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RESEARCH ARTICLE

New spectroscopy methods development for simultaneous estimation of Benazepril and Hydrochlorothiazide in pharmaceutical dosage form

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Benazepril (BENZ),
Hydrochlorothiazide (HCTZ),
Absorbance correction method (ACM),
UV spectrophotometer.

ABSTRACT:

A new, simple, rapid and novel spectrophotometric method has been developed for estimation of Benazepril (BENZ) and Hydrochlorothiazide (HCTZ) in bulk and combined pharmaceutical formulations using absorbance correction method (ACM). λmax of BENZ was found at 209nm and HCTZ was at270nm respectively. This method involved measurement of absorbance at two wavelengths of BENZ and HCTZ i.e., 240 nm and 270 nm for BENZ and 270nm and 240 nm for HCTZ. The combination is also estimated by ACM. It showed linearity of both the drugs at two wave lengths. Calibration curve was constructed at 240 nm and 270nm for BENZ and 270nm and 240 nm for HCTZ respectively. Beer's law obeyed in concentration range of 2-12 µg/ mL for BENZ and 4-14 µg/ mL for HCTZ respectively by the method. These methods were validated for precision, reproducibility, linearity and accuracy as per ICH guidelines. The proposed methods are recommended for routine analysis since they are rapid, simple, accurate, cost effective and also sensitive and specific. It involves neither heating nor use of any organic solvent for separation of the combination.

1. INTRODUCTION:

Benazepril hydrochloride (BENZ) is chemically 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-BENZazepine-1-acetic acid mono hydrochloride. The empirical formula of BENZ is $C_{24}H_{28}N_2O_5 \cdot HCl$ with a molecular weight of 460.96µg/mole [1]. It is an angiotensin converting enzyme. It is used as antihypertensive agent.

Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3,4-dihydro-2H-1,2,4-BENZothiadiazine-7-sulfonamide 1,1-dioxide. The empirical formula is $C_7H_8ClN_3O_4S_2$ with a molecular weight 297.73µg/mole [2]. It is a diuretic agent.

BENZ and HCTZ are official in the British Pharmacopeia and the Indian Pharmacopeia. Many methods have been reported in the literature for both determination of BENZ, individually and with other drugs in combination [3–17]. However, there is no UV spectrophotometric method (absorbance correction method) reported for the simultaneous estimation of BENZ and HCTZ 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 BENZ and HCTZ in tablet dosage form.

2. EXPERIMENTAL:

2.1 Instruments

A double-beam Shimadzu UV-visible spectrophotometer 1800 (Pharma spec), with wavelength accuracy of ±0.5µnm and a pair of 1cm matched quartz cells, was used to measure absorbance of the resulting solution. All weighing was done on Denver electronic balance. All

statistical calculations were carried out using Microsoft Excel 2007 analytical tool.

2.2 Materials and Methods

Analytically pure BENZ and HCTZ were procured as gift sample from Dishman Pharmaceuticals Ltd. and Cadila Pharmaceutical Pvt. Ltd. (Ahmedabad, India). 0.1M HCl (E. Merck, Mumbai, India) analytical grade was used as a diluent. Tablet formulation (Lotensin H, Novartis Pharmaceutical Pvt. Ltd.) containing labelled amount of 10mg of Benazepril hydrochloride and 12.5mg of hydrochlorothiazide was purchased from local market.

2.3 Preparation of Stock solutions

Accurately weighed 100 mg of BENZ and HCTZ standards was transferred to separate 100 mL volumetric flasks and dissolved in 0.1M HCl. The flasks were shaken, and the volume was made up to the mark with methanol to give solutions containing 1000 µg/mL BENZ and1000 µg/mL HCTZ, respectively.

2.4 Preparation of working Solutions:

Standard stock solution of BENZ was prepared by dissolving 10mg of BENZ in 10ml of 0.1M HCl to produce a concentration of 1000µg/ml. 1ml of this solution was taken and then diluted up to 10ml by using 0.1M HCl to produce a concentration of 100µg/ml. Again solutions were taken and then diluted by using 0.1M HCl to produce a concentration range 1- 20µg/ml.

Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using 0.1M HCl as a blank and the wavelength corresponding to maximum absorbances (λmax) were found to be 240nm and 270 nm of BENZ and HCTZ, respectively.(fig.1).

2.5 Analysis of Marketed Formulation

The pharmaceutical dosage form used in this study was Lotensin H tablets with a content of 10 mg BEN and 12.5mg HCT (as per USP) per tablet. Twenty tablets of brand Lotensin H 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 µg/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. APPLICATION OF THE METHOD:

3.1 Absorbance Correction Method

The value of λmax was determined by scanning the drug solution in the range 200-400nm was found to be at 270 nm and 240 nm respectively for HCTZ and BENZ. To construct Beer's plot for HCTZ and BENZ, working solutions of both the drugs were prepared in 0.1 M HCl.. Also Beer's plot was constructed for HCTZ and BENZ in solution mixture at different concentrations. Both the drugs followed linearity individually in HCTZ (2-12µg/ml) and BENZ (4-14 µg/ml) and in mixture with the concentration range HCTZ: BENZ is 2:5 in ratio.

The concentration of two drugs in the mixture can be calculated using following equation

$$A = abc$$

$$Cx = A1 / ab$$

$$Cx = A1 / ax1 * b \qquad (1)$$

$$A2 = A\ BENZ + A\ HCTZ$$

$$A2 = (ay2 * cy * b) + (ax2 * cx * b)$$

$$A2 = (ay2 * cy) + (ax2 * cx)$$

$$Cy = [A2 - (ax2 * cx)] / ay2 \qquad (2)$$

where A1, A2 are absorbance of mixture at 270 nm (λ1) and 240 nm (λ2), respectively, ax1 and ax2 are absorptivities of HCTZ at λ1 and λ2, respectively, ay1 and ay2 are absorptivities of BENZ at λ1 and λ2, respectively, cx and cy are concentrations of HCTZ and BENZ .

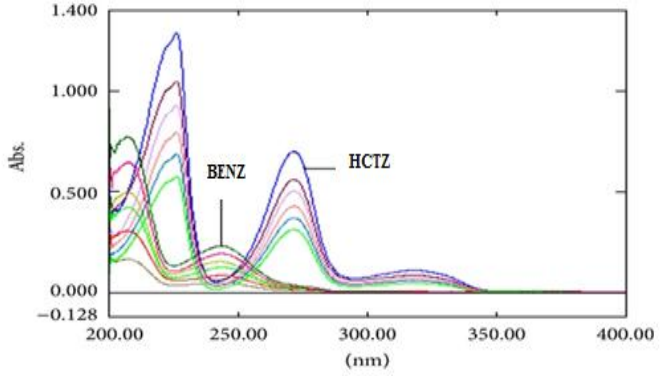

Fig . 1. Overlain spectrum of BENZ and HCTZ in 0.1 M HCl.

4. METHOD VALIDATION: [18]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for BENZ and HCTZ for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ)

4.1 Linearity: Developed analytical method shows linearity response over the range of 2–12 µg/mL for BENZ and 4–14µg/mL for HCTZ at 240 nm and 270 nm respectively.

4.2 Precision: The intraday and interday precision study of the proposed simultaneous equation spectrophotometric method was carried out by estimating responses three times on the same day and on three different days (first, second, and third days) for three different concentrations of BENZ (6, 8, and 10 µg/mL) and HCTZ (8, 10, 12 µg/mL), and the results are reported in terms of percentage relative standard deviation (%RSD).

4.3 Accuracy: The accuracy of the method was determined by calculating recoveries of BENZ and HCTZ by the method of standard additions. Known amount of BENZ (50%, 100%, and 150%) and HCTZ (50%, 100%, and 150%) was added to a pre quantified sample solutions, and the amounts of BENZ and HCTZ 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.

4.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 1-20 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value. Limit of Quantification: Limit of Quantification: The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula: LOQ = 3.3*LOD

5. RESULTS AND DISCUSSIONS:

An absorbance correction spectrophotometric method was successfully developed for the determination of BENZ and HCTZ from their combined dosage form. The proposed method shows good linearity in the concentration range of 2–12 µg/mL for BENZ and 4–14 µg/mL for HCTZ with correlation coefficient 0.9997 of BENZ and 0.9988 for HCTZ, respectively (Table 2). The % RSD values for BENZ and HCTZ 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 BENZ and HCTZ, respectively.

The %RSD values for interday study were found to be 0.30–0.81% and 0.18–0.40% for BENZ and HCTZ, respectively. The low RSD value indicates that the proposed method is precise (Tables 3). The detection limit of BENZ and HCTZ were 0.103 and 0.025 µg/mL, while quantitation limits of BENZ and HCTZ were 0.312 and 0.075 µg/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 BENZ and HCTZ by the method of standard additions. The percent recovery was found to be 100.0%–100.6% for BENZ and 99.8%–100.2% for HCTZ (Table 4). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine BENZ and HCTZ in tablet dosage form. The results obtained for BENZ and HCTZ were comparable with the corresponding labeled amounts (Table 5). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of BENZ and HCTZ in pharmaceutical dosage forms.

Table. 1: Summary of validation parameters.

Parameters	BENZ	HCTZ
Limit of detection(µg/mL)	0.103	0.025
Limit of quantitation(µg/mL	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%	0.18-40%
Repeatability[RSD,(n=6)]	1.32%	1.96%

Table 2: **Statistical data of BENZ and HCTZ.**

Parameters	BEN Z at 240 nm	HCTZ 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

Table 3: Determination of precision for BENZ and HCTZ

BENZ									
Conc. (µg/mL)		Intraday mean ± SD (n=3)		%RSD		Interday mean ± SD (n=3)		%RSD	
240nm	270 nm	240nm	270 nm	240 nm	270 nm	240nm	270 nm	240nm	270 nm
6	6	0.125±0.001	0.031±0.001	0.47	1.88	0.124±0.001	0.031±0.001	0.81	1.88
8	8	0.155±0.001	0.363±0.001	0.74	1.59	0.155±0.001	0.037±0.001	0.37	1.58
10	10	0.196±0.001	0.044±0.001	0.51	1.32	0.195±0.001	0.047±0.001	0.30	1.21
HCTZ									
8	8	0.035±0.001	0.443±0.001	0.17	0.13	0.034±0.001	0.444±0.002	1.68	0.34
10	10	0.042±0.001	0.526±0.001	0.24	0.19	0.042±0.001	0.525±0.002	1.25	0.40
12	12	0.217±0.282	0.634±0.002	0.18	0.24	0.054±0.001	0.633±0.001	1.06	0.18

Table 4: Determination of accuracy.

%Level	BENZ Added amount (µg/ml)	HCTZ Added amount (µg/ml)	BENZ Recovered amount (µg/ml)	HCTZ Recovered amount (µg/ml)	%Recovery	
					BENZ	HCTZ
50	6	6	6.04	6.00	100.6	100.2
100	8	8	8.01	7.98	100.1	99.81
150	10	10	10.00	9.98	100	99.9

Table 5. Assay results of marketed formulations

Tablet	BENZ Actual concentration (µg/ml)	HCTZ Actual concentration (µg/ml)	BENZ Obtained concentration (µg/ml)	HCTZ Obtained concentration (µg/ml	%Recovery	
					BENZ	HCTZ
Lotensin H	10	12.5	9.90	11.97	98.25	99.32

6. CONCLUSION:

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 BENZ and HCTZ. 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 BENZ and HCTZ in their pharmaceutical dosage form.

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