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Spectrophotometric Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Pharmaceutical Dosage Form using Mixed Hydrotropic Solubilisation Approach

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Abstract

Two validated UV spectrophotometric methods for the simultaneous estimation of Valsartan and Hydrochlorothiazide in pure powder and in two component tablet dosage form have been developed, utilising simultaneous equation and absorbance ratio method. The method is based on the measurement of absorbance of Valsartan and Hydrochlorothiazide at their respective wavelengths of 250.2 nm and 271.6 nm and at the is absorptive wavelength of 258.8 nm in a mixed hydrotropic blend of 25% w/v urea and 25% w/v sodium citrate. Linearity was observed in the concentration range of 4-28 µg/ml and 4-32 µg/ml for Valsartan and 2-14 and 4-32 µg/ml for Hydrochlorothiazide by method A and B respectively. The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

Key words: Valsartan, Hydrochlorothiazide, mixed hydrotropic blend, simultaneous equation method, Q absorbance method, validation

Introduction

The term hydrotropy has been used to designate the increase in solubility of poorly water soluble drugs in concentrated solutions of hydrotropic agents. A huge number of poorly water soluble drugs have been solubilized by use of various hydrotropic solutions [1-3]. Valsartan (VAL) is chemically: (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4tetrazol5yl) phenyl]phenyl}methyl) pentanamido] butanoic acid is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients [4]. Hydrochlorothiazide (HCT) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance [5]. It is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1, 1-dioxide. VAL and HCT are official in USP-NF 2007 [8] and IP 2007 [6], BP 2004 [7] respectively. Extensive literature survey revealed that few UV spectrophotometric methods [9,10], RP-HPLC methods [11] and HPTLC methods [12,13] has been reported. Since no spectrophotometric method is reported for simultaneous estimation of VAL and HCT in combination by these two methods using mixed hydrotropy approach precluding the use of organic solvents, the present work therefore describes a successful attempt to estimate both these drugs simultaneously by two simple UV spectrophotometric methods (simultaneous equation and Q absorbance method) using a hydrotropic blend containing 25% w/v urea and 25% w/v sodium citrate. The proposed methods were optimized and validated as per ICH guidelines [14].

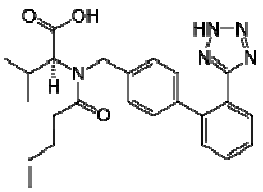

Fig.1 Valsartan

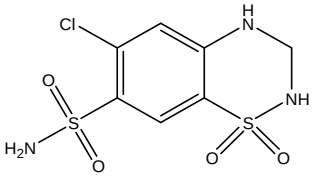

Fig. 2 Hydrochlorothiazide

Materials and Methods

Instrument

A UV/ VIS double beam spectrophotometer, (Shimadzu-1800) with matched quartz cells corresponding to 1 cm pathlength and spectral bandwidth of 2 nm connected to a computer loaded with Shimadzu UV Probe 2.42 software was used for all the spectrophotometric measurements in all proposed spectrophotometric methods.

Materials

Standard gift samples of Valsartan (VAL) and Hydrochlorothiazide (HCT) were procured from Lupin Pharmaceuticals, Pune. Combined Valsartan and Hydrochlorothiazide tablets were purchased from local market. All other chemicals used were of analytical grade.

Solvent used

A blend of urea and sodium citrate (25:25) in distilled water was used as a solvent in the study.

Preliminary solubility studies of VAL and HCT:

Solubility of VAL and HCT were determined at 28 ± 1 °C in a blend of urea and sodium citrate (25:25) solution, distilled water and buffer of pH 9 (pH of hydrotropic blend). Sufficient excess amount of each drug was added individually to screw capped glass vials of 30 ml capacity, containing distilled water, buffer and blend solution. The vials were shaken mechanically for 12 hours at 28 ± 1 °C in mechanical shaker (Lab Hosp). The solutions were allowed to equilibrate for next 24 hours and then centrifuge for 5 min at 2000 rpm (Remi Instruments Limited, Mumbai, India). The supernatant of each vial was filtered through whatman filter paper #41. Filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blanks.

Stock solutions

50 mg each of Valsartan and Hydrochlorothiazide were accurately weighed and transferred in 50 ml volumetric flasks separately, dissolved in 30 ml of urea and sodium citrate blend solution (25:25) and volume was adjusted to 50 ml with distilled water to obtain solution (1000 µg/ml) of each drug. Aliquot portions of the stock solutions were diluted individually with distilled water to get final concentration of 10 µg/ml for VAL and HCT respectively. These working standard solutions were scanned in the range of 400-200 nm against solvent blank. The absorption maxima of VAL was found at 250.2 nm while for HCT at 271.6 nm. The overlain spectra of VAL and HCT is shown in Fig. 3.

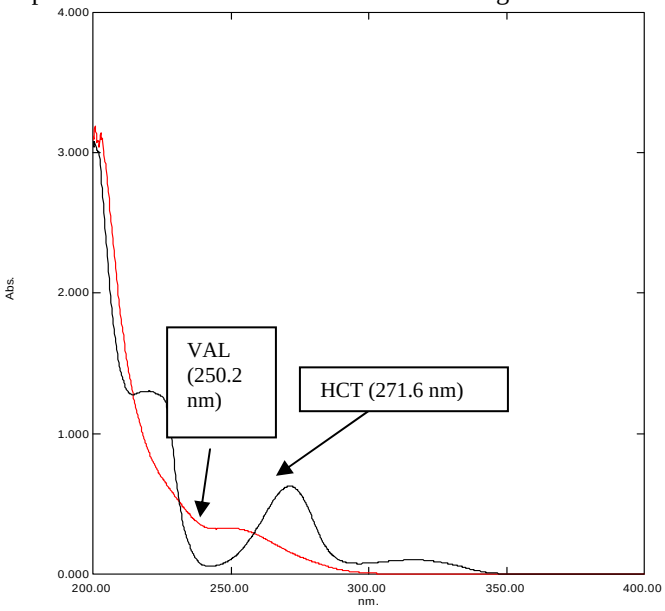

Fig. 3 Overlain zero order spectra of VAL and HCT

Determination of absorptivity value

The solutions of each drug in tripilicate were read against solvent blank at the selected wavelengths and A (1% 1 cm) value were calculated using below formula:

$$\text{Absorptivity, A (1\% 1 cm)} = \frac{\text{Absorbance at selected wavelength}}{\text{Concentration in g / 100 ml}}$$

Preparation of calibration curves

Stock solutions each of VAL and HCT having concentration of 100 µg/ml were prepared. Aliquots of each solution were appropriately diluted and the final dilutions were read at the selected wavelengths. The linearity of VAL and HCT was found to be in the concentration ranges of 4-28 and 4-32 µg/ml for VAL and 2-14 and 4-32 µg/ml for HCT by simultaneous equation (method A) and absorbance ratio (method B) respectively. The coefficients of correlation were found to be 0.9995 for VAL and 0.9996 for HCT by method A and 0.9998 and 0.9994 for VAL and HCT respectively by method B. The methods were first applied to standard laboratory mixture which yielded encouraging results and then were applied to marketed formulation.

Application of proposed method for physical laboratory mixture:

Mixture of VAL and HCT was prepared by dissolving 10 mg, diluted with 60 ml of urea and sodium citrate blend (25:25), sonicating it for 15 min and then make up the volume up to 100 ml to afford the concentration of 100 µg/ml. From the stock solution of VAL, 1.6 ml of VAL solution was diluted to get concentration of 16 µg/ml of VAL; and from the stock solution of HCT, 0.25 ml of HCT solution was diluted to get final concentration of 2.5 µg/ml of HCT. The solution was scanned in the range of 200 – 400 nm, absorbance of the sample solutions were recorded against blank. The concentrations (C_{VAL} and C_{HCT}) in sample solution were determined by using formulae given below, results are given in Table 1.

Table 1: Results of analysis of laboratory mixture

Method	Amount present (µg/ml)		Concentration found (µg/ml)		Percentage found (%)	
	VAL	HCT	VAL	HCT	VAL	HCT
A.	16	2.5	15.98	2.457	99.87	98.28
B.	16	2.5	15.891	2.469	99.32	98.4

Application of proposed method for analysis of tablets:

Twenty tablets were weighed and average weight was calculated. The tablets were triturated thoroughly and mixed. Tablet powder equivalent to 16 mg of VAL (~2.5 mg of HCT, on the basis of label claim) was transferred to 100 ml volumetric flask. 60 ml of urea:sodium citrate solution was added to the flask and stirred for 15 min to dissolve the drug. The content was filtered through Whatman filter paper (no.41) and volume was made upto 100 ml with distilled water. Filtrate was divided in 2 parts, A and B part. A was kept at room temperature for 48 hours to check the effect on stability of drugs in presence of urea and sodium citrate and also to note precipitation, if any during this period. Part B filtrate was appropriately diluted with distilled water to get a mixed standard containing 16 µg/ml VAL and 2.5 µg/ml HCT. The amount of each drug was estimated by proposed methods using the following formulae and the results of analysis are given in Table 2. After 48 hour, filtrate of part A was appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours.

Table 2: Results of analysis of tablet formulation

Method	Brand	Present amount (µg/ml)		Concentration found (µg/ml)		Percentage (%) found	
		VAL	HCT	VAL	HCT	VAL	HCT
A.	Valzaar H	16	2.5	15.872	2.514	99.2	100.56
B.	Valzaar H	16	2.5	15.894	2.50	99.34	100.0

Method A: Simultaneous equation method

This method of analysis is based on the absorption of drug X (Valsartan) and Y (Hydrochlorothiazide) at the wavelength maxima of the other. The quantification analysis was performed by using the following equations;

$$C_X = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \qquad C_Y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

Where Cx and Cy were the concentrations of VAL and HCT respectively in the diluted sample, ax_1 and ax_2 were absorptivities of VAL at λ_1 and λ_2, ay_1 and ay_2 were absorptivities of HCT at λ_1 and λ_2 respectively and A_1 and A_2 absorbances of mixed standard at 250.2 nm (λ_1) and 271.6 nm (λ_2) respectively.

Method B: Absorbance ratio

In absorption ratio method, absorbance of both the drugs were calculated at two selected wavelengths among which λ_1 is the wavelength of isobestic point (where both drugs show same absorbance) and λ_2 is the λ_{max} of either drug among the drugs to be analyzed. From the overlain spectra (Fig. 4.) wavelength

258.8 nm (λ_1- isobestic point) and 271.6 nm (λ_2- λmax of HCT) was selected for analysis. The concentration of individual drug components was calculated by using the following equation,

$$Cx = \frac{Qm - Qy}{Qx - Qy} * \frac{A_1}{ax_1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} * \frac{A_1}{ax_1}$$

$$\text{Where } Qm = \frac{A_2}{A_1}$$

A_1 is absorbance of mixed standard at λ_1 (isobestic point), A_2 is absorbance of mixed standard at λ_2 (λ_{max} of HCT)

$$Qx = \frac{ax_2}{ax_1}, \qquad Qy = \frac{ay_2}{ay_1}$$

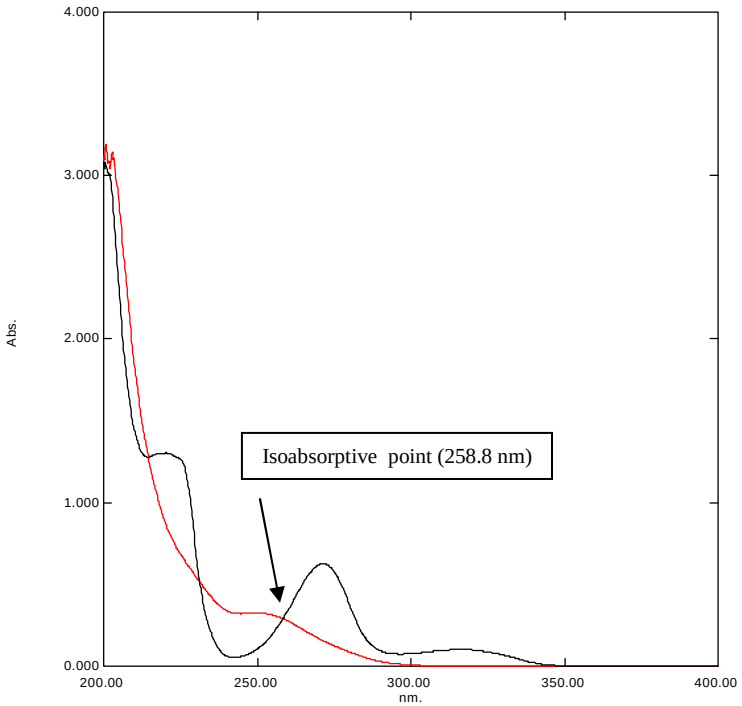

Fig. 4 Overlain Zero Order Spectra of VAL and HCT showing isoabsorptive point

Validation of proposed methods [14]

Linearity and Range:

To establish the linearity of the proposed method, three separate series of solutions of VAL and HCT were prepared from stock solution and analyzed. Least square regression analysis was done for the obtained data and shown in the table 3.

Accuracy:

It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of standard VAL and HCT was added to pre–analyzed sample (16 µg/ml of VAL and 2.5 µg/ml of HCT) and subjected them to the proposed methods. Results of Recovery studies were shown in Table 4.

Table 4: Data of Recovery studies

Method	Level of % Recovery	Initial concentration (µg/ml)		Concentration found (µg/ml)		% Recovery (Mean)*		% RSD	
		VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
A	80	16	2.5	28.79	4.48	99.99	99.75	0.058	0.23
	100	16	2.5	32.08	5.03	100.25	100.7	0.34	0.39
	120	16	2.5	35.205	5.5	100.01	99.99	0.32	0.17
B	80	16	2.5	28.75	4.48	99.82	99.76	0.25	0.74
	100	16	2.5	31.99	5.04	99.98	100.7	0.04	0.90
	120	16	2.5	35.25	5.44	100.15	98.9	0.16	0.62

* mean of 3 determinations

Precision: Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Variation of results within the same day (intraday), variation of results between consecutive days (interday) were analyzed and results are given in Table 3.

Ruggedness: Ruggedness was determined by two different analyst by preparing sample solution of VAL (16 µg/ml) and HCT (2.5 µg/ml) from stock solution using similar operational and environmental conditions and results are given in Table 5.

Table 5: Data of Ruggedness

Method	Drug	Concentration Found (%) ± RSD	
A		Analyst-I	Analyst-II
	VAL	99.92 ± 0.09	99.95 ± 0.09
	HCT	100.04 ± 0.007	99.38 ± 0.02
B	VAL	99.85 ± 0.07	99.75 ± 0.15
	HCT	99.36 ± 0.04	99.74 ± 0.23

Limit of Detection and Quantification (LOD and LOQ): The LOD and LOQ were estimated from the standard calibration curve. It is calculated using the formula LOD = 3.3 X σ/S and LOQ = 10 X σ/S where, σ is the standard deviation of the response and S is the slope of the calibration curve. Results are given in Table 3.

Table 3: results of validation parameters

Parameters	Method A		Method B	
	VAL	HCT	VAL	HCT
Linearity (µg/ml)	4-28	2-14	4-32	4-32
Correlation coefficient (r2)	0.9995	0.9996	0.9998	0.9994
Interday precision	0.40	0.43	0.58	0.37
Intraday precision	0.42	0.49	0.38	0.51
LOD (µg/ml)	0.22	0.14	0.122	0.045
LOQ (µg/ml)	0.69	0.44	0.37	0.102

Results and Discussion

The solubility of VAL and HCT in urea and sodium citrate blend solution was found to be more than 38 fold and 16 fold as compared to its solubility in distilled water respectively. The pH of blend solution was 9. To check the effect of pH on solubility of drugs, their solubility was also determined in buffer of pH 9. Solubilities of both the drugs in distilled water and buffer of pH 9 were almost same thus it is concluded that enhancement in solubility of VAL and HCT in blend solution was due to hydrotropic solubilization only. Fresh filtrate and 48 hours aged filtrate (kept at room temperature) of drugs were found to have same drug contents. Also there was no precipitation within 48 hours. This indicates that analysis can be accurately performed within 48 hour of extraction of the drug from tablet powder. In simultaneous equation method, VAL showed absorbance maxima at 250.2 nm and HCT at 271.6 nm. Linearity was observed in the concentration range of 4-28 µg/ml for VAL and 2-14 µg/ml for HCT. Correlation coefficient was found to be 0.9995 and 0.9996 for VAL and HCT respectively. The proposed Correlation method was applied for the determination of VAL and HCT in the marketed dosage and estimated as 99.2% and 100.56% respectively. In absorbance ratio method, from overlain spectra of VAL and HCT, two wavelengths were

selected at 258.8 nm (isoabsorptive point) and 271.6 nm (λmax of HCT). VAL and HCT follow linearity in the concentration range 3-21µg/ml and 3-21 µg/ml respectively. Correlation coefficient was found to be 0.9998 and 0.9994 for VAL and HCT respectively. The proposed method was applied for the determination of VAL and HCT in the marketed dosage and estimated as 99.34 % and 100.00 % respectively.

The recovery of drugs was determined at 80, 100 and 120 % levels for both methods. The percentage recovery was from 99.8 to 100.25% for VAL and 98.9 to 100.7 % for HCT. Precision, Ruggedness was performed as per ICH guidelines, results shows that % RSD < 2 % which is within the limit for all the methods. LOD and LOQ were found to be 0.22, 0.69 by simultaneous equation and 0.122, 0.37 by absorbance ratio for VAL and 0.14, 0.44 by simultaneous equation and 0.045, 0.102 by absorbance ratio for HCT.

Conclusion

A blend of urea and sodium citrate (25:25% w/v) was successfully used for simultaneous estimation of Valsartan and Hydrochlorothiazide. Two spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise, reproducible, ecofriendly, safe, cost-effective as they preclude the use of toxic organic solvents and can be employed successfully for the estimation of Valsartan and Hydrochlorothiazide in bulk and formulation.

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