Research Article

Quantification of Benazepril Hydrochloride and Hydrochlorothiazide in Tablet Dosage Form by Simultaneous Equation Spectrophotometric Method

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Simple, accurate, precise, reproducible, requiring no prior separation, and economical procedures for simultaneous estimation of benazepril hydrochloride (BEN) and Hydrochlorothiazide (HCT) in tablet dosage form have been developed. Simultaneous equation method employs for estimation of both drugs in methanol at 240 nm and 270 nm as two analytical wavelengths. BEN and HCT at their respective $\lambda_{\max}$ (240 nm and 270 nm) show linearity in a concentration range of 2–12 $\mu\text{g/mL}$ and 4–14 $\mu\text{g/mL}$. Recovery studies for BEN are 100.0–100.6% and 99.8–100.0% for HCT in the case of simultaneous equation method confirming the accuracy of the proposed method. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

1. Introduction

Benazepril hydrochloride (BEN) is chemically 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid monohydrochloride (Figure 1(a)). The empirical formula of BEN is $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_5\cdot\text{HCl}$ with a molecular weight of 460.96 g/mole [1]. It is an angiotensin converting enzyme. It is used as antihypertensive agent.

Hydrochlorothiazide (HCT) is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Figure 1(b)). The empirical formula is $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$ with a molecular weight 297.73 g/mole [2]. It is a diuretic agent.

BEN and HCT are official in the British Pharmacopoeia and the Indian Pharmacopoeia. Many methods have been reported in the literature for both determination of BEN, individually and with other drugs in combination [3–17]. However, there is no UV spectrophotometric method reported for the simultaneous estimation of BEN and HCT in pharmaceutical preparations in the literature survey. The

objective of the present work is to develop and validate new analytical methods for simultaneous determination of BEN and HCT in tablet dosage form.

2. Experimental

2.1. Materials and Methods. A double-beam Shimadzu UV-visible spectrophotometer 1700 (Pharma spec), with wavelength accuracy of $\pm 0.5\text{ nm}$ and a pair of 1 cm matched quartz cells, was used to measure absorbance of the resulting solution. All weighing was done on an electronic balance (Shimadzu BL-220H). All statistical calculations were carried out using Microsoft Excel 2007 analytical tool.

Analytically pure BEN and HCT were procured as gift sample from Dishman Pharmaceuticals Ltd. and Cadila Pharmaceutical Pvt. Ltd. (Ahmedabad, India). Methanol (E. Merck, Mumbai, India) analytical grade was used as a diluent. Tablet formulation (Lotensin HCT, Novartis Pharmaceutical Pvt. Ltd.) containing labelled amount of 10 mg of benazepril hydrochloride and 12.5 mg of hydrochlorothiazide was purchased from local market.

FIGURE 1: (a) Structure of hydrochlorothiazide. (b) Structure of benazepril hydrochloride.

2.2. Preparation of Solutions. Accurately weighed 100 mg of BEN and HCT standards was transferred to separate 100 mL volumetric flasks and dissolved in 50 mL Methanol. The flasks were shaken, and the volume was made up to the mark with methanol to give solutions containing 1000 µg/mL BEN and 1000 µg/mL HCT, respectively.

2.3. Methodology. Selection of analytical wavelengths was done by taking pure samples of BEN and HCT, which were separately dissolved in methanol, to give two solutions of 10 µg/mL, respectively. They were scanned in the wavelength range of 200–400 nm. From the overlain spectra (Figure 2), wavelengths of 240 and 270 nm were selected for the formation of simultaneous equations. For constructing a calibration curve, two series of different concentrations in range of 2–12 µg/mL for BEN and 4–14 µg/mL for HCT were prepared from stock solutions. The calibration curves were plotted at 240 and 270 nm. The absorptivities (A1%, 1 cm) of both drugs at both wavelengths were determined. These calculated values were the mean of five independent determinations. Concentrations in the sample were obtained by using the following equations:

$$C_x = \frac{A_1 a y_2 - A_2 a y_1}{a x_1 a y_2 - a x_2 a y_1} \quad (1)$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a y_1 a x_2 - a y_2 a x_1}$$

where A_1 and A_2 are absorbance of mixtures at 240 nm and 270 nm, respectively; $a x_1$ and $a x_2$ are absorptivities of BEN at λ_1 and λ_2 , respectively, $a y_1$ and $a y_2$ are absorptivities of HCT at λ_1 and λ_2 respectively. C_x and C_y are concentrations of BEN and HCT, respectively.

2.4. Method Validation. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [18].

2.5. Linearity and Range. Developed analytical method shows linearity response over the range of 2–12 µg/mL for BEN and 4–14 µg/mL for HCT at 240 nm and 270 nm respectively.

2.6. Precision. The intraday and interday precision study of the proposed simultaneous equation spectrophotometric

FIGURE 2: Overlain spectrum of BEN and HCT in Methanol.

TABLE 1: Summary of validation parameters.

Parameters	BEN	HCT
Limit of detection	0.103	0.025
Limit of quantitation	0.312	0.075
Accuracy (%)	100.0–100.6	99.8–100.2
Precision		
Intraday ($n = 3$)	0.47–0.74%	0.13–0.24%
Interday ($n = 3$)	0.30–0.81%	0.18–0.40%
Repeatability (RSD, $n = 6$)	1.32%	1.96%
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent suitability	Suitable for 24 hrs.	Suitable for 24 hrs.

TABLE 2: Statistical data of BEN and HCT.

Parameters	BEN at 240 nm	HCT at 270 nm
Linear range	2–12 µg/mL	4–14 µg/mL
Slope	0.0181	0.05
Intercept	0.0129	0.035
Standard deviation of intercept	0.0031	0.0010
Standard deviation of slope	0.0005	0.0019
Regression coefficient (R^2)	0.9997	0.9988

method was carried out by estimating responses three times on the same day and on three different days (first, second, and

TABLE 3: Determination of precision for BEN.

BEN	Conc. ($\mu\text{g/mL}$)	Intraday mean $\pm$ SD ($n = 3$)	%RSD	Interday mean $\pm$ SD ($n = 3$)	%RSD
At 240 nm	6	0.12267 ± 0.00058	0.47	0.124 ± 0.001	0.81
	8	0.15533 ± 0.00115	0.74	0.15533 ± 0.00058	0.37
	10	0.196 ± 0.001	0.51	0.19533 ± 0.00058	0.30
At 270 nm	6	0.03067 ± 0.00058	1.88	0.03067 ± 0.00058	1.88
	8	0.3633 ± 0.00058	1.59	0.03667 ± 0.00058	1.58
	10	0.04367 ± 0.00058	1.32	0.04767 ± 0.00058	1.21

TABLE 4: Determination of precision for HCT.

HCT	Conc. ($\mu\text{g/mL}$)	Intraday mean $\pm$ SD ($n = 3$)	%RSD	Interday mean $\pm$ SD ($n = 3$)	%RSD
At 240 nm	8	0.03467 ± 0.00058	0.017	0.03433 ± 0.00058	1.68
	10	0.042 ± 0.001	0.024	0.04267 ± 0.00058	1.35
	12	0.217 ± 0.28232	0.18	0.05433 ± 0.00058	1.06
At 270 nm	8	0.44333 ± 0.00058	0.13	0.44467 ± 0.00153	0.34
	10	0.526 ± 0.001	0.19	0.52567 ± 0.00208	0.40
	12	0.63367 ± 0.00153	0.24	0.63333 ± 0.00115	0.18

third days) for three different concentrations of BEN (6, 8, and 10 $\mu\text{g/mL}$) and HCT (8, 10, 12 $\mu\text{g/mL}$), and the results are reported in terms of percentage relative standard deviation (%RSD).

2.7. Accuracy. The accuracy of the method was determined by calculating recoveries of BEN and HCT by the method of standard additions. Known amount of BEN (50%, 100%, and 150%) and HCT (50%, 100%, and 150%) was added to a pre quantified sample solutions, and the amounts of BEN and HCT were estimated by measuring the response at the appropriate wavelengths. The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration level.

2.8. LOD and LOQ. Calibration curve was repeated 5 times, and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows:

$$\begin{aligned} \text{LOD} &= 3.3 \times \frac{\sigma}{S}, \\ \text{LOQ} &= 10 \times \frac{\sigma}{S}, \end{aligned} \quad (2)$$

where σ is the standard deviation of the y -intercept S is the slope of the calibration curve.

2.9. Solution Stability. Solution stability of the method was studied by observing the stability of both drug solutions at $25 \pm 2^\circ\text{C}$ for 24 h.

2.10. Analysis of Marketed Formulation. The pharmaceutical dosage form used in this study was Lotensin HCT tablets with a content of 10 mg BEN and 12.5 mg HCT (as per USP) per tablet. Twenty tablets of brand Lotencin HCT tablets were weighed and finely powdered. Accurately weighed tablet powder equivalent to 10 mg was taken in 100 mL volumetric

flask. Few mL of methanol was added and sonicated for 5 min. The volume was made up to the mark with methanol. Aliquot portion of this solution was further diluted to achieve final concentration of 10 $\mu\text{g/mL}$ for BEN and HCT. The absorbances were noted at respective wavelengths. The concentration of each drug in tablet formulation was determined using the above methods.

3. Result and Discussion

A simultaneous equation spectrophotometric method was successfully developed for the determination of BEN and HCT from their combined dosage form.

The proposed simultaneous equation method shows good linearity in the concentration range of 2–12 $\mu\text{g/mL}$ for BEN and 4–14 $\mu\text{g/mL}$ for HCT with correlation coefficient 0.9997 of BEN and 0.9988 for HCT, respectively (Table 2).

The %RSD values for BEN and HCT were found to be 1.32% and 1.96%, respectively. The low values of relative standard deviation (less than 2%) indicate that the proposed method is repeatable. The %RSD values for intraday study were found to be 0.47–0.74% and 0.13–0.24% for BEN and HCT, respectively. The %RSD values for interday study were found to be 0.30–0.81% and 0.18–0.40% for BEN and HCT, respectively. The low RSD value indicates that the proposed method is precise (Tables 3 and 4). The detection limit of BEN and HCT were 0.103 and 0.025 $\mu\text{g/mL}$, while quantitation limits of BEN and HCT were 0.312 and 0.075 $\mu\text{g/mL}$, respectively. The above data shows that a nanogram quantity of both the drugs can be accurately and precisely determined. The validation parameters are summarized in Table 1.

The accuracy of the method was determined by calculating recoveries of BEN and HCT by the method of standard additions. The percent recovery was found to be 100.0%–100.6% for BEN and 99.8%–100.2% for HCT (Table 5). The results of recovery studies indicate that the proposed method is accurate.

TABLE 5: Determination of accuracy.

% level	Amount of drug added ($\mu\text{g/mL}$)		Recovered amount of drug ($\mu\text{g/mL}$)		% recovery	
	BEN ($\mu\text{g/mL}$)	HCT ($\mu\text{g/mL}$)	BEN ($\mu\text{g/mL}$)	HCT ($\mu\text{g/mL}$)	BEN	HCT
50	6	6	6.04	6.00	100.6	100.2
100	8	8	8.01	7.98	100.1	99.89
150	10	10	10.00	9.98	100.0	99.9

TABLE 6: Assay results of marketed formulation.

Formulation	Actual concentration ($\mu\text{g/mL}$)		Amount obtained ($\mu\text{g/mL}$)		% BEN	% HCT
	BEN	HCT	BEN	HCT		
Tablet	4.0	5.0	3.90	4.97	98.25%	99.32%

The proposed validated method was successfully applied to determine BEN and HCT in tablet dosage form. The results obtained for BEN and HCT were comparable with the corresponding labelled amounts (Table 6). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of BEN and HCT in pharmaceutical dosage forms.

4. Conclusions

Sensitive, precise, and accurate simultaneous UV spectroscopic method was developed and validated. The proposed method is accurate, precise, reproducible, and economic and can be successfully used for routine analysis of simultaneous estimations of BEN and HCT. The method was validated as per the ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and limits of quantification (LOQ), and robustness. The proposed method can be used for quality control assay of BEN and HCT in their pharmaceutical dosage form.

Conflict of Interests

All authors do not have conflict of interests.

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