Original Article

Simultaneous estimation of captopril and hydrochlorothiazide in combined dosage forms

Abstract

Aim: This work deals with the simultaneous determination of captopril (CAP) and hydrochlorothiazide (HZ) in two-component solid dosage form, without prior separation, by three different techniques (simultaneous equation, absorbance ratio method, and first-order derivative method). **Materials and Methods:** This work was carried out on Shimadzu electron UV1800 double-beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200–400 nm. Methanol and distilled water are used as solvent. **Results:** The first method is the application of simultaneous equation. Where the linearity ranges for both the drugs were 5–35 µg/ml. The second method is the determination of ratio of absorbance at 271 nm, the maximum absorption of HZ and isobestic wavelength 209 nm, the linearity ranges for both the drugs were 10–120 µg/ml The third method is the first-order derivative method, where the CAP shows wavelength at 222 nm and HZ shows at 340 nm, and the linearity ranges for CAP and HZ were 1–30 µg/ml and 1–40 µg/ml, respectively. The proposed procedures were successfully applied for the simultaneous determination of both the drugs in commercial tablet preparation. The validity of the proposed methods was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 99.52±0.214 and 99.00±0.165 using the simultaneous equation method, 99.76±0.684 and 99.58±0.279 using the graphical absorbance ratio method, and 99.45±0295 and 99.21±0.678 using first derivative method, for CAP and HZ, respectively. **Conclusion:** The proposed procedures are rapid, simple, require no preliminary separation steps, and can be used for routine analysis of both drugs in quality control laboratories.

Key words:
Captopril, first derivative method, hydrochlorothiazide, Q-Analysis method, simultaneous equation method

Introduction

Captopril (CAP), (2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid, an active inhibitor of the angiotensin-converting enzyme (ACE) has been used for the treatment of hypertensive diseases[1] and moderate heart failure[2] as such or in combination with other drugs. Hydrochlorothiazide (HZ) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1,1-dioxide. It is the prototype of the thiazide group and antihypertensive drug.[3]

Literature survey reveals that CAP was determined by several methods including spectrophotometric,[4-8] HPLC,[9-11] flow injection biamperometric,[12] flow injection chemiluminescence,[13] potentiometric and visual titrimetric,[14] and FT-Raman spectroscopy.[15] HZ was determined by capillary electrophoresis,[16] electrochemical study,[17] spectrophotometric,[18-22] and HPLC.[23,24] Simultaneous estimation of CAP and HZ in combined dosage forms was also reported using spectrophotometric[25] and HPLC[26-28] methods. Nevertheless, the reported spectrometric method was first derivative spectrophotometric method only. The aim of this article was to explore the possibility of using techniques of

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Kareti Srinivasa Rao, Minakshi Panda, Nargesh Kumar Keshar, Sushil Kumar Yadav

Departments of Pharmaceutical Analysis and Quality Assurance, Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India

Address for correspondence:
Dr. K. Srinivasa Rao,
Roland Institute of Pharmaceutical Sciences,
Berhampur, Orissa - 760 010, India.
E-mail: ksrao108@gmail.com

simultaneous equation, the absorbance ratio (Q-analysis), and first derivative method for quantifying CAP and HZ simultaneously in their mixture forms. The proposed methods are simple, convenient, precise, accurate, and economical than the reported method.

Materials and Methods

Experimental

Instrument: This work was carried out on Shimadzu electron UV1800 double-beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200–400 nm. Afcoset ER 200A electronic balance was used for weighing the samples.

Materials

Standard gift samples of captopril (CAP) and hydrochlorothiazide (HZ) were obtained from Torrent pharmaceuticals Ltd, Baddi, Himachal Pradesh. Combined CAP and HZ tablets were purchased from local market. Methanol was purchased from MERCK Ltd.

Procedure

Simultaneous equation method (Method-I)

Standard stock solutions (1 mg/ml) of CAP and HZ were prepared by dissolving 100 mg of each in 20 ml methanol in a 100-ml volumetric flask and diluted to 100 ml with methanol. From this, suitable aliquots are taken and diluted with distilled water to get 10 µg/ml of CAP and 10 µg/ml of HZ. The absorption spectra of all the solutions were recorded between 200 and 400 nm. The absorbance were measured for CAP and HZ at 205 nm (λ_1) (maximum absorbance of CAP), 271 nm (λ_2) (maximum absorbance of HZ), and 209 nm (isobestic point). Wavelengths 205 nm and 271 nm were selected for the formation of simultaneous equation [Figure 1]. The absorbances were measured at the selected wavelengths. The molar absorptivity values were 678.5 at λ_1 and 580.8 at λ_2 for CAP and 898 at λ_1 and 496.8 at λ_2 for HZ. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations:

$$Cx = A_2ay_1 - A_1ay_2/ax_2ay_1 - ax_1ay_2$$

$$Cy = A_1ax_2 - A_2ax_1/ay_1ax_2 - ay_2ax_1$$

where A_1 and A_2 are absorbances of the mixture at λ_1 and λ_2, respectively, ax_1 and ax_2 are absorptivity of X at λ_1 and λ_2, respectively, ay_1 and ay_2 denote absorptivity of Y at λ_1 and λ_2, respectively, and Cx and Cy are concentrations of CAP and HZ, respectively.

The graphical absorbance ratio method (Q-analysis method) (Method-II)

In the quantitative assay of two components by Q-analysis method, absorbances were measured at two wavelengths, one being the isobestic wavelength and the other being wavelength of maximum absorption of one of the two components. From overlain spectra of CAP and HZ, absorbances were measured at the selected wavelength, i.e., 209 nm (isobestic wavelength) and 271 nm (wavelength of maximum absorption of HZ) [Figure 1]. The concentration of each component can be calculated by mathematical treatment of the following mentioned equation.

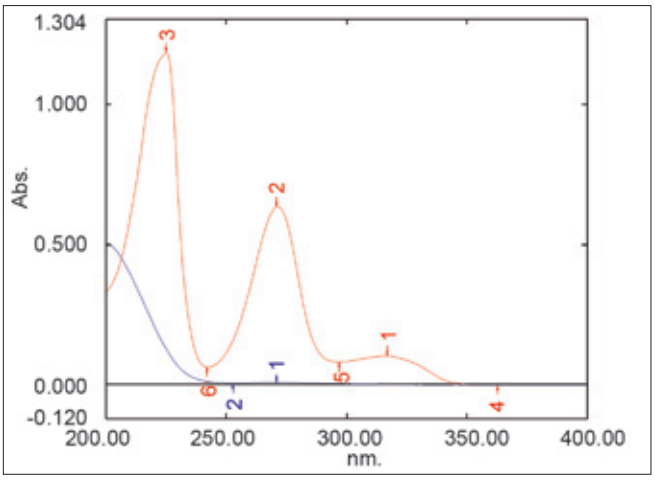

Figure 1: Overlain UV absorption spectrum of CAP and HZ (10 and 10 μg/ml) for Q-analysis method and simultaneous equation method

For CAP

$$C_1 = Qm - Qy/Qx - Qy.\ A_1/a$$

For HZ

$$C_2 = Qm - Qx/Qy - Qx.\ A_1/a$$

where, C_1 = concentration of CAP
C_2 = concentration of HZ
A_1 = absorbance of sample at isobestic wavelength (209 nm)
a = absorptivity of CAP and HZ at isobestic wavelength (209 nm)
Qx = absorptivity of CAP at 271 nm/absorptivity of CAP at 209 nm
Qy = absorptivity of HZ at 271 nm/absorptivity of HZ at 209 nm
Qm = absorptivity of sample solution at 271 nm/absorptivity of sample solution at 209 nm.

First-order derivative method (Method III)

Solutions of 10 µg/ml of CAP and HZ were prepared separately. Both the solutions were scanned in the spectrum mode from 200 to 400 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First-order derivative (n=1) was selected for analysis of both the drugs. The zero crossing wavelengths 222 nm and 340 nm were selected for CAP and HZ, respectively [Figure 2].

Preparation of calibration curve

Six mixed standards having concentration 1, 5, 10, 15, 20, 25, and 30 μg/ml of CAP and 1, 5, 10, 20, 30, and 40 μg ml of HZ, respectively, were prepared and scanned in the spectrum mode from 200 to 400 nm. The absorption spectra so obtained were derivatized to obtain first derivative order spectra. The absorbances of CAP and HZ were measured at 222 and 340 nm, respectively, and calibration curves of both the drugs were plotted separately. The concentration of individual drug present in the mixture was determined against calibration curve in quantitation mode.

Application of the proposed procedure for the determination of CAP and HZ in tablets

Twenty tablets were weighed, and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 100 mg of CAP and 100 mg of HZ was transferred to 100-ml volumetric flask. The powder was dissolved in 20 ml of methanol by intermittent shaking followed by sonication for 15 min and then the volume was made up to 100 ml with methanol. The solution was then filtered through a Whitman filter paper (No. 41). The solution was diluted further with distilled water to obtain 10 μg/ml of CAP and 10 μg/ml of HZ. The concentrations of both CAP and HZ were determined by measuring the absorbance of the samples at 205 nm (λ_{max} for CAP), 271 nm (λ_{max} for HZ), and 209 nm (isobestic point). The recorded data were then substituted in the equation, and the results obtained are summarized in [Table 1]. The analysis procedure was repeated three times. The selectivity of the proposed procedure was examined by determining the recovery of the two drugs by standard addition method [Table 2].

Results and Discussion

The proposed methods were found to be simple, accurate, economic, and rapid for routine simultaneous estimation of two drugs. The values of relative standard deviation are satisfactorily low, and recovery was closed to 100%, indicating reproducibility and accuracy of all methods. These methods also gave excellent result and can be used for routine analysis of these two drugs in combined dosage form.

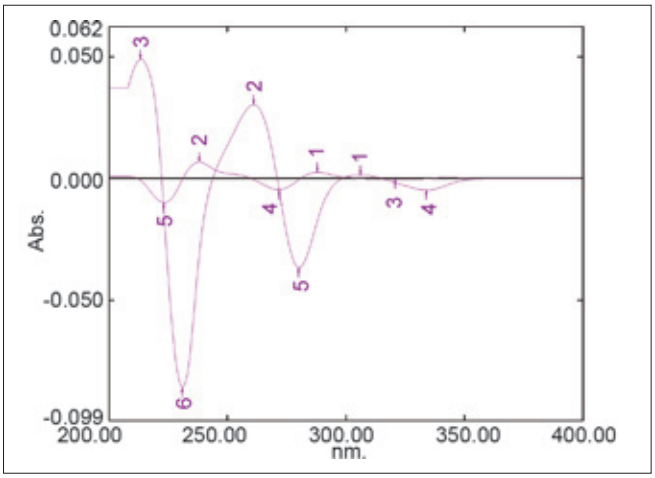

Figure 2: Overlain UV absorption spectrum of CAP and HZ (10 and 10 μg/ml) for first derivative method

Table 1: Determination of CAP and HZ in tablet using the proposed methods

	Simultaneous equation method Recovery (%)±SD		Graphical absorbance ratio method Recovery (%)±SD		First derivative method Recovery (%) ± SD	
	CAP	HZ	CAP	HZ	CAP	HZ
Capotril-H tablets	98.99±0.218	99.92±0.154	98.89±0.247	99.69±0.597	99.90±0.644	99.63±0.365
RSD %	0.002	0.001	0.069	0.005	0.006	0.003

SD – Standard deviation; RSD – Relative standard deviation; (n=3); CAP – Captopril; HZ – Hydrochlorothiazide

Table 2: Results of the application of the standard addition technique to the simultaneous determination of CAP and HZ in tablet by the proposed method

Claimed amount taken (μg/ml)		Standard added (μg/ml)		Recovery of added standard (%) ± SD					
				CAP			HZ		
CAP	HZ	CAP	HZ	SEM	Q-analysis	First derivative	SEM	Q-analysis	First derivative
10	10	8	8	99.61±0.216	99.81±0.579	99.88±0.301	99.02±0.154	99.88±0.257	99.53±0.694
10	10	10	10	99.89±0.116	99.55±0.667	99.46±0.297	98.99±0.169	99.09±0.317	99.09±0.688
10	10	12	12	99.08±0.311	99.92±0.807	99.01±0.289	99.01±0.172	99.78±0.264	99.02±0.654
Mean				99.52	99.76	99.45	99.00	99.58	99.21
SD				0.214	0.684	0.295	0.165	0.279	0.678

SD – Standard deviation; SEM – Simultaneous equation method; Q – Analysis, Graphical absorbance ratio method ; (n=3); CAP – Captopril; HZ – Hydrochlorothiazide

In simultaneous equation method, the overlay spectra of CAP and HZ show overlap that prevents the use of direct absorbance measurement for determination of both the drugs in their mixture. The Figure 1 shows that the λ_{max} for CAP at 205 nm and for HZ at 271 nm. The absorbance curves at the selected wavelengths were found to be proportional to the corresponding concentration of the two drugs in the range of 5–35 μg/ml for both the drugs as shown by the small intercept and correlation coefficient approaching unity in the regression equation [Table 3]. The absorptivity values of the drugs were determined at selected wavelength. The absorptivity is the ratio of mean absorbance of the drug at selected wavelength with the concentration of component in mg/ml. These absorptivity values were the mean of six independent determinations. A set of two simultaneous equations obtained by using mean absorptivity values are given below

$A_1 = 678.5\ C_{CAP} + 898\ C_{HZ}$ ---------- (at λ_{205})

$A_2 = 580.8\ C_{CAP} + 496.8\ C_{HZ}$ ---------- (at λ_{271})

where A_1 and A_2 are absorbance of the sample at 205n and 271 nm, respectively; 678.5 and 580.8 are the absorptivity values of CAP at 205 and 271 nm, respectively; 898 and 496.8 are the absorptivity values of HZ at 205 and 271 nm, respectively; C_{CAP} is the concentration of the CAP; and C_{HZ} is the concentration of HZ in mg/ml.

The proposed Q-analysis method is also a simple method. In this method, the absorbances of the sample solution at the two selected wavelengths were measured and few calculations were done.

The first derivative spectrophotometry method requires spectral data processing and hence can be applied only on recording spectrophotometers with such facilities. This method was used to totally eliminate the spectral interference from one of two drugs while eliminating the other drug. This was achieved by selecting the zero crossing point on the derivative spectra of one drug as the wavelength for the estimation of other drug. First derivative method is simple, less time consuming, no manual calculation, and gives marginally better result than Q-analysis method.

Validation of methods

The methods were validated with respect to linearity, limit of detection (LOD), limit of quantification, precision, accuracy, and selectivity/sensitivity.

For linearity, the calibration plots for each method were constructed after analysis of different concentration, and each concentration was measured six times. The regression equation and correlation coefficients of the mean of six consecutive calibration curves are given in [Table 3].

LOD (k = 3.3) and LOQ (k = 10) of the methods were established according to the ICH definitions ($C_1 = k\ S_0/s$), where C_1 is LOD or LOQ, S_0 is the standard error of blank determination, s is the slope of the standard curve, and k is the constant related to the confidence interval. The LOD, LOQ, and standard error of the methods are given in [Table 3].

Accuracy was investigated by analyzing three different concentrations of combined dosage form of CAP and HZ in linear range in six independent replicates. The data evaluated using equations are summarized in [Table 4]. Accuracy was expressed as bias (%). The bias values were close to zero [Table 4]. The relative standard deviation

Table 3: Data for calibration graph for CAP and HZ using simultaneous equation, graphical absorbance ratio method, and first derivative method

	Simultaneous equation method	Graphical absorbance ratio method	First derivative method
CAP			
Slope	0.0428	0.0118	-0.001
Intercept	0.0031	-0.0303	0.0005
Correlation coefficient	1	0.9962	0.999
Linearity range (μg/ml)	5–35	10–120	1–30
LOD (μg/ml)	0.367	1.21	-0.47
LOQ (μg/ml)	1.2	4.05	-1.5
HZ			
Slope	0.0439	0.0121	-0.003
Intercept	0.0147	0.0025	0.000
Correlation coefficient	0.9996	0.9999	0.999
Linearity range (μg/ml)	5–35	10–120	1–40
LOD (μg/ml)	0.39	1.2	-0.42
LOQ (μg/ml)	1.22	4.0	-1.4

CAP – Captopril; HZ – Hydrochlorothiazide

Table 4: Precision and accuracy of spectrophotometric method developed for analysis of tablet

	Simultaneous equation method	Graphical absorbance ratio method	First derivative method
CAP			
Amount found (mean % ± SD)	98.9±0.216	98.8±0.257	99.9±0.624
Accuracy, bias (%)	−1.1	−1.2	-0.01
Precision, RSD (%)	0.019	0.065	0.006
HZ			
Amount found (Mean % ± SD)	99.9±0.154	99.6±0.577	99.6±0.305
Accuracy, bias (%)	−0.1	−0.4	−0.38
Precision, RSD (%)	0.005	0.004	0.029

SD – Standard deviation; % Bias – [100 (found - added)/added]; RSD – Relative standard deviation; (n=6); CAP – Captopril; HZ - Hydrochlorothiazide

(RSD) values and also the low RSD values obtained from the analysis of pharmaceutical formulations indicated that the intermediate precision of the method was good.

Conclusion

The proposed method based on simultaneous equation, graphical absorbance ratio, and first-order derivative methods can be used for the simultaneous determination of CAP and HZ either in their combined dosage form or alone in their tablet preparation. The proposed methods are precise, accurate, and simple to perform. Also, no separation step is required. Hence, the proposed methods can be used for the routine analysis of CAP and HZ.

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