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HPTLC method for simultaneous estimations of ramipril and losartan potassium in pharmaceutical dosage form: development and validation considerations

Darshana A. Parmar[1], Dinesh V.Thakkar[1], Rashmin B. Patel[1*] and Mrunali R. Patel[2]

[1]*Department of Quality Assurance, A. R. College of pharmacy and G. H. Patel institute of Pharmacy, Vallabh Vidyanagar 388120, Anand, Gujarat, India*
[2]*Department of Pharmaceutics and Pharmaceutical Technology, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar 388121, Anand, Gujarat, India*

Abstract

This paper describes validated high-performance thin-layer chromatography (HPTLC) methods for simultaneous estimation of ramipril (RAM) and losartan potassium (LOS) in pure powder and formulation. The HPTLC separation was achieved on an aluminum-backed layer of silica gel $60F_{254}$ using methanol: ethyl acetate: toluene: glacial acetic acid (1:9:1:0.2 v/v/v/v) as mobile phase. Quantification in HPTLC method was achieved with UV detection at 210 nm over the concentration range of 300 – 1300 ng/spot for RAM and 3000 – 13000 for LOS, respectively, with recovery of 98.93 - 99.73 and 98.96 - 100.11 % for RAM and LOS, respectively. These methods are simple, specific, precise, sensitive and robust; they are applicable for the simultaneous determination of RAM and LOS in pure powder and formulation.

Keywords: HPTLC, Losartan potassium, Ramipril, Simultaneous estimation, Validation

Introduction

Ramipril (RAM) is chemically (2S,3aS,6aS)-1-[(2S)-2-[[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]octahydrocyclopenta[b]pyrrole-2-carboxylic acid used as antianginal, antihypertensive drug. Losartan potassium (LOS) is chemically monopotassium salt of 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4yl]methyl]-1*H*-imidazole-5-methanol used as anti-hypertensive drug by inhibiting angiotensin II receptor. The combined tablet dosage form of RAM and LOS is available in market and it is indicated in the treatment of hypertension [1-4].

Literature survey revealed that HPLC methods [1-4] have been reported for estimations of RAM and LOS in combine pharmaceutical dosage forms. Literature survey also revealed that various HPLC, HPTLC and spectrophotometric methods has been reported for the estimations of RAM or LOS individually and in combination with other drugs [5-17]. To our present knowledge, HPTLC method has not been explored for simultaneous determination of RAM and LOS in pharmaceutical dosage form till date. Therefore, it was felt necessary to develop a HPTLC method for determinations and quantitative estimations of RAM and

Correspondence to: Rashmin B. Patel, Department of Quality Assurance, A. R. College of pharmacy and G. H. Patel institute of Pharmacy, Vallabh Vidyanagar 388120, Anand, Gujarat, India
Phone and Fax: +91-2692-230788
Email: rbp.arcp@gmail.com

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LOS in pharmaceutical dosage form. In view of this, the present study describes development and validation of HPTLC method for simultaneous estimations of RAM and LOS in pharmaceutical formulation.

Materials and Methods

Chemicals and reagents: Pure drug samples of RAM and LOS were procured as gift samples from Redson pharmaceutical, (Ahmedabad, Gujarat, India). Commercial pharmaceutical tablets: Loram (5mg RAM and 50 mg LOS) (Unichem Laboratories, India) was procured from local market. Methanol, ethyl acetate, toluene and glacial acetic acid of (analytical reagent grade) and all other chemicals were purchased from Allied Chemical Corporation, (Vadodara, Gujarat, India).

Instruments and chromatographic condition: Chromatography was performed on 10 cm × 10 cm aluminum TLC plates precoated with silica gel of 250 µm thickness (Merck, Darmstadt, Germany). The TLC plates were prewashed by methanol and activated at 80 ºC for 5 min prior to chromatography. Samples were applied as 6 mm wide bands by means of CAMAG (Muttenz, Switzerland) Linomat V sample applicator fitted with 100 µl applicator syringe (Hamilton, Bonaduz, Switzerland). A constant application rate of 150 nL/s was employed and the space between two adjacent bands was 5 mm. The TLC plates were then conditioned for 20 min in a presaturated twin-trough glass chamber (10 cm × 10 cm) with the mobile phase of methanol: ethyl acetate: toluene: glacial acetic acid (1:9:1:0.2 v/v/v/v). The TLC plate was developed using mobile phase (ascending development) to a distance of 60 mm from the point of application at ambient temperature. Subsequent to the development, the TLC plates were dried and spots were visualized in CAMAG UV cabinet (Muttenz, Switzerland) with dual wavelength UV lamp (254 and 366 nm); densitometric scanning was performed at 210 nm with CAMAG TLC scanner III (Muttenz, Switzerland) operated in reflectance-absorbance mode and controlled by WinCats software (version 1.3.4). The slit dimensions were 5.0×0.45 mm at 20 mm/s scanning speed. The concentrations of compound were studied from the intensity of diffusely reflected light. Evaluation was based on linear regression of peak areas.

Preparation of mixed standard stock solution of RAM and LOS: Mixed standard stock solution was prepared by dissolving accurately weighed 10 mg of RAM and 100 mg LOS with 100 mL of methanol. Mixed standard stock solution of combined drugs was prepared containing 100 µg/mL RAM and 1000 µg/mL LOS.

Method validation: Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness [18].

Linearity: Each concentration in the range of 300-1300 ng/band for RAM (3, 5, 7, 9, 11, and 13 µL of stock solution) and 3000-13000 ng/band for LOS (3, 5, 7, 9, 11, and 13 µL of stock solution) spotted six times on individual TLC plates and response was measured after scanning. For evaluation of linearity, peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient [18, 19].

Precision: The intraday and inter-day precision of the method were determined by estimating the corresponding response three times on the same day and on three different days over a period of one week for three different concentrations of RAM (500, 700, and 900 ng/band) and LOS (5000, 7000, and 9000 ng/band). The precision of the method was evaluated by calculating the percent relative standard deviation (% RSD) [18].

Repeatability: Repeatability of sample application was assessed by spotting RAM (700 ng/band) and LOS (7000 ng/band) six times on TLC plate, developing the plate and recording peak area for the spots. The repeatability of the method was evaluated by calculating the percent relative standard deviation (% RSD) of mean peak area obtained from each spot of sample [18].

Accuracy: The accuracy of the method was determined by recovery studies using method of standard additions. To the pre-analyzed sample solution, a known amount of standard solution of pure drug was spiked at three different levels (80 %, 100 % and 120 %) of the target concentration. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay [18].

Sensitivity: Limits of detection (LOD) and quantitation (LOQ) were determined as signal-to-noise ratio using the equations $LOD=3.3\times N/B$ and $LOQ=10\times N/B$, where N is the standard deviation of the Y-intercept and B is the slope of the calibration curve [18].

Specificity: Specificity of the method was ascertained by analyzing standard drug and sample. The spot for RAM and LOS was confirmed by comparing the R_f and spectra of the spot with that of standard. The wavelength 210 nm for detecting peak purity of RAM and LOS was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions [18, 19].

Robustness: Robustness was evaluated by changing the composition of mobile phase (± 5 %) and spot stability by performing two-dimensional chromatographic development using the same mobile phase. Saturation time for the chromatographic development was changed by 10 min and the effect was observed [18, 19].

Analysis of RAM and LOS in marketed tablet formulation: To determine the content of RAM and LOS simultaneously in marketed tablet dosage form (LORAM *5, label claim 5 mg RAM and 50 mg LOS); twenty tablets were accurately weighed, average weight determined and grounded to fine powder. A quantity of powder equivalent to 10 mg (RAM) and 100 mg (LOS) was transferred into a 100 mL volumetric flask and extracted with methanol by sonication for 20 min. The solution was diluted to the mark with methanol to obtain a final concentration of 100 µg/mL of RAM and

1000 µg/mL LOS. The resulting solution was filtered using 0.45 µm filter. 5 µL of sample solution containing 500 ng of RAM and 5000 ng of LOS was applied on the TLC plate followed by development and scanning at 210 nm. The analysis was repeated for five times. The concentration of drugs was determined from the linear regression equation and % label claim was calculated.

Results and Discussion

Method development

Selection of best solvent system is the critical step in HPTLC method development. From the different solvent systems tried, mobile phase consisting of methanol : ethyl acetate : toluene : glacial acetic acid in the ratio 1:9:1:0.2 (v/v/v/v) resulted in better separation which gave symmetric peaks of RAM with R_f of 0.41 ± 0.01 and LOS with R_f of 0.70 ± 0.01, respectively (**Figure 1** and **2**). Well-defined bands were obtained when the chamber was saturated with mobile phase for 20 min at ambient temperature. For quantitative purpose, the densitometric scanning was carried at a wavelength of 210 nm where RAM and LOS exhibited good UV absorption (**Figure 3**).

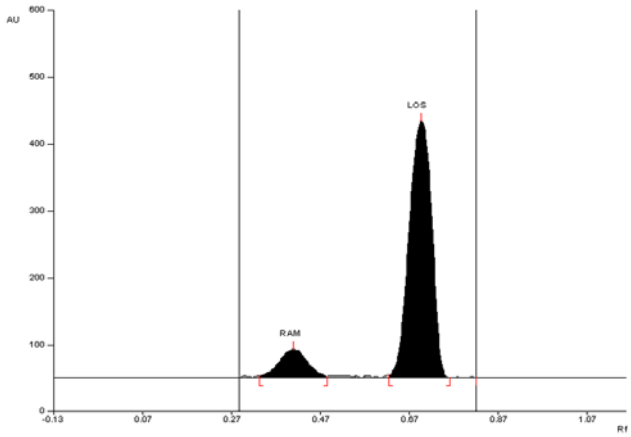

Figure 1 Chromatogram of standard RAM (700 ng/spot) and LOS (7000 ng/spot) using methanol: ethyl acetate: toluene: glacial acetic acid (1:9:1:0.2 v/v/v/v) as mobile phase.

Method validation

Linearity was observed over the concentration range of 300–1300 ng/band for RAM and 3000–13000 ng/band for LOS, respectively. The regression equations (n=6) were y=1.92x+583.60 for RAM and y=0.84x+7762.33 for LOS, respectively. The calibration curves for RAM and LOS using proposed HPTLC methods are shown in **Figure 4**. The correlation coefficients (r) were 0.998 and 0.997 for RAM and LOS, respectively (**Table 1**). If statistically proven, the calibration can be accepted as linear. However, declaring method linearity on the basis of correlation coefficient value as a sole proof of linearity is inappropriate. Different approaches for checking linearity objectively are reported and should be used. The simplest method of linearity test for HPTLC method is residual plot. Residuals are smallest possible differences, which may be acquired by reducing the distance between the experimental points and regression line. Residuals are

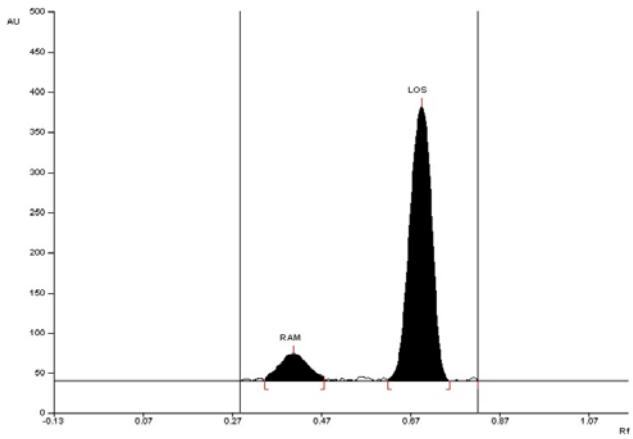

Figure 2 Chromatogram of RAM (500 ng/spot) and LOS in tablet formulation (5000 ng/spot) using methanol: ethyl acetate : toluene : glacial acetic acid (1:9:1:0.2 v/v/v/v) as mobile phase.

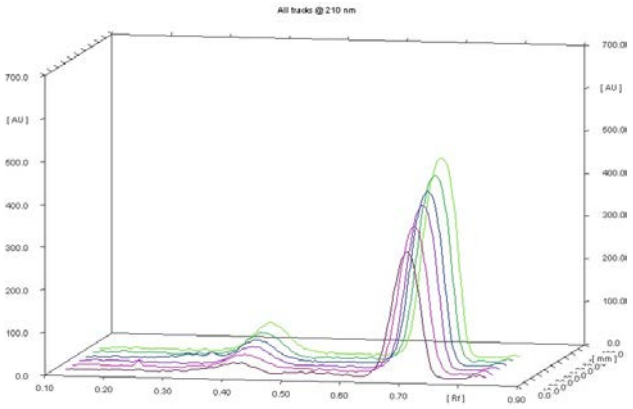

Figure 3 Three - dimensional overlay of HPTLC densitograms of calibration spots of RAM and LOS.

Table 1 Regression analysis of calibration curves for RAM and LOS for the proposed HPTLC method

Parameter	HPTLC method	
	RAM	LOS
Concentration range	300-1300 ng/spot	3000-13000 ng/spot
Slope	1.92	0.84
Standard deviation of the slope	0.045	0.004
Intercept	583.6	7762.3
Standard deviation of the intercept	41.22	11.79
Correlation coefficient	0.998	0.997

distributed randomly at both sides of the straight-line. Opposite deviations (positive and negative) would cancel each other out and square of residuals will be summarized and minimized giving its name least square regression. In this study, linearity was confirmed since residuals were randomly distributed around the regression line (no lack of fit) (**Figure 4**).

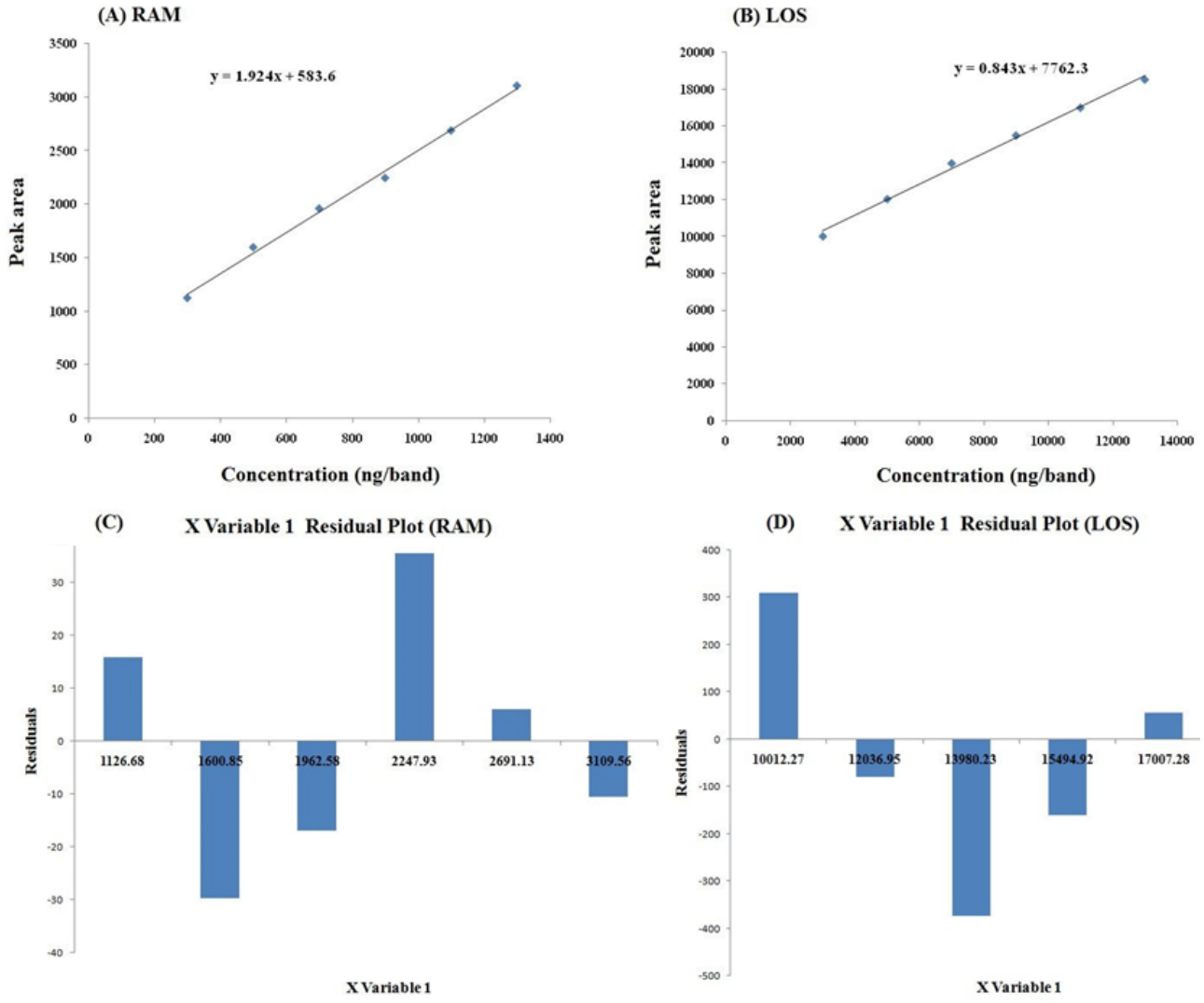

Figure 4 Calibration curves (A, B) and residual plots (C, D) for RAM and LOS using proposed HPTLC method.

Table 2 Summary of validation parameters for the proposed HPTLC method

Parameter	HPTLC method		Acceptance criteria
	RAM	LOS	
LOD[a]	70.71 ng/mL	46.14 ng/mL	< $conc._{min}$
LOQ[b]	241.83 ng/mL	139.86 ng/mL	< $conc._{min}$
Accuracy, %	98.93 - 99.73	98.96 - 100.11	95 - 102 %
Repeatability (RSD, %, n=6)	0.95	0.22	< 2 %
Reproducibility (RSD, %)			
Interday (n=3)	0.880 - 1.57	0.25 - 0.61	< 2 %
Intraday (n=3)	0.58 - 1.31	0.21 - 0.37	< 2 %
Specificity	specific	specific	specific
Robustness	robust	robust	robust

Intermediate precision was evaluated at different times on the same day and on different days. Low values of RSD (less than 2 %) obtained in the studies indicated that the method was precise and reproducible (**Table 2**). System precision of the proposed method was evaluated and % RSD for peak area were found to be 0.95 and 0.22 (less than 2 %) for RAM and LOS, respectively (**Table 2**). Replicate analyses of the standard and sample solutions showed good reproducibility, with RSD values less than 1.0 % for both repeatability and precision experiments.

When the method was used for accuracy and subsequent analysis of both drugs from the pharmaceutical dosage form, and spiked with 80, 100, and 120 % of additional pure drug, the mean recovery was found to be 98.93 - 99.73 % for RAM and 98.96 - 100.11 % for LOS, respectively (**Table 2**). Recovery values revealed the high efficiency of the HPTLC method for extraction of RAM and LOS without pretreatment.

The limits of detection (LOD) and quantification (LOQ) for RAM were 70.71 ng/band and 214.83 ng/band, respectively. For LOS the values were 46.14 ng/band and 139.86 ng/band, respectively (**Table 2**).

The peak purity of RAM and LOS were assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9995 and r (M, E) = 0.9993 for RAM and r (S, M) = 0.9994 and r (M, E) = 0.9996 for LOS. Good match was obtained between standard and sample spectra of RAM and LOS respectively. System suitability parameters for the proposed method are shown in **Table 3**.

No decomposition was observed in both first and second direction of the run for the both drugs on plate. No change in R_f values were observed when saturation time and mobile phase composition changed. Hence, method was found to be robust for estimation of LOS and RAM.

Table 3 System suitability test parameters for RAM and LOS for the proposed HPTLC method

Parameters	RAM ± % RSD[a] (n^b = 6)	LOS ± % RSD[a] (n^b = 6)
R_f	0.41 ± 0.01	0.7 ± 0.01
Area (average)	1962.58 ± 32.72	13980.23 ± 44.50
Peak purity	> 0.999	> 0.999

[a]RSD = relative standard deviation
[b]n = number of determination

Analysis of RAM and LOS in marketed formulation

The proposed developed and validated HPTLC method was successfully applied for quantitative estimation of RAM and LOS in their marketed formulations (LORAM). The assay results obtained were accurate, and precise as indicated by the good recovery and acceptable standard deviation values (**Table 4**). The good performance of the method indicates that it can be used for the determination of RAM and LOS in pharmaceutical formulations.

Table 4 Assay results for the combined dosage form using the proposed HPTLC method

Assay	RAM ± % SD[a] (n^b = 6)	LOS ± % SD[a] (n^b = 6)
Tablet	99.32 ± 1.03	100.58 ± 0.92

[a]SD = relative standard deviation
[b]n = number of determination

Conclusion

This developed and validated HPTLC method for simultaneous analysis of RAM and LOS in pharmaceutical preparations was very simple, rapid, accurate, and precise. The method was successfully applied for determination of RAM and LOS in their pharmaceutical tablet formulation. Moreover, it showed advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly analytical time reduction per sample. Hence, this method can be conveniently used for routine quality control analysis of RAM and LOS in their pharmaceutical formulation.

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