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Original Article

Development and Validation of Chemometric Assisted FTIR Spectroscopic Method for Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Pure and Pharmaceutical Dosage Forms

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ABSTRACT

Objectives: To develop a new Chemometric assisted Fourier transform infrared (FTIR) spectroscopic method for the simultaneous estimation of valsartan and hydrochlorothiazide in pharmaceutical dosage forms. **Methods:** The method involves the preparation of solid pellets of valsartan and hydrochlorothiazide using KBr and direct measurement using reduced path length cell. The wave numbers for quantitative estimation were selected with aid of chemometrics software and the spectra were measured in absorbance mode. **Results:** The infrared spectra showed different peaks with baseline correction, among which intense, clear and proportionate peaks were selected at 1600 cm^{-1} and 3361 cm^{-1} corresponding to amide (C=O) and amine (N-H) functional groups for valsartan and hydrochlorothiazide respectively for quantitative estimation were assessed using Chemometrics. Beer Lambert's law was obeyed over the concentration range of 5-25µg/mg for valsartan and 10-50 µg/mg for hydrochlorothiazide. The method was validated according to ICH guidelines. **Conclusion:** The developed method was cost effective and fulfilled most validation requirements in a range of concentrations suitable for quality control of both in pure and solid dosage form. This method can be used as an alternative method for HPLC, UV or pharmacopoeial methods as quality control check in pure and marketed formulations.

Key words: Chemometrics, FTIR, Hydrochlorothiazide, ICH guidelines, Method validation Valsartan.

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INTRODUCTION

Valsartan belongs to a class of antihypertensive agents called angiotensin II receptor blockers (ARBs).[1-3] Valsartan is a specific and selective type-1 angiotensin II receptor (AT1) antagonist which blocks the blood pressure increasing effects of angiotensin II via the renin-angiotensin-aldosterone system (RAAS). It is used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. The IUPAC name of valsartan is N-pentanonyl-N-[2´-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl]-L-valine. Its molecular formula and molecular weight are $C_{24}H_{29}N_5O_3$ and 435.5 g/mole respectively.[4-6]

Hydrochlorothiazide (HCTZ) acts as a diuretic and chemically it is 6-chloro-3, 4-dihydro-2H-1, 2, 4 benzothiadiazine- 7- sulphonamide 1, I-dioxide. A fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve compliance, improve hypertension control, decrease dose dependent side effects and reduce cost as the first line treatment of hypertension.[7]

Chemometrics is computer software which converts the raw data into useful information. It is a chemical discipline that uses mathematical and statistical methods to design or to select optimal measurement procedures and to provide maximum chemical information by analyzing chemical data. It is a multivariate data analysis which is highly visual approach that helps to identify and understand patterns in large and complex data sets. Easily accessible raw data plots are very useful tool in the analysis. Visual inspection using a line plot of several scans provides insights into the profile of the data and is a good first check that the data are consistent. It helps in isolating the poor scans or regions of high noise.

Literature review reveals that there are several UV-spectrophotometric, HPLC, HPTLC, QbD, LC-MS-MS, RP-HPLC, Paper chromatography, UPLC, Hydrotropic solublization and Fourier transform convolution emission methods has been reported so far for the simultaneous estimation of valsartan and hydrochlorothiazide drugs alone and in combination with other drugs. There are no chemometric assited FTIR spectroscopic methods[8-17] for simultaneous determination of valsartan and hydrochlorothiazide in pure and marketed formulations as per literature.[18-29]

MATERIALS AND METHODS

***Reagents and Chemicals*:** Valsartan used is a gift sample from Alembic Pharmaceuticals Ltd., Hyderabad and Hydrochlorothiazide from Abbott laboratories pharmaceutical products Ltd, Hyderabad. VALZAAR H® tablets (Torrent Pharmaceuticals Ltd.) were purchased from local market. All other chemicals used were of analytical grade.

Instrumentation: FTIR instrument- Shimadzu 8400S composed of DLATGS detector, IR solution software and HPLC instrument- Shimadzu LC 20AT, with a phenomenex C_{18} (150 mm × 4.6 mm) × 5 µm column, with the LC solution software were used in the analytical work.

Method Development:[30-32] FTIR spectroscopic method was developed by using solid sampling technique. The following parameters were optimized during method development.

Selection of measurement mode: IR spectra of valsartan and hydrochlorothiazide were taken in absorbance mode for quantitative analysis. Absorbance of peaks at different wave numbers corresponding to different functional groups was measured.

***Selection of apodization*:** The "apodization" function used to calculate the power spectrum through fourier transform of the interferogram. The apodization function gives effects on the resolution and the S/N ratio of spectra. As the resolution is higher, noise on the balance is larger. Apodization function "Happ-Genzel" was selected.

Selection of beam: Beam parameter is used to switch the beam operation. The parameter "internal" was selected for the measurement of sample in main unit.

***Selection of detector*:** The standard DLATGS detector is selected. The "standard" parameter was selected for DLATGS detector.

Selection of mirror speed-Mirror speed "2.8 (mm/sec)" was selected for the standard DLATGS detector.

Selection of sampling technique:[33] Liquid sample technique is not suitable as functional group peaks are not clear in IR spectra of valsartan and hydrochlorothiazide in chloroform and dimethyl sulphoxide (DMSO). Pressed pellet technique was selected for quantitative estimation, the concentrations of sample by pressed pellet technique were prepared with the aid of geometric mixing and were analyzed by using sample cells in FTIR.

IR Spectrum analysis for functional group assessment:[34] Functional group assessment was carried out using line spectra, matrix spectra in unscrambler version 10.5.1 X. IR spectra of valsartan and hydrochlorthiazide taken in absorbance mode were analyzed and functional peaks whose absorbance showed a proportionate increase in absorbance with an increase in conc. were selected for the quantitation of two drugs.

Preparation of standard solutions of Valsartan and Hydrochlorthiazide: To the accurately weighed 10 mg of valsartan and hydrochlorthiazide, 100 mg of dried KBr was mixed with the aid of geometric mixing individually. This forms the stock of 100µg/mg. From the stock (100 µg/mg) 12.5, 25, 50, 75, 100mg of Valsartan and was weighed accurately and diluted to 50mg with dried KBr to make the final concentration of 25, 50, 75, 100, 125µg/mg of valsartan respectively. From the stock (100µg/mg of HCTZ) 5, 10, 15, 20, 25mg of Hydrochlorothiazide weighed accurately and diluted to 50mg with dried KBr to make the final concentration of 10, 20, 30, 40, 50µg/mg of HCTZ respectively.

Method Validation[35]

The developed method was validated according to the ICH guidelines Q2 (R1): Validation of Analytical Procedures: Text and Methodology for the following parameters:

Linearity and Range: Each of the working standard solutions 25, 50, 75, 100, 125µg/mg of valsartan and 10, 20, 30, 40, 50µg/mg hydrochlorothiazide were prepared and analyzed in FTIR instrument. Absorbance of the peaks at 1600 cm^{-1} and 3361 cm^{-1} were recorded for these standard solutions. Standard calibration curves were plotted between concentration and absorbance. Linearity was established by regression analysis; regression equation and coefficient of determination are reported.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The sensitivity of proposed method for measurement of valsartan and hydrochlorothiazide was estimated in terms of LOD and LOQ. The limit of detection (LOD) and the limit of quantitation (LOQ) were determined using standard deviation method. Standard deviation and slope were calculated from the calibration curve established for linearity parameter.

***Sandell's sensitivity*-** The sandell's sensitivity was calculated using the following formula.

Sandell's sensitivity (π) = Concentration (µg/100mg) × 0.001/ absorbance value

***Precision*-** Precision of the method was established by reporting repeatability, interday precision.

***Repeatability*-** Repeatability was determined by analyzing 6 replicates of 50µg/mg of valsartan and 50µg/mg of hydrochlorothiazide concentration respectively. The % relative standard deviation (RSD) was calculated.

***Interday precision*-** Interday precision of the developed method was determined by analyzing three replicates of different concentration samples (50µg/mg, 75µg/mg, 100µg/mg for valsartan and 10µg/mg, 20µg/mg, 30µg/mg for hydrochlorothiazide) for two consecutive days. The % relative standard deviation (RSD) was calculated on day 1 and day 2.

***Accuracy*-** Accuracy may be reported as the percentage recovery of a known added amount of analyte to a sample or as the difference between the mean value obtained and the accepted true value of a sample, together with an associated confidence interval.

***For drug product*-** Accuracy study was carried out by calculating % recovery of valsartan and hydrochlorothiazide by standard addition method. Known amounts of standard mixture of valsartan and hydrochlorothiazide (32, 40 and 48µg/mg), were added respectively to a pre-quantified test mixture of VAL and HCTZ respectively (40µg/mg). The % recovery was calculated by measuring absorbance and fitting these values into the regression equation of the calibration curve. The % relative standard deviation (RSD) was calculated at each level.

***Assay of valsartan and hydrochlorothiazide tablets*-** Twenty tablets (Valzaar H® containing 160mg of valsartan and 12.5mg of hydrochlorothiazide) were triturated after taking their average weight. The tablet powder equivalent to 1 tablet was accurately weighed and made up to 100 mg with dried KBr and triturated well to get a concentration of 100µg/mg. Further dilutions were made from this stock mixture to prepare the pellet of desired concentration. The amount of two drugs present in the tablet is calculated using below formula.

$$\text{Assay} = \frac{\text{Concentration (µg/mg)} \times \text{dilution factor} \times \text{average weight of the tablet}}{\text{Weight of tablet powder taken (mg)} \times \text{label claim of drug}} \times 100$$

RESULTS AND DISCUSSION

***Development and optimization of FTIR method*:** IR spectra of standard valsartan and Hydrochlorthiazide were taken by pressed pellet technique using KBr and liquid sampling technique using chloroform and DMSO are shown in Figure 1 and 2.

The FTIR spectra of valsartan and hydrochlorothiazide standard prepared in potassium bromide disks exhibits numerous well defined bands, thus including that direct sample measurements, such as solid attenuated total reflectance (ATR) or diffuse reflectance (DRIFT), could be an alternative for fast sample analysis, but using also multivariate calibration techniques.

Various trails were performed for optimizing the FTIR conditions. The optimized conditions were found as below in Table 1. Valsartan IR spectrum showed peaks at 3380cm^{-1}, 2962 cm^{-1}, 1730 cm^{-1}, 1600 cm^{1}, 1473 cm^{-1} and 1105 cm^{-1}. Among which 1600 cm^{-1} group showed clear, intense peak which increased linearly as the concentration increases, was

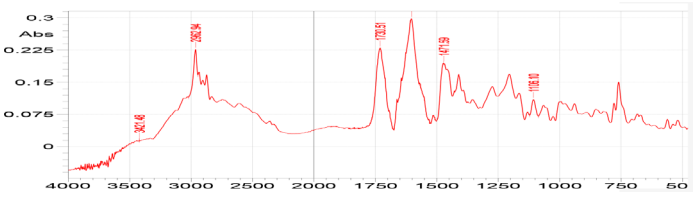

Figure 1: IR spectrum of Valsartan in absorbance mode.

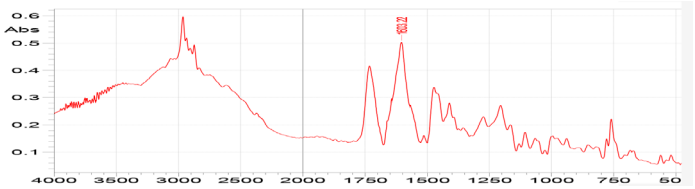

Figure 2: IR spectrum of Hydrochlorthiazide in absorbance mode.

Table 1: Optimized FTIR conditions.

Measurement mode	Absorbance mode
Apodization	Happ-Genzel
Method of Making Pellets	Direct mixing method
Final Weight of Pellets	50 mg
Frequency Range	400-4000 cm^{-1}
Peak selection	1600 cm^{-1} for Valsartan 3361 cm^{-1} for HCTZ
Number of Scans	45
Resolution	8.0 cm^{-1}

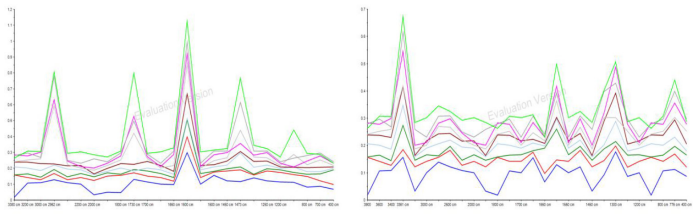

Figure 3: Line spectra of Valsartan and Hydrochlorthiazide.

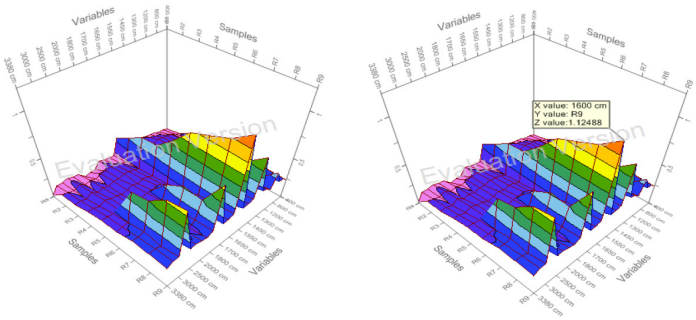

Figure 4: Matrix spectra of Valsartan.

selected for quantitative analysis of valsartan. Hydrochlorothiazide IR spectrum showed peaks at 3361cm^{-1}, 1600cm^{-1}, 1374cm^{-1} and 776cm^{-1}. Among which N-H (stretching) group showed clear, intense peak which increased linearly as the concentration increases was selected for

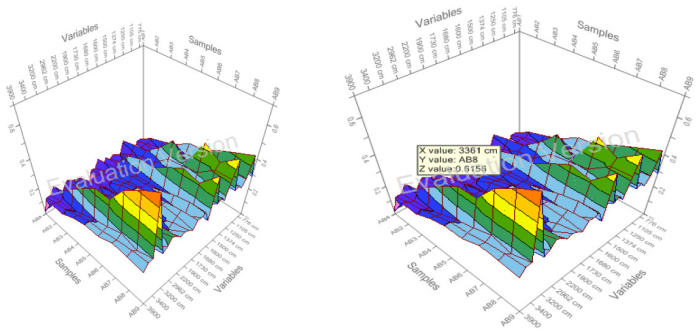

Figure 5: Matrix spectra of Hydrochlorthiazide.

Table 2: Standard calibration curve data for valsartan and hydrochlorothiazide.

S.No	Concentration (µg/mg)		Absorbance* at 1600 cm^{-1}	
	VAL	HCTZ	VAL	HCTZ
1	25	10	0.2978	0.1561
2	50	20	0.5029	0.2726
3	75	30	0.6919	0.4162
4	100	40	0.9171	0.5456
5	125	50	1.2483	0.6727

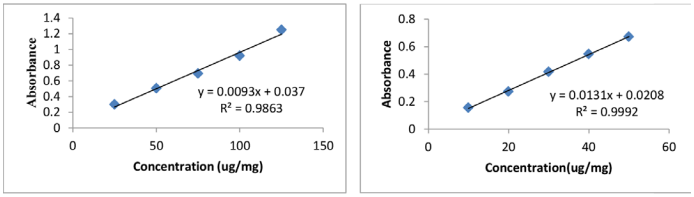

Figure 6: Calibration curve of Valsartan and Hydrochlorthiazide.

Table 3: Repeatability data of valsartan and hydrochlorothiazide.

Conc. (µg/mg)	Absorbance		Mean ± standard deviation (*n*=6)		% RSD	
	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ
50	0.672	0.619	0.676±0.000169	0.628±0.00764	1.92	1.12
50	0.658	0.630				
50	0.669	0.636				
50	0.686	0.618				
50	0.682	0.631				
50	0.694	0.635				

quantitative analysis of hydrochlorothiazide. Functional group peaks were selected for quantitative estimation using chemometrics software. The line and matrix spectra are shown in Figure 3-5.

The linearity of the method was established by performing linear regression analysis for the calibration curve constructed between concentration and absorbance. Calibration curve and calibration curve data of valsartan and hydrochlorthiazide is given in Table 2 and Figure 6. LOD and LOQ were found to be 3.71ug/mg and 11.25ug/mg for valsartan and 1.52ug/mg and 4.61ug/mg for hydrochlorothiazide respectively. This indicates the sensitivity of the method.

Table 4: Interday precision data of valsartan.

Conc. (µg/mg)	Absorbance		Mean ± standard deviation (*n*=3)		% RSD	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
50	0.682	0.674	0.678±0.01058	0.681±0.00754	1.54	1.10
	0.686	0.682				
	0.666	0.689				
75	0.701	0.702	0.702±0.00173	0.705±0.00308	0.24	0.43
	0.703	0.705				
	0.704	0.708				
100	0.915	0.921	0.916±0.00170	0.925±0.00406	0.185	0.48
	0.916	0.925				
	0.917	0.929				

Table 5: Interday precision data of hydrochlorothiazide.

Conc (µg/mg)	Absorbance		Mean ± standard deviation (*n*=3)		% RSD	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
30	0.401	0.409	0.401±0.00902	0.408±0.00703	1.8	1.7
	0.402	0.401				
	0.410	0.415				
40	0.532	0.542	0.535±0.00360	0.545±0.00360	0.67	0.66
	0.536	0.546				
	0.539	0.549				
50	0.619	0.620	0.628±0.00818	0.623±0.00608	1.3	0.97
	0.630	0.630				
	0.635	0.619				

Table 6: Recovery data for VAL and HCTZ drug product.

Spike level	Absorbance*		Concentration recovered		% Recovery	
	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ
80%	0.993	0.424	36.9	26.16	115%	81.7%
100%	1.234	0.619	46.19	42.41	109%	106%
120%	1.369	0.736	52.03	52.17	106%	108%

Table 7: Assay results of marketed tablets.

Brand name	Drug	Functional groups	Abs*	Label claim (mg)		% purity
				Actual	Found	
VALZAAR H®	Valsartan	1600 cm^{-1}	0.901	160	158.6	99.1%
	Hydrochloro thiazide	3361 cm^{-1}	0.619	12.5	12.48	99.8%

The precision of the developed analytical method was reported in terms of repeatability and Interday precision. The results were found to be within the limits i.e., <2%. Results are reported in Table 3-5.

Accuracy study was carried out by calculating % Recovery of the valsartan and hydrochlorothiazide by standard addition method respectively. Recovery data for VAL and HCTZ drug product are reported in Table 6.

Assay was performed for the tablets and the percentage purity was found to be within limits and results of marketed tablets are given in Table 7.

CONCLUSION

The developed chemometric assisted FTIR spectrophotometric method for analysis of valsartan and hydrochlorothiazide in pharmaceutical formulation was simple and cost effective, as FTIR technique needs small volume of solvent. The KBr pellet technique used for sample preparation in this method was precise and fulfilled most validation requirements in a range of concentrations suitable for quality control of both in pure and solid dosage form. This method can be used as an alternative method for HPLC, UV or pharmacopoeia methods as quality control check in pure and marketed formulations.

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CONFLICT OF INTEREST

The authors do not have any conflicts of interest to declare.

ABBREVIATIONS

FTIR: Fourier transform infrared; **ICH:** International council for harmonization; **PLSR:** Partial least square regression; **PCR:** Principal component regression; **PLSR:** Partial least square regression; **SVMR:** Support vector machine regression; **LWR:** Locally weighted regression; **PCA:** Principal component analysis; **PLSDA:** Partial least square discriminant analysis; **HCA:** Hierarchical cluster analysis; **kohonen SOM:** kohonen self-organizing map; **PLS:** Partial least square; **SDFCL:** SD fine chemicals limited; **KBR:** Potassium bromide; **VAL:** Valsartan; **HCTZ:** Hydrochlorothiazide; **LOD:** Limit of detection; **LOQ:** Limit of quantitation.

SUMMARY

A new Chemometric assisted FTIR spectroscopic method for simultaneous estimation of Valsartan and Hydrochlorothiazide in pure and pharmaceutical dosage forms was developed and validated. Literature review reveals that there are several UV-spectrophotometric, HPLC, HPTLC, QbD, LC-MS-MS, RP-HPLC, Paper chromatography, UPLC, Hydrotropic solublization and Fourier transform convolution emission methods has been reported so far for the simultaneous estimation of valsartan and hydrochlorothiazide drugs alone and in combination with other drugs. There are no chemometric assited FTIR spectroscopic methods for simultaneous determination of valsartan and hydrochlorothiazide in pure and marketed formulations this method involves the preparation of solid pellets of valsartan and hydrochlorothiazide using KBr with the aid of geometric mixing and direct measurement using reduced path length cell. The spectra were measured in absorbance mode and the equipment was configured to take spectra at 8 cm^{-1} resolution in IR range. Chemometrics applied to the FTIR spectra in the region of 1600$cm^{-1}$and 3361 cm^{-1} for VAL and HCTZ respectively. The proposed FTIR method reduces the solvent consumption and also eliminates the use of reagents. The developed method was simple cost effective precised and fulfilled most validation requirements in a range of concentrations suitable for quality control of

both in pure and solid dosage form. Thus the developed method offers a good alternative for the quantitative estimation of valsartan and hydrochlorothiazide in bulk and pharmaceutical dosage forms.

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