 2,01,491.67x+60,532.30 with a correlation coefficient (r2) of 0.9997 and Hydrochlorothiazide were y = 64,635.86x-74,607.10 with a correlation coefficient (r2) of 0.9994. The detection was performed at 236 nm at a flow rate of 1.0 ml/min. Benazepril had a retention duration of 3.4 min, while Hydrochlorothiazide had a retention time of 5.4 min. In reported method [32] the benazepril hydrochloride and Hydrochlorothiazide was 9.19 min and 3.10 min, respectively. The retention time was higher and require large amount of mobile phase. The validation in accordance with ICH principles shows the method's appropriateness for the quantitative determination of the substances. Statistics were used to validate the suggested techniques' dependability and analytical performance with regard to the constraints of linearity, range, precision, accuracy, detection and quantitation.

According to the Accuracy research, the percent recovery of BZP is 99.09-100.69% and that of HTZ is 98.27-101.88%, both of which are within the ICH criteria. Intraday and interday precision ensure that % RSD remained within ICH norms, i.e., NMT 2 for both BZP and HTZ. Benazepril had a limit of detection of 0.08g/ml-0.24g/ml and Hydrochlorothiazide had a limit of quantitation of 0.03 g/ml-0.10 g/ml. Robustness was examined using purposeful variation, i.e., a change in flow rate and a change in wavelength that was within 2% of RSD according to ICH norms. The ruggedness testing yields results within 2 % of the variance in Analyst examined. Sandoz BZP+HTZ Tablet 20/25 mg % test revealed Benazepril (99.77 %)-Hydrochlorothiazide (98.03 %). BZP and HTZ were treated to a variety of stresses such as acid, alkali, oxidation, dry heat, and photo light. Forced degradation studies provide knowledge about possible degradation pathways and degradation products of the active ingredients and help elucidate the structure of the degradants [33, 34]. Degradation products generated from forced degradation studies are potential degradation products that may or may not be formed under relevant storage conditions. Still, they assist in developing stability-indicating methods. The proposed methods separated the drug efficiently from its breakdown products and were thus regarded as good stability-indicating procedures.

CONCLUSION

The suggested chromatographic technique for identifying BZP and HTZ from pure and dosage forms was found to be straightforward, accurate, precise, quick, and specific. The mobile phase used in method development is easy to make and affordable. The formulation had good sample recoveries. Among all proven approaches, this strategy is the most cost-effective and has the lowest run time, allowing for quick analysis. As a result, this method may be simply and conveniently used for *in vitro* dissolution and routine analysis of Benazepril and Hydrochlorothiazide in Pharmaceutical dosage form.

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