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Development and Validation of a HPTLC Method for the Simultaneous Estimation of Ramipril and Valsartan

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Research Article

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Abstract

A simple, precise, accurate and rapid high-performance thin-layer chromatographic method has been developed and validated for the estimation of ramipril and valsartan simultaneously in combined dosage form. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of chloroform: ethyl acetate: methanol: glacial acetic acid (5.0:5.0:1.0:02 v/v/v/v). The detection of spots was carried out at 210 nm. The method was validated in terms of linearity, accuracy, and precision. The calibration curve was found to be linear between 0.4 to 2.0 µg/spot for ramipril and 0.2 to 1.0 µg/spot for valsartan. The limit of detection and the limit of quantification for ramipril were found to be 200 ng/spot and 640 ng/spot for ramipril and 100 ng/spot and 330 ng/spot respectively. The proposed method can successfully be used to determine the drug content of marketed formulations.

Keywords: *HPTLC, Validation, Ramipril, Valsartan.*

Introduction

The combination of ramipril and valsartan has recently been introduced in the market. Ramipril is an angiotensin conversion enzyme inhibitor which is chemically (2S,3aS,6aS)-1-{N-[(S)-1-Ethoxycarbonyl-3-phenylpropyl]L-alanyl}perhydrocyclopenta[b]pyrrole-2-carboxylic acid and valsartan is ACE inhibitor. Valsartan is an angiotensin II type 1 (AT_1) receptor antagonist which is chemically *N*-[*p*-(*o*-1*H*-Tetrazol-5-ylphenyl) benzyl]-*N*-valeryl-L-valine [1]. Valsartan is not official in IP, BP, and USP [1-3]. There are no reports on the simultaneous estimation by HPLC and HPTLC determination of this combination in marketed preparation. The objective of the present work was to develop an accurate, specific and reproducible method for the simultaneous estimation of ramipril and valsartan in pharmaceutical dosage form.

Materials and Methods [4-5]

Ramipril and valsartan working standards were procured as gift samples from Lupin Laboratories, Mumbai. Silica gel $60F_{254}$ TLC plates (E. Merck KGaA, Mumbai) were used as stationary phase. Capsules containing 5 mg ramipril and 80 mg valsartan were purchased with brand name Valent R. A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Camag TLC scanner 3, Camag WinCATS software, Camag twin-trough chamber and ultrasonicator were used during the study.

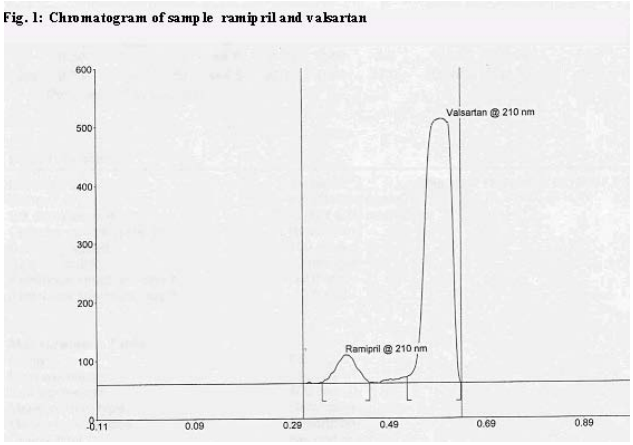
HPTLC method and chromatographic conditions [6-7]

In HPTLC method, several mobile solvent system were tried to accomplish a good chromatogram. The chromatographic estimations were performed using stationary phase, precoated silica gel $60F_{254}$ aluminium sheets (20 x 10 cm, prewashed with methanol, dried in oven at 50º for 5 min), mobile phase, chloroform: ethyl acetate: methanol: glacial acetic acid (5.0:5.0:1.0:02 v/v/v/v); dual chamber and plate saturation time of 30 min. Migration distance allowed was 80 mm; wavelength scanning was done at 210 nm. A solvent system that would give dense and compact spots with appropriate and significantly different R_f values was desired for quantification of ramipril and valsartan in pharmaceutical formulations. The mobile phase consisting of chloroform: ethyl acetate: methanol: glacial acetic acid (5.0:5.0:1.0:02 v/v/v/v) gives R_f values of 0.40 and 0.59 for ramipril and valsartan.

Calibration curve [8]

Aliquots of 2, 4, 6, 8, and 10 µl of standard solution of valsartan and 4, 8, 12, 16, and 20 µl of standard solution of ramipril were applied on the TLC plate. The TLC plate was dried, developed, scanned photometrically. The calibration curves were prepared by plotting peak area

versus concentration (µg/spot) corresponding to each spot. Chromatogram of ramipril and valsartan in which peak 1 and 2 are chromatograms of ramipril and valsartan respectively, and X-Axis indicates area under curve (AUC) while Y-Axis indicates R_f value (Figure-1).



Chromatogram of Sample Ramipril and Valsartan

Validation of the method [9-11]

The method was validated in term of linearity, accuracy, inter-day and intra-day, reproducibility and specificity and precision (%CV). The limit of detection (LOD) and limit of quantitation (LOQ) were also determined. The accuracy of the method was evaluated by carrying out recovery studies. Repeatability of the sample application was assessed by spotting ramipril and valsartan six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The %RSD for peak area values of ramipril and valsartan was found to be 0.21 and 0.40 respectively (Table-1).

Parameter	Result Ramipril	Valsartan
Linearity range (µg/spot)	0.4-2.0	0.2-1.0
Correlation coefficient	0.99850	0.99734
Precision (% CV)	1.601	2.150
Intra day (n=6)	0.21-0.44	0.13-.27
Inter day (n=6)	0.24-0.55	0.18-0.25
Repeatability of sample application (n=6)	200	100
Repeatability of peak area (n=6)	640	330
Limit of detection (ng/spot)	specific	specific
Limit of quantification (ng/spot)		

TABLE 1. VALIDATION PARAMETERS OF RAMIPRIL AND VALSARTAN

Results and Discussion [12-16]

Several mobile phase compositions were tried to resolve the peaks and get Rf value above 0.40. The developed HPTLC method is simple, precise, specific and accurate, and the statistical analysis proved that method is reproducible and selective for the analysis of ramipril and valsartan simultaneously in capsule formulation.

The assay value for marketed formulation was found to be within the limits as listed in the table 2. The low RSD value indicated the suitability of the method for routine analysis of ramipril and valsartan in pharmaceutical dosage forms.

TABLE 2. RECOVERY STUDY OF RAMIPRIL AND VALSARTAN

Label claim mg/capsule	Amount added (%)	Total amount added (mg)	Amount recovered* (mg) ± SD	%RSD
Ramipril 5	50	2.5	7.53±0.03	0.4
	100	5	9.89±0.04	0.4
Valsartan 80	50	40	119.6±0.95	0.79
	100	80	158.4 ±0.91	0.57

Recovery study of ramipril and valsartan. * indicates that each value is a mean ± standard deviation of three determinations.

To study the accuracy of the developed method, multilevel recovery studies were carried out. Sample stock solution from tablet formulation of 1 mg/ml and 100 µg/ml of ramipril and valsartan respectively were prepared. To the above prepared solution, 50%, 100% of the standard ramipril and valsartan was added. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within limits. The linear regression equations are y = 1043 + 6.344 * X for valsartan, and y = 85.57 + 2.101 * X for ramipril. The high percentage of recovery of drugs indicates that the method is accurate.

Repeatability of measurement of peak area was determined by spotting 6 µl of ramipril and 6 µl of valsartan solution on the TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and %RSD for measurement of the peak area of ramipril and valsartan was found to be 0.277 and 0.183 respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of ramipril and valsartan.

TABLE 3. INTRA-DAY AND INTER-DAY PRECISION STUDY (N = 3)

Concentration (ng/spot)		Intra-day (%RSD)		Inter-day (%RSD)	
Ramipril	Valsartan	Ramipril	Valsartan	Ramipril	Valsartan
400	200	0.444	0.277	0.554	0.223
800	400	0.325	0.205	0.395	0.242
1200	600	0.305	0.214	0.345	0.259
1600	800	0.417	0.170	0.466	0.172
2000	1000	0.212	0.136	0.249	0.184

RSD = Relative standard deviation

The intra-day and inter-day precision (RSD) were determined by analyzing standard solution in the concentration range of 0.2-1.0 µg/spot for valsartan and 0.4-2.0 for ramipril six times on the same day an over a period of 3 days. The intra-day and inter-day precision results are given in the table 3.

Linearity range for ramipril and valsartan was found to be in the range of 0.4-2.0 µg/spot and 0.2-1.0 µg/spot, with correlation coefficient of 0.99850 and 0.99734, respectively. The LOD and LOQ for ramipril and valsartan were found to be 200 ng/spot and 640 ng/spot for ramipril and 100 ng/spot and 330 ng/spot respectively for valsartan.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests