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Development and validation of HPTLC method for simultaneous estimation of Valsartan and Hydrochlorothiazide in tablet dosage form

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Abstract

A simple and sensitive high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of valsartan and hydrochlorothiazide in combined dosage forms. The chromatographic development was carried out on aluminum plates precoated with silica gel 60 F254 using a mixture of Chloroform: Methanol: Formic acid (4:1:0.05, v/v/v) as mobile phase. The detection was carried out densitometrically using UV detector at 264 nm in absorbance mode. This system was found to give separation for valsartan with Rf 0.76 and hydrochlorothiazide with Rf 0.44. The method was validated according to ICH Q2 (R1) guidelines. The calibration curve was found to be linear between 1000 – 7000 ng/spot and 200 – 1000 ng/spot for valsartan and hydrochlorothiazide, respectively with significantly high value of correlation coefficient (r^2 = 0.99). The limits of detection and quantitation were found to be 391 and 1185.1 ng/spot, respectively for valsartan and 57.37 and 173.8 ng/spot, respectively for hydrochlorothiazide. The percentage assay was found to be 99.25 and 101.2 while accuracy was accessed by percentage recovery and found to be 100.64 and 99.84 for valsartan and hydrochlorothiazide, respectively. The proposed method was found to be accurate, precise, reproducible, and specific and can be applicable for the simultaneous determination of valsartan and hydrochlorothiazide in tablet dosage form.

Keywords: Valsartan, Hydrochlorothiazide, High performance thin layer chromatography, Validation, Specificity, Accuracy.

INTRODUCTION

Valsartan (VAL), a nonpeptide, is N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-L-valine (Figure 1)[1]. It is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug.

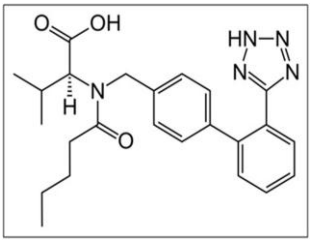

Figure 1. Structure of Valsartan

Hydrochlorothiazide (HCT) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide,1,1-dioxide, is a diuretic drug (Figure 2)[2].

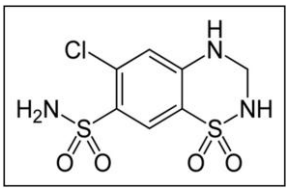

Fig 2. Structure of Hydrochlorothiazide

In patients with moderate hypertension, first-line therapy with the fixed-dose of valsartan/hydrochlorothiazide combination leads to BP normalization with high response rates. The rationale behind this drug combination is that in treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy, oral administration of valsartan with hydrochlorothiazide has been found more effective than use of either drug alone. Very few methods for determination of valsartan individually have appeared in the literature. Methods used include RP - HPLC analysis[3], comparison of UV and second derivative spectrophotometric method[4]

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and HPLC with liquid extraction [5]. There are several reports of the determination of hydrochlorothiazide individually like liquid chromatography–electrospray ionization tandem mass spectrometry[6], diffuse reflectance spectroscopy[7], chemiluminescence analysis[8] or in combination with other drugs, including use of UV[9, 10], ion pair chromatography[11] and spectrophotometry[12,13]. A literature survey has revealed there is no HPTLC method for analysis of valsartan and hydrochlorothiazide in pharmaceutical preparations. The intended purpose of this research was to establish accurate, precise, reproducible, and specific method and after validation in accordance with International Conference on Harmonization (ICH Q2 R1) guidelines[14] and the directives for good laboratory practice, to use the method for analysis of the drug content of tablets.

MATERIALS AND METHODS

Instruments

Camag HPTLC system comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag flat bottom and twin-trough developing chamber (10 X 10 cm), UV cabinet, Camag winCATS software, Hamilton syringe (100 µl), analytical balance were used in the study.

Reagents and Materials

VAL and HCT bulk powders were kindly gifted by Lupin Pharmaceuticals Pvt. Ltd.(Pune), India and Jubilant Organosys Ltd.,(Mysore), India respectively. Silica Gel 60 F254 TLC plates (10 X 10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were used as stationary phase. The commercial fixed dose combination tablet "VALENT-H" containing VAL 80 mg, HCT 12.5 mg was procured from Lupin Pharmaceuticals Pvt. Ltd.(Pune).Methanol, Chloroform (AR grade, Fisher Scientific, India) and Formic Acid (S. D. Fine Chemicals Ltd., Mumbai, India) were used for mobile phase preparation and as solvents.

Preparation of stock standard solution

VAL (64 mg) and HCT (10 mg) were weighed accurately, transferred to 10 ml volumetric flask, dissolved with 5 ml methanol. Above solution was diluted to 10 ml with methanol to get 6400 µg/ ml of VAL and 1000 µg/ml of HCT.

Chromatographic conditions

The experiment was performed on silica gel 60 F_{254} aluminum sheets (10 X 10 cm) as stationary phase, using mobile phase comprised of Chloroform: Methanol: Formic acid (4:1:0.05 v/v). TLC plates were prewashed with methanol and activated in an oven at 120ºC for 15 min prior to chromatography. The solutions were applied on TLC plate in the form of bands of 8 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. The space between two bands was fixed at 14 mm. Ascending development to 70 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber saturated with the mobile phase for 15 min. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS software. Both components show reasonably good response at 264 nm keeping the slit dimension of 6.00 x 0.30 mm and scanning speed of 20 mm/s (Figure 3).

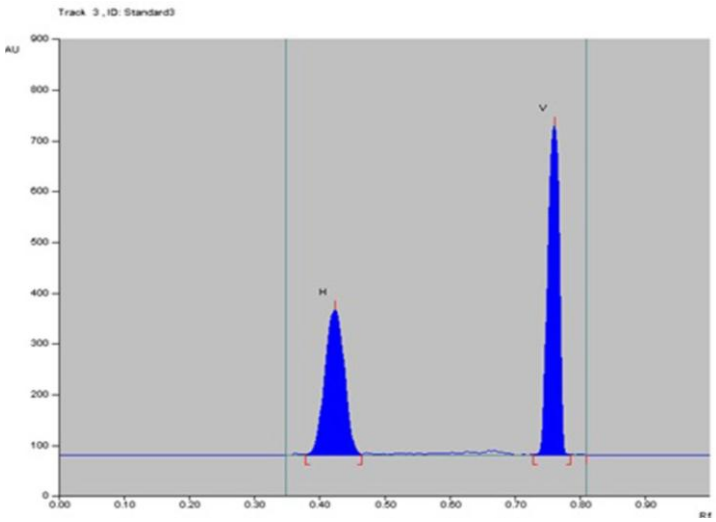

Figure 3. Separation of VAL and HCT

Linearity (Calibration curve)

Calibration curves were plotted over the concentration range of 1000 – 7000 ng/spot and 200 – 1000 ng/spot for VAL and HCT, respectively. But the tablet ratio was 80:12.5 i.e. 6.4:1 for VAL and HCT. Thus, for method development the concentration range selected for VAL was 1280 – 6400 ng and for HCT was 200 – 1000 ng according to tablet ratio. Standard solutions equivalent to 1280, 2560, 3840, 5120 and 6400 ng $spot^{-1}$ of VAL by using standard stock solution 6400 µg/ml and Standard solutions equivalent to 200, 400, 600, 800 and 1000 ng $spot^{-1}$ of HCT by using standard stock solution 1000 µg/ml were spotted on the HPTLC plates by over

spotting. Sample was applied in form of bands by using Linomat 5. Then the plate was developed in previously saturated chamber with the mobile phase Chloroform: Methanol: Formic acid (4:1:0.05 v/v). After the development, plate was scanned at 264 nm (Figure 4).

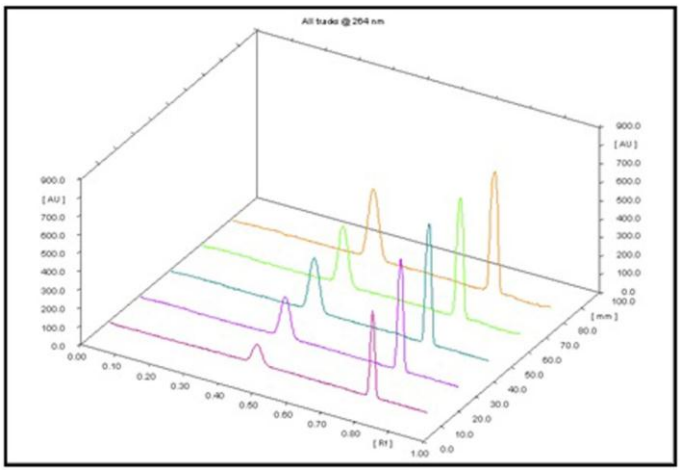

Figure 4. Linearity peaks for VAL and HCT

Application of the Proposed Method for Estimation of the Drugs in standard mixture

Appropriate volume 1 ml of VAL from stock solution (6400 µg/ml) and 1 ml of HCT from stock solution (1000 µg/ml) were withdrawn in 10 ml volumetric flask and volume was adjusted up to the mark with methanol. This standard mixture having concentration 640 µg/ml of VAL and 100 µg/ml of HCT was used for application on plate. 5 µl of this standard mixture was applied as bands on the plate to obtain concentration 3200 µg/ml of VAL and 500 µg/ml of HCT respectively. The plate was developed as per the standard conditions and it was repeated for six times to get reproducibility. The concentrations of VAL and HCT were calculated by using regression equation and the percentage was calculated. Results are shown in Table 1.

Table 1: Application of proposed method for standard mixture

Drug	Concentration applied (ng)	% Amount found (n = 6)	*% R.S.D.*
VAL	3200	99.96	0.58
HCT	*500*	*100.06*	*0.62*

Application of the Proposed Method for Estimation of the Drugs in Tablet:

Twenty tablets were weighed separately and powdered. Amount of powder equivalent to 80 mg of VAL and 12.5 mg of HCT was transferred to a 10 ml calibrated volumetric flask and extracted with 8 ml Methanol for 10 minutes by shaking mechanically. The volume was made with methanol and solution was filtered through Whatman filter paper no.1. From the filtered solution 1ml was taken and diluted to 10 ml with methanol which provided concentration 800 µg/ml and 125 µg/ml of VAL and HCT respectively. A 5 µL sample solution was spotted on the chromatographic plate to obtain concentration of 4000 ng and 625 ng of VAL and HCT respectively. Chromatographic plate was developed and scanned according to developed method. The same procedure was repeated for six times to get reproducibility and percentage amount was calculated by using regression equation. The results of the assay are shown in **Table 2.**

Table 2: Application of proposed method for analysis of tablets*

Tablet sample	Concentration applied (ng)	% Amount found (n = 6)	*% R.S.D.*
VAL	4000	99.25	0.98
HCT	625	101.2	0.84

*Official limit of VAL and HCT tablets is not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of valsartan ($C_{24}H_{29}N_{53}O_3$) and hydrochlorothiazide ($C_7H_8ClNO_4S_2$) according to IP 2010.

Validation of HPTLC method:

Validation of the proposed method was carried out with various parameters such as precision, accuracy, specificity, LOD, LOQ, robustness and ruggedness.

Precision:

Precision studies were performed by using standard solutions containing the three concentrations that are 2560, 3840, 5120 ng/spot $^{-1}$ of VAL and 400, 600, 800 ng/spot $^{-1}$ of HCT.

Repeatability:

The precision of the method in terms of repeatability was determined by analyzing three concentrations per three replicates of VAL standard solutions and HCT standard solutions. Above three concentrations of both VAL and HCT were applied on plate and chromatographic development was carried out repeating each three times. Depending on peak areas

obtained for each concentration, standard deviation and percentage relative standard deviation was calculated.

Intermediate precision:

Intermediate precision was assessed by analyzing VAL standard solutions and HCT standard drug solutions on three consecutive days over a period of one week. The same concentrations as used in repeatability were applied to plate and it was developed repeating each three times. Same parameters were calculated for intermediate precision. The results of the repeatability and intermediate precision are as shown in **Table 3.**

Table 3: Results of repeatability and intermediate precision (Precision data)

Drug	Amount taken (ng)	Repeatability (n =3)		Intermediate precision (n = 3)	
		SD	% R.S.D.	SD	% R.S.D
VAL	2560	0.836 x 10^3	0.116	0.926 x 10^3	1.299
	3840	1.398 x 10^3	0.168	1.332 x 10^3	1.554
	5120	1.837 x 10^3	0.209	1.237 x 10^3	1.283
	Mean	1.357 x 10^3	0.164	1.165 x 10^3	1.412
HCT	400	0.464 x 10^3	0.081	1.654 x 10^3	2.023
	600	1.231 x 10^3	0.177	1.409 x 10^3	1.764
	800	1.713 x 10^3	0.209	1.578 x 10^3	1.598
	Mean	1.136 x 10^3	0.155	1.547 x 10^3	1.795

Accuracy:

The accuracy of the method was determined by the use of standard additions at three different levels i.e. multiple level recovery studies. Sample stock solution of tablet formulation of 80 mg VAL and 12.5 mg HCT was prepared as stated before. On chromatographic plate, 2 µl of this standard solution was applied to get 1600 ng of VAL and 250 ng of HCT. To this application, 80%, 100% and 120% of the standard drug solutions were spiked that included 1280 ng, 1600 ng, 1920 ng of VAL and 200 ng, 250 ng, 300 ng of HCT respectively. Each level was repeated thrice and the percentage recoveries were calculated as given in **Table 4.**

Table 4: Results of recovery studies (Accuracy data)

Recovery level	Initial amount (ng)		Concentration of excess drug added (ng)		% Recovery (n = 3)	
	VAL	HCT	VAL	HCT	VAL	HCT
80 %	1600	250	1280	200	99.92	99.47
100 %	1600	250	1600	250	100.2	99.25
120 %	1600	250	1920	300	101.8	100.8
Mean					100.64	99.84

Specificity:

For the determination of interference of excipients, microcrystalline cellulose and starch were added in concentration 1000 ng to each standard stock solution of VAL and HCT. The bands were applied on plate and plate was developed according to standard procedure and results were interpreted. Also, the peak purity of both drugs extracted from tablet and standard VAL as well as standard HCT extracted from bulk was assessed by correlating the spectra at the peak start (S), peak apex (A) and at the peak end (E) positions.

LOD and LOQ:

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations 1 and 2.

$LOD = 3.3 \times \sigma / S$ ..... (1)

$LOQ = 10 \times \sigma / S$ ..... (2)

where σ is the standard deviation of intercept, S is the slope of the calibration curve.

Reproducibility and Robustness:

Reproducibility of the method was checked by performing the chromatographic assays with the help of two various laboratories and the variations in the results were checked. Robustness was checked by changing the chamber (20 cm x 10 cm) and migration distance (80 mm) for the mobile phase. Plates were developed according to proposed method and peak areas were recorded. To compare the data, percentage relative standard deviation was calculated for each parameter.

RESULTS AND DISCUSSION

HPTLC method was developed for the simultaneous estimation of VAL and HCT in tablet dosage form. The intended purpose of this research was to establish accurate, precise, reproducible, and specific method and after validation in accordance with ICH guidelines to use the method for analysis of the drug content of tablets.

The method was based on separation of the two drugs followed by densitometric measurement of their spots at 264 nm. The separation was carried out on Merck TLC aluminium sheets of silica gel 60 F_{254} using Chloroform: Methanol: Formic acid (4:1:0.05 v/v) as mobile phase. Linearity was assessed by visual inspection of plot of concentration versus peak area. The graphs were found to be linear in the range 1280 – 6400 ng/spot with correlation coefficient value 0.999 for VAL and 200 – 1000 ng/spot with correlation coefficient value 0.999 for HCT. The sensitivity of method was assessed by determining LOD and LOQ. For VAL, LOD and LOQ was found to be 391 and 1185.1 ng respectively. For HCT, the LOD and LOQ was found to be 57.37 and 173.8 ng, respectively.

According to ICH guidelines, assay procedure should include application of method to an analyte of known purity which was carried out by using standard mixture and purity was found to be 99.96 % for VAL and 100.06 % for HCT. Next step is to apply the procedure to marketed formulation and compare the results with standard. Thus, the proposed method was applied to pharmaceutical formulation that is tablet VALENT-H and percentage label claim of VAL and HCT was found to be 99.25 % and 101.20 % respectively which was within specified limits as per standards stated in IP 2010.

Accuracy was carried out in terms of recovery at 80 %, 100 % and 120 % level including 3 concentrations per 3 replicates. The mean percentage recovery was found to be 100.64 % for VAL and 99.84 % for HCT. It was observed that the calculated results of percentage recovery were expressing closeness of agreement between official limits. Hence method can be said to be as accurate according to ICH guidelines.

The method was found to be precise as observed by results obtained in repeatability and intermediate precision which expresses the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under prescribed conditions.

Reproducibility was assessed by means of an inter laboratory experiments which gave similar estimations for both drugs. Robustness of the method was assessed by studying two parameters (change in chamber and change in migration distance) and effects on the results were examined. % R.S.D. values less than 2 showed the reliability of an analysis with respect to deliberate variations in method parameters.

The peak purity spectra comparison of standard and tablet sample at the peak start (S), peak apex (A) and at the peak end (E) positions was showing that the analyte chromatographic peak is not attributable to more than one components having r2 value 0.999 for the peak purity which satisfies the criteria for specificity of the proposed method according to ICH guidelines. The summary of validation parameters are as shown in **Table 5.**

Table 5: Summary of validation

Parameters	**VAL**	**HCT**
Linearity range (ng)	1280-6400	200-1000
Regression equation	Y = 1.452X + 440.1	Y=7.72X + 783.8
Correlation coefficient (r^2)	0.999	0.999
% Recovery (n = 3)	100.64	99.84
LOD (ng)	391	57.37
LOQ (ng)	1185.1	173.8
Standard error	$0.7285x10^{3}$	$0.7752x\ 10^{-3}$
Precision (% R.S.D.)		
Intra- day (n = 3)	0.164	0.155
Inter-day (n = 3)	1.142	1.795
Specificity (%R.S.D.)		
Addition of MCC	0.8	0.6
Addition of starch	0.6	0.7
Reproducibility (%R.S.D.)		
Lab I (n=3)	0.7	0.8
Lab II (n=3)	1.0	1.1
Robustness (%R.S.D.)		
Change in chamber (n=3)	0.9	1.1
Change in migration distance (n=3)	0.8	1.2

CONCLUSION

The proposed HPTLC method was found to be simple, sensitive, accurate, precise, reproducible, specific, robust, and economical and can be used for the routine simultaneous estimation of VAL and HCT in pharmaceutical formulations.

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CONFLICT OF INTEREST STATEMENT

There are no competing interests amongst authors. The authors declare that there are no conflicts of interest.

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