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Research Article

H-POINT STANDARD ADDITION METHOD FOR SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF IRBESARTAN, HYDROCHLOROTHIAZIDE AND TELMISARTAN IN TABLETS

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ABSTRACT

Simultaneous spectrophotometric method described for the determination of Irbesartan, Hydrochlorothiazide and Telmisartan in tablets using absorbance correction – H-Point Standard addition method (HPSAM). A simple and novel method absorbance correction – HPSAM is reported for simultaneous estimation of three drugs using UV-visible spectrophotometry without any prior separation of samples. The linear range was 0.5-3 µg/mL for Irbesartan, Hydrocholrothiazide and Telmisartan. The relative standard deviation (RSD) for the simultaneous determination of 1 µg/mL of Irbesartan and 2 µg/mL of Hydrochorothiazide and Telmisartan by applying HPSAM was 0.819, 0.388 and 1.365 respectively. The reported methods HPSAM can be claimed as green analytical chemistry as it does not involve the use of organic solvents and hence can be utilized for the routine analysis of these drugs from tablets.

Keywords: Irbesartan, Hydrochlorothiazide, Telmisartan, HPSAM, green analytical chemistry.

INTRODUCTION

Irbesartan (IRB) an ARB and is widely used for the treatment of hypertension and heart failure in clinical patients. Angiotensin II is an octapeptide regarded as the main effector of AT1 receptor in renin–angiotensin system1.

Hydrochlorothiazide (HCZ), is widely used in antihypertensive pharmaceutical preparations and is chemically known as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide[2–4].

Telmisartan (TEL), 4 - [(2-n-propyl-4-methyl-6-(1-methyl benz imidazole-2-yl)-benz imidazole-1-yl) methyl] –biphenyl -2-carboxylic acid, is a selective angiotensin II type 1 receptor (AT1R) blocker, which belongs to the group of angiotensin II receptor antagonists[5]. Figure 1 represents the structure of IRB, HCZ and TEL.

A literature survey reveals a variety of spectrophotometric and chromatographic methods such as UV derivative, the simultaneous equation method, colorimetric determination, High Performance Thin Layer Chromatographic method (HPLC), ratio derivative and a stability indicating HPLC methods have been reported for the determination of LOS, AML and HCZ in pharmaceutical dosage forms individually or in combination with other drugs[6–36].

In this paper the authors report a novel technique called absorbance correction H-point standard addition method (HPSAM). This technique also overcomes the spectral interference without prior separation of components and also gives a general linearity range which can be applied for analysis of samples of any concentration.

In 1988, Bosch-Reig and Campins-Falco presented a new technique; the HPSAM based on the principle of dual wavelength spectrophotometry and the standard addition method[37]. The greatest advantage of HPSAM is that it can remove errors resulting from the presence of an interfering and blank reagent. However the method faces a drawback of being applicable only for determination of two drugs. Hence the authors propose a new technique – absorbance correction HPSAM to perform quantitative analysis of three overlapping analytes without prior separation.

MATERIALS AND METHODS

Apparatus

A Perkin Elmer (Lamda 25) Spectrophotometer controlled by UV Winlab software and equipped with a 1 cm pathlength quartz cell was used for UV-Vis spectra acquisition. IRB, HCZ and TEL reference standards were kindly supplied by Madras Pharmaceuticals, Chennai, India. The tablets Irovel H(Sun Pharma, Label Claim – 150 mg IRB and 12.5 mg HCZ), Xarb H (Nicholas Piramal, Label Claim – 150 mg IRB and 12.5 mg HCZ) and Telista H (Lupin Pharmaceuticals, Label Claim – 40 mg TEL and 12.5 mg HCZ) were procured from local pharmacies. All other chemicals were of analytical reagent grade and procured from SD Fine chemicals, Mumbai, India.

EXPERIMENTAL

Standard Solutions

Standard stock solutions (1000 µg/mL) of IRB, HCZ and TEL were prepared separately in the diluent 0.1M NaOH and water in the ratio 20:80 v/v. These solutions were taken and then diluted to 10 ml with water to give a final analyte concentration desired.

Sample Preparation

Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred into a 100 ml volumetric flask including the diluent. The flask was sonicated for 15 mins and diluted to the mark with diluents. An aliquot of the solution was centrifuged at 5000 rpm for 10 mins. Appropriate amount of clear supernatant was transferred into a 10 mL flask and diluted with water. Then the absorbance values of these solutions were measured.

Calibration of Telmisartan

The calibration graphs of TEL were constructed in three selected wavelengths 216.32, 228.41 and 295.12 nm and the results are shown in Table 1.

H-Point Standard Addition Method:

An aliquot of the solution containing 20 µg/mL IRB, 10 µg/mL HCZ and 30 µg/mL TEL were added into 10ml volumetric flask and made up to the mark with water. The solution was then allowed to stand for 10 min at room temperature. After that, a portion of the solution was transferred into a quartz cell to measure its absorbance at appropriate wavelengths. Synthetic samples containing different concentration ratios of IRB, HCZ and TEL were prepared and standard additions of IRB up to 8 µg/mL were performed. Simultaneous determination of IRB, HCZ and TEL with absorbance correction and HPSAM was performed (measuring the absorbance at 295.12 nm for direct determination of TEL according to Beer's law and also calculating the corrected absorbance at 216.32 and 228.41 nm for HPSAM). The linear range for TEL determination is between 2-10 µg/mL in 295.12 nm. The concentration ranges of IRB and HCZ for construction of HPSA calibration graphs was 1-5 µg/mL for both the drugs.

Requirements for applying HPSAM

For the simultaneous estimation of three analytes, three wavelengths were selected as λ_1, λ_2 and λ_3. Two wavelengths, λ_1, and $\lambda_2$were selected from the spectrum of the interferent (Y) at which the absorbance values remains same and the absorbance of the analyte (X) shows a linear response. Third wavelength $\lambda_3$was selected from the second interferent (Z), at which both the analyte and the first interferent are free of interferences. The contribution made by the second interferent at λ_1, and λ_2 is overcome by absorbance correction method as follows:

$$A_{corr,\lambda 1} = A_{mix,\lambda 1} - r_1 \times A_{mix,\lambda 3} \quad (1)$$

$$A_{corr,\lambda 2} = A_{mix,\lambda 2} - r_2 \times A_{mix,\lambda 3v} \quad (2)$$

Where $A_{mix,\lambda 1}$, $A_{mix,\lambda 2}$ and $A_{mix,\lambda 3}$ are the absorbances of sample (ternary mixture) at λ_1, λ_2 and λ_3 respectively. $A_{corr,\lambda 1}$ and $A_{corr,\lambda 2}$ are the net absorbances due to the contribution of second interferent (Z) at λ_1 and λ_2 respectively, that are used for the construction for H-Point Standard addition graph (HPSA graph). The values r_1 and r_2 are the slope ratios of second interferent, Z calibration graphs:

$$r_1 = \text{Slope in } \lambda_1 / \text{Slope in } \lambda_3 \quad (3)$$

$$r_2 = \text{Slope in } \lambda_2 / \text{Slope in } \lambda_3 \quad (4)$$

Wavelength Selection

To select the appropriate wavelengths for using HPSAM the following principles were applied. At these selected wavelengths the analyte signals must be linear with the concentrations and the interference signal must remain equal, in the case where the analyte concentrations are changed, the analytical signal obtained from the mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths (λ_1 and

λ_2) must be as large as possible in order to get good accuracy and sensitivity.
In this case there were several pairs of wavelengths. The wavelength pair of 216.32 and 228.41 nm (λ_1 and (λ_2) was chosen for the study. Standard solutions of IRB and HCZ were initially tested to validate the applicability of the chosen wavelengths.

RESULTS & DISCUSSION

HPSAM

In this system, IRB was selected as an analyte and HCZ as an interferent. Several wavelength pairs were examined and the wavelength pair of 216.32 and 228.41 nm was selected. The third wavelength (λ_3) was selected at 295.12 nm from the spectrum of TEL at which the other two drugs are free from interference.
Under the optimum conditions described above, simultaneous determination of IRB, HCZ and TEL was performed by applying correction absorbance and HPSAM. To check the reproducibility of the method, six replicate measurements of IRB, HCZ and TEL were performed. The concentration of TEL was obtained directly from the absorbance of samples using calibration graph (295.12 nm). After removing the contribution of TEL absorbance from the total absorbance at 216.32 and 228.41 nm, the concentration of interfering component (HCZ) was calculated in each test solution by means of the calibration method using standard solutions and the ordinate value of H-point (A_H). The concentration of IRB was directly obtained (-C_H). The relative standard deviation (RSD) for the simultaneous determination of 1 µg/mL of Irbesartan and 2 µg/mL of Hydrochorothiazide and Telmisartan by applying HPSAM was 0.819, 0.388 and 1.365 respectively (Table 3).

Accuracy of HPSAM

For Synthetic Mixture
In order to ensure the method's accuracy several synthetic mixtures with different concentration ratio of IRB, HCZ and TEL were analyzed with the proposed method. The results are given in Table 4. As can be seen in Table 2 and 3, the accuracy and precision of the method are satisfactory. Fig 3a and 3b shows the H-Point standard addition plots for several synthetic test solutions.
For Pharmaceutical Dosage form
In order to check the applicability of the method, the tablet dosage form was analysed by the proposed method. The results are shown in Table 4 and found to be accurate.

Application

To evaluate the analytical applicability of all the proposed method it was applied to the simultaneous determination of IRB, HCZ and TEL in pharmaceutical preparations containing any two compounds. The results are given in Table 4. The good agreement between the results with the composition values indicated by the suppliers indicates the successful applicability of the proposed methods for simultaneous determination of IRB, HCZ and TEL in pharmaceutical preparations.

CONCLUSION

It was observed that IRB, HCZ and TEL in their mixture have the overlapping absorption spectra in the spectral region of 200 and 350 nm. Therefore, the simultaneous spectrophotometric determination of IRB, HCZ and TEL substances in their synthetic and commercial tablets was performed by using HPSAM. The proposed method does not require prior separation step and spectral derivation for the simultaneous analysis of IRB, HCZ and TEL. Hence the proposed method can be used for the routine analysis and quality control of the marketing tablet formulation containing IRB, HCZ and TEL substances.

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Table 1: Linearity results of all three drugs at selected Wavelengths

Drug	λmax (nm)	r^2	Slope	Intercept	Linear range (µg/mL)
TEL	216.32	0.9989	0.1103	0.0037	2-10
	228.41	0.9987	0.1095	0.0009	
	295.12	0.9982	0.0512	0.9982	
IRB	216.32	0.9993	0.0533	-0.003	1-5
	228.41	0.9995	0.0423	-0.003	
HCZ	216.32	0.9998	0.0452	0.0012	1-5
	228.41	0.9997	0.0473	0.0013	

IRB – Losartan potassium, HCZ – Hydrochlorothiazide and
TEL – Amlodipine Besylate

Table 2: Results of several experiments for the analysis of Irbesartan, Hydrochlorothiazide and Telmisartan mixtures at different concentration ratios by absorbance correction – HPSAM

Regression equation	r^2	Amount present (µg/ml)			Amount Found (µg/ml)		
		IRB	HCZ	TEL	IRB	HCZ	TEL
$A_{216.32}$=0.0682C+0.3025 $A_{228.41}$=0.0464C+0.2781	0.9992 0.9986	1.0	2.0	2.0	1.0	1.93	2.0
$A_{216.32}$=0.0794C+0.3022 $A_{228.41}$=0.0514C+0.2795	0.9972 0.9981	1.0	2.0	2.0	0.9	1.93	1.96
$A_{216.32}$=0.0708C+0.2162 $A_{228.41}$=0.0482C+0.1777	0.9990 0.9980	2.0	1.0	3.0	2.0	0.96	2.88
$A_{216.32}$=0.0756C+0.4097 $A_{228.41}$=0.0518C+0.3442	0.9987 0.9984	3.0	1.6	1.4	3.0	1.61	1.39
$A_{216.32}$=0.0893C+0.3956 $A_{228.41}$=0.0578C+0.3187	0.9927 0.9944	2.6	1.2	1.6	2.6	1.28	1.53
$A_{216.32}$=0.0794C+0.3022 $A_{228.41}$=0.0514C+0.2795	0.9972 0.9981	1.0	2.0	2.0	1.0	1.93	2.02
$A_{216.32}$=0.0695C+0.2980 $A_{228.41}$=0.0488C+0.2639	0.9992 0.9949	1.6	1.6	2.0	1.6	1.66	2.20
$A_{216.32}$=0.0675C+0.4735 $A_{228.41}$=0.0509C+0.4249	0.9994 0.9993	3.0	2.0	2.0	3.0	2.09	2.06
$A_{216.32}$=0.0758C+0.2851 $A_{228.41}$=0.0564C+0.2276	0.9993 0.9991	3.0	0.5	2.0	3.0	0.5	2.1
$A_{216.32}$=0.0762C+0.1380 $A_{228.41}$=0.0548C+0.1418	0.9966 0.9961	0.5	1.0	3.0	0.48	1.0	3.02

IRB – Irbesartan, HCZ – Hydrochlorothiazide and TEL – Telmisartan
A – absorbance at respective wavelength, C – Concentration of unknown sample,
r^2 – correlation coefficient

Table 3: Results for five replicate analysis of Irbesartan, Hydrochlorothizide and mixtures by absorbance correction HPSAM

Regression equation	r^2	Amount present (µg/ml)			Amount Found (µg/ml)		
		IRB	HCZ	TEL	IRB	HCZ	TEL
$A_{216.32}$=0.0794C+0.3002 $A_{228.41}$=0.0514C+0.2811	0.9972 0.9981	1	2	2	1	2	2.002
$A_{216.32}$=0.0848C+0.2979 $A_{228.41}$=0.0531C+0.2763	0.9971 0.9970	1	2	2	0.98	1.985	1.966
$A_{216.32}$=0.0812C+0.3029 $A_{228.41}$=0.0520C+0.2806	0.9947 0.9956	1	2	2	1	1.985	1.966
$A_{216.32}$=0.0805C+0.3113 $A_{228.41}$=0.0498C+0.2845	0.9959 0.9989	1	2	2	1	2	1.966
$A_{216.32}$=0.0811C+0.3078 $A_{228.41}$=0.0497C+0.2797	0.9961 0.9970	1	2	2	1	2	2.031
$A_{216.32}$=0.0796C+0.3101 $A_{228.41}$=0.0516C+0.2828	0.9971 0.9947	1	2	2	1	2	2.002
Mean RSD					0.996 0.819	1.995 0.388	1.988 1.365

IRB – Irbesartan, HCZ – Hydrochlorothiazide and TEL – Telmisartan
A – absorbance at respective wavelength, C – Concentration of unknown sample,
r^2 – correlation coefficient

Table 4: Simultaneous determination of Irbesartan, Hydrochlorothiazide and Telmisartan from formulations using absorbance correction HPSAM

Formulation	Label Claim (mg/tab)			Amount Found* (mg/tab)			% Recovery*		
	IRB	HCZ	IS**	IRB	HCZ	IS**	IRB	HCZ	IS**
IROVEL H (Sun Pharma)	150	12.5	50 (TEL)	150.62	12.48	50.11	100.41	99.84	100.22
XARB H (Nicolas Piramal)	150	12.5	50 (TEL)	151.32	12.51	49.34	100.88	100.08	98.68
Telista –H (Lupin)	TEL	HCZ	IS**	TEL	HCZ	IS**	TEL	HCZ	IS**
	40	12.5	10 (IRB)	40.44	12.47	10.08	101.1	99.76	100.8

*Mean of six estimations
** Internal Standard Used

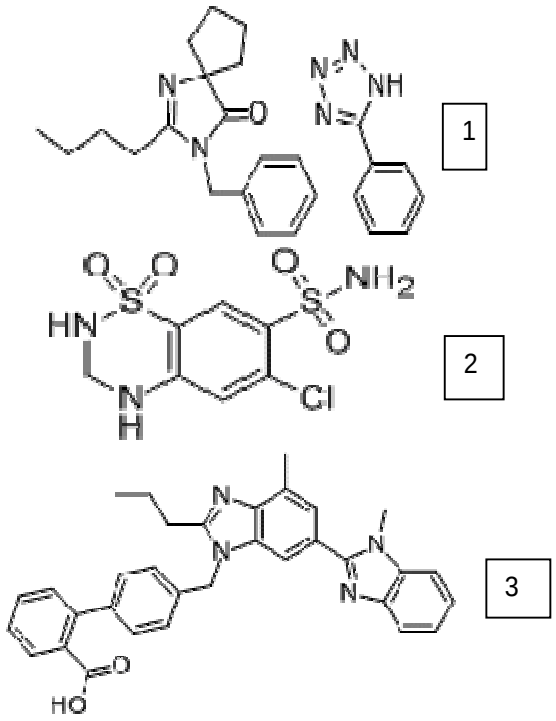

Fig. 1: Structure of Irbesartan [1], Hydrochlorothiazide [2] and Telmisartan [3]

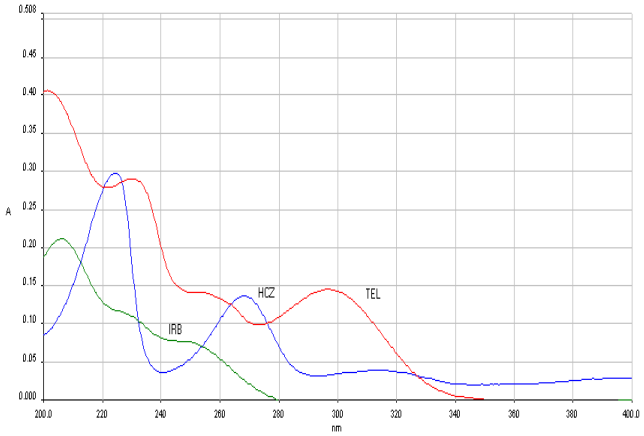

Fig. 2: Overlain absorption spectra of IRB, HCZ and TEL

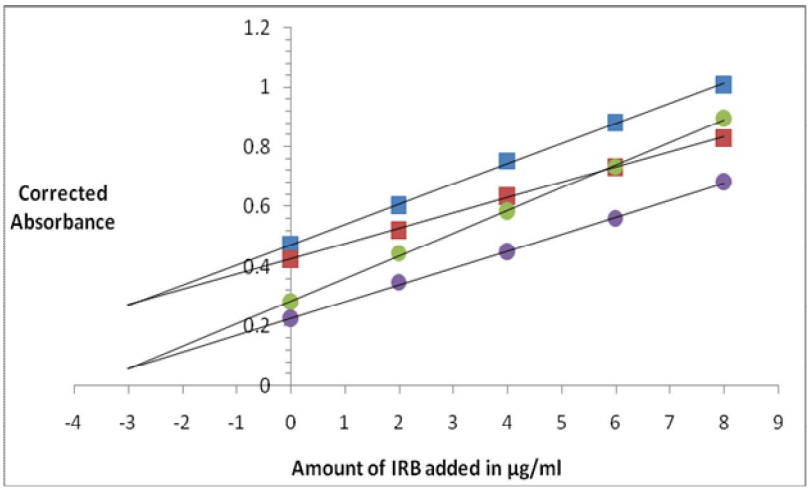

Fig. 3a: H-Point standard addition plot for the simultaneous determination of Irbesartan and Hydrochlorothiazide with constant concentration of Irbesartan (3 µg/ml) and different concentrations of Hydrochlorothiazide (1) 2 and (2) 0.5 µg/ml

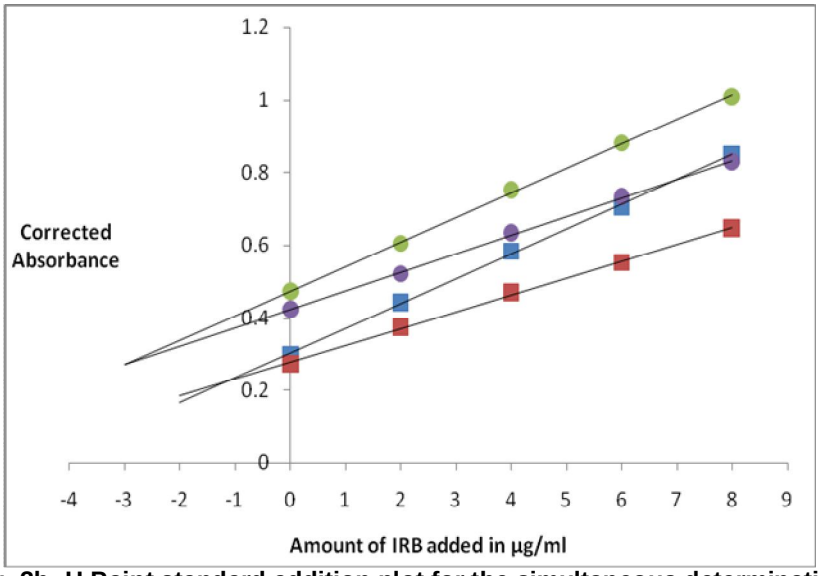

Fig. 3b: H-Point standard addition plot for the simultaneous determination of Irbesartan and Hydrochlorothiazide with constant concentration of Hydrochlorothiazide (4 µg/ml) and different concentrations of Irbesartan (1) 3 µg/ml and (2) 1 µg/ml

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