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Original Research Article

Stability-Indicating HPLC Method for the Simultaneous Determination of Valsartan and Ezetimibe in Pharmaceuticals

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Abstract

Purpose: *To develop a simple, accurate, sensitive, precise and robust reverse-phase HPLC stability-indicating method for the simultaneous estimation of valsartan and ezetimibe in combined tablet formulation.*
Methods: *A stability indicating method for the simultaneous estimation of valsartan and ezetimibe in combined tablet formulation using a RP-HPLC was developed and validated as per ICH guidelines using a symmetry C18 column with a mobile phase comprising phosphate buffer and acetonitrile (58:42 v/v, pH 3.15) with a flow rate of 0.8 mL/min at 230 nm. Stress degradation studies were performed in acidic, alkaline, oxidation and photolysis conditions to demonstrate the stability-indicating power of the method.*
Results: *The contents of valsartan and ezetimibe were in the range of 99.77 ± 0.10 and 99.30 ± 0.43 % in the marketed formulation, 99.77 ± 0.08 and 99.29 ± 0.38 for the test formulation, respectively. The correlation coefficient for both valsartan and ezetimibe was 0.999 and recovery was in the range of 98 – 102 %. The limit of detection (LOD) was 0.2 and 0.3 µg/mL for valsartan and ezetimibe, respectively, while limit of quantification (LOQ) was 1 µg/mL for both valsartan and ezetimibe, respectively.*
Conclusion: *The proposed method is simple, precise, accurate, reproducible, specific and reproducible used for the quantitative determination of valsartan and ezetimibe in bulk and dosage formulations.*

Keywords*: Valsartan, Ezetimibe, Validation, Stability-indicating, Pharmaceuticals*

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INTRODUCTION

Valsartan (VAL) (3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]amino] -butanoic acid) is an angiotensin II receptor antagonist used in the treatment of high blood pressure, congestive heart failure (CHF), and post-myocardial infarction (MI). It acts by blocking the action of angiotensin, dilates blood vessels and hence reduces blood pressure. Ezetimibe (EZE) ((3R, 4S)-1-(4-fluorophenyl)-3-((3S)-3-(4-fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxyphenyl)-2-azetidinone) is an anti-hyperlipidemic medication used to lower cholesterol levels. It is a class of lipid-lowering agent that selectively inhibits the intestinal absorption of cholesterol and related phytosterols.

Literature survey on the methods for valsartan indicates that several methods based on different techniques like spectrometric methods [1-3] and chromatographic methods [4-8] either individually or in combination with other drugs other than

Ezetimibe. Additionally, the literature survey on EZE reveals spectrometric method [9-12] and chromatographic method [13-15] for the determination of ezetimibe individually or in combined dosage forms. Simultaneous spectrophotometric determination of valsartan and ezetimibe in pharmaceuticals has been reported [16]. Though a spectrophotometric method is less time consuming, it is inadequate in identification and separation of the potential impurities formed during its storage; hence a stability indicating HPLC method is essential. To the best of our knowledge, there are no chromatographic methods reported elsewhere for the combination of VAL and EZE.

The aim of the present study was to develop a simple, accurate, sensitive and precise RP-HPLC stability-indicating method for the simultaneous estimation of valsartan and ezetimibe in combined tablet formulation.

EXPERIMENTAL

Apparatus and reagents

The HPLC system (Alliance, Milford USA) consisted of 2695 Separation module and 2998 Photodiode array detector with a thermostat for column and auto-injector. Chromatograms were automatically obtained from the system software, Empower.

All the chemicals were procured from E. Merck. Reference standard of valsartan (Lot # TRC-200306011) and ezetimibe (5-YM-118-1) were obtained from Toronto Research Chemicals, Canada. Valsartan and ezetimibe powders were obtained from Matrix Pvt. Ltd and Glenmark Pvt. Ltd., India, respectively. The commercial formulation of valsartan (Valzar 80 mg Lot# 06039005) was from Torrent Pharmaceutical Ltd, and ezetimibe (Zeteze 10 mg Lot# 1997604) from Ranbaxy Laboratories Ltd procured from the pharmacy.

The combined tablet formulation of valsartan and ezetimibe was prepared [16] and subjected to a stability studies in 3 different conditions namely 25 °C and 60 %RH, 30 °C and 65 %RH and 40 °C and 75 %RH as per the International Conference on harmonization (ICH) guidelines. The samples were drawn at periodic interval (1, 2, 3, 4.5 and 6 months) and the critical parameters like assay and release were ascertained.

Method development was performed on the symmetry C18 column using HPLC system (Waters, USA). The optimized condition for the mobile phase consists of phosphate buffer (pH = 3.15) filtered through 0.45 µm filter and acetonitrile in the ratio of 58: 42 (v/v) at a flow rate of 0.8 mL/ min and λ_{max} was 230 nm. Terbinafine HCl structurally similar, in part, to valsartan and ezetimibe was used as the internal standard to avoid any error during the injection. Detection was done using a photo diode array (PDA) detector at 230 nm.

Degradation studies

Stress degradation of the method was performed to measure the analyte response in the presence of its potential impurities. Stress testing of the individual drug substance and the combination was performed to measure the resolution factors of the drug peak from its nearest resolving peak and also from all other peaks. The drugs were subjected to acidic, alkaline, oxidising and photolytic conditions. For acidic degradation, the drugs were subjected to 1 N hydrochloric acid at 80°C for 1 h, for the alkaline degradation the drugs treated with 1 N sodium hydroxide at 80°C for 1 hr. Oxidative studies were carried out using 10% hydrogen peroxide for 1 hr. Photo degradation was performed by exposing the solution to light for 72 h. After the completion of the treatment, the solutions were left to return to room temperature, neutralised (for acidic and basic degradation) and diluted with the mobile phase to obtain the final concentration of 100 µg/mL.

Sample solution for formulation assay

Twenty tablets of a combination of Ezetimibe 10 mg (Zeteze) and Valsartan 80 mg (Valzaar) were weighed and finely powdered. A quantity of powder equivalent to 80 mg of Valsartan and 10 mg of Ezetimibe was transferred to a 100 mL volumetric flask containing 80 mL of mobile phase, sonicated for 20 min and the volume was made to the mark with the mobile phase before centrifuging at 2000 rpm for 20 min. The solution was filtered through 0.45 µm millipore filter and diluted after the addition of IS to obtain 80, 10 and 100 µg/ml of VAL, EZE and IS, respectively. The tablet sample solution was injected and the chromatograms were recorded. The peak area ratio of each of the drugs to the internal standard was calculated and the amount present per tablet was established. Similarly the sample for the in house combined dosage formulation was also prepared and the amount was calculated using the formula.

$$\text{Assay (\%)} = \frac{Ru}{Rs} \times \frac{Cstd}{Csam} \times \frac{Avg\ Wt}{Label\ claim} \times P \times 100. \quad (1)$$

where R_u= peak area response of valsartan (or ezetimibe) in the sample solution; R_s = Peak area response of valsartan (or ezetimibe) in the Bracketing Standard injections; C_{std} = standard concentration of valsartan (or ezetimibe) (mg/mL); C_{sam} = sample concentration of valsartan (or ezetimibe) (mg/mL); Avg wt = Average weight of number of tablets taken for analysis (mg/tab); *P* = purity factor of the standard; Label claim = label claim of valsartan (or ezetimibe) per tablet (mg/tab)

Sample solution for the determination of drug release (for valsartan)

The dissolution for the combined formulation of Valsartan (80 mg) and Ezetimibe (10 mg) dosage was carried out as per USP Chapter <711> requirement [17] using phosphate buffer (pH 6.8) as dissolution medium, vessel volume of 1000 mL equilibrated to 37± 2°C. Accurately six tablets were weighed and transferred each into a dissolution vessel containing 1000 mL of dissolution media bath and the system was agitated at a paddle speed of 50 rpm. The samples were drawn after 1, 3, 6, 8, 10 and 12 h, filtered and injected onto HPLC for analysis.

Sample solution for the determination of drug release (for ezetimibe)

The dissolution for the combined formulation of Valsartan (80 mg) and Ezetimibe (10 mg) dosage was carried out as per USP Chapter <711> requirement, using acetate buffer (pH 4.5) with 0.45% sodium lauryl sulphate as the dissolution medium, vessel volume of 500 mL and 37± 2°C as vessel temperature. Six tablets were weighed and transferred each into a dissolution vessel containing 500 mL of dissolution media bath and the system was agitated at a paddle speed of 50 rpm. The samples was drawn after 5, 10, 15, 30, 45 and 60 minutes, filtered and injected into HPLC for analysis.

Standard combined Solution for % release

The standard solution prepared consists of 80 µg/mL of Valsartan and 10 µg/mL of Ezetimibe in the mobile phase. The percentage dissolved was calculated using the formula

$$\text{Dissolved (\%)} = \frac{R_u}{R_s} \times C_s \times P \times \frac{V}{\text{Label claim}} \times 100 \quad \ldots (2)$$

where R_u, = peak area response of Valsartan or Ezetimibe in the Sample solution; R_s, = peak area response of valsartan or ezetimibe in the standard injections (mg/mL); C_s, = standard concentration in mg/mL of valsartan or ezetimibe (mg/mL); V, = volume of dissolution medium (mL); *P*, = purity factor of the standard; and label claim = label claim of valsartan or ezetimibe per tablet (mg/ tab).

Method validation

The method was validated as per the ICH guidelines [18] and the method validation parameters such as linearity, precision, accuracy, robustness, limit of detection, limit of quantification and specificity were studied.

Linearity and range

Calibration curve was constructed with various concentrations of VAL and EZE in the range of 1 - 200 µg/mL. The linearity graph was plotted with response against concentration.

Recovery

The accuracy of the method was determined by recovery study carried out by the standard addition method. The recovery of the sample solution was studied by spiking a known quantity of the drug in the range of 60-100 µg/mL for valsartan and 8-12 µg/mL for ezetimibe.

Precision

System precision for the method was calculated from the coefficient of variance for five replicate injections of the standard. Method precision was calculated for six different preparations of the samples. The intermediate precision was performed by carrying out the method precision on a different chromatographic system (Agilent 1100 series, USA).

Statistical analysis

The results obtained for the assay were subjected to statistical analysis using two-tailed Student's t-test using MS Excel 2007. Differences were considered significant at $p < 0.05$.

RESULTS

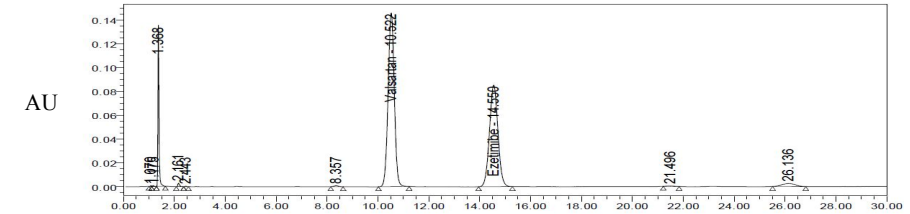
The HPLC method chromatographic parameters were optimised to obtain the best resolution and peak shape for the VAL and EZE. The phosphate buffer and acetonitrile in the ratio of 58:42 (v/v) was attained as the optimal condition for well resolved peak and system suitability parameter at 230 nm. The concentration of the Valsartan and Ezetimibe was optimized to obtain a signal to noise ratio of greater than 3000, the tailing of less than 1.5, theoretical plates of greater than 3000 and resolution of greater than 2 (USP-NF 35).

The method was validated as per the ICH guidelines [17] and the method validation parameters such as linearity, precision, accuracy, robustness, limit of detection, limit of quantification and specificity were studied as presented in the table 1.

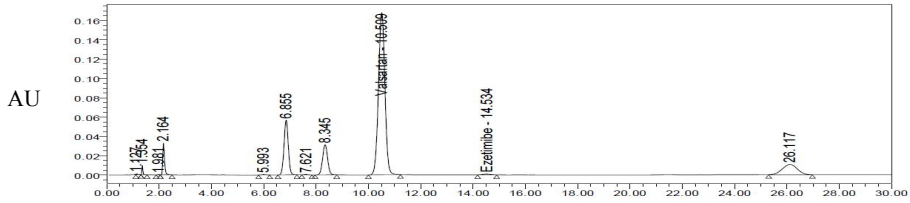
Table 1: Validation parameters for HPLC method

Parameter	VAL	EZE
Wavelength (nm)	230	230
Correlation coefficient (r^2)	0.999	0.999
Slope (m)	0.015	0.012
Recovery (%)	99.9- 101.5	98.4- 101.8
Limit of detection (µg /mL)	0.2	0.3
Limit of quantification (µg /mL)	1	1
No. of data points	5	5

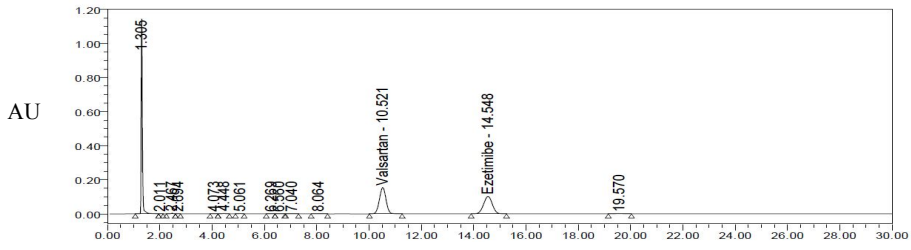
The correlation coefficient of both valsartan and ezetimibe was 0.999 at 230 nm. The coefficient of variance for the precision was < 1.5 % (0.33 and 0.40 % for valsartan and ezetimibe, respectively) and the intermediate precision was found to be less than 1 %; low value of coefficient of variation indicated high precision of the method. Recovery was within the range of 100 ± 2 % indicating the accuracy of the method (Figure 1). It also indicated the absence of interference of excipients in the determination of valsartan and ezetimibe by the proposed method in the formulation or in samples.



(a) Representative chromatograms of Ezetimibe, Valsartan combination in acidic



(b) Representative chromatograms of Ezetimibe, Valsartan combination in alkaline



(c) Representative chromatograms of Ezetimibe, Valsartan combination in Oxidation

Figure 1: Representative chromatograms of ezetimibe/valsartan combination in (a) acidic (b), alkaline and (c) Oxidation (You need to increase the font size of the y- and x-axis laels, units and annotations to at least font otherwise your manuscript will be rejected).

Table 2: Assay results for Valsartan and Ezetimibe in tablets formulations

Sample drug content	Amount found (mg, mean ± SD)				Calculated value of *t*
	***MF*[c]**	***CV (%)***	***DF***	**CV (%)**	
Valsartan (80 mg)	79.81 ± 0.08 [#](99.77% ± 0.10)	0.1	79.82 ± 0.07 [#](99.77% ± 0.08)	0.08	0.95
Ezetimibe (10 mg)	9.93 ± 0.04 [#](99.30% ± 0.43)	0.43	9.93 ± 0.09 [#](99.29% ± 0.38)	0.39	0.96

SD = standard deviation; [c] commercial formulation; DF = formulation prepared in-house; [#] the percent drug content ± SD

Limit of detection (LOD) and limit of quantification (LOQ)

LOD for the VAL and EZE using signal to noise ratio was 0.2 and 0.3 µg/mL, respectively, while the LOQ was 1 µg/mL for both VAL and EZE.

Robustness

The flow rate, column oven temperature and the mobile phase composition were varied by ± 2 mL, ± 5 °C and ± 1 %, respectively, and changes in the areas of the peaks of interest were recorded. The peak areas were not significantly affected and hence the method was robust.

Degradation behaviour

Forced degradation studies of both the drugs namely Valsartan and Ezetimibe were carried out individually and in combination under different stress conditions like acid hydrolysis, alkaline hydrolysis, hydrogen peroxide oxidation and photolysis. The results are shown in Figure 1.

Acid hydrolysis

The acid hydrolysis performed using 1N HCl at 80 ºC for 1 h for both valsartan and ezetimibe indicated degradation. The major degradation products for Ezetimibe were observed at relative retention time (RRT) of 0.14 and 1.80 min; for valsartan, the degradation product was observed at RRT of 0.12 min. These impurities were also detected in the combination of Ezetimibe and Valsartan.

Alkaline hydrolysis

The alkaline hydrolysis condition was performed using 0.1N NaOH at 80 ºC for 1 h both ezetimibe and valsartan. Degradation of ezetimibe was found to occur profusely than valsartan. The major degradation products for Ezetimibe were observed at RRT of 0.14, 0.36, 0.47, 0.57 and 1.8 min and for the Valsartan the degradation product was observed at RRT 0.12 min.

Oxidation

In the oxidation condition with 10 % H_2O_2 for 1 h, both ezetimibe and valsartan did not show any oxidative stress degradation peak in the chromatogram.

Photolysis

There was no major degradation observed for both valsartan and ezetimibe and hence they were not sensitive to light.

Assay data for pharmaceutical formulation

The developed method was applied to analyse a commercial product and a tablet formulation developed in-house, both of which contained both Valsartan and Ezetimibe. Drug contents of both the standard and samples were calculated for a concentration of 80 µg/mL Valsartan and 10 µg/mL Ezetimibe. The results obtained were subjected to statistical analysis using two tailed Student's t-test in MS Excel 2007. The results of this study concluded that the means of two groups were statistically not different from each other. This is evident from the t-values displayed in Table 2.

Assay results for stability test

The assay results for both valsartan and ezetimibe are as displayed in Table 3.

The results for the dissolution studies of ezetimibe and valsartan are as displayed in Table 4. percent release of drug remained almost unchanged.

DISCUSSION

The method developed was optimised and validated according to ICH guidelines and found to be linear in the range of 1-200 µg/mL for both Valsartan and Ezetimibe. The degradation studies were performed to indicate the stability

Table 3: Assay result (%) for the stability studies of Valsartan and Ezetimibe

Time point	40°C/75% RH		30°C/65% RH		25°C/60% RH	
	Val	*Eze*	*Val*	*Eze*	*Val*	*Eze*
Initial	NA	NA	NA	NA	100.2	99.4
1	99.6	99.2	NA	NA	NA	NA
2	99.8	99.1	NA	NA	NA	NA
3	99.5	98.9	99.7	99.6	99.8	99.1
4.5	99.2	98.6	99.5	99.5	100	99.4
6	99.1	98.4	99.2	99.1	99.5	99.2

NA = Not applicable

Table 4: Dissolution data for the stability studies

Drug	Medium	Time (in min)	Average release* (%)															
			Time point (in months)/conditions															
			Initial	1	2	3	4.5	6	1	2	3	4.5	6	1	2	3	4.5	6
				40°C/75% RH					30°C/65% RH					25°C/60% RH				
Ezetimibe		5	23	24	27	25	NA	21	NA	NA	26	22	27	NA	NA	24	NA	25
	pH 4.5 acetate buffer	10	45	42	46	49	NA	43	NA	NA	42	47	41	NA	NA	42	NA	44
		15	62	68	64	63	NA	59	NA	NA	63	61	62	NA	NA	65	NA	63
		30	83	85	83	86	NA	81	NA	NA	82	85	83	NA	NA	84	NA	80
		45	93	92	89	90	NA	93	NA	NA	87	91	88	NA	NA	85	NA	84
		60	96	97	99	96	NA	95	NA	NA	94	96	96	NA	NA	98	NA	96
Valsartan		60	20	22	18	24	NA	21	NA	NA	21	19	20	NA	NA	23	NA	25
	pH 6.8 Phosphate buffer	180	40	43	38	41	NA	42	NA	NA	46	42	39	NA	NA	37	NA	35
		360	60	63	61	56	NA	59	NA	NA	55	60	54	NA	NA	62	NA	64
		480	75	78	75	70	NA	74	NA	NA	72	75	77	NA	NA	78	NA	74
		600	90	92	89	90	NA	93	NA	NA	87	91	88	NA	NA	85	NA	84
		720	98	97	99	96	NA	95	NA	NA	94	96	96	NA	NA	98	NA	96

**Average release of 6 vessels; NA= Not available*

indicating power. All the degradation products were well resolved from the Valsartan and Ezetimibe by more than 2. Bhatia et al [19] reported the structure of the impurity formed during acid degradation studies for Valsartan as VALAD (2-methyl- N-[{2H-tetrazol- 5yl} biphehyl-4-yl] methyl} propane-1-amine). There are no other reports on the structure of other impurities formed during degradation.

The assay method was applied to formulations, both marketed and formulations and the assay values were in the range of 98 – 102 %. The stability studies were performed under various ICH conditions and the developed method was also successfully used to determine the content and release of valsartan and ezetimibe in pH 6.8 and pH 4.5 buffers, respectively.

The stability study was performed for 6 months in various conditions (long-term, intermediate and accelerated). There was no significant change in the parameters. Though drug content decreased from 100.2 to 99.2 % and 99.4 to 99.2 % for valsartan and ezetimibe, respectively, with time the values were found to be within the acceptable limit of 90 to 110 % [18]. The decrease in content might be attributed to the increase in moisture content (4.5 to 5.5 % and 4.3 to 5.0 % for Valsartan and Ezetimibe respectively). (This is Discussion, not results. Move to Duscussion section. The small difference in the release value can be attributed to tablet to tablet variation. It shows that there is no interaction between the drugs and the excipients in the presented dosage form. Hence, the product was stable over a period of 6 months.

CONCLUSION

An isocratic stability indicating HPLC method was successful developed for the simultaneous identification and determination of valsartan and ezetimibe in formulations and in the presence of degradation products. The proposed method is simple, accurate, precise, robust, rugged and specific. The developed HPLC method could be employed for the analysis of the drugs in bulk, dosage forms either individually or in combination of the two drugs as well as for dissolution of the two drugs from dosage forms. The combined dosage formulation of valsartan and ezetimibe is stable in all the three conditions of accelerated, intermediate and long-term over a period of six months. Since the formulation does not absorb moisture significantly, no packaging requirement with special critical conditions would be required.

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