

## STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN TABLETS

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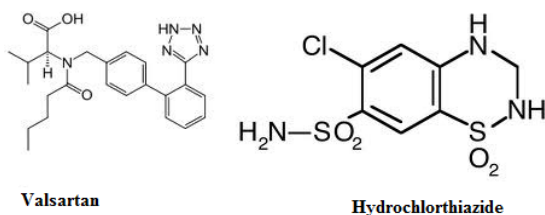
### ABSTRACT

A simple, selective, rapid, precise and stability-indicating HPTLC method has been developed for the quantitative simultaneous estimation of Valsartan and Hydrochlorothiazide in combined pharmaceutical dosage form and validation was done. The proposed HPTLC method involves the use of HPTLC plates (Merck) pre-coated with silica gel 60F<sub>254</sub> on aluminium sheets and a mobile phase comprising of chloroform: ethyl acetate: acetic acid (5:5:0.2 v/v/v). Densitometric analysis of both the drugs was carried out in the absorbance mode at 248 nm. The method has been successfully applied for estimation of Valsartan and Hydrochlorothiazide in combined tablets dosage form. Both the drugs were subjected to acid-alkali hydrolysis, oxidation and photolytic degradation and both of them were found to be susceptible to acidalkali hydrolysis, oxidation and photolytic degradation. Linearity of Valsartan was found to be within the range of 800-5600 ng/spot ( $r^2 = 0.9996$ ) and for Hydrochlorothiazide the range was found to be 125-875 ng/spot ( $r^2 = 0.9975$ ), with significant high values of correlation coefficient for both the drugs. The limit of detection and limit of quantitation were found to be 124.71 ng/spot and 377.91 ng/spot for Valsartan and 47.67 ng/spot and 114.46 ng/spot for Hydrochlorothiazide. The percentage recovery of Valsartan and Hydrochlorothiazide was ranged from 99.93 to 99.99 and 99.97 to 99.98 respectively. The %R.S.D. values for intraday precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise. The validation studies were carried out fulfilling International Conference on Harmonisation (ICH) requirements. The method was validated for the precision, robustness and recovery. As method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

**Keywords:** HPTLC, Telmisartan, Ramipril, Simultaneous estimation, Validation, Valsartan, Hydrochlorothiazide.

### INTRODUCTION

Valsartan, chemically known as (S)-3-methyl-2- (N-[[2'- (2H-1, 2, 3, 4-tetrazol-5-yl) biphenyl-4-yl]methyl]pentanamido) butanoic acid<sup>1</sup>, is an angiotensin II receptor antagonist and is used in the treatment of high blood pressure, congestive heart failure (CHF) or post-myocardial infarction (MI)<sup>2</sup>. It is official in United State Pharmacopoeia. Hydrochlorothiazide, chemically known as 6-chloro-1, 1 dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide, is a first-line diuretic<sup>3</sup> which is official in British Pharmacopoeia (**Fig-1**). A combination of VAL and HTZ in the form of a tablet or capsule formulations is widely used for the moderate to serve antihypertensive, not controlled by a single agent. The official monographs describe the procedure for individual assay of VAL and HTZ. The literature survey reveals that several methods were reported, including spectroscopic and chromatographic methods for the individual estimation of VAL and HTZ or combination with other drugs.<sup>4,5,6</sup> but, no stability indicating assay method has been reported for the quantitative determination of VAL and HTZ in combined dosage form. The simultaneous equation method<sup>7,8</sup> was exploited for quantitative determination of VAL and HTZ in combined dosage form. Absorption correction methods<sup>9</sup> for estimation of valsartan with amlodipine and hydrochlorothiazide in combine dosage form has been reported. A stability-indicating LC method<sup>10</sup> has been reported for simultaneous determination of Telmisartan and Hydrochlorothiazide in combined pharmaceutical dosage form in the presence of their degradants. Hence we attempted to develop a simple, accurate, precise and economic analytical method. This project work describes a stability indicating HPTLC method for simultaneous estimation of VAL and HTZ in combination tablets.



**Fig. 1: Structure of Valsartan and Hydrochlorothiazide**

### MATERIAL AND METHODS

#### Instrument

A Camag, Linomat V Sample Applicator with Camag 100  $\mu$ l Syringe; Camag Twin trough Glass Chamber (20 x 10); Camag TLC Scanner III with Wincat software.

#### Reagents and chemicals

Pure drug samples of VAL and HTZ were provided as a gift sample by Torrent Pharmaceutical Industries Ltd, Ahmedabad, India. Commercial pharmaceutical preparation Valzaar-H (Torrent Pharmaceutical Industries Ltd) was procured from local pharmacy. All the chemicals used were obtained from S. D. Fine Chem. Ltd., Mumbai used without further purification.

#### Preparation of standard stock solution and calibration curve

Standard stock solution was prepared by dissolving 8 mg of VAL and 1.25 mg of HTZ in ethanol and volume was adjusted to 10 mL with the same, to give a solution concentration of 800 ng/ $\mu$ L and 125 ng/ $\mu$ L of VAL and HTZ, respectively. For linearity purpose, stock solution was applied on the plate using micro-liter syringe with the help of Linomat V applicator, to give spots of concentrations 800-5600 ng/spot and 125-875 ng/spot for VAL and HTZ, respectively. Calibration curve was obtained by plotting peak-area on ordinate and corresponding concentration on abscissa.

#### Chromatographic conditions

The experiment was performed on silica gel 60F<sub>254</sub> HPTLC plates using mobile phase comprising of chloroform: ethyl acetate: acetic acid in the ratio of (5:5:0.2 v/v/v). The plates were prewashed with methanol and activated in an oven at 60°C for 1 hour before use. The sample solutions were applied as 1  $\mu$ l per spot and sharp bands of 6 mm width with the help of Camag Linomat V sample applicator in speed of 10 second/ $\mu$ l. After application of samples the plates were dried in oven and ascending development to distance of 90 mm was performed in a saturated 20cm x 10cm Camag twin trough chamber for 15 minutes at room temperature. Subsequent to the

development, the HPTLC plates were air dried and densitometric scanning was performed at 248 nm using Camag TLC scanner III with help of Wincats software.

#### Linearity and range

Adequate dilutions were made from stock solutions to get five concentrations of mixed standards contains 800-5600 ng/spot for

VAL and 125-875 ng/spot for HTZ respectively using double distilled water. All these five dilutions were applied as spots of 1  $\mu$ l each on a TLC plate. After development of the plate on the selected mobile phase it was dried in hot air oven. The plate was then scanned and quantified at 248 nm. The densitograms were recorded and the measured peak areas were plotted against concentrations.

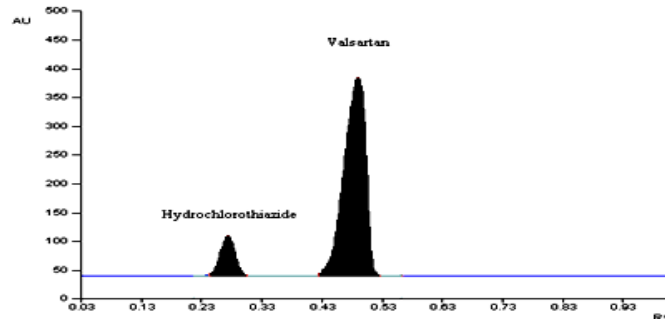


Fig. 2: A typical chromatogram of VAL (R<sub>f</sub>0.25) and HTZ (R<sub>f</sub>0.46)

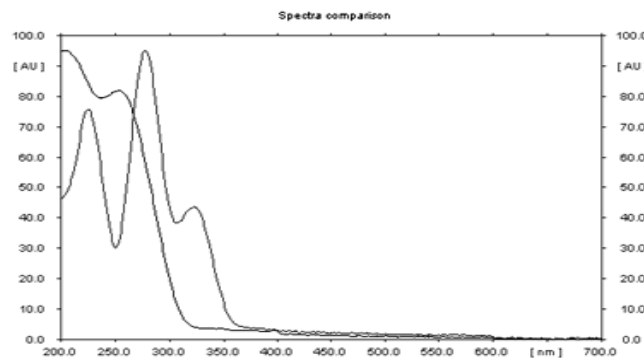


Fig. 3: Overlain absorbance spectrum of VAL and HTZ

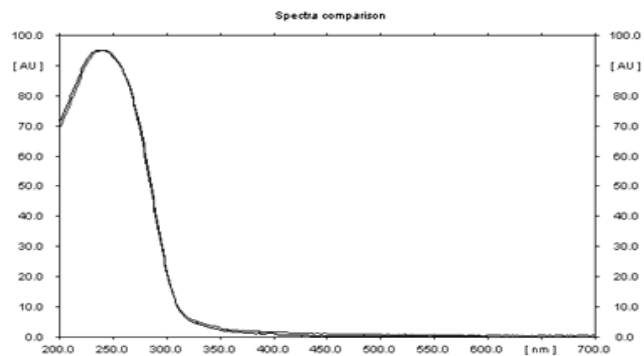


Fig. 4: Peak purity spectrum of VAL scanned at peak-start, peak-apex and peak-end positions of the spot (correlation > 0.99)

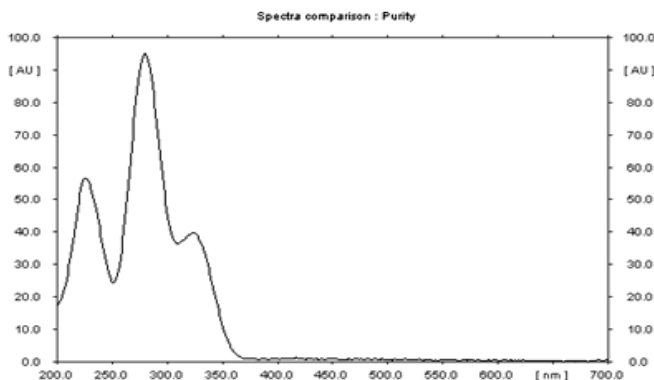


Fig. 5: Peak purity spectrum of HTZ scanned at peak-start, peak-apex and peak-end positions of the spot (correlation > 0.99)

### Application of developed method to estimate the drugs in tablet formulations

Accurately weighed and powdered 20 tablets of VAL and HTZ combination (Valzaar-H), each containing 80 mg of VAL and 12.5 mg of HTZ as per label claim. A batch of twenty tablets was used for the assay. Six replicates of solutions, containing 80 mg of VAL and 12.5 mg of HTZ were obtained by dissolving the powdered material in ethanol, which was sonicated for 20 min. The solutions were filtered and diluted to 100 mL with methanol, and applied on the plate to give 800 ng/spot of VAL and 125 ng/spot of HTZ, respectively. The plate was developed as described above and the spots were scanned at 248 nm. The results of assay are summarized in Table-1.

### Validation

The linearity was studied in the concentration range of 800-5600 ng/spot and 125-875 ng/spot for VAL and HTZ, respectively. Linear regression data is shown in Table 2.

Repeatability of the method was determined by analyzing six samples of same drug concentrations on a TLC plate. Chromatographs were recorded and area of each spot was measured.

Intra-day precision was determined by analyzing both the drugs at three different concentrations and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for three consecutive days. To ensure the accuracy of method, recovery studies were performed by standard addition method at 80 %, 100 % and 120 % concentration levels of the label claims, to the pre-analyzed samples and their contents were re-analyzed, using the proposed method. The results obtained are given in Table 3.

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula,  $LOD = 3.3 \times \sigma/S$  and  $LOQ = 10 \times \sigma/S$ , where  $\sigma$  is residual standard deviation of regression line and  $S$  is slope of corresponding regression line. The LOD and LOQ were found to be 124.71 ng/spot and 377.91 ng/spot for VAL and 47.67 ng/spot and 114.46 ng/spot for HTZ, respectively. The drugs were separated with good resolution by the developed solvent system (Fig. 2). The  $R_f$  values for VAL and HTZ were found to be 0.25 and 0.46, respectively (Fig. 3). The peak-purity of VAL and HTZ was determined by comparing their respective spectra at the peak start (S), peak apex (M) and at peak end (E) positions of the spot. Correlation  $r(S, M) = 0.9999$ ,  $r(M, E) = 0.9995$  for VAL was observed (Fig. 4). For HTZ correlation  $r(S, M) = 0.9998$ ,  $r(M, E) = 0.9999$  was observed (Fig. 5).

Ruggedness of the developed method was established by two different analysts, who assayed the formulations, using similar operational and environmental conditions. The results are summarized Table 4.

### Degradation study using HPTLC technique

Forced degradation studies<sup>11</sup> are an important part of the drug development process. Although the concept of stress testing is not new to the pharmaceutical industry, they are regularly been performed to determine stability and half-life of the drug products. The purpose of stability testing is to provide evidence on how quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and various storage conditions.

#### Acidic degradation

To 5 ml of stock solutions of both drugs, 10 ml of 1N HCl was added and refluxed at 60°C for 3 hrs.

#### Basic degradation

To 5 ml of stock solutions of both drugs, 10 ml of 1N NaOH was added and refluxed at 60°C for 3 hrs.

#### Oxidative degradation

To 5 ml of stock from each, added separately 10 ml of 30% H<sub>2</sub>O<sub>2</sub> and the mixture was refluxed at 60°C for 3 hrs.

#### Photolytic degradation

About 250 mg of each of VAL RS and HTZ RS and 350 mg of powdered tablet formulation were taken in separate petridishes, and kept under direct sunlight for 24 hrs.

#### Estimation of degradation

The degraded samples were applied as spots of 1 µl each after suitable dilutions on separate TLC plates. The peak areas of VAL, HTZ and of degraded products were measured by densitometric scanning at 248 nm. Table 5 shows the percentage of degradation under each stress conditions.

### RESULTS AND DISCUSSION

The present study was carried out to develop a simple and rapid HPTLC method for the estimation of VAL and HTZ using percolated TLC plates. The  $R_f$  value of VAL and HTZ was found to be at 0.25 and 0.46 respectively. The peaks were well resolved and symmetrical in shape. Linearity experiment was performed and the response was found to be in the concentration range of 800-5600 ng/spot and 125-875 ng/spot with the correlation coefficient 'r' values of 0.9996 and 0.9975 for VAL and HTZ respectively. Accuracy of method was determined by recovery studies (n=3). There was good repeatability of the proposed method as the precision of the method was less than 2% for both the drugs. The mean percentage recovery was found to be 99.93 - 99.99% for VAL and 99.97 - 99.98 for HTZ. This method is rapid, precise and accurate and hence can be employed for the routine quality control analysis.

Table 1: Assay of VAL and HTZ in tablets

Component	Label Claim (mg)	Amount Found mg ± SD (n=6)	% Label Claim	% RSD
VAL	80	79.87 ± 0.009	99.84	0.0921
HTZ	12.5	12.45 ± 0.20	99.66	0.2092

Table 2: Results of Linearity Studies

Parameter	VAL	HTZ
Linearity range (ng/spot)	800 - 5600	125 - 875
Slope	3.746	7.641
Y-intercept	2968.2	535.36
Correlation coefficient (r)	0.9998	0.9975

Table 3: Results of Recovery Studies

Component	Initial concentration (ng/spot)	Excess drug added (ng)	Drug recovered ng ± SD (n=3)	% Recovery	%RSD
VAL	3200	2560	2559.12 ± 5.69	99.93	0.02117
	3200	6400	6359.25 ± 10.9	99.93	0.1714
	3200	3840	3814.5 ± 0.77	99.99	0.0201
HTZ	500	400	392.59 ± 0.77	99.97	0.1961
	500	500	495.33 ± 0.27	99.99	0.0545
	500	600	594.24 ± 0.38	99.98	0.0639

Table 4: Results of Ruggedness Studies

Component	Analyst I		Analyst II	
	% Label Claim	%RSD	%Label Claim	%RSD
VAL	99.97	0.0483	99.92	0.0571
HTZ	99.92	0.1867	99.89	0.1129

Table 5: Results of degradation studies

Stress condition	% degraded (Valsartan)		% degraded (Hydrochlorothiazide)	
	Standard	Formulation	Standard	Formulation
Acid	8.77	5.89	5.98	4.55
Alkali	9.85	6.64	2.23	2.01
Oxidative	7.98	4.58	11.83	7.36
Photolytic	No-degradation	-	No-degradation	-

## CONCLUSION

This study found that HPTLC method for determination of Valsartan and Hydrochlorothiazide in combined dosage form is accurate, precise, reproducible and linear. This method has been wrathful for estimation of Valsartan and Hydrochlorothiazide in combined dosage form and also report force degradation study for same combination.

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