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METHOD DEVELOPMENT FOR THE ESTIMATION OF LOPINAVIR AND RITONAVIR AND IN COMBINATION BY USING RP-HPLC

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ABSTRACT

The estimation of Lopinavir and Ritonavir was done by RP-HPLC. The Phosphate buffer was pH 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C18 column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Lopinavir and Ritonavir were found to be from 100-500 µg/ml of Lopinavir and 10-50µg/ml of Ritonavir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Lopinavir and Ritonavir. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Key Words: RP-HPLC, Lopinavir and Ritonavir

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INTRODUCTION

Lopinavir (ABT-378) is an antiretroviral of the protease inhibitor class. It is marketed by Abbott as Kaletra, a co-formulation with a sub-therapeutic dose of ritonavir, as a component of combination therapy to treat HIV/AIDS.The drug is soluble in water. [1-4] Lopinavir inhibits the HIV viral protease enzyme. This prevents cleavage of the gag-pol polyprotein and, therefore, improper viral assembly results. This subsequently results in non-infectious, immature viral particles. IUPAC Name of ritinovir 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 4yl]methyl}) carbamoyl]amino}utanamido]-1,6-diphenylhexan-2-yl]carbamate.[5,6] Ritonavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.[7]

MATERIALS AND METHODS

Table 1: Instruments used

SL.No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	Labindia UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Lopinavir	Mylon
2	Ritonavir	Cipla
3	KH_2PO_4	Finer chemical Ltd
4	Water and Methanol for HPLC	Lichrosolv (Merck)
5	Acetonitrile for HPLC	Molychem
6	Ortho phosphoric Acid	Merck

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wave length selection: UV spectrum of 10 µg / ml Lopinavir and Ritonavir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

Preparation of Phosphate buffer: Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase: Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PAD or detector.
Temperature : Ambient
Column : Inertsil ODS (4.6 x 150mm, 5µm)
Buffer : 6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with ortho phaosparic acid.
pH : 3.0
Mobile phase : 30% buffer 70% Methanol
Flow rate : 0.8 ml per min
Wavelength : 260 nm
Injection volume : 10 µl
Run time : 10min.

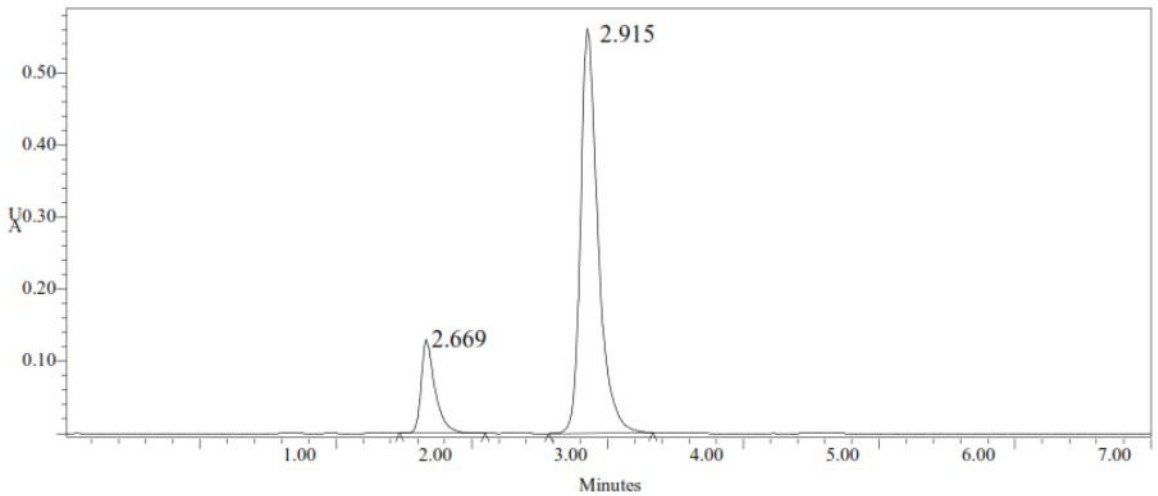

Fig.1: System suitability

Table 3: Results of system suitability parameters for Lopinavir and Ritonavir

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Lopinavir**	3.525	810802	213642		1.0	3527
2	**Ritonavir**	2.984	681469	154566	2.4	1.1	3115

Precision: Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table 4: Results of method precession for Lopinavir and Ritonavir

Name: Lopinavir

	Name	RT	Area
1	Lopinavir	3.557	819305
2	Lopinavir	3.547	807157
3	Lopinavir	3.544	804070
4	Lopinavir	3.537	808474
5	Lopinavir	3.534	804505
Mean			808702
Std. Dev.			6203.7
% RSD			0.77

Name: Ritonavir

	Name	RT	Area
1	Ritonavir	3.019	691143
2	Ritonavir	3.011	685431
3	Ritonavir	3.004	683543
4	Ritonavir	2.997	683564
5	Ritonavir	2.994	683532
Mean			685443
Std. Dev.			3289.7
% RSD			0.48

Accuracy: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 5: Accuracy (recovery) data for Lopinavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	644765	5.0	5.036	100.7%	99.84%
100%	803722	10.0	10.003	100.0%	
150%	962917	14.4	14.224	98.780%	

Table 6: Accuracy (recovery) data for Ritonavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	544711	5.3	5.34	100.8%	100.51%
100%	675935	10	10.10	100.01%	
150%	812764	14.2	14.45	99.68%	

Linearity: The linearity range was found to lie from 100µg/ml to 500µg/ml of Lopinavir, 10µg/ml to 50µg/ml of Ritonavir are shown below.

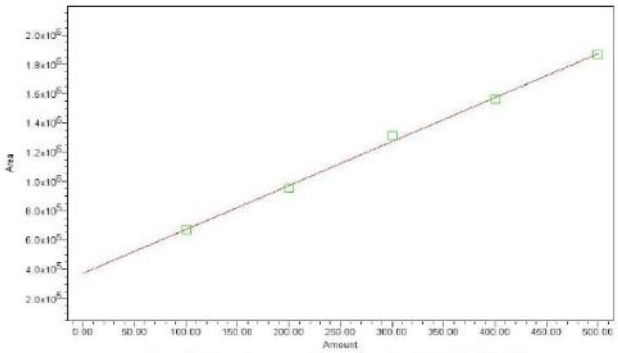

Fig. 2: Calibration graph for Lopinavir at 260 nm

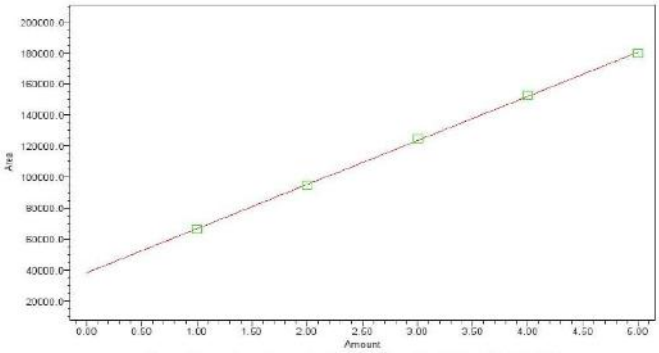

Fig. 3: calibration graph for Ritonavir at 260 nm

Limit of Detection: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

Table 7: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Lopinavir	52	152	2.9
Ritonavir	52	156	3

Limit of quantification (LOQ): The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Table 8: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Lopinavir	52	522	10.03
Ritonavir	52	524	10.1

CONCLUSION

A novel ananlytical method was developed the detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Lopinavir and Ritonavir were found to be from 100-500 µg/ml of Lopinavir and 10-50µg/ml of Ritonavir. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Lopinavir and Ritonavir. LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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