Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND ION PAIR CHROMATOGRAPHIC TECHNIQUE FOR ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE

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ABSTRACT

 Two new simple, sensitive, rapid, accurate and reproducible methods (UV-spectrophotometric and ion pair chromatography) have been developed for simultaneous estimation of valsartan (VAL) and hydrochlrothiazide (HCTZ) from their tablet dosage form. The first method involves multiwavelength spectrophotometric method (Method 1) in which interference of HCTZ at 245nm (wavelength for estimation of VAL) was removed by recording absorbance difference at 245nm and 301 nm whereas HCTZ was estimated directly from its absorbance at 316 nm at which VAL shows no absorbance. Linearity of the response was demonstrated by VAL in the concentration range of 5-45 µg/ml with a square correlation coefficient (r^2) of 0.9998. Linearity of the response was demonstrated by HCTZ in the concent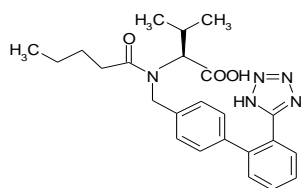ration range of 2-18 µg/ml with a square correlation coefficient (r^2) of 0.9994. The second method utilizes ion pair chromatography (Method 2) on a HIQ sil ODS column (250 mm$_*$ 4.6 mm i.d.) using methanol: 0.0025 M orthophosphoric acid: (70:30 by volume) having pH 4.6: 0.1% hexane sulphonic acid as mobile phase with UV detection at 259nm over concentration range for VAL is 240-0 µg/ml, and for HCTZ is 75-0µg/ml. Losartan potassium was used as the internal standard. The suggested procedures were checked using laboratory prepared mixtures and were applied successfully for the analysis of their tablet dosage form. The results of analysis were statistically analysed. Both the methods were validated as per ICH Q2B guidelines.

KEYWORDS: valsartan, hydrochlrothiazide, Losartan Potassium Ultraviolet spectrophotometry, Multiwavelength method, RP-HPLC, Validation

1. INTRODUCTION

Valsartan (VAL) is angiotensin II receptor blocker. Chemically VAL is N-[-P-(0-1 H- Tetrazole-5yl phenyl) Benzyl]-N-Valeryl -L-Valine [1].Several analytical methods such as liquid chromatography-tandam mass sprectrometry [2], micellar electrokinetic chromatographic method [3], capillary zone electophoresis [4], pKa [5], HPLC [6-7] determination methods are reported for estimation of valsartan from their respective single dosage forms. Ratio spectra derivative, inverse least square technique [8], and HPLC by using buffer, First derivative spectrophotometry [9], has been reported for simultaneous estimation of valsartan and hydrochlorothiazide from their combined dosage forms.
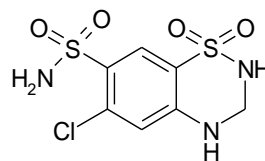
Hydrochlrothiazide (HCTZ) chemically known as 6-chloro -3,4 dihydro -2H,-1,2,4,Benzothiadiazine-7-

Sulfonamide,1,1-dioxide, is a diuretic and is useful in the treatment of mild to moderate essential hypertension [10].The drug is mentioned in Martindale; The Extra Pharmacopoeia[11]. A survey of literature revealed different analytical methods including Gas chromatography-mass spectrometric method [12], electrochemical [13], time resolved chemiluminence [14], micellar electrokinetic method[15], High Performance Liquid Chromatography[16-18], Capillary electrophoresis[4] etc. Few spectrophotometric methods have also been reported[19-20]. Fix dose combination containing VAL and HCTZ is available in tablet dosage form in market. In the present work an attempt has been made to develop and validate a simple, sensitive and reproducible spectrophotometric method and ion pair chromatographic method with greater precision, accuracy, and sensitivity for the quality control and routine analysis of VAL and HCTZ in tablet dosage form.

Valsartan

$C_{24}H_{29}N_5O_3$

Mol. Wt. 435.5

Hydrochlorothiazide

$C_7H_8Cl\ N_3O_4\ S_2$

Mol. Wt. 297.7

Chemical structure of valsartan and hydrochlorthiazide

2. EXPERIMENTAL

2.1 Apparatus

a) A PC based Jasco V-530 recording spectrophotometer with spectral bandwidth (resolution) of 2 nm and Wavelength accuracy ± 0.3 nm (with automatic wavelength correction) was employed for all measurements using a matched pair of 10mm quartz cell.b) The HPLC system was a PC based Jasco series comprising of a pump PU-2080 and a UV-2070 detector. Manual injections were carried out using a Rheodine injector with a fixed 20 µl

2.2. Materials

2.2.1. Pure samples

Valsartan and hydrochlorothiazide were kindly supplied by Lupine pharma Ltd., Pune and Macloide pharma

external loop. The chromatographic separations were performed on a 5 µm HIQ sil ODS column (250 mm x 4.6 mm i.d.), operating at ambient temperature, using a mobile phase consisting of methanol: 0.0025 M orthophosphoric acid (70:30 by volume) having pH 4.6: 0.1% hexane sulphonic acid. c) Shimadzu AY 120 analytical balance was used for weighing. d) PCi Ultrasonicator was used for sonication.

Ltd., Mumbai, India. respectively. Their purity was found to be 100.65 ± 0.71 and 99.23 ± 0.82 respectively.

2.2.2. Market samples

Marketed sample of VAL and HCTZ (Valent H) in their combined tablet dosage form. of Merck Limited Aurangabad, India. Batch No. A 6011WB was used for analysis. Each tablet contains 80mg of VAL and 12.5 mg of HCTZ.

2.2.3. Chemical and reagents

For spectrophotometric work methanol (Loba chemie Pvt. Ltd. Mumbai, India) of pure analytical grade. For HPLC work double distilled water, Methanol and Orthophosphoric acid, and hexane sulphonic acid sodium salt (Spectrochem Pvt. Ltd. Mumbai,India) were of HPLC grade.

2.3. Preparation of standard and sample solutions

2.3.1. Standard stock solutions

2.3.1.1. For spectrophotometric method

VAL and HCTZ standard stock solution (1000 µg. m l $^{-1}$ each of VAL and HCTZ) were prepared by weighing accurately 100 mg of VAL and HCTZ separately into two 100-ml volumetric flasks and dissolved in 40 ml of methanol. The mixture was sonicated for 10 minutes and volume was made up to 100 ml with the same solvent.

2.3.1.1. For HPLC method

Standard stock solutions containing VAL and HCTZ were prepared by transferring 100 mg of VAL into 50 ml volumetric flask and dissolved in 30ml of methanol and ultrasonicated for 10 minutes. The final volume of the

2.4. Laboratory prepared mixtures

2.4.1. For spectrophotometric method

Accurate aliquots of VAL and HCTZ were transferred from working solutions (100 µg ml^{-1}) into a series of seven 10-ml volumetric flasks and volume was made up to the mark with methanol. Ten mixed standard solutions containing concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 µg/ml of VAL and 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 µg/ml of HCTZ were obtained.

2.4.2. For HPLC method

In to a series of six 10 ml volumetric flasks, 2.4 to 0 ml of VAL (1000µg ml^{-1}) and 0 to 7.5 ml of HCTZ solution

solution was made up to 50 ml with methanol to get stock solutions containing 2000 µg ml^{-1} of VAL. 100 mg of HCTZ separately into 100 ml volumetric flasks and dissolved in 40ml methanol and ultrasonicated for 10 minutes. The final volume of the solution was made up to 100 ml with methanol to get stock solutions containing 1000 µg ml^{-1} of HCTZ.

Losartan potassium (LP) was selected as an internal standard. Standard stock solution containing LP was prepared by dissolving 10 mg of LP in 40 ml of methanol in a 100 ml volumetric flask. It was then ultrasonicated for 10 minutes and then final volume of solution was made up to 100 ml with methanol to get 100 µg ml^{-1} of LP.

2.3.2. Working solutions

One ml of the stock solutions of VAL and HCTZ standard stock solution were transferred in two separate 100ml volumetric flasks and diluted to the mark with methanol to get a final concentration of 100µg/ml each of VAL and HCTZ for the spectrophotometric work. For HPLC method, 50 ml of the Standard stock solutions containing VAL transferred into separate 100-ml volumetric flasks and 10 ml of HCTZ were transferred into separate 100-ml volumetric flasks and diluted to the mark with mobile phase to get a final concentration of 1000 µg/ml of VAL and 100 µg/ml HCTZ.

(100 µg ml^{-1}) was pipetted and to each flask 2 ml of (100 µg ml^{-1}) of LP as internal standard was added and then final volume of all the solutions was made up to 10 ml with mobile phase. Seven mixed standard solutions containing concentrations of 240, 200, 160, 120, 80,40, and 0 µg/ml of VAL and 0,12.5, 25, 37.5, 50, 62.5, and 75 µg/ml HCTZ and 20 µg/ml of LP were obtained.

2.5. Procedures

2.5.1. Spectrophotometric method

For estimation of VAL, multiwavelength spectrophotometric method employing 245 nm and 301nm as analytical wavelengths was used; the two wavelengths were

chosen to eliminate interference of HCTZ at the sampling wavelength of VAL. For estimation of HCTZ, 316 nm was selected as the analytical wavelength, as VAL shows no absorption at this wavelength.

2.5.1.1. Linearity

Absorption spectras of ten mixed standard solutions prepared as above containing concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45μg/ml of VAL and 18, 16, 14, 12, 10, 8, 6, 4, 2, and 0 μg/ml of HCTZ were recorded in the range of 200-400 nm with scanning speed of 1000 nm min^{-1}, 1.0 nm data pitch and 2 nm bandwidth against methanol as blank. Calibration curve for VAL was constructed against concentration by taking absorbance differences at 245 nm and 301 nm from the spectra of mixed standards whereas the calibration curve for HCTZ was plotted against concentration using absorbance at 316 nm and the regression equations were generated.

2.5.1.2. Assay of laboratory-prepared mixtures

Absorption spectras of different laboratory-prepared mixtures containing different concentrations of the two drugs were recorded. For the determination of

2.5.2. HPLC method

2.5.2.1. Linearity

Accurate aliquots equivalent to 240-0 μg/ml of VAL (1000 μg. ml^{-1}) and 0-75 μg/ml of HCTZ (100 μg. ml^{-1}) from their working solutions were transferred into one set of series of six 10 ml volumetric flasks. To each flask 2 ml of (100 μg. ml^{-1}) of LP as internal standard was added and completed to volume with the mobile phase. A 20 μl of sample solution was injected into the chromatographic system using fixed volume loop injector and chromatograms were recorded. The flow rate was maintained at 1 ml min^{-1} at ambient temperature and the elluents were monitored at 259 nm. The separation was done on a C18 column using methanol: 0.0025 M orthophosphoric acid (70:30 by volume) having pH 4.6: 0.1% hexane sulphonic acid. Calibration curves for both VAL and HCTZ were plotted and the corresponding regression equations were generated.

2.5.2.2. Assay of laboratory-prepared mixtures

concentration of the VAL absorbance difference was recorded at 245 nm and 301 nm whereas for determination of concentration of HCTZ absorbance was noted at 316 nm. The concentration of each drug was calculated from the corresponding regression equation.

2.5.1.3. Assay of marketed pharmaceutical preparation (Valent H tablet)

A commercially available tablet formulation containing VAL 80mg and HCTZ 12.5mg was analyzed using this method. Twenty tablets were accurately weighed and average weight was calculated.

These tablets were ground to a fine powder and an accurately weighed tablet powder equivalent to 10mg of VAL was transferred to a 100 ml volumetric flask and to this 2 mg of pure HCTZ was added. 60ml of methanol was added to the flask. The solution was sonicated for 10 min. at 25 ^{0}C. The solution was then filtered through Whatmann filter paper No.41 and the volume was made up to 100 ml with methanol. The solution was analysed using the method described under assay of laboratory-prepared mixtures (as in 2.5.1.2.).

Chromatograms of different laboratory-prepared mixtures containing different concentrations of the two drugs were recorded. The chromatographic conditions were applied for each laboratory-prepared mixtures and the concentrations of VAL and HCTZ were calculated by substituting in the regression equations.

2.5.2.3. Assay of marketed pharmaceutical preparation (Valent H tablet)

From the triturate of 20 tablets, an amount equivalent to 80 mg of VAL and 12.5 mg of HCTZ was weighed, transferred to a 50 ml volumetric flask and dissolved in 30 ml methanol. It was then ultrasonicated for 10 minutes. The solution was filtered through 0.22 μ membrane filter and then final volume of the solution was made up to 50 with methanol. Appropriate aliquots within the Beer's law limit were withdrawn into 10 ml volumetric flasks and to each of them 2ml of (100 μg. ml^{-1}) of LP as internal standard was added. The volume was made up to the mark with

methanol and analyzed by the proposed method using the procedure described under linearity (2.5.2.1.). The concentration of VAL and HCTZ present in the sample solution was calculated by using regression equation generated from calibration curve of respective drugs.

3. Results and Discussion

The development of an analytical method for simultaneous estimation of drugs without previous chemical separation in multicomponent pharmaceutical formulations has received considerable attention in recent years because of their importance in quality control of drugs and drug products.

This work is devoted for the analysis of VAL and HCTZ which are available together in the from of tablets.

Spectrophotometric method

Multiwavelength absorption spectrophotometric method was developed for analysis of VAL and HCTZ. The method utilizes ten mixed standard solutions involving scanning at 245 nm, 301 nm and 316 nm as sampling wavelengths. From the overlain spectra it was noted that there is no wavelength over the scanning range of 200 to 400 nm where VAL can be accurately quantified without substantial interference of HCTZ. Thus quantification of VAL was achieved by taking the absorbance difference at 245 nm and 301nm. HCTZ was directly estimated at 316 nm without interference of VAL. Overlain spectra of individual drugs and mixed standards are shown in Fig. 1 and 2.

Regression analysis for multiwavelength spectrophotometric method was carried out (Table I) and the linearity of the calibration graph and adherence of the method to Beer's law were validated by high value of the correlation coefficient (r), 0.9998and 0.9994 for VAL and HCTZ respectively. The results of Quantitative determination of VAL and HCTZ in tablets were found in good agreement with the labeled amount of both the drugs(Table II). In addition, coefficient of variation (CV) for the determination of VAL and HCTZ was 0. and 0.

Closeness of the amount found to the labelled amount and the low coefficient of variation value showed

Therefore the aim of this work was to develop simple analytical methods for the simultaneous determination of VAL and HCTZ. This was achieved by development of one multiwavelength spectrophotometric and one ion pair chromatographic method. The methods were validated according to International Conference on Harmonization Q2B guidelines [39] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Nine point calibration curves were generated with appropriate volumes of working standard solutions for UV and six point calibration curves were generated with appropriate volumes of working standard solutions for HPLC methods.

that the proposed method was accurate and precise. Recovery study conducted by the proposed spectrophotometric method was performed by spiking the powdered tablets with appropriate amounts of stock solution. The results of the recovery analysis are also represented in Table III. High recoveries, 98.77 ± 0.42 and 101.07 ± 1.02 and low standard deviation confirmed the suitability of the proposed method for the determination of VAL and HCTZ respectively in tablet formulation.

Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %R.S.D. The intermediate precision was studied by comparing the assays on 2 Different days and the results documented as standard deviation and %R.S.D. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from six determinations over four concentration levels covering the specified range was determined. Results of inter-day and intra-day precision are given in table IV. The repeatability of the method was determined by assaying six sample solutions. (Given as Table V).
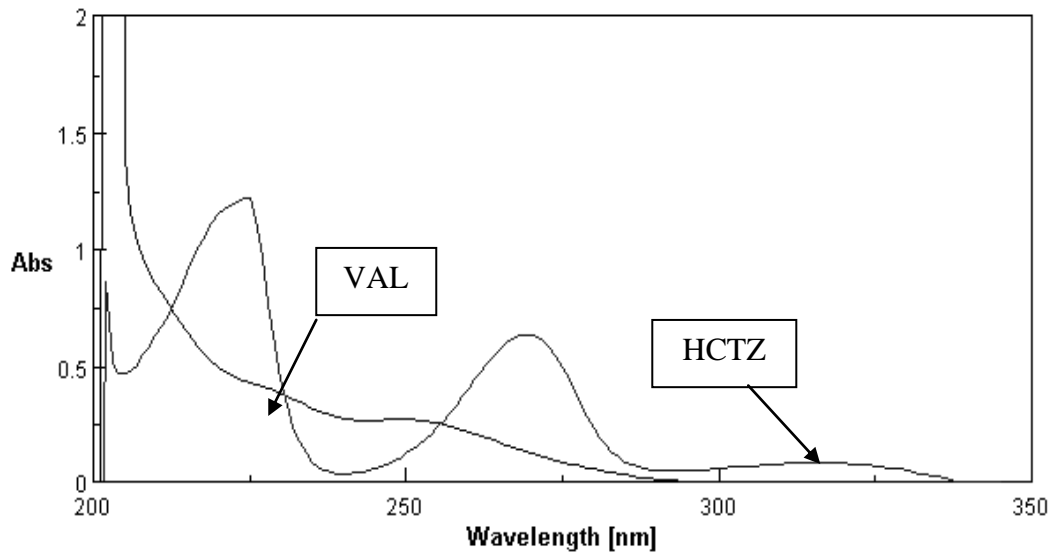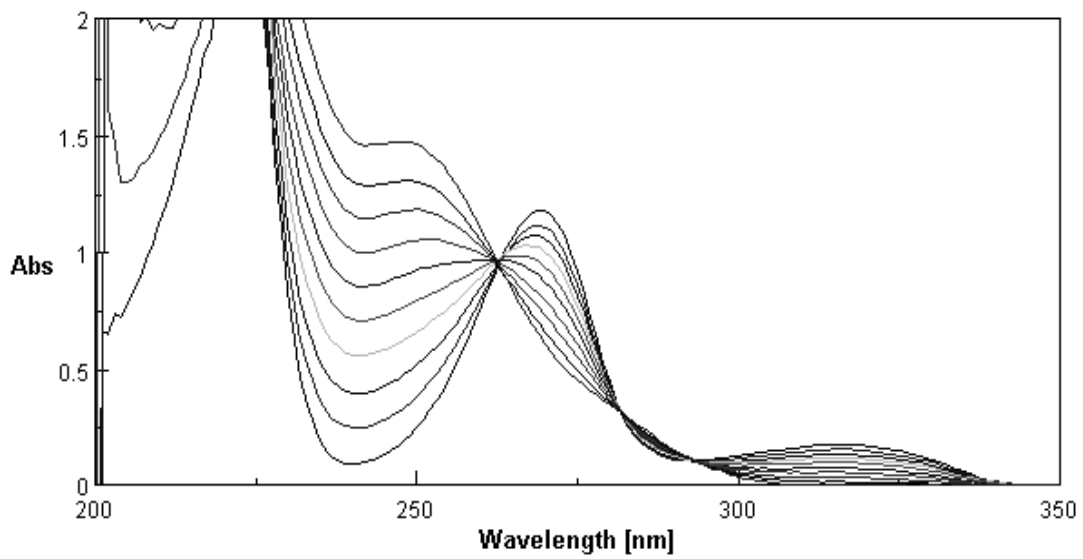
Fig.1 Overlain spectra of VAL and HCTZ.

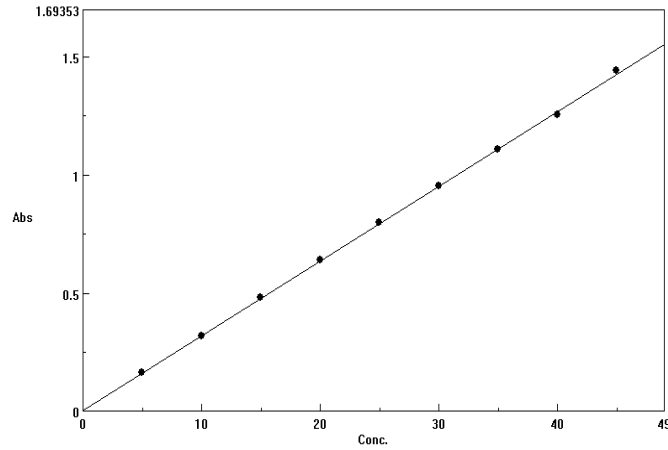**Fig.2 Overlain spectra of mixed standards of VAL and HCTZ.**
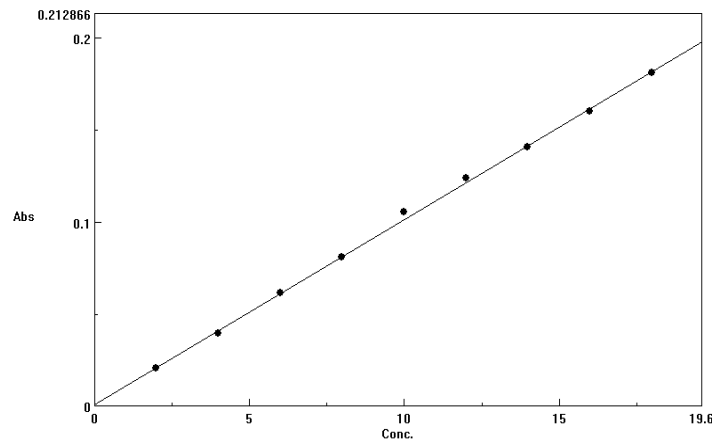
Fig.3 Calibration curve for VAL.
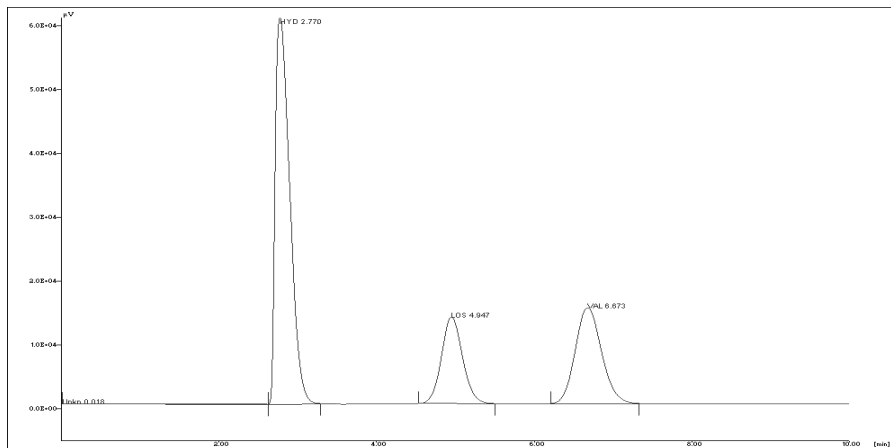

Fig.4 Calibration curve for HCTZ.

Fig. 5 Chromatogram of Physical mixture.

The method specificity was assessed by comparing the UV spectra obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in methanol without drug). The excipients chosen are the ones used commonly in tablet formulation, which included lactose, starch, microcrystalline cellulose, PVP, and magnesium stearate. The drug to excipient ratio used was similar to that in the commercial formulations.The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantitation (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as (Given as Table VI)

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

and

$$LOQ = 10\sigma / S$$

Where σ is the standard deviation of the lowest standard concentration and S is the slope of the calibration curve.An appropriate number of blank samples (6 determinations) were scanned in the selected range and the standard deviations of these responses were calculated. Low LOD as 0.36, 0.12 and LOQ as 1.19, 0.41 for VAL and HCTZ respectively shows the high sensitivity of the method.

Table I: Optical characteristics (Method 1)

Parameters	VAL	HCTZ
Beer's law limit(μg/ml)	5-45	2-18
Molar extinction coefficient (Moles/lit)		
Correlation coefficient	0.9998	0.9994
Regression equation (Y*)		
Slope (B)	0.0315	0.0100
Intercept (A)	0.0059	0.0012

*Y= A + B*C, where C is the concentration in μg/ml and Y is absorbance unit.

TABLE II: Results of Analysis of tablet formulation (Method 1)

Analyte	% Concentration estimated* (Mean ± S.D.)	Coefficient of variance
VAL	100.81 ± 1.23	1.220
HCTZ	99.63 ± 1.47	1.476

* Average of six determinations; SD = Standard Deviation.

Table III: Recovery studies (Method 1)

Analyte	% Concentration estimated* (Mean ± S.D.)	Coefficient of variance
VAL	98.77 ± 0.42	0.425
HCTZ	101.07 ± 1.02	1.009

*Average of six determinations; SD = Standard Deviation.

Table IV: Intraday and Interday precision (Method 1)

Day	% label claim Estimated* (Mean ± %RSD)		Coefficient of variance		% Recovery Estimated* (Mean ± %RSD)		Coefficient of variance	
	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ
Day 1	101.24 ± 0.71	100.98± 1.25	0.70	1.23	100.33 ± 0.85	101.36 ± 1.37	0.84	1.35
Day2	99.98± 0.97	100.49 ± 1.46	0.97	1.45	99.27± 0.73	101.71 ± 1.03	0.73	1.01
Day2	99.77± 0.77	100.68 ± 0.63	0.77	0.62	100.16 ± 0.48	102.31± 0.93	0.47	0.90

*Average of five determinations; %RSD = % Relative Standard Deviation.

Table V: Repeatibility (Method 1)

Label claim (mg/tab)	% label claim Estimated * (Mean ±SD)		%Recovery Estimated* (Mean ±SD)	
	VAL	HCTZ	VAL	HCTZ
80,12.5	100.15 ± 1.78	100.15± 0.53	100.83 ± 0.47	100.78 ± 0.49

* Average of five determinations; SD = Standard Deviation

Table VI: Limit of Detection and Limit of Quantitation (Method 1)

LOD (µg/ml) *		LOQ (µg/ml) *	
VAL	HCTZ	VAL	HCTZ
0.36	0.12	1.19	0.42

* Data obtained by six determinations.

TableVII: Optical characteristics (Method 2)

Parameters	Values for VAL	Values for HCTZ
Beer's law limit(µg/ml)	0 – 240	0 – 75
Correlation coefficient	0.9984	0.9987
Regression equation (Y*)		
Slope (B)	0.0540	0.0660
Intercept (A)	0.0110	0.1039

*$Y = A + B*C$, where C is the concentration in µg/ml and Y is absorbance unit.

Table VIII: Results of analysis (Method 2)

Label claim VAL & HCTZ (mg/tab)	Label claim Estimated (%)* (Mean ± %R.S.D.)		Recovery Estimated (%)* (Mean ± %R.S.D.)	
	VAL	HCTZ	VAL	HCTZ
80, 12.5	99.27 ± 0.77	99.42 ± 1.02	99.47 ± 1.06	98.85 ± 1.07

*Average of five determinations; R.S.D., Relative Standard Deviation.

Table IX: System Suitability Parameters (Method 2)

Sr. No.	Parameters	VAL	HCTZ
1.	Theoretical Plates	2017.19	998.37
2.	Assymetry	1.09	1.67
3.	Resolution	3.22	
4.	Retention Time in minutes	6.6	2.7
5.	Selectivity	1.35	
6.	Calibration Curve (µg/ml)	40-240	12.5-75
7.	Limit of Detection (µg/ml)	3.87	0.15
8.	Limit of Quantitation (µg/ml)	12.77	0.53

Ion pair chromatographic method

A Ion pair chromatographic method was developed for estimation of VAL and HCTZ, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. In order to affect the simultaneous elution of the two components under isocratic conditions, different chromatographic conditions (organic modifier, flow rate, ionic strength, pH) were investigated. RP-HPLC system consisting of HIQ Sil-C_{18} -10 column (250 mm x 4.6 mm i.d.) provided good resolution for separation of VAL and HCTZ. The mobile phase for the two drugs was selected based on its polarity. Mobile phases containing methanol alone or acetonitrile alone were found to elute the two compounds unresolved. Various ratios of Methanol: acetonirtrile: water were found to produce the chromatograms with very close retention times, poor resolution. Mobile phase containing methanol: water (70:30) gives tailng effect. Best resolution was achieved at the mobile phase composition of methanol:0.0025 M orthophosphoric acid (70:30 by volume) having pH 4.6: 0.1% hexane sulphonic acid where the peaks of valsartan , hydrochlorothiazide, and losartan potassium were clearly resolved. Flow rate of 0.5 ml/min. resulted in greater retention times and 1.2 ml/min. resulted in very close retention times with poor resolution. A flow rate of 1 ml/min. resulted in elution of all drugs within 10 minutes.

` The sampling wavelength was selected after scanning the drug solutions in the mobile phase having concentration of 25 μg ml^{-1} in the UV range of 200 – 400 nm on a UV spectrophotometer. 259 nm was selected as suitable wavelength for estimation. Losartan potassium was found to be a suitable internal standard for this study under the selected chromatographic conditions. Chromatogram of physical mixture is shown in Fig.5. while Optical characteristics are shown in Table VII. The method was specific as none of the excipients interfered with the analytes of interest. Hence, the method was suitably employed for assaying the commercial formulation containing VAL and HCTZ. A six-point calibration curve was constructed with working standards and was found linear ($r2 \geq 0.9984$, 0.9987) for VAL, HCTZ over their calibration ranges.

The proposed method was applied to the determination of VAL and HCTZ in their pharmaceutical preparation. The results indicate satisfactory accuracy and precision of the method. The statistical data obtained after replicate determinations (n = 5) is shown in Table VIII. The % recovery ± S. D. (n = 5) of the added VAL and HCTZ was 99.47 ± 1.06 and 98.85 ± 1.07, respectively (Table VII.). System suitability parameters are shown in Table IX.

4 ACKNOWLEDGEMENT

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