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HPTLC method development and validation for the estimation of Lopinavir and Ritonavir in capsule dosage form

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ABSTRACT

A simple, selective, linear, precise and accurate HPTLC method was developed and validated for rapid assay of Lopinavir and Ritonavir in capsule dosage form. The separation was achieved on aluminum plate 60F254, (10 × 10 & 20 × 10 cm) with 250 µm thickness as the stationary phase and the mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2, v/v). The solvent system was found to give compact spot for Lopinavir and Ritonavir (Rf values of 0.81 and 0.53 min). Densitometry analysis was carried out in the absorbance mode at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with respect to peak area in the concentration range 100-600 µg and 100-700 µg of Lopinavir and Ritonavir (with r = 0.9999 and 0.9999). The method was validated for limit of detection, limit of quantitation, accuracy, precision, robustness and recovery. The result and statistical analysis proves that the developed method is reproducible and selective for the estimation of said drug. The proposed method can be successfully applied for the estimation of Lopinavir and Ritonavir in capsule dosage forms.

Keywords: Lopinavir and Ritonavir; HPTLC; Densitometric analysis Validation.

INTRODUCTION

The chemical name for Lopinavir is (2S)-N-[(2S,4S,5S)-5-[2-(2,6-dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphennylhexane-2yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide. Whereas the chemical name for ritonavir is 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3 thiazol -4-yl]methyl})ncarbonyl] amino} butanamido]-1,6-diphenylhexane-2-yl] carbamate. The mechanism of action of lopinavir and ritonavir is to inhibit the HIV viral Protease enzyme. This prevents cleavage of the gag-pol polyprotein and therefore results in improper viral assembly and subsequently results in non-infectious, immature viral particles. There are various methods for estimation of lopinavir and ritonavir by spectroscopy, HPLC and HPTLC in single and some in combination dosage forms. The present developed method is simple, precise, specific and accurate and validated as per ICH guidelines. The statistical analysis proved that method is reproducible and selective for the analysis of Lopinavir and Ritonavir in bulk drug and capsule formulation using HPTLC.

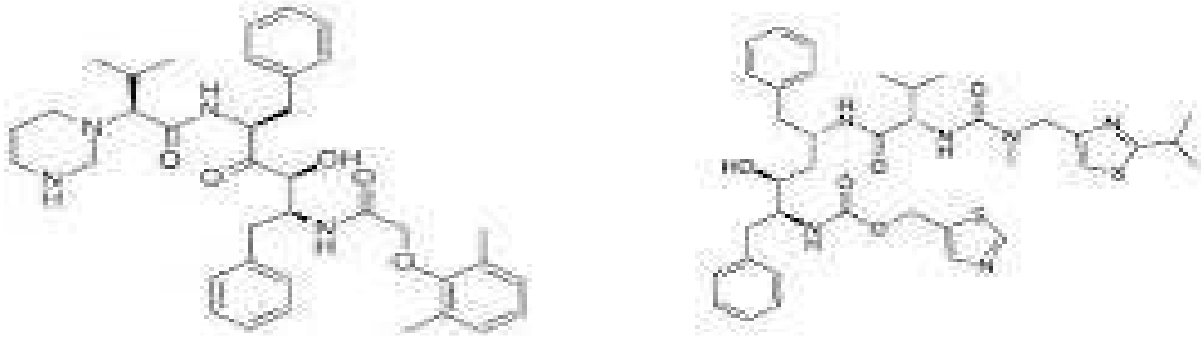

Fig. 1: Chemical structures of Lopinavir and Ritonavir

MATERIALS AND METHODS

Instrumental and analytical conditions:

Standard experimental conditions were optimized in view to develop an assay method to quantify Lopinavir and Ritonavir as in its capsule dosage form. Samples was spotted in the form of band of 2 mm with CAMAG microlitre syringe on pre-coated silica gel aluminum plate 60F254, (10 × 10 & 20 × 10 cm) with 250 µm thickness; using CAMAG LINOMAT V semiautomatic sample applicator and LINOMAT V automatic sample applicator with help of (Hamilton-100 µl Switzerland) syringe. The plates were prewashed with methanol so as to remove adhere impurity and activated at room 120°C for 5 min prior to chromatography. Samples were applied as band at a distance of 8 mm from lower edge and the distance between two bands was 4 mm. The mobile phase consisted of chloroform: methanol: ammonia (8:2:0.2v/v/v) was optimized for good resolution with compact spots. The length of chromatogram run was 80 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorption mode at 254 nm. Reagents and chemicals: Analytically pure Lopinavir and Ritonavir and capsule formulation was gifted by Cipla Pharmaceutical Limited, Kurkumbh. All chemicals and reagents used were of AR grade, from Merck Chemicals (Mumbai, India). Preparation of Analytical solutions: Preparation of mobile phase: Mobile phase was prepared by mixing 8 ml chloroform, 2 ml of methanol and 0.2 ml of ammonia. Preparation of standard stock solution: The stock solutions (1000 µg/ml) of LOP and RIT was prepared by accurately dissolving 10 mg of the drugs with sufficient methanol in 10 ml volumetric flask and then the volume was made separately to 10 ml with methanol. Preparation of standard solution: 5.0ml of LOP and RIT stock solution further diluted to 10 ml with methanol to get final concentration of 0.5 µg/µl of LOP and RIT. Then further take 5 ml and diluted to 10 ml to get concentration 0.25 µg/µl. Preparation of sample stock solution: Twenty capsule were weighed and average weight was calculated. The capsules were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of LOP and RIT was transferred to 10 ml volumetric flask. To it add 5 ml of methanol shake well and sonicated for 10 min. The resultant solution was filtered through 0.45µm membrane filter, diluted to volume with methanol to get stock sample solution containing 10 µg/µl of LOP and RIT.

Preparation of standard stock solution

The stock solution (1000 µg/ml) of LOP and RIT were prepared by accurately dissolving 10 mg of both the drugs with sufficient methanol in 10 ml volumetric flask and then the volume was made separately to 10 ml with methanol.

Preparation of working standard solution (mixed)

1ml of standard stock solution was further diluted to 10ml with methanol so as to give final concentration 100µg/ml.

Preparation of sample solution

Twenty capsules were emptied and find out the average weight. An accurately weighed quantity of powder equivalent to 133.3mg and 33.3 mg of LOP and RIT was transferred to 10 ml volumetric flask. To it add 5 ml of methanol shake well and sonicate for 10 min. The resultant solution was filtered through 0.45µm membrane filter, diluted to volume with methanol to get stock sample solution containing 5 mg/ml and 1.2 mg/ml of LOP and RIT

Analysis of the marketed formulation:
The marketed formulation was analyzed using the developed method. The chromatogram of marketed formulation sample showed only two peaks at Rf value of 0.81 and 0.53 for LOP and RIT respectively as similar with bulk drug LOP and RIT indicating that there is no interference of the excipients present in the tablet formulation. The content of LOP and RIT was calculated by comparing peak areas of sample with that of the standard. The drug content was found to be 100 ±0.25 % for LOP and 100.2 ± 0.90 % for RIT for six replicate determinations.

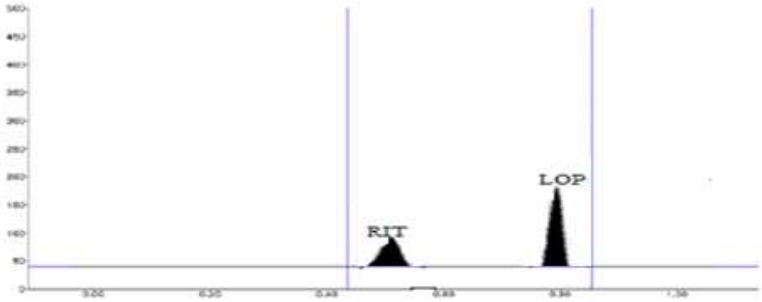

Fig. 2: Chromatogram represents Lopinavir and Ritonavir in combination

Method development and validation of HPTLC:
Linearity:
1,2,3,4,5,6 and 7 µl of mixed working standard solution were applied to TLC plate so as to give concentration 100,200,300,400,500,600 and 700 ng/spot for LOP and 100,200,300,400,500,600 and 700 ng/spot for RIT.

The plate was developed in mobile phase, dried in the flow of hot air and densitometrically scanned under standard chromatographic condition. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

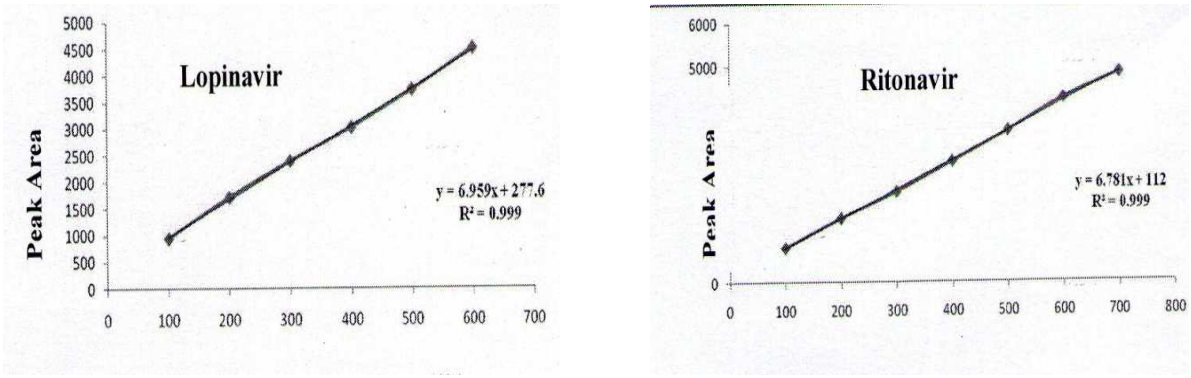

Fig. 3: Linearity curve of Lopinavir and Ritonavir

Precision:
Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Interday precision and intraday precision were determined both in terms of repeatability (injection and analysis). The intermediate precision of method was checked by repeating the study on different days. The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same band. The intermediate precision of method was checked by repeating the study on different days.

Accuracy:
The recovery studies were carried out by adding known amount of standard to samples at 80, 100 and 120% level and analyzed by the proposed method, in triplicate. This was done to check the recovery of the drug at different levels in the formulations by optimized method.

Limit of detection and limit of quantitation:
The limits of detection and quantitation of the developed method were calculated for LOP and RIT using the formula as given below. Limit of Detection=3.3 x σ/S and Limit of Quantitation=10 x σ/S Where, "σ" is the standard deviation of the response, "S" is the slope of the calibration curve.

Specificity:
The specificity of the method was ascertained by analyzing the standard drug and sample with respect to Rf value and spectra. The peak purity of LOP and RIT was assessed by comparing the spectra of diluents, mobile phase, standard and sample.

RESULTS AND DISCUSSION

The present investigation reported a new HPTLC method development and validation of estimation of LOP and RIT. The method developed was proceeding with wavelength selection. The optimized wavelength was 254nm.In order to get the optimized HPTLC method various mobile phases were used. The mobile phase consisted of an aqueous solution of chloroform: methanol: ammonia (8: 2: 0.2 v/v) was used and the Rf value was about 0.81 and 0.53. The specificity of the method was determined for presence of components that may be unexpected to be present. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the capsule dosage form. The linearity was determined in analyte concentration range of 100-600 and 100-700 µg spot. The calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.999 and 0.999 for LOP and RIT. (Table 1, Fig. 3) the precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The repeatability, interday and intraday were calculated for LOP and RIT (Table 2) the accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined for LOP and RIT and was found to be 99.79 and 100.6% (Tables 3). A typical chromatogram showing the separation of LOP and RIT is shown in fig 4.

Table 1: Regression Statistics for analysis of Lopinavir and Ritonavir

Parameter	Data of Lopinavir	Data of Ritonavir
Range	100-600 ng/spots	100-700 ng/spot
Correlations coefficient	0.9995	0.9993
Regration equation	y=6.959x + 277.6	y=6.781x + 112
LOD	6.58 ng/spot	11.52 ng/spot
LOQ	34.11 ng/spot	26.22 ng/spot

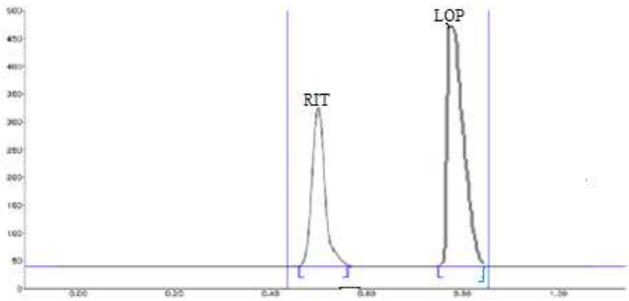

Fig 4: Representative chromatogram of LOP and RIT

Table 2: % RSD for Lopinavir and Ritonavir

Sr.No.	Concentrations of Lopinavir ng/spot	Concentrations of Ritonavir ng/spot	% RSD of Lopinavir	% RSD of Ritonavir
1	300	300	0.24	0.126
2	400	400	0.372	0.535
3	600	600	0.482	1.2

Table 3: Recovery study of Lopinavir and Ritonavir

	Recovery level	Initial amount (ng)	Amount added (ng)	Amount recovered	% Recovery (ng)	SD	% RSD
Lopinavir	80	200	160	159.6	99.89	2.64	0.46
	100	200	200	198.4	99.2	2.735	0.43
	120	200	240	240.7	100.3	1.59	0.22
			Mean		99.79	0.794	0.8
Ritonavir	80	100	80	79.92	99.96	0.09	0.658
	100	100	100	102	102	0.12	0.771
	120	100	120	120	100	0.15	0.901
			Mean		100.6	1.2	1.24

CONCLUSION

The developed HPTLC method enables accurate, precise and specific for determination of Lopinavir and Ritonavir. Statistical analysis proves that the method is reproducible and selective for routine analysis of Lopinavir and Ritonavir in pharmaceutical dosage form without interference from excipients.

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