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Research Article

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF IRBESARTAN AND HYDROCHLOROTHIAZIDE IN TABLETS

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ABSTRACT
Two simple, sensitive, rapid, accurate and economic spectrophotometric methods were developed for the estimation of Irbesartan and Hydrochlorothiazide in two-component solid dosage form. First method is based on the simultaneous equation, and second method is based on Q analysis (absorbance ratio method). Irbesartan has absorbance maxima at 250 nm, and Hydrochlorothiazide has absorbance maxima at 270.6 nm in methanol. In first method, the linearity was obtained in the concentration range 2-36 µg/ml for Irbesartan and 1-18 µg/ml for Hydrochlorothiazide. The concentration of drugs in mixture was determined using simultaneous equations. In the second method, the linearity was obtained in the concentration range of 1-24 µg/ml for both drugs. The concentrations of the drugs in mixture were determined by using ratio of absorbances at isoabsorptive point and at the λ_{max} of one of the drugs. The results of analysis have been validated statistically and by recovery studies.
KEYWORDS: Irbesartan, Hydrochlorothiazide, Simultaneous equation method, Absorbance ratio method

INTRODUCTION

Irbesartan (IRB), 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-1, 3-diazaspiro [4.4] non-1-en-4-one[1], an angiotensin II antagonists, used to treat hypertension and congestive heart failure[2]. Hydrochlorothiazide (HCTZ), 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine- 7-sulfonamide 1, 1-dioxide[3], is a popular diuretic drug that acts by inhibiting the kidney's ability to retain water. HCTZ is indicated in the management of hypertension either as the sole therapeutic agent, or in combination with other antihypertensive.

IRB is official in USP. The USP[4] describe HPLC method for estimation of IRB. Literature survey reveals HPLC[5] and spectrophotometric method[6] for its determination. HCTZ is official in IP, BP, and USP. The IP[7] and USP[8] describe HPLC method for its determination and the BP[9] describes HPLC and spectroscopic method. Literature survey reveals HPLC[10,11], ESI-MS[12], HPTLC[13], spectrophotometric methods[13,14] and electrochemical method[15] for its determination in dosage forms and biological fluids. The combination of two drugs is not official in any pharmacopoeia; hence no official method is available for the estimation of IRB and HCTZ in combined dosage forms. Literature survey reveals HPLC [16], HPTLC[17] and derivative spectroscopic method[18] for simultaneous estimation in combined dosage forms, but there is no simple spectrophotometric method available for estimation of these drugs in combined dosage forms. The present manuscript describes two simple, sensitive, accurate, rapid and economical methods for simultaneous estimation of IRB and HCTZ in tablet dosage form.

MATERIALS AND METHODS

Apparatus

A Shimadzu 1700 UV/Visible spectrophotometer with two 1 cm quartz cells was used for all absorbance measurements. Spectra were automatically obtained by UV-Probe system software. CP224S analytical balance (Sartorius, Gottingen, Germany) and Ultrasonic cleaner (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials

IRB and HCTZ bulk powder was kindly gifted by Cadila Healthcare Ltd., Ahmedabad, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Development of the methods

The solutions of IRB and HCTZ were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm.

Method I (Simultaneous equation method)

From the spectra, it was found that IRB has no $\lambda_{max.}$ The satisfactory absorbance was found at 250 nm wavelength, so it was selected for its determination. The λ_{max} of HCTZ was found to be 270.6 nm.

Method II (Absorbance ratio method)

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one is an isoabsorptive point and the other being the λ_{max} of one of the two components. From the overlay spectra of two drugs, it is evident that IRB and HCTZ show an isoabsoptive point at 255.6 nm. The second wavelength used was 270.6 nm which is being the λ_{max} of HCTZ.

Preparation of standard stock solutions

Accurately weighed portions of IRB (10 mg) and HCTZ (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentrations of IRB (100 µg/ml) and HCTZ (100 µg/ml).

Validation of the proposed methods

Linearity

Method I (simultaneous equation method)

Eight standard solutions having concentration 2, 4, 8, 12, 16, 24, 32 and 36 µg/ml for IRB and eight standard solutions having concentration 1, 2, 4, 6, 8, 12, 16, and 18 µg/ml for HCTZ were prepared in methanol using the standard stock solution. The absorbance of resulting solutions was measured at 250 nm and 270.6 nm, and the calibration curves for both drugs were plotted at these wavelengths.

The absorptivity coefficients of these two drugs were determined using calibration curve equation. Two simultaneous equations were formed using these absorptivity coefficient values. $A1 = 129\times Cx + 760\times C_Y$, $A2 = 317\times Cx + 136\times C_Y$, where Cx and C_Y are concentrations of IRB and HCTZ respectively in g/100 ml in the sample solution. A1 and A2 are the absorbances of the mixture at 270.6 nm and 250 nm, respectively. The concentration of Cx and C_Y can be obtained as $Cx = [(A2\times 760 - Al\times 136)]/ 223376$ and $C_Y = [(Al\times 317 - A2\times 129)]/ 223376$.

Method II (Absorbance ratio method)

Eight standard solutions having concentration 1, 2, 3, 6, 8, 12, 18 and 24 µg/ml for IRB and HCTZ were prepared in methanol, and the absorbances at 255.6 nm (isoabsorptive point) and 270.6 nm (λ_{max} of HCTZ) were measured, and the calibration curves for both drugs were plotted at these wavelengths.

The absorptivity coefficients of these two drugs were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using equations $Cx = Q_M - Q_Y/Q_X - Q_Y \times A_1/ax_1$, $C_Y = A_1/ax_1 - Cx$, where A_1 and A_2 are absorbances of mixture at 255.6 nm and 270.6 nm; and ax_1 and ay_1 are absorptivities of IRB and HCTZ respectively at 255.6 nm; ax_2 and ay_2 are absorptivities of IRB and HCTZ respectively at 270.6 nm; and $Q_M = A_2/A_1$, $Q_X = ax_2/ ax_1$ and $Q_Y = ay_2/ay_1$.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of IRB and HCTZ (24 µg/ml and 2 µg/ml for Method I and 12 µg/ml for each in Method II) without changing the parameters for the both methods.

Intermediate Precision (reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of standard solutions of IRB and HCTZ (4, 12, 36 µg/ml and 4, 12, 18 µg/ml, respectively) for simultaneous equation method and (4, 12, 24 µg/ml and 4, 12, 24 µg/ml for each) for absorbance ratio method. The results were reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the methods was determined by calculating recoveries of IRB and HCTZ by the standard addition method. Known amounts of standard solutions of IRB and HCTZ were added at 50, 100, 150 % level (For Method I) and 50, 75, 100% level (For Method II) to prequantified sample solutions of IRB and HCTZ (12 µg/ml and 1 µg/ml, respectively). The amounts of IRB and HCTZ were estimated by applying obtained values to the simultaneous equation and equation of absorption ratio method.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines[19].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma /S$$

Where, σ = the standard deviation of the response and S = Slope of calibration curve.

Analysis of IRB and HCTZ in combined dosage forms

Twenty tablets were weighed, their average weight was determined, and crushed in mortar. Powder equivalent to 24 mg of IRB and 2 mg of HCTZ were weighed and transferred to 100 ml volumetric flask and mixed 50 ml

of methanol and sonicated the solution for 30 min. The volume is made up to mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitabley diluted to get a final concentration of 24 µg/ml of IRB and 2 µg/ml of HCTZ. For method I, the absorbencies of the sample solution, i.e, A_1 and A_2 were recorded at 270.6 nm and 250 nm respectively, and concentration of two drugs in the sample were determined using the equations Cx = [(A2×760-Al×136)]/ 223376 and Cy = [(Al×317-A2×129)]/ 223376. For method II, the absorbance of the sample solution, i.e. A_1 and A_2 were recorded at 255.6 nm (isoabsorptive point) and 270.6 nm (λ_{max} of HCTZ) respectively, and ratios of absorbencies were calculated, i.e. A_2/A_1. Relative concentration of two drugs in the sample was calculated using the equations $Cx = Q_M-Q_Y/Q_X-Q_Y \times A_1/ax_1$, $C_Y = A_1/ax_1-Cx$. The analysis procedure was repeated three times with tablet formulation. The result of analysis of tablet formulation is shown in Table 3.

RESULT AND DISCUSSION

The proposed methods were found to be simple, accurate, economical and rapid for the routine simultaneous estimation of two drugs. The values of standard deviation and coefficient of variation was satisfactory, and recovery studies ranging from 99.9-102.4% (for IRB) and 98.5-100.6% (for HCTZ) were indicative of the accuracy of the proposed methods (Table 1 and Table 2).

In simultaneous equation method (Method I), two wavelengths of respective absorbance maxima, i.e., 250 nm for IRB and 270.6nm for HCTZ, were used for the analysis of the drugs. The criteria for obtaining maximum precision by this method were calculated and found to be outside the range of 0.1-2. In absorbance ratio method (Method II), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelengths, which was fulfilled in case of both these drugs.

The validation parameters were studied at all the wavelengths for both the methods. Accuracy was determined by calculating the recovery, and the mean was determined. Precision was calculated as repeatability (standard deviation and relative standard deviation) and inter- and intra-day variation (% RSD) for both the drugs. Both the methods were successfully used to determine the amounts of IRB and HCTZ present in the tablets. The results obtained were in good agreement with the corresponding labeled amount (Table 3). By observing the validation parameters, both the methods were found to be simple, sensitive, accurate and precise (Table 4 and Table 5). Hence both the methods can be employed for the routine analysis of these two drugs in combinations.

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Table 1: Recovery data of method (Simultaneous equation method)

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	%Mean Recovery ± %RSD (n=3)
IRB	12	6	18.4	102.4 ± 0.83
	12	12	23.98	99.9 ± 1.33
	12	18	30.43	100.7 ± 1.32
HCTZ	1	0.5	1.52	101.3 ± 0.85
	1	1	2.01	100.5 ± 0.42
	1	1.5	2.49	99.6 ± 0.85

Table 2: Recovery data of method II (Absorbance ratio method)

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	%Mean Recovery ± % RSD (n=3)
IRB	12	6	17.94	99.62 ± 0.60
	12	9	21.00	100.0 ± 0.49
	12	12	23.95	99.81 ± 0.36
HCTZ	1	0.5	1.51	100.6 ± 1.50
	1	0.75	1.74	99.40 ± 0.91
	1	1	1.97	98.53 ± 0.60

Table 3: Analysis of IRB and HCTZ in tablets

Method	Drug	Labeled amount (mg)	Amount found (mg)	% Amount found ± %RSD (n=3)
I	IRB	300	301.2	100.4 ± 0.78
	HCTZ	25	25.29	101.2 ± 1.00
II	IRB	300	299.90	99.9 ± 0.70
	HCTZ	25	25.20	100.8 ± 1.61

Table 4: Regression analysis data and summary of validation parameter for the Simultaneous equation method

Parameters	IRB		HCTZ	
Wavelength (nm)	270.6	250	270.6	250
Beer's Law Limit (µg/ml)	2 – 36	2 – 36	1 – 18	1 – 18
Regression equation (y = mx + c)	y= 0.0129x + 0.0307	y= 0.0317x + 0.0303	y= 0.076x + 0.009	y=0.0136x + 0.0083
Slope (m)	0.0129	0.0317	0.0760	0.0136
Intercept (c)	0.0307	0.0303	0.0090	0.0083
Correlation Coefficient (r^2)	0.9987	0.9989	0.9988	0.9990
LOD[a] (µg/ml)	0.60	0.50	0.08	0.27
LOQ[b] (µg/ml)	1.80	1.51	0.25	0.82
Repeatability(%RSD[c], n=6)	1.13	0.55	1.09	2.02
Precision (RSD) %				
Interday (n=3)	0.50-1.20	0.30-1.05	1.01-1.59	0.88-2.46
Intraday (n=3)	0.30-1.85	0.39-0.95	0.49-1.29	0.80-1.90

[a] LOD = Limit of detection. [b] LOQ = Limit of quantitation. [c] RSD = Relative standard deviation, %

Table 4: Regression analysis data and summary of validation parameter for the Absorbance ratio method

Parameters	IRB	HCTZ	Isoabsorptive point
Wavelength (nm)	270.6	270.6	255.6
Beer's Law Limit (µg/ml)	1 – 24	1 – 24	1 – 24
Regression equation (y = mx + c)	y=0.0146x+0.0099	y=0.0712x+0.023	y=0.0273x+0.015
Slope(m)	0.0146	0.0712	0.0273
Intercept(c)	0.0099	0.023	0.015
Correlation coefficient(r^2)	0.9981	0.9992	0.9982
LOD[a] (µg/ml)	0.33	0.09	0.30
LOQ[b] (µg/ml)	1.00	0.28	0.90
Repeatability (%RSD, n=6)	1.27	0.71	1.19
Precision (RSD[c], %)			
Interday (n = 3)	0.73 – 1.54	0.46 – 1.34	0.39-1.90
Intraday (n = 3)	0.42- 1.54	0.46-0.92	0.34-1.16

[a] LOD = Limit of detection. [b] LOQ = Limit of quantitation. [c] RSD = Relative standard deviation, %

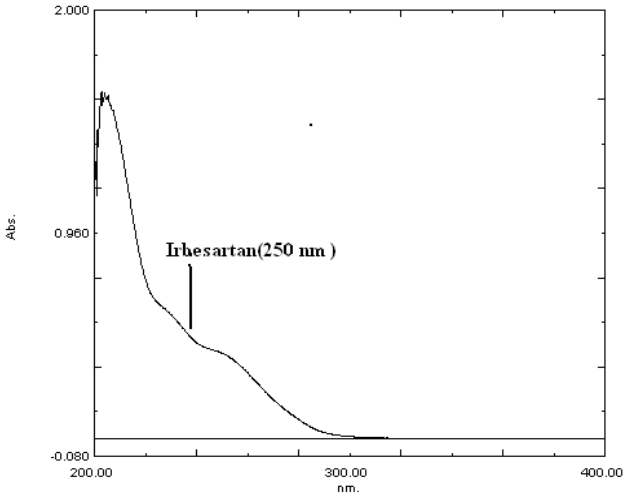

Figure 1: **Representative spectra of IRB showing λ at 250 nm**

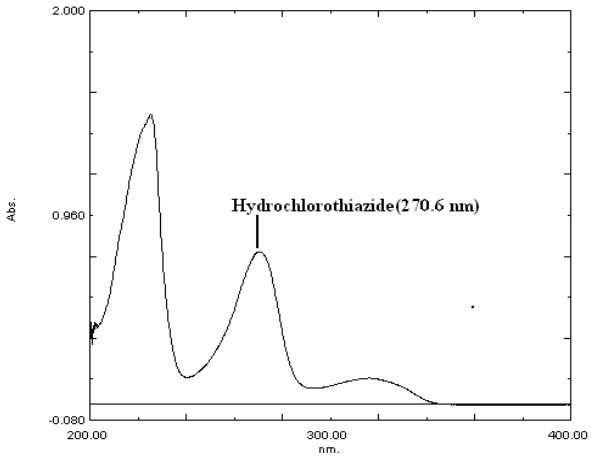

Figure 2: Representative spectra of IRB showing λ_{max} at 270.6 nm

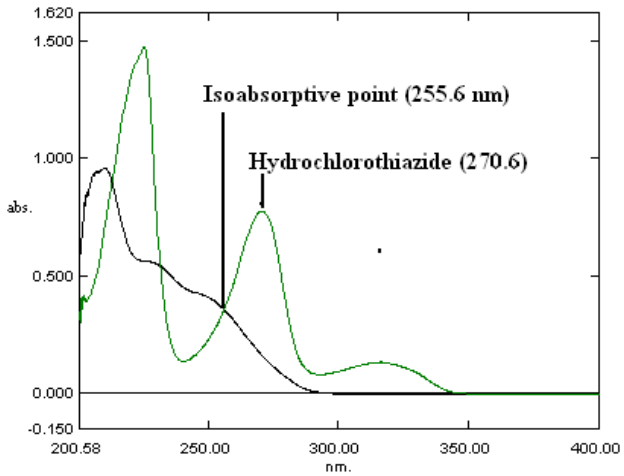

Figure 3: Overlain spectra of IRB and HCTZ showing Isoabsoptive point (255.6 nm)

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