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SIMULTANEOUS DETERMINATION OF LOPINAVIR AND RITONAVIR BY VALIDATED RP-HPLC METHOD IN BULK AND DOSAGE FORMS

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ABSTRACT

An accurate RP-HPLC procedure was developed and validated for the separation and simultaneous quantification of lopinavir and ritonavir in pure and tablets. The separation was carried out using phenomenex C_{18} (250 x 4.6 mm, 5 μm particle sizes) column, with a mobile phase consisting of methanol(90%) and phosphate buffer (pH 7.8) (10 % v/v). The flow rate was set at 1.0 ml/min and monitored at 215 nm using UV detector. At 3.7 min and 4.7 min, lopinavir and ritonavir were given peaks, respectively. The quantification was done in the range of 100-600μg/ml for lopinavir and ritonavir individually. The liquid chromatography procedure was validated for linearity, accuracy, precision, and robustness. All these analytical validation parameters were observed and the % RSD was determined which indicates the usefulness of the method for determination of both in bulk and tablets.

KEYWORDS: Lopinavir, Ritonavir, Validation, RP-HPLC, Methanol, phosphate Buffer (pH7.8)

I INTRODUCTION

Lopinavir is chemically known as (2*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide and its empirical formula is $C_{37}H_{48}N_4O_5$ with molecular weight of 628.80[Indian pharmacopoeia, 2007]. Ritonavir is chemically known as 1,3-thiazol-5-ylmethyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[(2*S*)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino} butanami-do]-1,6-diphenylhexan-2-yl]carbamate and its empirical formula is $C_{37}H_{48}N_6O_5S_2$ and its molecular weight is 721.00 [Indian Pharmacopoeia 2007].

Both Lopinavir and ritonavir inhibits the HIV viral protease enzyme. The chemical structures for lopinavir and ritonavir were shown in fig 1 & 2. HPTLC [Sulebhavikar VA *et al*., 2008], HPLC [Seshachalam U *et al*., 2007, Phechkrajang MC *et al*., 2009, Ponnilavarasan I *et al*., 2010, Chiranjeevi K *et al*., 2011, Suneetha A *et al*., 2011], LC–MS/MS [Temghare AG *et al*., 2009, Myasein F *et al*., 2009], UV Spectrophotometric methods

[Vaishali N, Kishore B 2010, Vaishali P *et al*., 2010, Ponnilavarasan I *et al*., 2010, Chiranjeevi K *et al*., 2011] have been reported for determination of lopinavir and ritonavir.

II EXPERIMENTAL

Materials

Triple distilled water of HPLC grade, methanol of HPLC grade, 0.2 M potassium dihydrogen phosphate (KH_2PO_4) and 0.2 M sodium hydroxide (NaOH), which are of AR grade were used in the analysis.

Method

SCHIMADZU LC 20 AD (prominence) with column-Phenomenex C_{18} (250×4.6mm, 5µ particle size), injector-Rheodyne, UV-Visible spectrophotometer (PerkinElmer lambda 25) were used for the analysis.

Chromatographic conditions

Mobile phase–methanol: phosphate buffer (7.8) (90:10); pump-LC20 AD; mode of operation –isocratic; temperature–ambient; flow rate–1.0ml/min and UV detection at 215 nm were opted for the operation.

Drug samples and reagents

Reference standard samples of lopinavir and ritonavir were procured from Hetero labs, Hyderabad. Commercial sample of lopinavir and ritonavir tablets (V-LETRA, Ranbaxy Ltd., India) were received from the market.

III STANDARDIZATION OF METHOD

Preparation of mobile phase

Methanol and phosphate buffer pH 7.8 (prepared as per IP 2007) were filtered through 0.45 µm membrane filter and sonicated for 20 min before use.

Preparation of stock solutions

Stock solutions of lopinavir and ritonavir were prepared by dissolving 25 mg of each drug taken in a separate 25 ml volumetric flasks in methanol and volume is made to 25 ml with methanol, sonicated for about 15 min. From the individual stock solutions, working standard solutions were prepared in a concentration range of 100-600µg/ml for lopinavir and ritonavir.

Estimation of the drugs from tablet dosage forms

Twenty tablets of V-LETRA, containing lopinavir (200 mg) and ritonavir (50 mg) were weighed and finely powdered. The amount of powder equivalent to 50 mg of ritonavir was weighed, transferred into 100 ml volumetric flask and dissolved in the mobile phase by sonication for about 15 min. This solution was filtered through 0.45 µm membrane filter paper. From the filtrate different aliquots were taken in separate 10 ml volumetric flasks. The contents of the flask were made up to the volume with methanol and mixed well. Then these samples were injected and peaks were recorded.

Calibration curve

Standard plots were prepared for each drug by measuring different volumes of stock solutions into 10 ml volumetric flasks and diluted to volume with diluent, so that 100-600 µg/ml of lopinavir/ritonavir were obtained. Six replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

IV RESULTS AND DISCUSSIONS

Method optimization

The developed RP-HPLC method utilizes methanol and buffer (pH-7.8) in the ratio of (90:10) as a mobile phase and column phenomenex C_{18} as a stationary phase. The suitable parameters were chosen after several trails with buffers of different pH values and various compositions of methanol, acetonitrile and buffer. The trails revealed that with the decrease in methanol concentration with buffer (pH7.8), the peak obtained was broad and showed severe tailing. The peaks obtained with a composition of methanol (90%) and phosphate buffer (10%) was proved to be most suitable of all the combinations since the peaks obtained were better defined and resolved and free from tailing. To determine the effect of flow rate, the method was performed at different flow rates of 0.5 ml/min, 0.7 ml/min, 1.1 ml/min and 1.2 ml/min. The optimum flow rate of 1ml/min was chosen finally. The retention times obtained for lopinavir and ritonavir were 3.7 and 4.7 min, respectively and chromatograms of standard solution and sample solution were shown in figures 4 and 5.

Method validation

Validation of said procedure was carried out and the summary of validation parameters was tabulated in table 3. Linearity was established between concentration and peak area values by injecting varying quantities of the standard solution and calculating the peak area values. There exists a linear relationship showing concentrations ranging from 100-600 µg/ml for lopinavir and ritonavir individually with peak area values. From the data obtained, correlation coefficient for the lopinavir is 0.9996 and for ritonavir was found to be 0.9998. Linear regression data for calibration curves were shown in table 1.The resulting linearity plots were shown in the figures 4 and 5. Repeatability expresses the precision of the procedure where the % R.S.D for lopinavir was 0.366 and ritonavir was 1.01. As per the summary of the validation results, presented in table 2, the number of theoretical plates for lopinavir was found to be 6270 and ritonavir were found to be 5269 and tailing factor for lopinavir was found to be 0.7 and for ritonavir was found to be 0.5. The number of the theoretical plates was high and tailing factor was very less indicating the efficient performance of the column. Recovery studies were performed at 80%, 100%, and 120% levels of the known level in the sample. Average recovery of the analyte for both lopinavir and ritonavir was found to be good as given in table 3.

V CONCLUSION

It is clear from the present study that the prescribed method of analysis is simple, accurate, specific and precise in operation and can be employed for routine batch analysis of combination of lopinavir and ritonavir in tablets.

ACKNOWLEDGEMENT

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Fig 1 Chemical structure of lopinavir

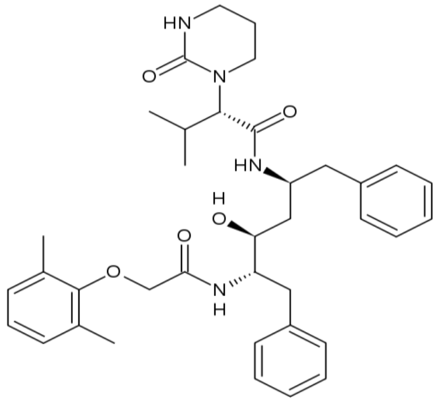


Fig 2 Chemical structure of ritonavir

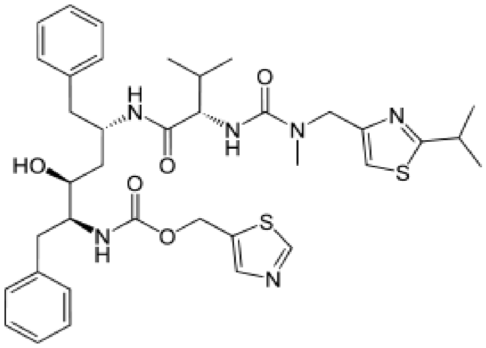


Fig 3 chromatogram of standard solution of lopinavir and ritonavir.

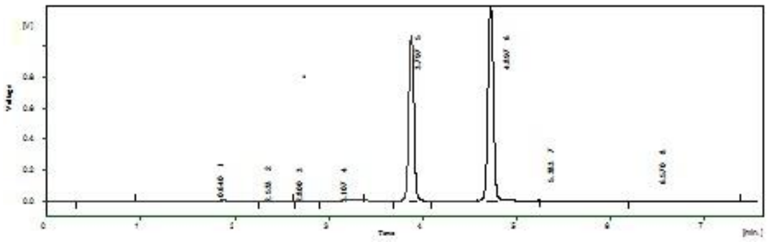
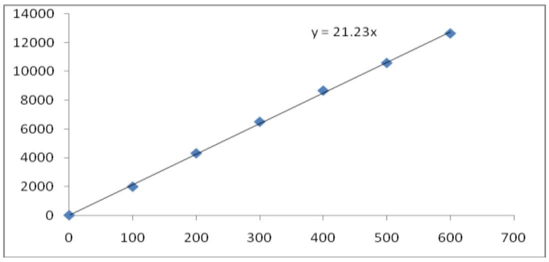


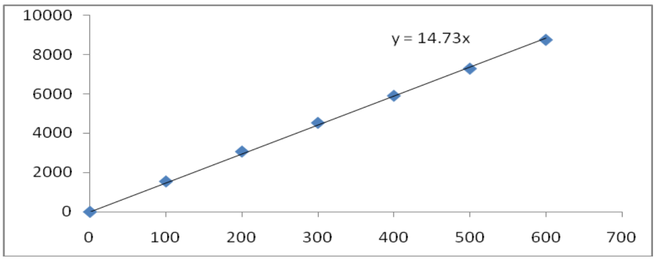
Fig 4 ***Linearity plot of lopinavir***



Area

Concentration(μg/ml)

Fig 5 Linearity plot of ritonavir



Area

Concentration(µg/ml)

Table 1 Linear regression data for calibration curves

Statistical analysis		
Drugs	**ritonavir**	**lopinavir**
Slope	14.73	21.23
Correlation coefficient	0.9998	0.9996
Standard deviation	46.92	24.23
Linearity range, µg/ml	100-600	100-600
%RSD	1.01	0.366

Table 2. Validation summary

Validation Parameters	Result	
	Lopinavir	**Ritonavir**
Retention time	4.701	4.06
Theoretical plates	6270	5269
Tailing factor	0.7	0.5
LOD(mcg/ml)	17.61	15.808
LOQ(mcg/ml)	53.34	47.905
Resolution	0.7	

Table 3 *Results of HPLC assay and recovery studies*

Sample	Amount claim (mg / tablet)	Amount found (mg / tablet)	% Recovery*
Lopinavir	10	10.08	100.8
Ritonavir	10	9.95	99.8