INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 2 | Apr - Jun - 2019
Journal Home page: www.ijpar.com

Research article *Open Access*

Method development and validation for the simultaneous determination of Ritonavir and Lopinavir by RP-HPLC and by UV- Spectrophotometry

[1]B.Poornima*, [2]P.Jayachandra Reddy, [3]D.S.S.N.Neelima

[1]HOD, Associate Professor, Department of Pharmaceutical Analysis, Krishna Teja Pharmacy College, Tirupati

[2]Principal, Professor, Department of Pharmaceutical Analysis, Krishna Teja Pharmacy College, Tirupati, Andhra Pradesh

[3]Scientific & Applied Research Center, Hyderabad, Telangana State.

***Corresponding Author: B.Poornima**

ABSTRACT

In the present work, RP-HPLC, UV spectroscopy method have been developed and validated for simultaneous determination of Ritonavir and Lopinavir in Tablet dosage form. The RP-HPLC method, C18 (250x4.6mm, i.d 5µm) as a stationary phase and mobile phase of methanol: water (85:15) at 1ml/min flow rate was used for the separation of both the compounds with a detection of 225nm. Linearity was obtai ned in the concentration range of 5-50µg/ml for Ritonavir and 20-200µg/ml for Lopinavir. The retention times of Ritonavir and Lopinavir was 4.8 and 5.9 min. In the second method i.e UV-Spectrophotometric simultaneous determination was done at 219nm and 245nm. Calibration graphs were established for 10-50µg/ml for Ritonavir and 20-200µg/ml for Lopinavir. These methods were economic and time saving. The newly developed methods were successfully utilized for the Quantitative estimation of Ritonavir and Lopinavir in pharmaceutical dosage forms, both the methods have been extensively validated as per ICH guidelines.

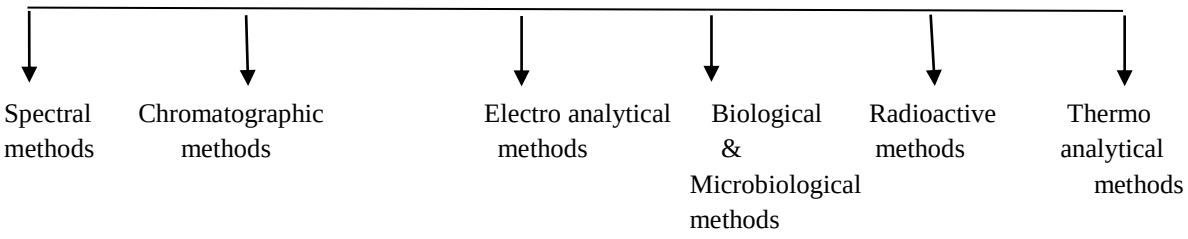
Keywords: Ritonavir, Lopinavir, Methanol, Water, Waters C18 Column RP-HPLC, Validation

INTRODUCTION

Pharmaceutical analysis is the interdisciplinary subject and it is derived from various branches of sciences like chemistry, physics, microbiology, nuclear science and electronics which deals with analysis of biological, chemicals, foods, drugs and pharmaceuticals qualitatively and quantitatively.

Pharmaceutical Analytical Techniques are categorized into:

Pharmaceutical Analytical Techniques



High Performance Liquid Chromatography

High pressure liquid chromatography, High performance liquid chromatography, High speed liquid chromatography, High price liquid chromatography, High efficiency liquid chromatography. HPLC is one of the most powerful tools in analytical chemistry, with the ability to separate, identify and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC can be applied to any sample, such as pharmaceuticals, food, nutra ceuticals, cosmetics, biological fluids and environmental matrices [1].

Principle of HPLC

This chromatographic process has been classically defined as a separation that is achieved by distributing the solute mixture between two phases, a mobile phase and stationary phase. Those solutes that are preferentially distributed in the stationary phase remain in the column longer than those that are preferentially distributed in the mobile phase. In fact, the solutes are distributed between the two phases to different extents because the molecular interactive forces between the solute molecules and those of the two phases are different for each individual solute. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase [2].

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages [3].

- ✓ Speed
- ✓ Greater sensitivity
- ✓ Improved resolution
- ✓ Reusable columns
- ✓ Ideal for the substances of low viscosity
- ✓ Easy sample recovery, handling and maintenance.
- ✓ Precise and reproducible

TYPES OF HPLC TECHNIQUES

Based on Modes of Chromatography

- ✓ Normal Phase- High Performance Liquid Chromatography (NP-HPLC)
- ✓ Reversed Phase- High Performance Liquid Chromatography (RP-HPLC)

Based on principle of separation

- Adsorption chromatography
- Ion Exchange Chromatography
- Ion pair chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Based on internal diameter of the column

- Micro HPLC (0.3-1mm i.d)
- Semi-micro HPLC (1-3mm i.d)
- Conventional HPLC (4-8mm i.d)
- Semi-preparative HPLC (10-20mm i.d)
- Preparative HPLC (20-50mm i.d)
- Process HPLC (>50mm i.d)

Normal Phase Chromatography

NP-HPLC separation is a competitive process. Analyte molecules compete with the mobile phase molecules for the adsorption sites on the surface of the stationary phase. This method separate analytes based on adsorption to a stationary surface chemistry and by polarity [4]. NP-HPLC uses a polar stationary phase and a non-polar mobile phase, and works efficiently for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase.

Adsorption strengths increases with increased analyte polarity and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increase the elution time. The adsorption strengths and hence 'K' value (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatic < organic < halogen compounds < sulphides < ethers < esters < aldehydes and ketones < amines < sulphones < amides < carboxylic acids. The strength depends not only on the functional groups in the analyte molecule but also steric factors. The effect of sterics on interaction strength allows this method to resolve (separate) structural isomers [5].

Reversed Phase Chromatography

RP-HPLC is the most popular mode of chromatography. RP-HPLC has a non-polar stationary phase and polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. Today, reversed-phase column chromatography accounts for the vast majority of analysis performed in liquid chromatography. The less water-soluble compounds are better retained by the reversed phase surface. The retention time decreases in the following order: Aliphatic > induced dipoles (e.g. CCl_4) > permanent dipoles (e.g. $CHCl_3$) > weak Lewis bases (Ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids) etc. Also the retention increases as the number of carbon atoms increases [6].

HPLC INSTRUMENTAL PARAMETERS

A. Total retention time (t_R): It is the time required by the sample to migrate from column inlet to detector.

B. Dead volume (t_0): The time required by the inert substance to migrate from column inlet to column end without being retained by stationary phase.

C. Plate number

$$N = 3500 \times L\ (cm) / dp\ (\mu m)$$

Where L = length of column

dp = diameter of particles in μm

D. HETP (Height Equivalent Theoretical Plate)

HETP = L / N (Low HETP = High efficiency)

Where L = length of column

N = plate number

E. Column efficiency

$$N = a\ (t_R / w)^2$$

Where W = width of peak

N = no. of theoretical plates

t_R = retention time

F. Resolution: It indicates the quality of separation of adjacent peaks.

$$Rs = 2\ (t_2 - t_1) / (w_1 + w_2)$$

Where t_1 & t_2 = retention time of 1st and2nd adjacent peaks

w_1 & w_2 = width of bands

G. Capacity factor (k')

It indicates the degree of separation.

$$K' = t_R - t_0 / t_0$$

K' value should be 1-10

If k' < 1.0 - inadequate separation

If k' > 10 - band broadening

If k' > 30 - no separation

H. Separation / selectivity factor

It should not be equal to 1

$$\alpha' = k'_2 / k'_1 \text{ (or) } t_2 - t_0 / t_1 - t_0$$

I. Asymmetry factor

It indicates peak tailing and fronting.

$$As = B / A$$

J. 10% Asymmetry

$$AsF = BC / AC$$

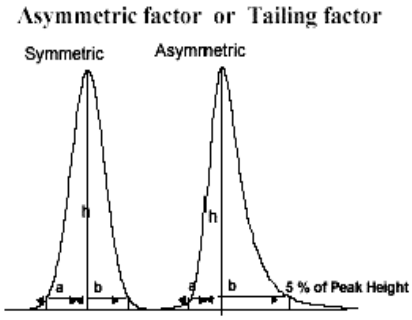


Fig.1 Asymmetry

Introduction to Spectrophotometry

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. It is one of the powerful most tools available for the study of molecular structure and is used in the analysis of wide range of samples. Molecules absorb radiation, in discrete packets, which are also called quanta of energy [7]. The spectrum is the response corresponding to the physical properties of a compound when it is subjected to various wavelengths. When one considers a molecule, it found that it is associated with several different types of motion. The molecule as a whole rotates, the bonds undergo vibrations and even the electrons may jump from one energy level to other. Each of these kinds of motion is quantized. Thus the particular motion occurs only when the exact "packet of energy" belonging to the region under study is supplied to the molecule. Spectroscopy is the measurement of these interactions and a machine which performs such measurements is known as a spectrometer. Four spectroscopic techniques namely UV-Visible, Infrared, NMR and Mass spectroscopy are generally used for structural elucidation [8].

Lambert-Beer's Law

Lamberts Law

The relationship between absorption of radiation and length of the path through the absorbing medium was formulated by Lambert (1768). "When beam of monochromatic light passes through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light" [9].

Beer's Law

It is analogous to Lamberts law. The intensity of a beam of monochromatic radiation decreases exponentially as the concentration of absorbing species increases arithmetically. These two laws are readily combined to give a convenient expression as

$$\log Io/It = Kbc$$

Where, Io= Intensity of incident radiation,

It=Intensity of transmitted radiation,

K=constant,

b= thickness of the absorbing medium and c= concentration of the solution.

This is the fundamental equation for Colorimetry or Spectrophotometry. The term log Io/It is called as absorbance (A) or optical density (OD). Therefore,

$$A = \log Io/It = Kbc$$

K is replaced with ε when concentration is taken in moles/lit. Hence, A= εbc, ε is known as molar absorptivity and expressed in Lmol¯1cm-1. This equation indicates that the absorbance is directly proportional to the concentration of the solution. The term It/Io is called as transmittance (T). Hence,

$T = It/Io$

Now, $\%T = It/Io \times 100$

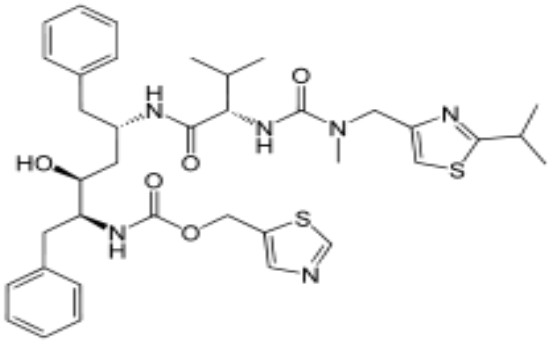
Rearranging, $Io/It\ t = 100 / \%T$

Applying logs $\log Io/It = \log 100 - \log (\%T)$

i.e, $A=2- \log (\%T)$

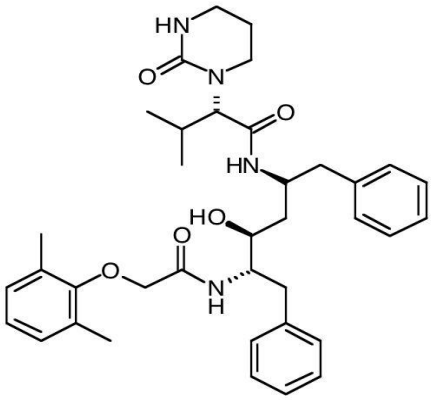
Hence, if the transmittance of a solution is known, then its absorbance is calculated.

Ritonavir



Ritonavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Ritonavir binds to the protease active site and inhibits the activity of the enzyme [10]. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

Lopinavir



Lopinavir inhibits the HIV viral protease enzyme by forming an inhibitor-enzyme complex therapy by preventing the cleavage of the gag-pol polyproteins [11]. Immature, non infectious viral particals are subsequently produced.

MATERIALS AND METHODS

RP-HPLC Method Development & Validation of selected API'S Ritonavir and Lopinavir.

Preparation of standard drug solutions

Accurately about 10mg of the Ritonavir (RTV) and Lopinavir (LPV) pure drugs were weighed and transferred into 10ml clean, dried standard volumetric flasks separately. The volume was made up to the mark with methanol. 1ml of the above solutions was transferred into a 10ml flask separately and the volume was made up to the mark with mobile phase to obtain 100µg/ml concentration of LPV and RTV. From these stock solutions 20µg/ml of RTV & LPV were prepared and used for the optimization of mobile phase. All the stock solutions were stored at 23ºC [12].

Optimization of chromatographic conditions

Drug solubilises were examined in literature and found to be soluble in methanol and water. Drugs pKa were known from literature as 13.98 & 14.23 for LPV & RTV, so the pH of the mobile phase adjusted to 3.5. The detection wavelength was optimized in the double beam spectrophotometer, by scanning sample in the range of 200-400nm. From the overlaid spectrum of LPV

& RTV, 225nm was selected for the simultaneous quantification in HPLC method.

A different mobile phase ratio of methanol and water starting from pure methanol to gradual increment of aqueous phase in steps was performed in C18 (150X4.6mm, 5µ) column and the results were showed in table.11 and figure.2. Our objective of the chromatographic method development was to achieve a total run time of chromatogram <10min, peak tailing factor <1.3 with good retention and resolution [13].

Method Validation

Method validation was performed in terms of specificity, linearity, Accuracy, Precision, LOD, LOQ, robustness and system suitability.

Assay of Formulation

Twenty tablets of KALETRA were weighed & finely powdered and extracted with methanol in a 10ml volumetric flask, and 5ml of methanol was added to the same. The flask was sonicated for 15min and volume was made up to the mark with methanol. The above solution was filtered using Whatman No.1 filter. 1ml was transferred into a 10ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 20µg/ml of RTV and 80µg/ml of LPV. The solution was injected under above chromatographic conditions and peak area was measured. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated [14].

UV Spectrophotometric method development and validation of selected API's-Ritonavir and Lopinavir

Development and validation of UV Spectrophotometric method for the determination of Lopinavir and Ritonavir in tablet formulation. UV-visible spectrophotometric method for estimation of Lopinavir and Ritonavir by 2nd order derivative method [15].

METHOD DEVELOPMENT

sa) Determination of solubility: Lopinavir and Ritonavir solubility was tested in different organic and aqueous solvents. Both are soluble in methanol.

b) Preparation of stock solution: The stock solutions of concentration 1000µg/ml of LPV and 1000µg/ml of RTV prepared by dissolving 10mg of each drug in 10 ml of methanol

c) Determination of analytical wavelength: Accurately measured 0.4 ml of the stock solution of Lopinavir was transferred to a 10 ml volumetric flask. Then, it was diluted up to the mark with methanol to obtain a solution of strength 40 µg/ml. in same way 20 µg/ml of Ritonavir prepared. The resulting solution was scanned in the UV region of 200-400 nm using methanol as the blank. The zero-order spectrum was then converted to the first-derivative spectrum using UV probe software (scaling factor-5, delta λ-2). The wavelength at which there was an absorption minimum was selected as the analytical wavelength.

d) Selection of suitable wavelengths for Lopinavir and Ritonavir estimation: Maximum absorbance wavelength for Ritonavir is 239nm and maximum absorbance wavelength for Lopinavir is 210nm.by observing the both spectra of Lopinavir and Ritonavir, 241nm selected for Ritonavir because at 241nm Lopinavir show 0' absorbance.219nm selected for Lopinavir because at 219nm Ritonavir show 0'absorbance.so that we can analyse both drugs [16].

METHOD VALIDATION

Method validation was performed in terms of linearity, Accuracy, Precision, LOD, LOQ, specificity.

RESULTS AND DISCUSSION

Table 1. Optimization of mobile phase for Estimation of RTV & LPV

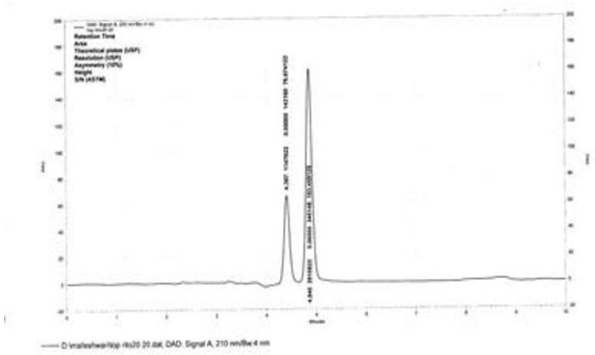
S. No	Mobile phase composition (meoth:water) 1 ml/min	Retension times(min)		Asymmetry	
		Ritonavir	Liponavir	Ritonavir	Liponavir
1	90:10	4.4	4.8	1.04	1.05
2	80:20	1.8	2.4	0.9	1.11
3	70:30	2.07	3.3	1.11	1.07

4	75:25	1.3	2.86	0.99	1.09
5	**85:15**	**4.8**	**5.9**	**1.04**	**1.05**

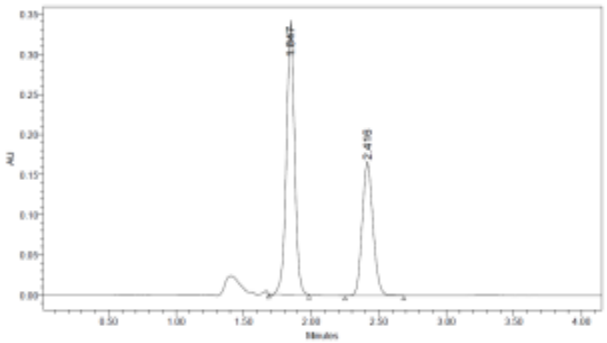
RTV - 20µg/ml, LPV - 20µg/ml,

Table 2. Optimized Chromatographic conditions

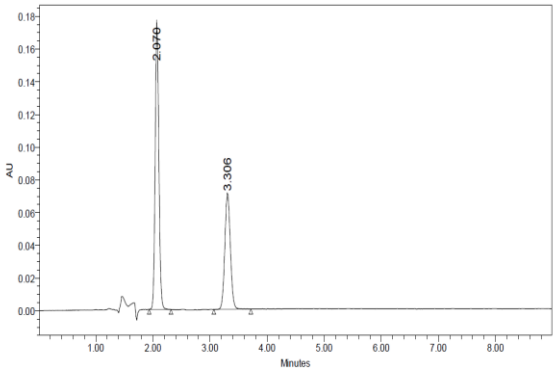
S.No	Parameters	Conditions
1	Mobile Phase Optimized	Methanol : Water - 85:15
2	Stationary Phase	C_{18} (250 × 4.6 mm i.d.,5 µm)
3	Flow Rate	1 ml / min
4	Run Time	10 min
5	Column Temperature	23^{o}C
6	Volume of Injection	20 µL
7	Detection Wavelength	225 nm
8	Retention time of Drug (min)	RTV - 4.8, LPV - 5.9



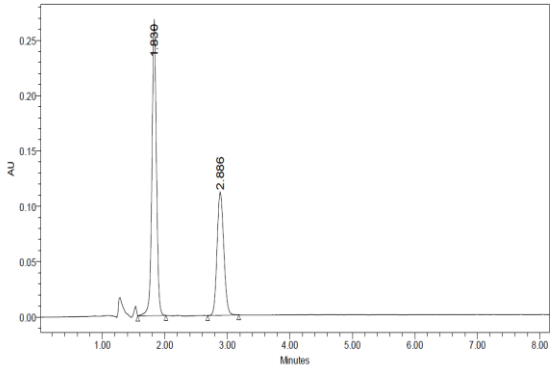
Trail-1



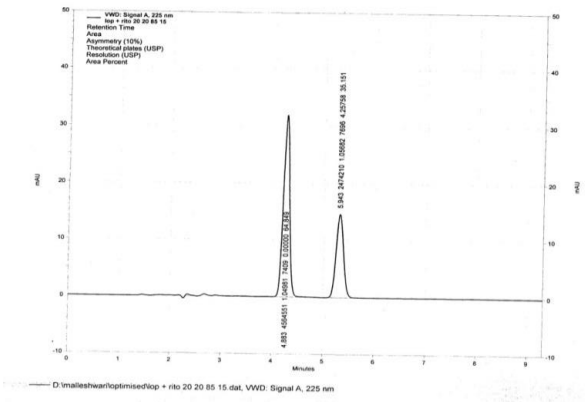
Trail-2



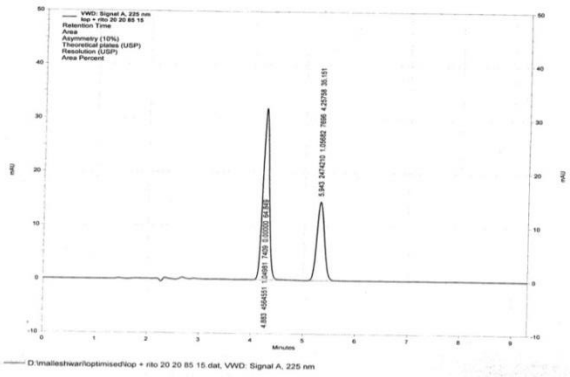
Trail-3



Trail-4



Trail-5



Optimized

Trail 1(methanol: water 90:10) Trail 2 (methanol: water 80:20)
Trial 3 (methanol: water 70:30) Trial 4 (methanol: water 75:25)
Trial 5 (methanol: water 85:15) Optimized (Methanol: water 85:15)

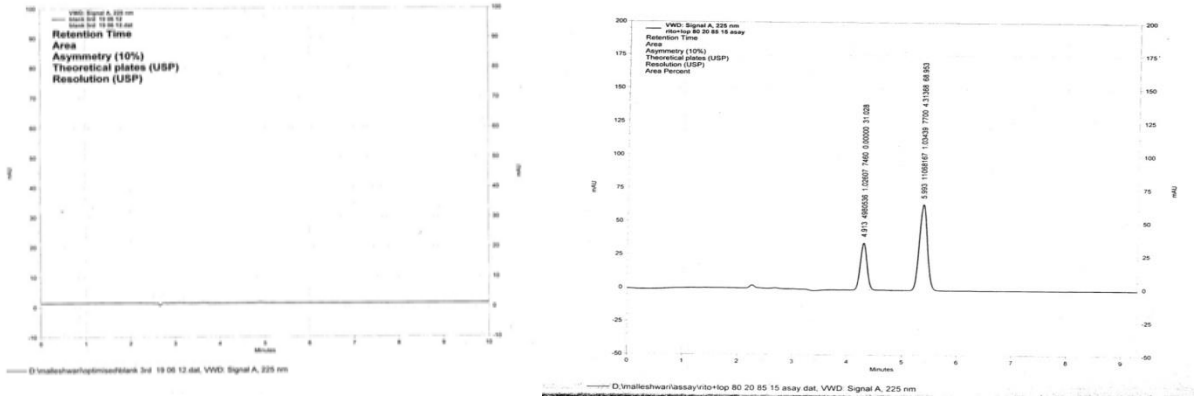
Fig. 2 Trails

METHOD VALIDATION

The present method was validated based on ICH Q2 (R1)parameters. The following parameters were validated for the proposed method based on ICH Q2 (R1) guidelines.

Specificity

Specificity of the method was found out through non-interference of the blank, internal standard and mobile phase. The method showed excellent specificity. On the basis of this chromatogram, we can say that there is no interference of blank. The result chromatogram is shown in the Fig. No 3 and reports are tabulated in the Table No.3.



Blank chromatogram **Sample peak**

Fig.3 Blank & Sample Chromatograms

Table No.3 Results of blank interference for Specificity

S. No	Blank injection	Observation
01	Blank injection	No interference
02	Sample	response Was absorbed

DISCUSSION

The specificity study was performed for blank and the chromatogram shows that there is no interference in the retention time of the analytes.

Linearity

Table 4. Linearity of ritonavir and Lopinavir

S.No	Ritonavir			Lopinavir		
	Con(µg/ml)	Peak area Mean±SD	%RSD	Con(µg/ml)	Peak area Mean±SD	%RSD
1	5	1642618±154.3	0.9	20	2583567±161.9	0.6
2	10	2902847±108.3	0.3	40	399083567±105.18	0.8

3	15	4231894±68.64	1.6	60	6683876±2642.35	0.9
4	20	5703577±998	1.2	80	8365465±10047.54	0.3
5	25	6851666±666	1.4	100	12470110±52915.5	0.4
6	30	8123717±680	0.8	120	14477651±66602.74	0.6
7	35	9620840±132	1.3	140	17220437±89416.87	1.2
8	40	10909098±56.3	0.5	160	18240337±121654.6	0.6
9	45	12503592±59.3	0.4	180	19734981±3999.5	1.4
10	50	13986160±51.3	0.3	200	21293957±40925.7	1.3

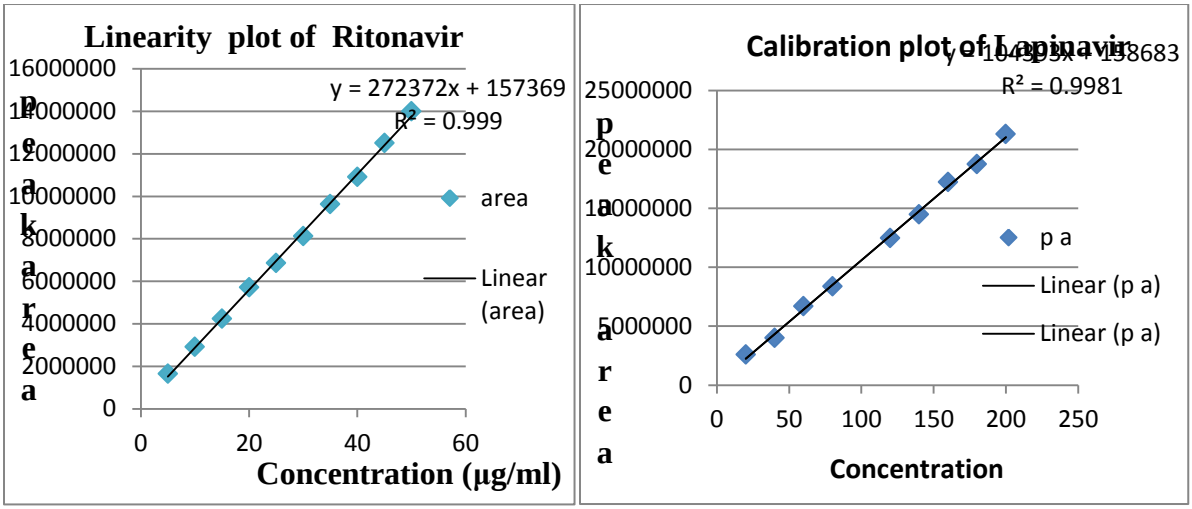


Fig.4 Calibration plots of Ritonavir & Lapinavir

DISCUSSION

The linearity study was performed for Ritonavir and Lopinavir range of 5- 50µg/ml and 20-200 µg/ml respectively. The correlation coefficient was found to be 0.998 and 0.999. respectively.

Assay of Formulation

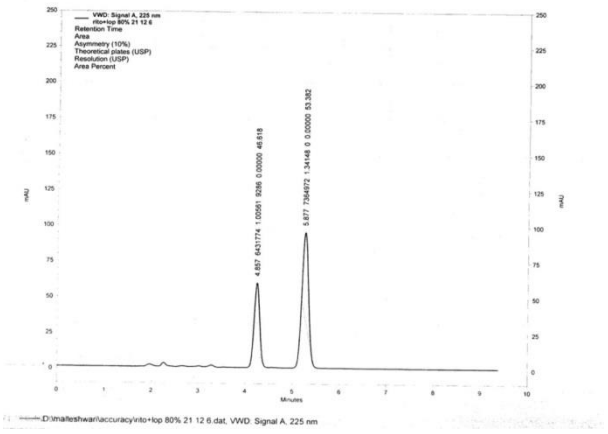
Table 5. Assay of Formulation

S.No	Formulation	Labelled claim (mg)	Amount found (mg)	Recovery Mean ± SD	% RSD
1	**Kaletra**	RTV-50mg	49.88	99.8 ±0.2	0.61
		LPV-200mg	197.13	98.5 ± 0.3	0.29

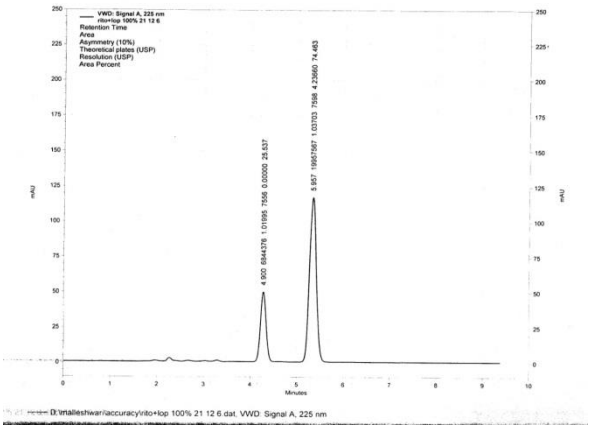
Table 6. Recovery Studies

S.No	Name of the drug	Pre analysed sample (µg/ml)	Recovery level	Amount of drug added(µg/ml)	Total amount found	%recovery	%RSD
1	RTV	20	80%	16	35.36	98.2	0.6
			100%	20	40.70	101.75	0.8
			120%	24	45.29	102.94	1.4
2	LPV	80	80%	64	141.42	98.2	1.2
			100%	80	162.95	100.58	1.4
			120%	96	178.64	101.5	1.5

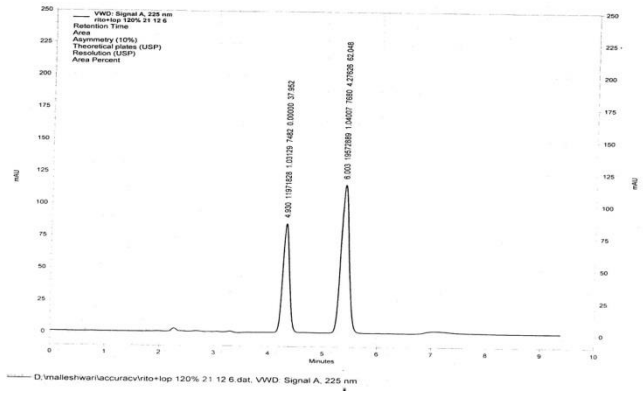
ACCURACY



Accuracy 80%



Accuracy 100%



Accuracy 120%

DISCUSSION

The accuracy study was performed for the range of 80%, 100%, and 120%. The % recovery of Ritonavir and Lopinavir was found to be 98.2-102.94 % & 98.2-101.5% respectively (NLT-98& NMT-102).

Precision

Table 7. Precision studies

	RTV Con(µg/ml)	%RSD	LPV Con(µg/ml)	%RSD
Intra day	10(LQC)	0.4	40(LQC)	1.7
	20(MQC)	0.2	80(MQC)	0.6
	30(HQC)	0.5	120(HQC)	0.6
Inter day	10(LQC)	0.18	40(LQC)	0.8
	20(MQC)	0.71	80(MQC)	1.4
	30(HQC)	0.66	120(HQC)	1.8

DISCUSSION

The precision is checked to ensure that the analytical system is precise. The % RSD was found to be NMT-2%.

System Suitability

Table 8. Results of System suitability parameters

S. No.	System Suitability Parameters	Observations		Proposed Acceptance Criteria
		Ritonavir	Lopinavir	
1.	% Relative Standard Deviation for replicate injections of analyte peak in standard solution	0.71	1.4	Should be not more than 2.0%
2.	Symmetry factor for analyte peak in standard solution	1.04	1.05	Should be not more than 2.0
3.	Plate count for analyte peak in standard solution	7440	7755	Should be not less than 2000

Robustness

Table 9. Robustness studies

S.No	Parameter	Standard	Modification	Retension time(min)	
				RTV	LPV
1	Flow rate	1ml/min	0.8ml/min	6.1	7.5
			1.2ml/min	4.0	4.9
2	Wavelength	225nm	223nm	4.9	6.0
			227nm	4.9	6.0
3	Mobile phase	85:15	83:17	5.8	7.5
			87:13	4.6	5.6

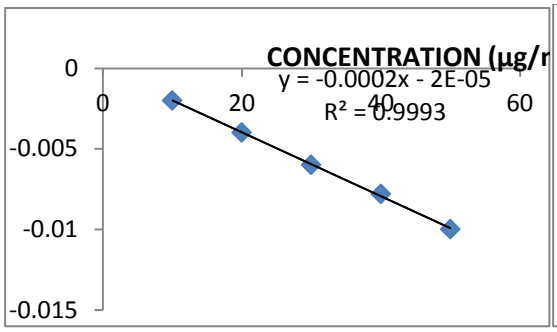
DISCUSSION

As per above observations, it was found that the % Relative standard deviation of replicate injections of standard preparations with ±0.1 flow rate, ±5nm wavelength and ± 2ml Mobile phase ratio was found to be 2.0%. Also system suitability parameters such as Tailing, Tangent / Column efficiency and %RSD of replicate injections of standard preparation, are meeting the requirements. Hence the proposed method is robust for the estimation of Ritonavir and Lopinavir an even with variation of different flow rate**,** wavelength and Mobile phase ratio.

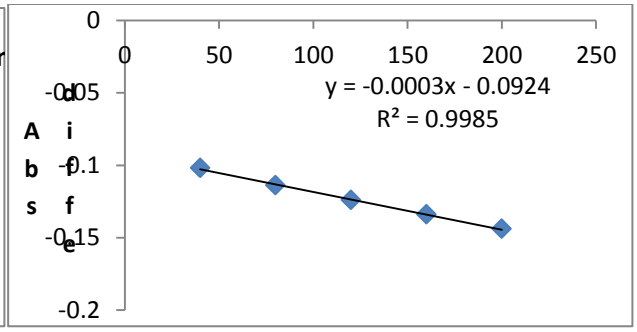
UV SPECTROPHOTOMETRIC METHOD

Table 10. Linearity of RTV & LPV

S.No	RTV Concentration (µg/ml)	Absorbance difference $d^2A/d\lambda^2$±SD	LPV Concentration (µg/ml)	Absorbance difference $d^2A/d\lambda^2$±SD
1	10	-0.002±0.01	40	-0.0102±0.002
2	20	-0.004±0.013	80	-0.114±0.007
3	30	-0.006±0.022	120	-0.123±0.005
4	40	-0.079±0.049	160	-0.135±0.004
5	50	-0.010±0.052	200	-0.144±0.011



Calibration plot of Ritonavir



Calibration plot of Lopinavir

Table 11. Precision studies

	RTV Con(µg/ml)	%RSD	LPV Con(µg/ml)	%RSD
Intra day	10(LQC)	1.65	40(LQC)	1.4
	30(MQC)	1.9	120(MQC)	1.5
	50(HQC)	1.3	200(HQC)	1.6
Inter day	10(LQC)	1.6	40(LQC)	1.8
	30(MQC)	1.9	120(MQC)	1.24
	50(HQC)	1.7	200(HQC)	1.9

Table 12. Recovery studies

S.No	Name of the drug	Pre analysed sample (µg/ml)	Recovery level	Amount of drug added(µg/ml)	Abs difference ($d^2A/d\lambda^2$)	Total amount found	%recovery
1	RTV	20	80%	16	-0.0068	35.36	99.27
			100%	20	-0.008	40.70	101.75
			120%	24	-0.094	45.29	102.94
2	LPV	80	80%	64	-0.114	141.42	100.43
			100%	80	-0.132	162.95	101.79
			120%	96	-0.146	174.64	102.30

Table 13. Assay of formulation

S.No	Formulation	Labeled claim (mg)	Amount found (mg)	Percentage of labelled amount Mean ± SD	% RSD
1	**Kaletra**	RTV-50mg	49.86	99.8 ±0.2	0.91
		LPV-200mg	198.26	102.30 ± 0.3	1.4

DISCUSSION

The selected drugs Ritonavir and Lopinavir were estimated simultaneously by UV- visible spectroscopy method. The detection was carried out at wavelength 219,241 nm with second derivative spectroscopy method. The method was validated for all validation parameters as per ICH guidelines. The linearity ranges for RTV& LPV were 10– 50 µg/ml & 40– 200 µg/ml and with r^2 values of 0.999 & 0.998. The % RSD for method and system precision was < 2%. The method has been validated in assay of tablet dosage forms. The accuracy of the method was validated by recovery studies and was found to significant and under specification limits, with % Recovery 99.27– 102.94 % for RTV and 100.43 – 102.30% for LPV. The assay results of RTV & LPV were found to be 99.8 % & 102.30 %. The method was also passes the specifications for robustness parameters.

SUMMARY AND CONCLUSION

Table 14. Summary of HPLC method

S.No	Parameter	Value	
		RTV	**LPV**
1	Mobile phase ratio	Methanol: water - 85:15	
2	Flow rate	1ml/min	
3	Detection wavelength	225nm	
4	Retention time	4.8	5.9
5	Run time	10 min	
6	Asymmetry	1.04	1.05
7	Linearity	5-50µg/ml	20-200µg/ml
8	LOD	1.7µg/ml	5. 7µg/ml
9	LOQ	27.6µg/ml	83.79µg/ml
10	Precision	0.71	1.4
11	% Recovery	98-102.94	98.2-101.5
12	Regression equation	Y=27237x+15736	Y=10439x+15868
		R^2=0.9998	R^2=0.9997
13	Theoretical plates	7440	7755

Table 15. Summary of UV- Spectrophotometer method

S.No	Parameter	Value	
		RTV	**LPV**
1.	λmax	239nm, 210nm	210nm
2.	Beer's law(µg/ml)	10-50	20-200
3.	Regression equation	Y=0.000x-2E-05	Y=0.000x-0.092
4.	a. Slope	0.000	0.000
	b. Intercept	2E-05	-0.092
	c. correlation co-efficient	r^2=0.999	r^2=0.998
5.	Assay	99.8	102.3
6.	Precision	%RSD< 2	%RSD< 2
7.	% recovery	99.27-102.94%	100.43-102.30%

CONCLUSION

RP-HPLC and UV methods have been developed for simultaneous estimation of Ritonavir and Lopinavir in tablet formulation. It was shown that the methods were linear, accurate, precise, reproducible, economical, selective and specific providing the reliability of the methods. It produces symmetric peak shapes, good resolution, and reasonable retention times for both the drugs .The method was fully validated and showing satisfactory data for all the method validation parameters tested. The recoveries achieved are good by both the methods. Hence, this method can be applicable for the simultaneous estimation of Ritonavir and Lopinavir in quality control studies for routine analysis.

BIBILIOGRAPHY

[1]. Touchstone; Joseph, C.; History of Chromatography, Journal of Liquid Chromatography, 16(8), 1993, 1647-1665.

[2]. Ditz, R.; Liquid chromatography History, Preparative Chromatography, Edited by H. Schmidt-Traub, 2005.

[3]. International Conference on Harmonization (ICH) of Technical Requirement for the Registration of pharmaceuticals for Human Use, Validation of procedures: Methodology, adopted in 1996. Geneva.

[4]. Snyder, Lioyd.R. Kirkiand, Joseph.J. Giajch, Joseph.L. Practical HPLC Method Development, 2, 686-705.
[5]. Dong, M.W. Modern HPLC for Practicing scientists, John wiley series, 2006.
[6]. Indian pharmacopoeia, 2, 2007, 1075-1076.
[7]. K.Anand babu, B.Jayakar, G.Vinod kumar. Simultaneous estimation of Ritonavir and Lopinavir in pharmaceutical dosage form by RP-HPLC method. Journal of pharmacy research 4(9), 2011, 2985-2986.
[8]. I.Ponnilavarasan, A.Rajsekaran, J.G.Dhruman, D.Kalaiarasi, RP-HPLC method for simultaneous estimation of antiretroviral drugs Lopinavir and Ritonavir in tablet dosage form, digest journal of nanomaterials and biostructures, 5(3), 2010, 771-778.
[9]. A.Suneetha, S.Kathirvel and G.Ramachandrica. A validated RP-HPLC method for estimation of Lopinavir and Ritonavir in combined dosage form. International journal of pharmacy and pharmaceutical sciences, 3(1), 2011.
[10]. M.Jagadeeswaran, N.Gopal, K.pavan kumar, T.Siva kumar. Quantitative estimation of Lopinavir and Ritonavir in tablet dosage forms by RP-HPLC method Am Pharma Tech Res. 2(2), 2012.
[11]. Hatal Paresh Thakkar,Ketul Harsad bhai, A first-derivative spectrophotometric method for the estimation of Lopinavir in tablets. 1(3), 2010, 22-25
[12]. K. Vinod Kumar, M. Sudhakar, Y. Padmanabha Reddy, P.Malleshwari, Sk. Hafeez. RP-HPLC method development and validation for simultaneous estimation of lopinavir and ritonavir in dosage form and in plasma 3(9), 2014, 1-8.
[13]. V. P. Nagulwar and K.P. Bhusari Development of uv spectrophotometric first order derivative method for the simultaneous estimation of ritonavir and lopinavir in combined tablet dosage form , 3(7), 2012, 2317-2320
[14]. K. Chiranjeevi, K. P. Channabasavaraj, B. Lakshminarayana And B. Kalyan Kumar, Development and validation of RP-HPLC method for quantitative estimation of ritonavir in bulk and pharmaceutical dosage forms 2.2, 2011, 336-340.
[15]. K.Chiranjeevi, K.P.Channabasavaraj, P.Srinivas Reddy, P.T.Nagaraju, Development And Validation Of Spectrophotometric Method For Quantitative Estimation Of Ritonavir In Bulk And Pharmaceutical Dosage Forms 3(1), 58-62,
[16]. Laura Dickinson, Marta Boffito, David Back, Laura Else, Nils von Hentig,Geraint Davies, Saye Khoo, Anton Pozniak, Graeme Moyle and Leon Aarons Sequential Population Pharmacokinetic Modeling of Lopinavir and Ritonavir in Healthy Volunteers and Assessment of Different Dosing Strategies., 55(6), 2011, 2775-2782.