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Reversed Phase HPTLC Method Development and Validation for Quantitative Estimation of Valsartan in Drug Substance and Drug Product

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Abstract
Reversed phase high performance thin layer chromatographic method is developed and validated for quantitative estimation of drug substance Valsartan. The CAMAG HPTLC system, operated by software win CATS (Ver.3.2SP2), was used. The mobile phase was comprising of mixture of Methanol: Water (7.0:3.0 V/V). Dried Plates were scanned at 257 nm in absorbance mode. In HPTLC densitogram, Rf was in the range of 0.48 -0.52. Further, developed high performance thin layer chromatographic method validated as per current ICH Q2 guidelines which are for mainly used foe analytical method validation. And accepted globally. The response of Valsartan found in the range of 300 to 900 ng/band. The Limit of detection of Valsartan was 125.2 ng/band while limit of quantification was a 158.0 ng/band. The method is very simple, cost effective, user friendly, non-cumbersome, in line with current guidelines and can be used for quality control purpose in drug substance as well drug product.

Keywords
HPTLC, Reversed Phase HPTLC, Valsartan, Bulk drug, ICH, analytical method development and validation.

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INTRODUCTION
A nonpeptide angiotensin receptor antagonist, valsartan specifically prevents angiotensin II from attaching to the angiotensin II type 1 receptor. Large-scale research on post-myocardial infarction, heart failure (HF), and hypertension has shown valsartan's effectiveness, tolerance, and safety. [1]. Valsartan not only lowers blood pressure in people but also lowers the composite endpoint of mortality and morbidity in chronic heart failure when compared to a placebo. It is also being studied for its potential to postpone or stop the development of diabetes in individuals with impaired glucose tolerance. [2]. The chemical description of valsartan is N-(1-oxopentyl)-N-[[2'-(1 H-tetrazol-5-yl) [1, 1'-biphenyl]- 4- yl] methyl]"L-valine., having molecular weight is 435.5 with empirical formula C24H29N5O3. Valsartan is a fine powder that ranges from white to almost white. It is marginally soluble in water and soluble in methanol and ethanol. [3]

Many analytical methods have been reported in literature for determination of Valsartan with UV technique [4], Biological fluids [5,7], Liquid extraction [6], LC with UV [8], By HPTLC [9] Valsartan in combination with other drugs by HPTLC [10]. The novelty of the presented method is that it uses reversed phase high performance thin layer chromatographic plates. Further, the method is economical because it does not involve use of multiple solvents and thus saves enormous time engaged in mobile phase preparation and saturation. It has also many advantages over HPLC with respect to cost, speed, time, and simplicity of analysis. The limit of detection was 125.2 while limit of quantification value was 158.0 ng/ band. Till date, no reversed phase HPTLC method has been cited for

valsartan and attempt was made to report same in present work. The method was found to be simple, accurate, precise, robust, and cost effective for the determination of Valsartan from drug substance and drug product.

MATERIALS AND METHODS

Instrumental:

It is shown in **Table-1**.

Instrument Device	Specification
Make and model	CAMAG, Switzerland
Sample applicator	CAMAG Linomat V
Densitometric scanner	CAMAG TLC scanner 3
Sampling mode	Manual with Linomat applicator
Syringe	Hamilton (100 µL)
Planar stationary phase- Plates	HPTLC silica gel 60 RP-18 $F_{254}S$ (Merck).
Chromatographic development chamber	CAMAG, twin trough chamber
Drying device	Portable hot air dryer
Detection	Ultraviolet (UV) detector
Software	Vision CATS 3.2SP2

Chemicals and Reagents

Methanol was procured from Rankem Ltd. (HPLC grade with 99.0% purity) Valsartan API and standard was gifted from Shruutas pharmaceutical Pvt Ltd. (99%purity). Valsartan marketed sample (Valzaar -40 mg) were purchased from market .

EXPERIMENTAL

Experimental work is presented in two segments, namely analytical method development (AMD) and analytical method validation (AMV).

ANALYTICAL METHOD DEVELOPMENT (AMD)

Method development procedures

HPTLC method was developed for estimation of Valsartan. The details of experimental work are presented in Table 2.

Standard solution of Valsartan and sample solution of powder obtained from marketed tablet formulation were prepared as per afore mentioned procedures.

Optimisation of chromatographic conditions

Based on literature survey, polarity and solubility of Valsartan, many preliminary trials were conducted for selection of mobile phase composition; some are tabulated in Table 3.

Methanol: Water (7:3 V/V) was selected as optimized mobile phase composition such as sample application volume, band width and chamber saturation time. Relative humidity, migration time, temperature, separation technique, etc. were also optimized by performing lab studies. All optimized chromatographic conditions are tabulated in Table 4. Densitogram obtained using these optimized chromatographic conditions for Valsartan is shown in Figure 2, Rf value for Valsartan was found to be 0.51.

RESULTS AND DISCUSSION

Linearity

Linearity study done by plotting Valsartan peak area vs various sample concentration. The calibration graph shown a linear response and range observed 300-900 ng/band (Figure 3).

Precision

Intra-day precision

Precision study done at three different concentration levels from low (300 ng/band), mid (600 ng/band) to high (900 ng/band) within the same day at three different time intervals (session 1, 2, 3).

Inter-day precision

Three days in a row, it was conducted using the same homogenous sample and at the same concentration levels. The percentage RSD values for precision within and between days were found to be within an acceptable range., as shown in Table VII and VIII, respectively.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Values of LOD and LOQ of valsartan were calculated as per ICH Q2 guidelines [11] and tabulated in Table 9.

Accuracy

The accuracy of the method was determined by performing recovery studies. Known amounts of Valsartan analyte were added to samples in triplicate at three different concentration levels, representing 80%, 100%, and 120% of the target concentration. The results of these recovery trials are summarized in Table 9.

Table 2: Method development Stages

Sr. No.	System/Method/ Step	Steps followed
1.	Standard solution preparation	Ten milligrams of Valsartan were dissolved in ten milliliters of methanol to create a standard stock solution with a concentration of 1000 µg/mL (1000 ppm).
2.	Stationary phase selection	TLC plates with RP Silica Gel 60 F254 precoated: The chromatographic layer consisted of plates covered with a uniform 200-micrometer layer of porous (60-Å pore size) a polymeric binder, and a fluorescence indicator (F254) measuring 10 cm by 10 cm.
3.	Layer prewashing	Precoated TLC plates were prewashed with methanol to remove adsorbed material impurities which include water vapors and other volatile substances from the atmosphere when they get exposed in the lab environment.
4.	Layer preconditioning	Prewashed plate with methanol up to 90% and dried in oven before sample application.
5.	Preparation of sample solution for drug product	The three milligrams of Valsartan in five tablets were weighed and ground into a fine powder. 10 mg of Valsartan powder was added to a 10 mL volumetric flask, dissolved in methanol, and then the volume was increased to 10 mL using methanol. To ensure the drug is completely dissolved, it was sonicated for 30 minutes in an ultrasonication bath. The clear sample solution was obtained by filtering the solution through 0.45µm Whatman filter paper. Methanol was used to dilute it further, achieving a concentration of 100µg/mL.
6.	Selection of detection wavelength	On an HPTLC plate of appropriate size, a 100µg/mL (100 ppm) solution of Valsartan was placed. The plate was scanned densitometrically between 200 and 400 nm using a CAMAG HPTLC scanner 3.
7.	Optimization of chromatographic conditions	To choose and optimize the composition of the mobile phase and the chamber saturation duration, numerous conditions were conducted.

Table 3: Selection of the composition of the mobile phase through trials

Sr. No.	Mobile Phase Components	Composition (V/V)	Remark
1.	Methanol: Acetonitrile	1:1	**Acetonitrile is toxic and considering less toxic ad reproducibility, Methanol: Water (7:3) V/V) was selected as optimized mobile phase composition.**
2.	Acetonitrile: Water	1:1	
3.	Methanol: Water	7:3	

Table 4: Final Chromatographic Conditions

Sr. No.	Parameters	Final developed conditions
1.	Stationary phase	Merck's 10x10 cm (200 µm) precoated HPTLC plates with RP Silica Gel 60 F254
2.	Mobile phase composition	Methnaol : Water (7: 3 V/V)
3.	Sample application. i)Application volume ii) Band Length iii) Distance between the tracks	 6 µL 8 mm 15 mm
4.	Saturation time	20min
5.	Relative humidity (%)	55±5
6.	Temperature (°C)	25±2
7.	Separation technique	Ascending

8.	Total Quantity of mobile phase	10 mL
9.	Migration distance (MD)	70 mm
10.	Migration time/run time (MT)	15 min
11.	Detection wavelength (UV)	257 nm

Table 5: Analytical method validation: Parameters and procedures followed

Sr. No.	Parameters	Procedure Followed
1.	Linearity	ICH guidelines recommend using at least five concentrations to calculate linearity. The regression coefficient (R2) is determined by charting the peak area against the standard concentration.
2.	Specificity	To ensure the identity of an analyte, specificity tests should be performed in accordance with ICH. The Rf value and densitogram of the sample (tablet extract) and standard Valsartan were compared to confirm the method's specificity.
3.	Precision	**Precision was conducted at two levels, as follows** **Repeatability**: To determine repeatability, a minimum of nine determinations that covered the procedure's claimed range were used. (e.g., three concentrations/three replicates each). **Intermediate Precision**: The purpose of intermediate precision was to investigate how the precision of the analytical process was affected by random events, such as days. Precision investigations were conducted both within and between days by obtaining nine measurements of three concentrations and three replicates each, at three distinct times during the same day and on three separate days, respectively. Standard deviation and relative standard deviation (coefficient of variation) are used to report precision for each type of precision that is investigated.
4.	Limit of Detection (LOD) and Limit of Quantification (LOQ)	The standard deviation of the response and the slope of the calibration graph were used to calculate the values of the Limit of Detection (LOD) and Limit of Quantitation (LOQ). Equations were used to help with the quantification. For LOD = 3.3 sigma /s, LOQ = 10 sigma /s, where s = calibration curve slope and sigma = replication standard deviation.
5.	Accuracy	The percentage recovery in the current work was determined by conducting recovery assays in triplicate at three different concentration levels—10%, 20%, and 30%—using a known quantity of Valsartan reference solution. After these samples were analyzed, the outcomes were contrasted with what was anticipated.
6.	Robustness	The ability of an analytical process to withstand minor but deliberate changes in method parameters and to maintain its dependability under typical operating conditions is known as its robustness. The following parameters were intentionally altered to assess the robustness of the created analytical method: 1. Composition of Mobile Phase 2. Chamber saturation time.

Table 6: The calibration plot's linear regression data

Sr. No.	Parameter	Results
1.	Range	300-900 ng/band
2.	R^2	0.999
3.	y- intercept	589.8
4.	Slope	223.8

Table 7: Intra-day precision studies

		Valsartan			Inference
	Concentration levels	Low	Mid	High	
	Concentration (ng/band)	300	600	900	
Peak area	Session 1	0.00299	0.00609	0.00912	
	Session 2	0.00302	0.00615	0.00916	Acceptable % RSD, Hence Precise
	Session 3	0.00301	0.00614	0.00914	
	Average Peak area	0.00300	0.00612	0.00914	
	Standard Deviation	1.52	3.21	3.05	
	% RSD	0.50	0.52	0.33	

Table 8: Inter-day precision studies

		Valsartan			Inference
	Concentration levels	Low	Mid	High	
	Concentration (ng/band)	300	600	900	
Peak area	Session 1	0.00307	0.00616	0.00923	Accept-able % RSD, hence Precise
	Session 2	0.00306	0.00610	0.00914	
	Session 3	0.00303	0.00614	0.00918	
	Average Peak area	0.00305	0.00613	0.00918	
	Standard Deviation	2.08	3.06	4.50	
	% RSD	0.68	0.49	0.49	

Table 9: Detection limit and Quantification limit

Sr. No.	Parameters	Readings obtained
1.	LOD	125.2 ng/band
2.	LOQ	158.0 ng/band

Table 10: Recovery /Accuracy

Drug	Level of percentage recovery %	Amount present in extract (ng/band)	Amount of standard added (ng/band)	Total amount (ng/band)	% Recovery	Average % Recovery	% RSD
Valsartan	10	600	60	660	99.67	99.44%	0.41
	20	600	120	720	98.70		0.21
	30	600	180	780	99.97		0.34

Table 11: Robustness results

	% RSD			
Amount in ng/spot	**Mobile phase composition ± 10% (V/V)**		**Saturation time ± 5 min**	
	-10%, Methanol: Water (7.3:2.7 v/v)	**+10%, Methanol: Water (6.7:3.3 v/v)**	**+5 min**	**-5 min**
600	**0.58**	**0.82**	**0.30**	**0.23**

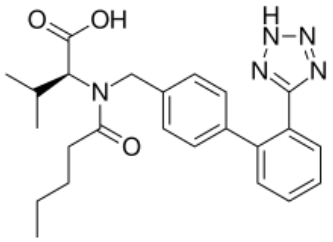
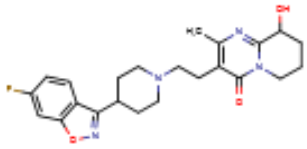


Figure 1: The Chemical structure of Valsartan



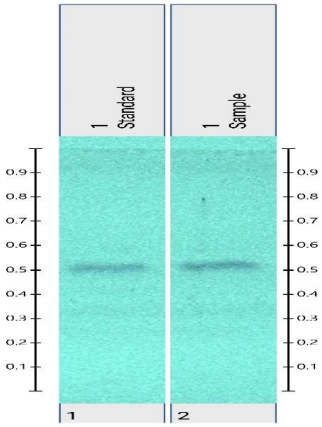


Figure 2: Scan at 254nm

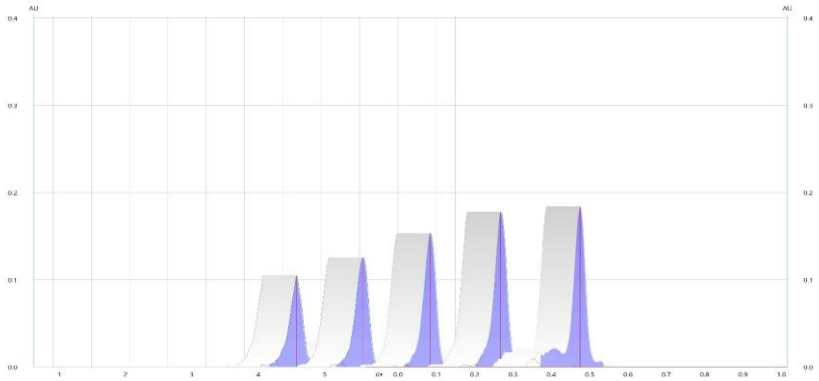


Figure 3: Linearity

CONCLUSION

The developed method was found to be very simple, fast, and cost effective as well as sensitive. The method was validated and found to be specific, linear, accurate, precise and robust.as per ICH Q2 guidelines Hence the HPTLC method can be conveniently adopted for routine analysis of the Valsartan in drug substance as well as drug product.

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