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Stability Indicating LC Method for the Estimation of Benazepril HCl and Hydrochlorthiazide in Pharmaceutical Dosage Form

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Abstract

A rapid, specific and sensitive reverse phase high performance liquid chromatographic method has been developed and validated for analysis of benazepril hydrochloride and hydrochlorothiazide in both bulk and pharmaceutical dosage form. A sunfire C-18, 250×4.6 mm i.d. and 5 µm particle size column with mobile phase containing water: methanol (55:45, v/v, pH 7). The flow rate was 1.0 mL min^{-1} and effluents were monitored at 233 nm. The retention time of benazepril hydrochloride and Hydrochlorthiazide was 9.19 min and 3.10 min respectively. Benazepril hydrochloride and hydrochlorthiazide was subjected to acid and alkali hydrolysis, chemical oxidation, wet hydrolysis, dry heat degradation and sun light degradation. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed LC method. The proposed method was validated with respect to linearity, accuracy, precision and robustness. The method was successfully applied to the estimation of benazepril hydrochloride and hydrochlorthiazide in tablet dosage forms.

Keywords: Benazepril hydrochloride; Hydrochlorthiazide; Liquid chromatography; Forced degradation; Validation

Introduction

Benazeprilhyrochloride(BEN)ischemically3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl] amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benza zepine-1-acetic acid monohydrochloride (Figure 1A). The empirical formula of BEN is $C_{24}H_{28}N_2O_5$•HCl with a molecular weight 460.96 g/mole. It is a angiotensin converting enzyme. It is used as antihypertensive agent. Hydrochlorthiazide (HCT) is chemically 6-chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Figure 1B). The empirical formula is $C_7H_8ClN_3O_4S_2$ with a molecular weight 297.73 g/mole. It is a diuretic agent [1-3].

In the proposed study, attempt has been made to develop sensitive stability indicating RP-LC method for the estimation of BEN and HCT in bulk and pharmaceutical dosage form.

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of BEN which includes high performance liquid chromatography (HPLC) (1), and HCT which includes potentiometry (1), HPTLC (High performance thin layer chromatography) (2), UV–visible simultaneous estimation method (3), RP-HPLC has been reported for the estimation of BEN with another drug combination instead of HCT in pharmaceutical dosage form [4,5]. RP–HPLC, HPTLC, and UV–Visible spectrophotometric methods has been reported for estimation of HCT with another drug combination instead of BEN in bulk and pharmaceutical dosage form [6-15]. LC and HPTLC method has been reported for simultaneous estimation of BEN and HCT in bulk and pharmaceutical dosage form [16,17]. In the proposed study, attempt has been made to develop stability indicating liquid chromatographic method for the estimation of BEN and HCT in pharmaceutical dosage form as per ICH guide lines [18,19].

Experimental

Instrumentation

High performance liquid chromatography: The liquid chromatographic system of Perkin Elmer, containing HPLC isocratic pump (515), UV detector and rheodyne injector with 20 µl fixed loop was used. A Sunfire C18 column with 250×4.6 mm i.d. and 5 µm particle size was used as stationary phase.

Reagents and materials: Analytically pure benazepril hydrochloride (BEN) and hydrochlortiazide (HCT) was procured from Dishman Pharmaceutical Pvt. Ltd and Cadila pharmaceutical Ltd, (Ahmedabad, India). Methanol, water (E. Merck, Mumbai, India) used for the preparation of mobile phase was of LC grade. Triethylamine (Sissco research laboratories, Mumbai, India) was of analytical reagent grade. Tablet formulation A (Lotencin HCT–(10 mg Benazepril hydrochloride and 12.5 mg Hydrochlortiazide), Novartis Pharmaceutical was purchased from local market.

Preparation of mobile phase and stock solution: Mobile phase was prepared by mixing 550 ml of Water with 450 ml of methanol in 1000 ml volumetric flask. The pH was adjusted to 7.0 using triethylamine (1%) and the solution was filtered through Whatman filter paper No.42 (0.45 µm) and it was sonicated for 15 min prior to use for degassing. This solution was used as a mobile phase.

BEN and HCT were weighed accurately (25 mg each) and transferred to separate 25 ml volumetric flasks containing few ml of methanol. Volumes were adjusted up to the mark with methanol to yield a solution containing 1000 µg/ml of BEN and HCT respectively. Aliquot (1.0 ml) from the above solutions of BEN and HCT were appropriately diluted with methanol to obtain working standards stock solution of 100 µg/ml of BEN and HCT respectively.

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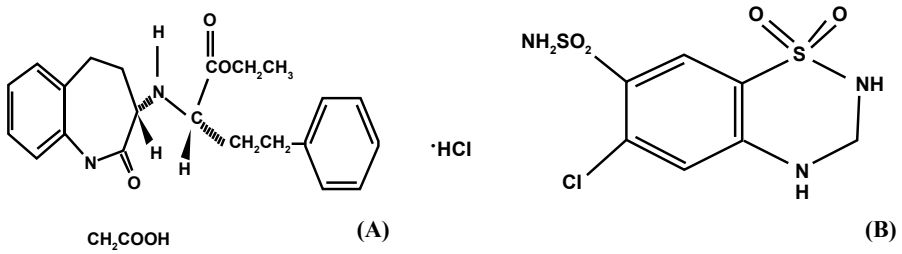

Figure 1: Chemical Structure of (A) Benazepril hydrochloride (B) Hydrochlorthiazide.

Chromatographic conditions: A reversed phase C18 column (Sunfire) equilibrated with mobile phase comprising of water:methanol (55:45 v/v; pH 7) was used. Mobile phase flow rate was maintained at 1 ml/ min and effluents were monitored at 233 nm. A 20 µL of sample was injected using a fixed loop, and the total run time was 10 min. All the chromatographic separations were carried out at controlled room temperature (25 ± 2°C).

Calibration curves for BEN and HCT: Appropriate aliquots of BEN and HCT working standard solutions were taken in different 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentrations 0.1, 0.5, 1, 5, 10, 20 µg/ml of BEN and 0.5, 1, 5, 10, 20, 30 µg/ml of HCT respectively. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting peak area versus concentrations of the drug. The non-weighted linear regression equation was computed for BEN and HCT.

Analysis of Marketed Formulations: Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg BEN and 12.5 mg of HCT was taken in 100 ml volumetric flask. A few ml of methanol was added to the above flask and the flask was sonicated for 10 min. The solution was filtered using Whatman filter paper No.42 (0.45 µm) and volume was made up to the mark with the methanol. Aliquot (0.4) was transferred to a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 4 µg/ml of BEN and 5 µg/ml of HCT. This solution was used for the estimation of BEN and HCT. Both the solutions were sonicated for 10 min. Solutions were injected as per the above chromatographic conditions and peak areas were recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

Validation of method : The method was validated as per ICH guideline for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy: Known amount of BEN (0, 0.5, 1, 1.5 µg/ml) and HCT (0, 1, 2, 3 µg/ml) was added to a pre quantified sample solutions. The amount of BEN and HCT was estimated using linear regression equation.

Precision: The instrument precision was evaluated by injecting the solution containing BEN (0.1, 1, 10 µg/ml) and HCT (0.5, 5, 20 µg/ml) six times repeatedly and peak area was measured. The results are reported in terms of % relative standard deviation. The intra-day and inter-day precision study of BEN and HCT was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of BEN (0.1, 1, 10 µg/ml) and HCT (0.5, 10, 20) within the calibration range and the results are reported in terms of % relative standard deviation (%RSD).

Specificity: The specificity was estimated by spiking commonly used excipients (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Limit of detection and quantification: The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD=3.3×σ/S and LOQ=10×σ/S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness: Robustness of the method was studied by deliberately changing the experimental conditions like flow rate, percentage of organic phase, and also by observing the stability of the sample solution at 25 ± 2° for 24 h. The sample solution was assayed at every 2 h interval up to 24 h.

Forced degradation study: Stress degradation study using acid and alkali hydrolysis, chemical oxidation, wet hydrolysis exposure to sun light and dry heat degradation was carried out and interference of the degradation products was investigated. BEN and HCT was weighed (10 mg) and transferred to 10 ml volumetric flasks and expose to different stress conditions.

Heat induced alkali hydrolysis: To the 10 ml volumetric flask, 10 mg of BEN and HCT was taken and 2 ml of 0.1 N NaOH was added to perform heat induced base hydrolysis. The flask was heated at 80°C for 4 hrs and allowed to cool to room temperature. Solution was neutralized with 0.1 N HCl and volume was made up to the mark with methanol. 0.1 ml of aliquots was taken from the above solution and diluted with mobile phase to obtain final concentration of 10 µg mL^{-1} of BEN and HCT.

Heat induced acid hydrolysis: To the 10 ml volumetric flask, 10 mg of BEN and HCT was taken and 2 ml of 0.1 N HCl was added to perform heat induced acid hydrolysis. The flask was heated at 80°C for 4 hrs and allowed to cool to room temperature. Solution was neutralized with 0.1 N NaOH and volume was made up to the mark with methanol. 0.1 ml of aliquot was taken from the above solution and diluted with mobile phase to obtain final concentration of 10 µg mL^{-1} of BEN and HCT.

Heat induced wet hydrolysis: To the 10 ml volumetric flask, 10 mg of BEN and HCT was taken and 2 ml of HPLC grade water was added to perform heat induced wet hydrolysis. The flask was heated at 80°C for 4 hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.1 ml of aliquot was taken from the above solution and diluted with mobile phase to obtain final

concentration of 10 µg mL^{-1} of BEN and HCT.

Heat induced oxidative stress degradation: To heat induced perform oxidative stress degradation, 10 mg of BEN and HCT was taken in 10 ml volumetric flask and 2 ml of 6% hydrogen peroxide was added. The mixture was heated in a water bath at 80°C for 4 hrs and allowed to cool to room temperature and volume was made up to the mark with methanol. 0.1 ml of aliquot was taken from above solution and diluted with mobile phase to obtain final concentration of 10 µg mL^{-1} of BEN and HCT.

Photolytic degradation: Analytically pure 10 mg of drugs were exposed to sunlight for 72 hrs. The solid was allowed to cool and transferred to volumetric flask (10 ml) and dissolve in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted with the mobile phase to obtain final concentration of 10 µg mL^{-1} of BEN and HCT. All the solutions were injected in the liquid chromatographic system and chromatograms were recorded.

Result

Validation of the proposed methods

Linearity: Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. The calibration curve for BEN was found to be linear in the range of 0.1-20 µg/ml with a correlation coefficient of 0.9923. The calibration curve for HCT was found to be linear in the range of 0.5-30 µg/ml with a correlation coefficient of 0.9977. The regression data shown in table confirms the linearity of the method over the concentration range studied (Table 1). Summary of validation parameters shown in (Tables 2 and 3).

Precision: Repeatability was determined by performing injection repeatability test and the % RSD values for BEN and HCT were found to be 0.30-1.68 and 0.20-1.43 respectively.

The intraday and interday precision studies were carried out on the same day and three different days. The results are reported in terms of %RSD. The low % RSD values indicate that the method is precise.

Parameters	BEN	HCT
Linearity range (µg/ml)	0.1-20 µg/ml	0.5-30 µg/ml
Correlation coefficient (r)	0.9923	0.9977
Slope	24072	30111
Standard deviation of slope	491.8421	3.34664
Intercept of regression	20734	6899.1
Standard deviation of intercept	485.9614	132.5796

Table 1: Statistical analysis data of calibration curve.

Parameters	BEN	HCT
Limit of Detection	0.065	0.0145
Limit of Quantitation	0.1	0.5
Accuracy (%)	98.66-99.32	98.93-99.73
Repeatability (%RSD, n=6)	0.30-1.68	0.20-1.43
Precision Intraday (n=3) Interday (n=3)	0.11-0.19 1.11-1.72	0.11-0.48 1.22-1.70
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hrs	Suitable for 24 hrs

Table 2: Summary of validation Parameters of RP-HPLC.

%Level	Amount of sample taken (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery
0%	1	0	98.66	98.66 ± 0.57
50%	1	0.5	1.49	99.2 ± 0.91
100%	1	1	1.97	98.83 ± 1.55
150%	1	1.5	2.49	99.32 ± 1.06

Table 3: Accuracy study of BEN by proposed RP-HPLC method.

%Level	Amount of sample taken (µg/ml)	Amount of standard drug added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery
0%	1.25	0	1.24	99.2 ± 0.5
50%	1.25	0.625	1.85	98.93 ± 1.32
100%	1.25	1.25	2.49	99.73 ± 0.38
150%	1.25	1.875	3.10	99.2 ± 0.76

Table 4: Accuracy study of HCT by proposed RP-HPLC method.

Method parameter	Normal condition	Deliberate changes	% RSD of peak area (n=3)	
			BEN	HCT
Flow rate	1.5 ml/min	0.8 ml/min	0.95	0.84
		1.2 ml/min	0.68	0.71
Mobile phase ratio	Water: Methanol (55:45)	53: 47	0.82	0.29
		57: 43	0.56	0.63
pH of mobile phase ratio	7	7.2	0.92	0.81
		6.8	0.65	0.76

Table 5: Robustness study by proposed RP–HPLC method.

Accuracy: Accuracy of an analytical method is the closeness of the test results to the true value. It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into pre-analysed sample solutions. The recoveries were found to be 99.2-99.32%, and 99.0-99.44% for BEN and HCT respectively (Tables 3 and 4).

Limit of detection and limit of quantification: The detection limits for BEN and HCT were 0.065 µg/ml and 0.014 µg/ml respectively, while quantitation limits were 0.1 µg/ml and 0.5 µg/ml respectively. The above data shows that a nano gram quantity of both the drugs can be accurately and precisely determined.

Specificity: The specificity study was carried out to check the interference from the excipients used in the formulation by preparing synthetic mixture containing the drug and excipients. The chromatogram showed peaks for the drug without any interfering peak.

Robustness: The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in table. The low value of relative standard deviation was indicating that the method was robust (Table 5).

Forced degradation study: Chromatogram of base hydrolysis performed at 80°C for 4 hrs reflux showed degradation of BEN and HCT at retention time (RT) 9.2 min and 3.12 min respectively (Figure 3).

Chromatogram of acid hydrolysis performed at 80°C for 4 hrs reflux showed degradation of BEN and HCT at retention time (RT) 9.4 min and 3.07 min respectively (Figure 4).

The chromatogram of oxidized BEN and HCT with 6% hydrogen peroxide at 80°C for 4 hrs reflux showed degradation of BEN and HCT with at retention time 9.3 min and 3.09 min (Figure 5).

The chromatogram of BEN and HCT exposed to dry heat at 80°C

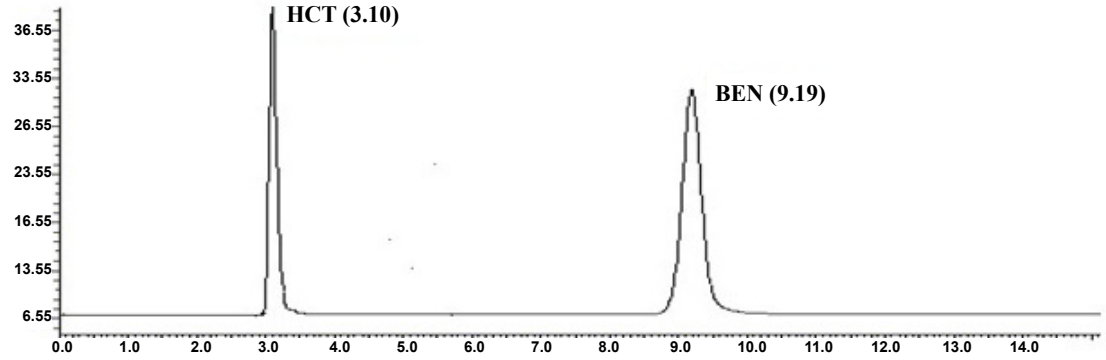

Figure 2: Chromatogram of standard BEN (10 µg/ml) and HCT (10 µg/ml) at flow rate of 1 ml/min in mix standard.

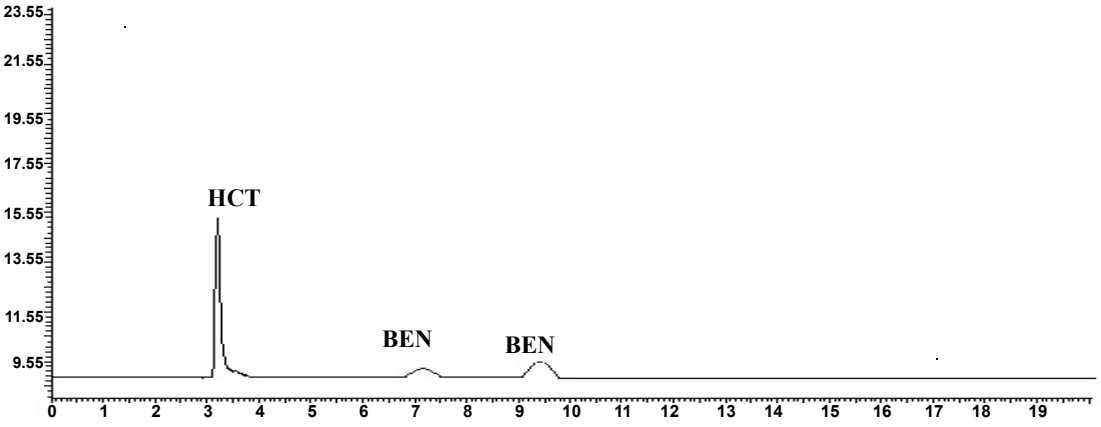

Figure 3: Chromatogram of base treated BEN (10 µg/ml) and HCT (10 µg/ml) at 80°C for 4 hrs in mixture.

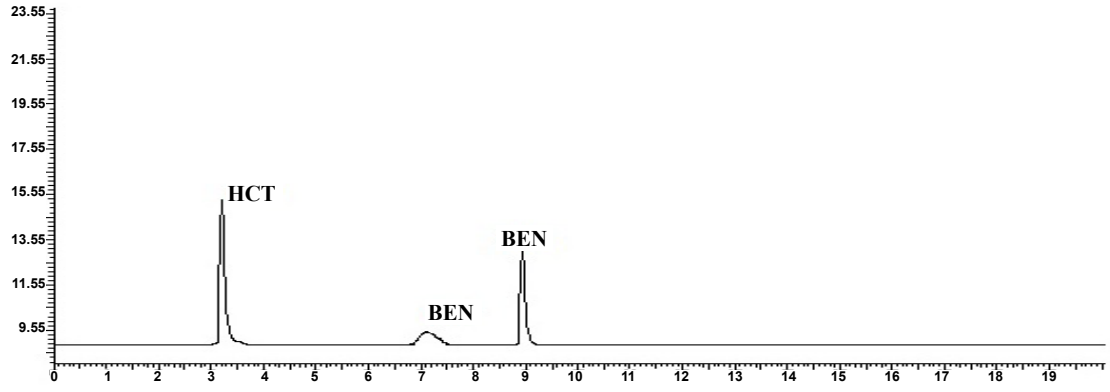

Figure 4: Chromatogram of acid treated BEN (10 µg/ml) and HCT (10 µg/ml) at 80°C for 4 hrs.

for 4 hrs showed degradation of BEN and HCT at retention time (RT) 9.10 min and 3.11 min. respectively (Figure 6).

The chromatogram of BEN and HCT expose to sun light for 72 hrs showed degradation of BEN and HCT at retention time (RT) 9.07 min and 3.14 min respectively (Figure 7 and Table 6).

Solution stability: The solution stability study showed that BEN and HCT were evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that solution was stable up to 24 hrs at room temperature

Analysis of marketed formulations: The proposed method was successfully applied to the determination of BEN and HCT in their combined dosage form. The % recovery was found to be more than 99.0% for all the drugs which were comparable with the corresponding labelled amounts. No interference from the excipients present in the marketed tablet formulation was observed (Tables 7 and 8).

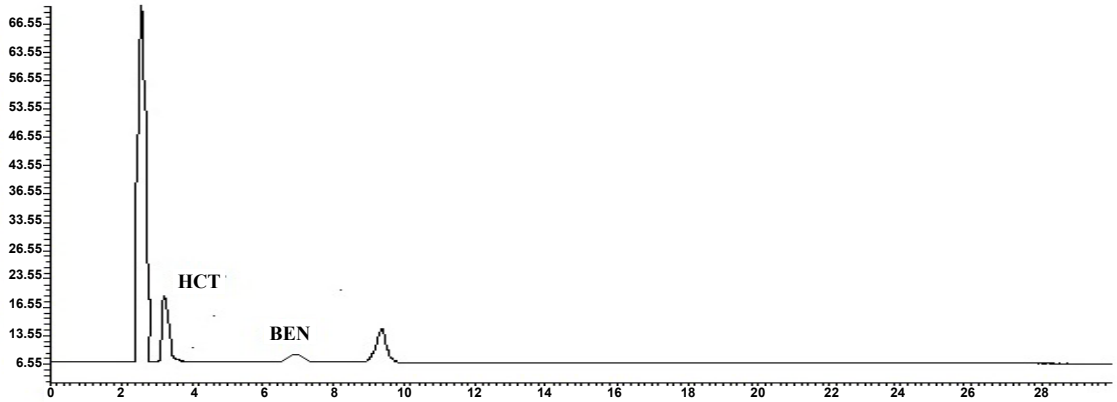

Figure 5: Chromatogram of hydrogen peroxide degraded sample of BEN (10 µg/ml) and HCT (10 µg/ml) at 80°C for 4 hrs.

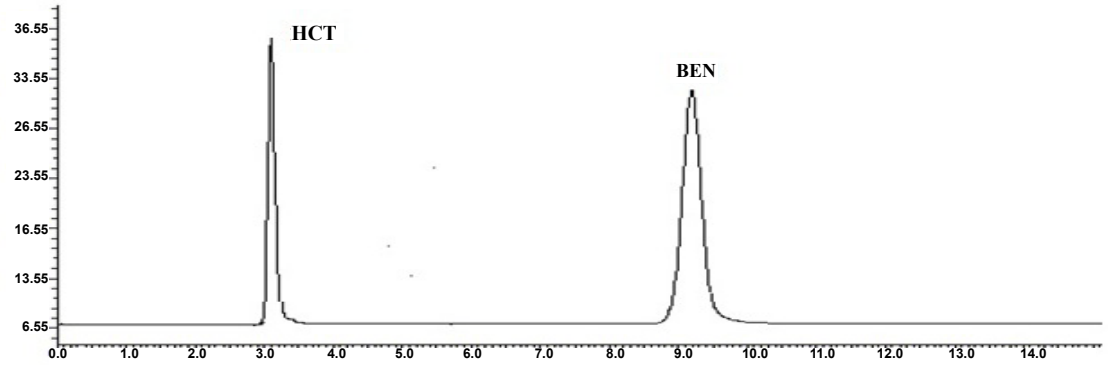

Figure 6: Chromatogram of dry Heat degraded sample of BEN (10 µg/ml) and HCT (10 µg/ml) at 80°C for 4 hrs.

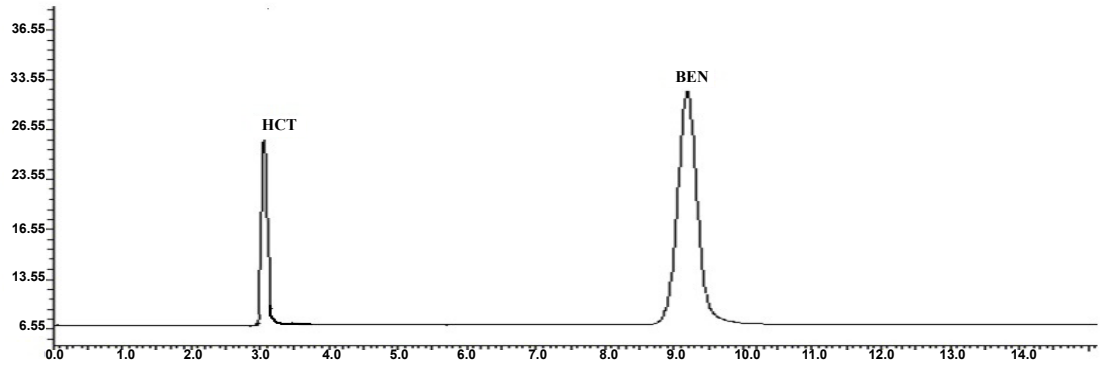

Figure 7: Chromatogram of photo degraded sample of BEN (10 µg/ml) and HCT (10 µg/ml) at 80°C for 72 hrs

Discussion

Optimization of mobile phase

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation. The drug solutions containing BEN (10 µg/ml), HCT (10 µg/ml) and their mixture were chromatographed at a flow rate of 1 ml/min with the following mobile phases.

Various mixtures containing water and methanol were tried as mobile phases in the initial stage of method development. Methanol: Water (50:50), Methanol: Water (80:20), Methanol: Water (70:30), Methanol: Water (60:40) was tried as mobile phase but satisfactory resolution of drug and degradation peaks were not achieved.

The mobile phase methanol: water (55:45, v/v pH adjusted with 1% solution of TEA) was found to be satisfactory and gave symmetric peak for BEN and HCT. The retention time for proposed method was found

Parameter	BEN	HCT
Retention time (min)	9.19	3.10
Theoretical plates	3595.43	5133.94
Tailing factor	1.33	1.02
Base width (sec)	12.57	30.88
Resolution	9.36	

Table 6: System suitability parameter.

Formulation	Actual concentration (µg/ml)		Amount obtained (µg/ml)		% BEN Mean ± SD (n=3)	% HCT Mean ± SD (n=3)
	BEN	HCT	BEN	HCT		
Tablet (Lotensin HCT)	4	5	3.98	5.01	99.5% ± 0.41	100.2% ± 0.58

Table 7: Assay Results of Marketed Formulation.

Conditions	Time (hrs)	Recovery (%)		Retention time of degradation products	
		BEN	HCT	BEN	HCT
Base (0.1 N NaOH)	4	62.89	18.45	9.2	3.1
Acid (0.1 N HCl)	4	87.98	10.97	9.4	3.07
6% H_2O_2	4	82.42	14.03	9.3	3.09
Photo oxidation	72	97.62	12.80	9.07	3.14
Dry Heat	4	99.97	44.12	9.10	3.11

Table 8: Forced degradation study of BEN and HCT for the proposed Method.

to be 9.19 min for BEN and 3.10 min for HCT as shown in Figure 2.

Method validation

The calibration curve was found to be linear over the range of 0.1-20 µg/ml for BEN and 0.5-30 µg/ml for HCT. Instrument precision was determined by performing injection repeatability test and the %RSD value for BEN and HCT was found to be 0.30-1.68% and 0.20-1.43. The intra-day and inter-day precision studies were carried out. For BEN the intra-day study %RSD values were found to be 0.11-0.19% and for inter-day precision study %RSD values were found to be 1.11-1.72%. For HCT the intra-day study %RSD values were found to be 0.11-0.48% and for inter-day precision study %RSD values were found to be 1.22-1.70%. The low %RSD values indicate that the method is precise.

The accuracy of the method was determined by calculating recoveries of BEN and HCT by method of standard additions. The recovery of BEN and HCT was found to be 98.66-99.32% and 98.93-99.73, respectively. The values indicate that the method is accurate.

The detection limits for BEN and HCT was found to be 0.07 µg/ml and 0.02 µg/ml while quantitation limits was found to be 0.1 µg/ml and 0.5 µg/ml, respectively. The above data shows that a nanogram quantity of the drug can be accurately and precisely determined. System suitability test was carried out on freshly prepared standard stock solution of BEN and HCT.

The liquid chromatogram of the placebo used in the specificity study did not give any interfering peak in the chromatogram, which suggests that the proposed LC method is both selective and specific.

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. The low value of percentage relative standard deviation was indicating that the method was robust.

The solution stability study revealed that BEN and HCT in mixed standard solution were found to be stable for 24 h without detection of degradation. The percentage recoveries of both the drugs were found to be satisfactory.

Forced degradation study

The degradation study thereby indicated that BEN and HCT was susceptible to acid hydrolysis, base hydrolysis, oxidation (6% hydrogen peroxide), photo degradation, and dry heat. No degradation products from different stress conditions affected determination of BEN and HCT.

Conclusion

As compared with the published, the proposed method is more sensitive. Proposed study describes stability indicating LC method for the estimation of BEN and HCT in bulk and their pharmaceutical dosage form. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of BEN and HCT without any interference from the excipients. The method was successfully used for the determination of drug in their pharmaceutical formulation. Also the above results indicated the suitability of the method for acid, base, oxidation, dry heat and photolytic degradation study. As the method separates the drugs from its degradation products, it can be used for analysis of stability samples. The method is suitable for the routine analysis of BEN and HCT in tablets. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals.

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