Simultaneous Estimation of Ramipril and Valsartan Using Planar Chromatography in Finished Pharmaceuticals

Nitin Dubey [a], *, Nidhi Dubey [b], Mayank Mandhanya [a], Nishant K. Shah [C]

a. College of Pharmacy, IPS Academy, Indore [M.P.], India.

b. School of Pharmacy, D.A.V.V., Indore [Madhya Pradesh], India

c. Shobhaben Pratapbhai Patel School of Pharmacy and Management, Mumbai, India

---

Abstract

Method for simultaneous estimation of ramipril and valsartan a chromatographic methods have been developed. The first method was based on HPTLC separation. Method was based on high performance thin layer chromatographic separation of two drugs using silica gel 60 F_{254} as stationary phase and toluene: acetonitrile: formic acid: glacial acetic acid (6.0:4.0:0.3:0.1 v/v/v/v) as mobile phase, followed by densitometric estimation at 225 nm. The drugs were satisfactorily resolved with R_f values 0.27 and 0.69 with a linear dynamic range of 400–2000 ng/spot for ramipril and valsartan respectively. Methods was validated and found to be simple, precise, specific, sensitive and accurate. The method was successfully applied for the determination of both the drugs from combined dosage form.

Keywords: *Planar Chromatography, Ramipril; Valsartan; HPTLC; Quantitative analysis*

Introduction

Recent trends indicate that Planar chromatography (HPTLC) is the technique of choice for estimation of analytes in sample matrix [6-9]. Ramipril (RAM) [(2S, 3aS, 6aS)-1[(S)-N-[S-1-Carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta pyrrole-2-carboxylicacid, 1-ethylester] is angiotensin-converting enzyme [ACE] inhibitor and prescribed for treatment of congestive heart failure and hypertension. Ramipril is official in British pharmacopoeia [1, 2]. Valsartan (VAL) [3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino]-butanoic acid] is angiotensin II receptor antagonist, and prescribed for treatment of hypertension. Combined dosage form of these two drugs is used for management and treatment of hypertension.

Literature survey revealed that various analytical methods like UV, LC, LC-MS [3-6] have been reported for the estimation of RAM alone or in combination with other drugs. Various LC [7-10] methods have been reported for the estimation of VAL in combination with other drugs in biological fluids and pharmaceutical dosage forms. But no method is reported for simultaneous estimation of ramipril and valsartan in combined dosage form.

HPTLC is a versatile analytical technique that requires less expensive instrumentation and expertise. The main advantages of HPTLC are the simultaneous separation of several samples on a single plate and in situ purification and separation of analytes. Moreover, HPTLC analysis needs small amounts of developing solvent and minimal sample

preparation. Use of UV/fluorescent detectors coupled with software enables HPTLC on par with HPLC with respect to the sensitivity of analyte detection. The ease of operation and shorter period of analysis is an added advantage of HPTLC [13, 14].

The apparent lack of a method for the estimation of RAM and VAL in finished pharmaceuticals (combined capsule dosage form) prompted us to develop accurate, specific and sensitive liquid chromatographic method. In the present study, simultaneous quantification of ramipril and valsartan in capsules by HPTLC method was developed.

Experimental

Instrumentation

UV spectral measurements were recorded in Perkin Elmer, U.S.A, Lambda 19, UV-Visible spectrophotometer. HPTLC system (Camag, Muttenz, Switzerland) consisted of a linomat V sample applicator equipped with a 100 µl Hamilton syringe and a scanner III operated using WINCATES software, Camag, loaded on a personal computer. HPTLC plates (20 cm×20 cm), pre-coated with silica gel $60F_{254}$ were purchased from E Merck (Darmstadt, Germany). Plates were pre-developed with methanol and dried at room temperature before application of the reference standards and samples. All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

Chemicals and reagents

Pure drugs RAM and VAL were obtained as gift samples from Lupin pharmaceuticals Ltd., Ankleshwar and Vasudha pharmaceuticals Ltd., Hyderabad respectively. Capsule preparations were procured form local market and designated as RV-1 and RV-2. Analytical reagent grade O-phosphoric acid and potassium dihydrogen phosphate were obtained from S. D. Fine chemicals, Mumbai, India. Toluene, formic acid and glacial acetic acid were of analytical reagent grade and procured from S. D. Fine chemicals, India.

HPTLC method

Preparation of standard stock solutions

Stock solutions were prepared by accurately weighing 25 mg each of RAM and VAL and transferring to two separate 25 ml volumetric flasks containing a few ml of methanol. The flasks were swirled to dissolve the solids. Volumes were made up to the mark with methanol to yield a solution containing 1000 µg/ ml of RAM and VAL. Aliquot from the stock solution of RAM and VAL were appropriately diluted with mobile phase to obtain solutions of 100 µg/ ml of each.

Chromatographic development

Plates were developed using a mobile phase consisting of toluene: acetonitrile: formic acid: glacial acetic acid (6:4:0.3:0.1, v/v/v/v). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 30 min at 25 ^{0}C ± 2 C. Ten milliliters of the mobile phase was used for each development and was allowed to migrate a distance of 80 mm. After development, the HPTLC plates were dried completely.

Densitometric analysis

Densitometric scanning was performed in the absorbance mode under control by winCATS planar chromatography software. The source of radiation was the deuterium lamp, and bands were scanned at 225 nm. The slit dimensions were 5 mm length and 0.45 mm width, with a scanning rate of 20 mm/s. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using a linear regression equation.

Method Validation

Validation of the developed HPTLC method was carried out according to International Conference on Harmonization (ICH) guidelines Q2 (R1) for accuracy, precision, repeatability, limit of detection, limit of quantitation and solution stability study [15].

Linearity

Linearity of the method was evaluated by constructing calibration curves at six concentration levels over a range of 400–2000 ng/band. The calibration curves were developed by plotting peak area versus concentration (n = 6) with the help of the winCATS software.

Precision

The intra-day and inter-day precision study of RAM and VAL was carried out by estimating the corresponding responses three times on the same day and on three different days for three different solutions containing RAM (400, 1200, 2000 ng/spot) and VAL (400, 1200, 2000 ng/spot) in mixture, and the results are reported in terms of RSD. The instrumental precision was evaluated by injecting mixed solutions containing three different concentrations of RAM (400, 1200, 2000 ng/spot) and VAL (400, 1200, 2000 ng/spot) six times and results are reported in terms of RSD.

Accuracy

The accuracy of the method was determined by calculating recoveries of RAM and VAL by method of standard additions. Known amount of RAM (0, 400, 800, 1200 ng/spot) and VAL (0, 400, 800, 1200 ng/spot) were added to a pre quantified sample solution, and the amount of RAM and VAL were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Detection limit and Quantitation limit

Limit of detection and limit of quantification were calculated using following equation as per ICH guidelines.

LOD = 3.3 ×σ /S; LOQ = 10 ×σ /S; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Solution stability study

Robustness method was studied by observing stability of the sample solution at 25 ± 2°C for 24 h.

Analysis of marketed formulation

The contents of 20 capsules (labeled claim: 5 mg of RAM and 80 mg of VAL per capsule), were weighed and finely powdered. The powder equivalent to 2.5 mg of RAM and 40 mg of VAL was accurately weighed and transferred to 50 ml volumetric flask containing 25 ml methanol and sonicated for 5 minutes. The flask was shaken and volume made up to the mark with methanol. To ensure complete extraction of the drug, the resulting solution was care fully centrifuged at 4000 rpm for 20 min and supernatant was filtered using What man filter paper (No.41). The aliquote1 ml of above solution was transferred to volumetric flask of 100 ml. Further 0.75 ml of 100 µg/ml of standard stock solution of RAM was added to this volumetric flask and volume made up to the mark by methanol to get a solution containing 80 µg/ml of RAM and 80 µg/ml of VAL respectively. 10µl of sample was spotted by using a semi automatic sampler of capacity 100 µl on to HPTLC plates.

Results and discussion

HPTLC method

To develop the HPTLC method of analysis of RAM and VAL in combined dosage form for routine analysis, selection of the mobile phase was carried out on the basis of polarity. A mobile consisting of toluene: acetonitrile: formic acid: glacial acetic acid (6: 4: 0.3: 0.1 , v/v/v/v) gave good resolution between RAM and VAL in combined dosage form. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation as chamber saturation time of less than 30 min and solvent migration distances greater than 80 mm resulted in diffusion of the analyte band. Therefore, toluene: acetonitrile: formic acid: glacial acetic acid (6: 4: 0.3: 0.1 , v/v/v/v) mobile phase with a chamber saturation time of 30 min at 25 ^{0}C and solvent migration distance of 80 mm was used. These chromatographic conditions produced a well-defined, resolved band of RAM and VAL with Rf 0.27 ± 0.02 for RAM and 0.69 ± 0.03 for VAL respectively (Figure 1).

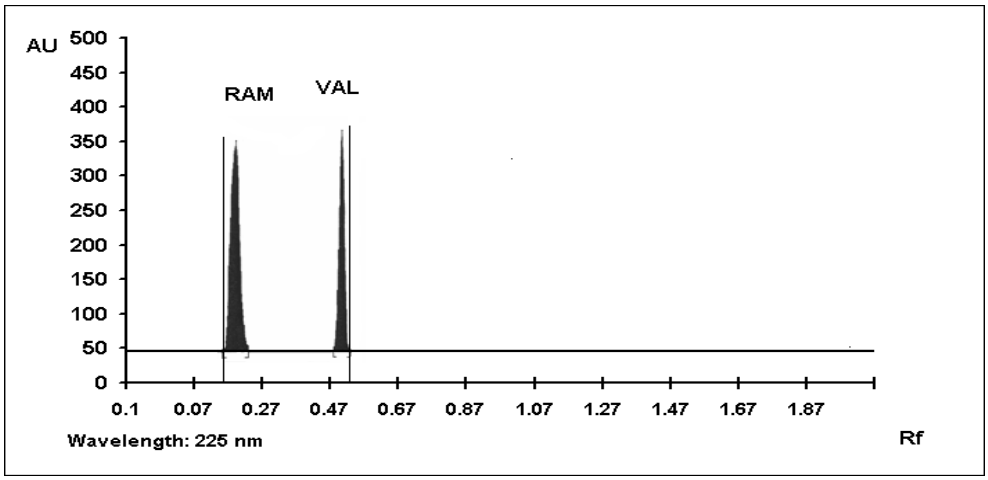

Figure 1: HPTLC densitogram showing well resolved spot for RAM and VAL.

The method was found to be linear in a concentration range of 400–2000 ng/band (n = 6) for both the drugs. Instrument precision was determined by performing repeatability test and the RSD values for RAM and VAL were found out. The intra-day and inter-day precision studies were carried out and the results are reported in terms of RSD (Table I). The low RSD values indicate that the method is precise.

Table 1 Method validation parameters for estimation of RAM and VAL by the proposed methods.

	HPTLC method	
Parameters	**RAM**	**VAL**
Instrumental precision (RSD,%, n=7)	0.78	0.67
Precision (CV, n=3)		
Intraday	1.28-1.68	1.43-2.72
Interday	1.31-1.71	1.50-2.79
Repeatability (RSD,%, n=5)	0.015	0.021
Limit of detection	130 ng/spot	80 ng/spot
Limit of quantitation	400 ng/spot	250 ng/spot
Specificity	Specific	Specific
Selectivity	Selectivity	Selectivity
Linearity		
Range	400-2000 ng/spot	400-2000 ng/spot
Correlation coefficient	0.9997	0.9992

The accuracy of the method was determined by calculating recoveries of RAM and VAL by method of standard addition. The recoveries were found to be 97.79 – 98.36 % and 98.12 – 100.56 % for RAM and VAL, respectively. The values indicate that the method is accurate. The detection limits for RAM and VAL were 130 ng/spot and 80 ng/spot, respectively, while quantitation limits were 400 ng/spot and 250 ng/spot, respectively.

The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The solution stability study revealed that RAM and VAL stock solutions were stable for 24 h without detectable degradation.

Marketed formulation was analyzed using proposed method which gave percentage recovery of 98.16–101.20 for RAM and 98.45 – 99.87 % for VAL respectively. there was no interfereing band observed in the chromatogram.

Conclusion

Proposed study describes HPTLC method for the estimation of RAM and VAL in combined dosage forms. The method were validated and found to be simple, sensitive, accurate, precise. The methods were successfully applied for determination of drugs in their pharmaceutical formulations. Compared to RP-HPLC method HPTLC method is simple, time consuming and can be used for the routine analysis of RAM and VAL from combined dosage forms.

Acknowledgements
Authors are grateful to Lupin pharmaceuticals Ltd., Ankleshwar and Vasudha pharmaceuticals Ltd., Hyderabad, India for providing gift sample of ramipril and valsartan, respectively.

References:

1. Anonymous (2004) *The Physician desk reference*, Govt. of India, pp 1216-1221.

2 Martindale (2002) *The complete drug reference*, Pharmaceutical press, pp 1224, 1239-1240, 1245.

3 Majed, A. l., Belal, F. and Al-warthan, A. A. (2001) *Spectroscopy Letters* 34(2), 211 – 220.

4 Tracqui, A., Kintz, P. and Mangin, P. (1995) *Journal of forensic science* 40 (2), 254-262.

5 Belal, F., Al-zaagi, I. A., Gadkareim, E. A. and Bounassif, M. A. A. (2001*) Journal of pharmaceutical and biomedical analysis* 24 (3), 335-342.

6 Dubey, N., Dubey, N., Mehta, R. S. and Saluja, A., K., (2009) *Journal of AOAC International* 92 (3), 779-784.

7 Dubey, N., Dubey, N., Mehta, R. S. and Saluja, A., K., (2009) *Journal of AOAC International* 92 (6), 1617-1621.

8 Dubey, N., Dubey, N., Mehta, R. S. and Saluja, A., K., (2009) *Journal of AOAC International* 92 (4), 1021-1026.

9 González, L., Alonso, R. M.and Jiménez, R. M. (2000) *Chromatographia* 52 (11-12), 735-740.

10 Dubey, N., Jain, D. k., Jadhwani, S. (2012). *Journal of Planar Chromatography: Modern TLC* 25(2), 162-167.

11 Lindsay, S. (1992) *High Performance Liquid Chromatography (Analytical Chemistry By Open Learning)*, John Wiley and sons Inc., pp 30-45.

12 Skoog, D. A., Holler, F.J., Nieman, T.A. (2006) *Principles of instrumental analysis*, Brooks Cole, pp 728-744.

13 Fried, J. B. (2003) *Handbook of thin layer chromatography*, Marcel Dekker, NewYork .

14 Sethi, P.D. (1996) *High performance thin layer chromatography*, CBS publishers, New Delhi, pp 1-68.

15 Carstensen, J.T. (1990) *Drug Stability*, Marcel Dekker, NewYork.