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

Chhalotiya UK*, Varsha LP, Dimal AS, Kashyap KB and Sunil LB

Department of Pharmaceutical Analysis, Indukaka IpcoWala College of Pharmacy, Gujarat, India

Abstract

A rapid, specific and sensitive reverse phase high performance liquid chromatographic method has been developed and validated for analysis of benazepril hydrochloride and hydrochlorthiazide 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

Benazepril hydrochloride (BEN) is chemically 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzepine-1-acetic acid monohydrochloride (Figure 1A). The empirical formula of BEN is C_{24}H_{27}N_{3}O_{5}·HCl with a molecular weight 460.96 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 benzepril 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.

Experimental

Instrumentation

High performance liquid chromatography: The liquid chromatographic system of Perkin Elmer, containing HPLC isocratic pump (515), UV detector and rhedynie 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 hydrochlorthiazide (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 Hydrochlorthiazide), 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.

Received January 31, 2014; Accepted March 26, 2014; Published March 30, 2014

Citation: Chhalotiya UK, Varsha LP, Dimal AS, Kashyap KB, Sunil LB (2014) Stability Indicating LC Method for the Estimation of Benazepril HCl and Hydrochlorthiazide in Pharmaceutical Dosage Form. J Chromatograph Separat Techniq S: 216. doi: 10.4172/2157-7064.1000216

Copyright: © 2014 Chhalotiya UK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 mark with 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, 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 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 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

Figure 1: Chemical Structure of (A) Benazepril hydrochloride (B) Hydrochlorthiazide.
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                | 8999.1               |
| Standard deviation of intercept | 485.9614             | 132.5796             |

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

| Method parameter          | Normal condition | Deliberate changes | % RSD of peak area (n=3) |
|---------------------------|------------------|--------------------|--------------------------|
| Flow rate                 | 1.5 ml/min       | 0.8 ml/min         | 0.95 (0.76)              |
| Mobile phase ratio        | Water: Methanol  | 53: 47             | 0.82 (0.29)              |
| pH of mobile phase ratio  | 7.2               | 6.8                | 0.65 (0.76)              |

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

| Method parameter          | Normal condition | Deliberate changes | % RSD of peak area (n=3) |
|---------------------------|------------------|--------------------|--------------------------|
| Flow rate                 | 1.5 ml/min       | 0.8 ml/min         | 0.95 (0.76)              |
| Mobile phase ratio        | Water: Methanol  | 53: 47             | 0.82 (0.29)              |
| pH of mobile phase ratio  | 7.2               | 6.8                | 0.65 (0.76)              |

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

| Deliberate changes | % Level | Amount of sample taken (µg/ml) | Amount of standard drug added (µg/ml) | Amount of drug recovered (µg/ml) | % Recovery |
|--------------------|---------|-------------------------------|---------------------------------------|---------------------------------|------------|
| 1.25               | 0%      | 0.04                         | 98.66                                 | 98.68 ± 0.57                    | 99.2 ± 0.91 |
| 1.25               | 50%     | 0.54                         | 1.49                                  | 99.2 ± 0.91                     | 99.2 ± 1.32 |
| 1.25               | 100%    | 1.64                         | 1.97                                  | 98.83 ± 1.55                    | 99.0-99.44  |
| 1.25               | 150%    | 2.64                         | 2.49                                  | 99.32 ± 1.06                    | 99.2 ± 0.76 |

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

| Method parameter          | Normal condition | Deliberate changes | % RSD of peak area (n=3) |
|---------------------------|------------------|--------------------|--------------------------|
| Flow rate                 | 1.5 ml/min       | 0.8 ml/min         | 0.95 (0.76)              |
| Mobile phase ratio        | Water: Methanol  | 53: 47             | 0.82 (0.29)              |
| pH of mobile phase ratio  | 7.2               | 6.8                | 0.65 (0.76)              |

Table 6: Robustness study by proposed RP–HPLC method.
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).
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
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 (% 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.

Acknowledgement

The authors are thankful to Dishman pharmaceutical Ltd and Cadila Pharmaceuticals Ltd., Ahmedabad for providing gratis sample of Benazepril Hydrochloride and Hydrochlorthiazide. The authors are very thankful Indukaka Ipcowala College of pharmacy, new vallabh vidyanagar, an and, for providing necessary facilities to carry out research work.

References

1. British Pharmacopoeia (2011) The department of health, social services and public safety. 2: 225-226.
2. Indian Pharmacopoeia (2007) The Indian pharmacopoeia commission, Ghaziabad, Ministry of Health & Family welfare 2: 576-578.
3. Pawar PY, Joshi RS, Sandhan V, Wagh S, Jangale K (2011) Simultaneous spectrophotometric estimation of Amlodipine Besylate and Benazepril HCl in pure and pharmaceutical dosage form, Der Pharmacia Lettre 3: 397-403.
4. Naidu KR, Kale UN, Shingare MS (2005) Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product. Journal of Pharmaceutical and Biomedical Analysis 39: 147-155.
5. Sarat M, Murli PK, Rambabu C (2012) Development and validation of RP-HPLC method for simultaneous estimation of amlodipine and benazepril hydrochloride in combined dosage form by RP-HPLC. International Journal of Chem Tech Research 2: 21-25.
6. Patel G, Patel S, Prajapati D, Mehta R (2010) RP-HPLC method for simultaneous estimation of amlodipine besylate and hydrochlorothiazide in combined dosage forms. International Journal of Pharmaceutical Sciences 3: 49-35.
7. Rao LA, Bhaskara RV (2011) Simultaneous estimation of valsartan and hydrochlorothiazide in tablets by RP-HPLC method. Intentional Journal Pharmaceutical & Industrial Researh 1: 170-174.
8. Kharoof M, Malikieh N, Abualhasan M, Shubitah R, Jaradat N, et al. (2012) Tablet formulation and development of a validated stability indicating HPLC method for quantification of valsartan and hydrochlorothiazide combination. International Journal of Pharmacy and Pharmaceutical Sciences 4: 683-687.
9. Chubukwar AR, Jagdale SC, Kuchekar BS, Lokhande PD, Shinde SN, et al. (2010) Development and validation of a RP-HPLC method for simultaneous estimation of amlodipine and hydrochlorothiazide in combined dosage form.
estimation of hydrochlorothiazide and irbesartan. Der Pharma Chemica 2: 148-156.
11. Rosangluaia, Shanmugasundaram P, Malarkodi V (2011) Validated HPTLC method for simultaneous estimation of irbesartan and hydrochlorothiazide in a tablet dosage form. Der Pharma Chemica 3: 310-317.
12. Singh B, Patel DK, Ghosh SK (2009) Development of RP-HPLC method for simultaneous analysis of metoprolol succinate and hydrochlorothiazide in a tablet formulation. Tropical Journal of Pharmaceutical Research 8: 539-543.
13. Kumbhar ST, Chougule GK, Tegeli VS, Gajeli GB, Thorat VS, et al. (2011) A Validated HPTLC method for simultaneous quantification of nebivolol and hydrochlorothiazide in bulk and tablet formulation. International Journal of Pharmaceutical Sciences and Drug Research 3: 62-66.
14. Gupta Y, Shrivastava A, Duggal D, Patel A, Agrawal S (2009) A new RP-HPLC method for simultaneous estimation of nebivolol hydrochloride and hydrochlorothiazide in dosage forms. Journal Young Pharmacist 1: 264-269.
15. Hapse SA, Wagh VS, Kadaokar PT, Dokhe MD, Shirsath AS (2012) Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. Der Pharma Chemica, 4: 10-14.
16. Alaa EG, Ahmed A, Laila AF, Marwan MS (2001) Application of LC and HPTLC-densitometry for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide. Journal of Pharmaceutical and Biomedical Analysis 25: 171-179.
17. Pandeti IE, Parisi-Poulou M (1999) Simultaneous determination of benazepril hydrochloride and hydrochlorothiazide by micro-bore liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 21: 1017-1024.
18. Validation of Analytical Procedures: Methodology. ICH Harmonized Tripartite Guidelines 2005.
19. ICH Harmonized tripartite Guidelines, Stability testing of New Drug Substances and products, Q1A(R2), Feb 2003.