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Research Article

Development method validation of RP-HPLC method for simultaneous determination of lopinavir and Ritonavir in bulk and formulation dosage

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ABSTRACT
A reverse phase high performance liquid chromatography method was developed for the simultaneous determination of in tablet dosage form. The determination was performed by the using of two phases one is stationary phase its a thermo hypresil BDS C18 column having 250x4.6mnx 5micrometer and another mobile phase contain (30:70) volumes of water and acetonitrile was mixed and used as mobile phase adjust P.H 7.9 using sodium dihydrogen dihydrate .the flow rate was 2ml min^{-1} effuent were monitored at 210nm.The retention time of lopinavir and Ritonavir 8.452 and 10.169 respectively and lopinavir and Ritonavir other replicate standard system suitability parameters are within the limits and uniform .validation parameters those are selectivity linearity (correlation coefficients is 1.0000) ,recovery of lopinavir99.6% and Ritonavir 100.3%as per USP acceptence criteria is 98% and 102% ,precision % RSD is less than 1 and also robustness results were uniform they were no marked changes so method is highly validated it use full for pharmaceutical analysis like quality control ,stability and other studies
Keywords- RP-HPLC, lopinavir ,Ritonavir, Method development

INTRODUCTION

Lopinavir is chemically known as (2*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6dimethylphenoxy)acetamido]-4-hydroxy-1,6-iphenylhexan-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 a molecular weight of 628.80. [1] Lopinavir inhibits the HIV viral protease enzyme and prevents cleavage of the gagpolpolyprotein.[2] This subsequently results in non-infectious, immature viral particles. The chemical structure was shown in figure.

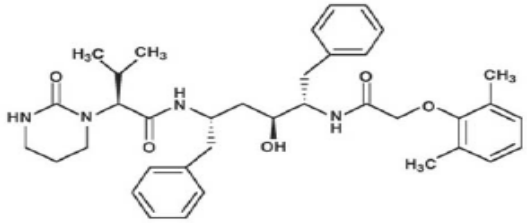

Fig: Lopinavir

Ritonavir was the first protease inhibitor used clinically [3] and chemically it is (5S,8S,10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-is(phenylmethyl)-2,4,7,12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester of molecular formula $C_{37}H_{48}N_6O_5S_2$, and its molecular weight is 720.95. Its structure is shown in figure. It is official in Indian Pharmacopoeia [4] and United States Pharmacopoeia [5].

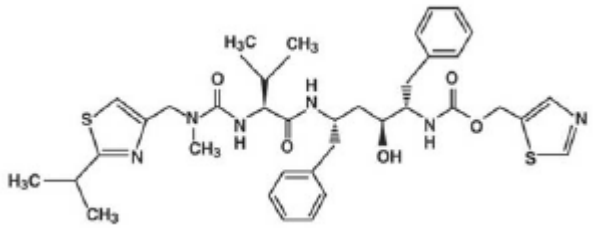

Fig: Ritonavir

Lopinavir and ritonavir is a well known composition used as anti-HIV drugs and was developed by Abbott Laboratories to improve pharmacokinetics and to reduce HIV resistance. [6] Different methods were reported in estimation of individual [7, 8, 9] as well as combination of Lopinavir and ritonavir. Simultaneous determination of Lopinavir and ritonavir dosage form were also

reported like HPLC[11], LC/MS[12], RP-HPLC[13], UV-Spectroscopy[14]. Therefore an attempt was made to develop a new rapid and sensitive RP-HPLC method and to validate as per ICH-guidelines[15].

MATERIALS AND METHODS

Drugs and instruments: Water e2695 alliance HPLC&agilent1100 and1200 system connected with PDA detector G1315B Chemostation ver.3.02,EZchrom software, Acetonitrile HPLC grade purchased from Aman chemicals Hyderabad ,HCL, NAOH, H_20_2,those are A. R. grade purchased from Asha analytical, Hyderabad.

Standard preparation : Accurately weighted 200mg of lopinavir ,25mg ritonavir is transfer into 100ml volumetric flask and make up with diluent sonicate for 10mintues kept 5mintues filtered 0.45micrometers filter paper .

Sample preparation: Accurately weighed 10tablets and calculate average weight of those tablets and crushed with motor to take tablet powder equal to single tablet weight and transfer into (equivalent weight 200mg) 100ml volumetric flask add 25ml of diluents and sonicate 15mintues then filter through 0.45micrometers filters and make up with diluents forther concentration add diluent as per test method .

Chromatographic conditions: Mobile phase ratio (30:70) ,Cosmosilc18column ,flow rate 2ml/ min, temperature 25^{o}C, wavelength 210nm

System suitability: System suitability is performed by six replicate standards inject into HPLC .it can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision .the USP defines parameters that can used to determine the system suitability prior to analysis .these parameters are retention time plate count ,resolution ,tailing and %RSD.

Selectivity: selectivity of the method was carried by out standards of lopinavir and Ritonavir were inject into HPLC after that commercial product and placebo ,excipients are one after one .it determines interference excipients peaks with analyte peaks.

Linearity: method linearity was determined by prepare five replicate standard solutions of those drugs in different (50%, 100%,150%)concentration were inject in to the HPLC. plot the graph standard areas verses concentration levels.

Accuracy (recovery studies): Recovery studies were carried out by prepare triplicate standard solutions in 50%,100%,150%concentrations levels and pre analyse the amount of samples.

Precision: Method precision was performed by prepare six replicate samples from single formulation and inject into HPLC at the same manner after 24 hours or day to day variation prepare six replicate samples from same formulation and inject into HPLC observe uniformity of test result and calculate the %RSD .

Robustness: Method robustness was determined by the small changes in chromatographic conditions like as 0.2ml flow rate +5^{o}C Temperature and inject the sample observe the results there were no marked changes compare to the other analysis.

RESULT AND DISCUSSION

System suitability parameters of standard 1 and standard 2 five replicate injection results are given below **table1 and 2** also chromatogram **figure 1**. Those result all are within the limit and also uniform % RSD is 0.5 so it proves method is suitable for analysis.

Result of selectivity was proved by the **figure 1 and 2.** These figures are standard chromatogram of lopinavir and Ritonavir second one is market formulation of lopinavir and Ritonavir they were not observed excipients and placebo peaks interference with analyte peaks so method is highly selective. Linearity of the results were given **tables 2, 3** and calibration curves are shown **figures 3,4.** Three different concentration levels of six replicate samples area was very linear and correlation coefficient was 1.000 it proves method is linear. Method accuracy results of lopinavir and Ritonavir are given **table 4 and 5**. Three spiked level (50%,100%,150%) known amount of drugs were compare to recovery of lopinavir99.6% and Ritonavir 100.3% as per ICH acceptance criteria of accuracy was 98% and 102% so it proves method is highly accurate. Intermediate precision of lopinavir and Ritonavir results were presented in **table-6**. Inter day and intraday of those runs parameters like retention time , tailing , resolution and plate count all are uniform and area %RSD was less than 1. Robustness results were given **table 7**.They were no significant changes observed at deliberate changes in temperature and flow rate trails then method is robust .

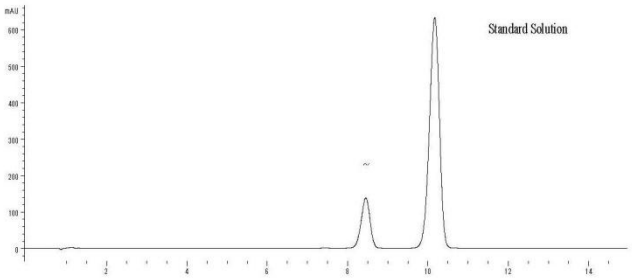

Figure No-1: Typical chromatogram of Standard solution

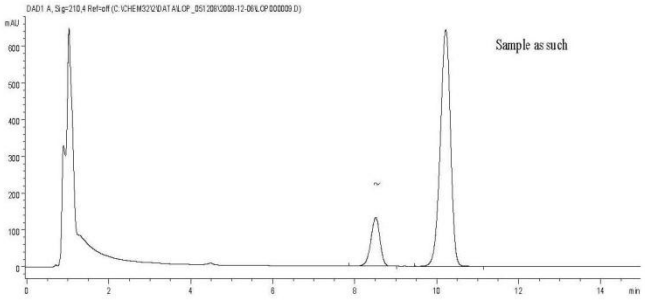

Figure No- 2: typical chromatogram of Sample as such

Table-1 Standard results

Injection no	Retention time	Area response	Retention time	Area response
	LOPINAVIR	LOPINAVIR	RITONAVIR	RITONAVIR
1	11.237	11149.9	9.269	2085.84814
2	11.307	11187.2	9.327	2089.01636
3	11.334	11162.5	9.349	2086.30176
4	11.351	11161.1	9.363	2083.90869
5	11.372	11150.4	9.378	2093.15918
6	11.402	11155.2	9.403	2080.01294
Mean	11.334	11161.1	9.348	2086.4
RSD	0.5%	0.1%	0.5%	0.2%
STANARDDEVATION		1.0%to2.0%	1.0%to2.0%	

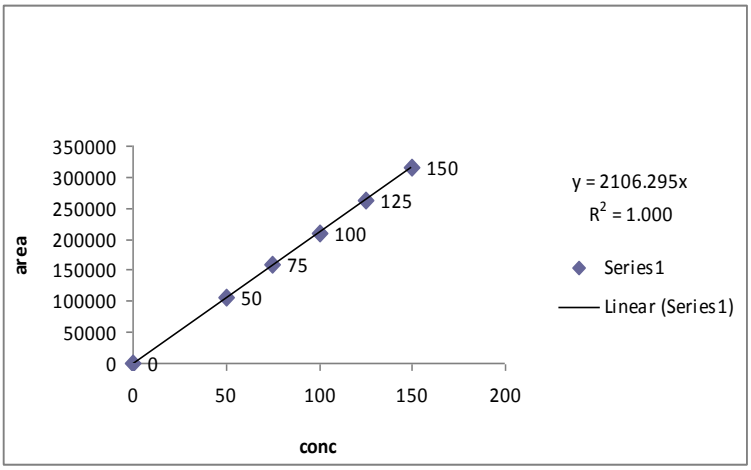

Figure-3 caliberation curve of lopinavir

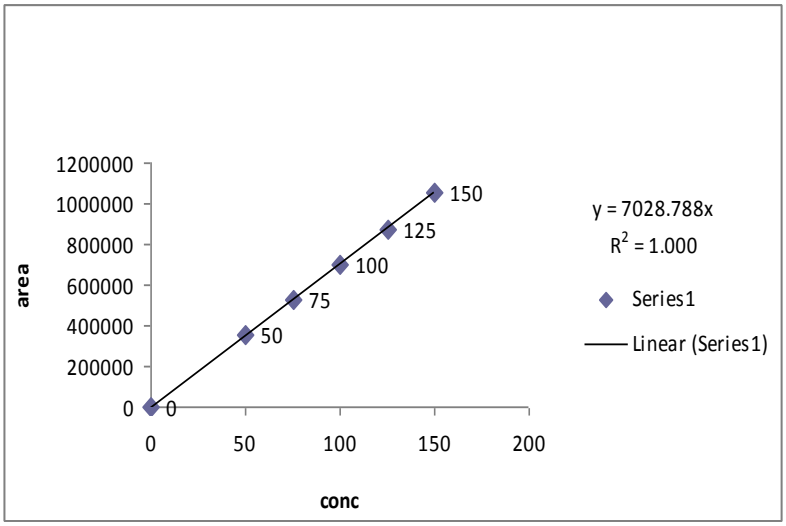

Figure-4 caliberation curve of ritonavir

Table- 2 : Linearity results of lopinavir

S.NO	Conc.	Area
1	0	0
2	50	106332
3	75	158636
4	100	210448
5	125	263039
6	150	315604

Table-3: Linearity results of ritonavir

S.NO	Conc.	Area
1	0	0
2	50	352105
3	75	526232
4	100	701595
5	125	876101
6	150	1057496

Table -4Accuracy results of lopinavir

Spiked level	Sample weight	Area response	*mg added	mg recovered	%recovery	Mean
50%	100	5836	96.35	96.37	100	99.6
50	100	5790	96.26	95.62	99.3	99.6
50	100	5776	95.39	95.39	99.6	99.6
100	100	11661	192.60	192.60	100.0	99.9
100	100	11678	192.61	192.61	100.1	99.9
100	100	11331	187.72	187.72	99.7	99.9
150	300	17193	282.18	283.18	100.6	100.6
150	300	16700	274.46	275.77	100.5	100.6
150	300	17919	293.51	295.89	100.8	100.6

Table-5 Accuracy results of Ritonavir

Spiked level	Sample weight	Area response	*mg added	mg recovered	%recovery	Mean
50%	100	1148	24.93	25.15	100.9	100.6
50%	100	1142	24.92	25.03	100.4	100.6
50%	100	1139	24.82	24.97	100.6	100.6
100%	100	2307	49.72	50.55	101.7	100.6
100%	100	2305	49.81	50.50	101.4	100.6
100%	100	2233	49.57	48.91	98.7	100.6
150%	300	3398	74.65	74.45	99.7	99.5
150%	300	3299	73.65	72.27	98.9	99.5
150%	300	3542	73.09	77.60	100.0	99.5

Table-6 Results of lopinavir and ritonavir intermediate precision

Drug	%RSD(intra day)	%RSD(inter day)
Lopinavir	0.2%	0.4%
Ritonavir	0.6%	0.6%

Table-7 Results of lopinavir and Ritonavir robustness

		Robustness			
		Lopinavir Tailng factor	RSD	Ritonavir Tailing factor	RSD
Original conditions	$+5^0$c -5^0c	1.045	0.1%	1.0403	0.2%
Flow change	-0.2ml/min	1.062	0.2%	1.062	0.1%
	+0.2ml/min	1.101	0.3%	1.096	0.4%

CONCLUSION

We had run various trail runs at different chromatographic conditions finally we founded the above conditions are suitable for development and validation for simultaneous estimation of lopinavir and Ritonavir in bulk and formulation dosage forms .This HPLC new method was very simple and accurate and also we observed validation parameters all are within the limits and %RSD is very low so it will be use full for routine analysis of quality control ,stability and further studies.

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REFERENCES:

1. Indian Pharmacopoeia, **2007**, Vol: 2, 685.
2. WHO Drug Information **2010;** Vol. 24, No. 1,
3. Katzung B.G., Basic and Clinical Pharmacology, 10th edn, McGraw Hill, New York, **2007**, 790.
4. Indian Pharmacopoeia, vol. III, **2007**, 1058.
5. United States Pharmacopoeia 30, National Formulary 25, **2007**, 3143.
6. Kaletra®. Abbott laboratories.
7. John R, Edna P, Dianne C. An isocratic reversed-phase high-performance liquid chromatographic method with ultraviolet detection at 205 nm has been validated for the determination of Indinavir, Ritonavir and Lopinavir (ABT 378) in human plasma. *J Chrom B* **2002**; 775:225–30.
8. Seshachalam U, Haribabu B and Chandrasekhar KB. A novel validated LC method for quantitation of lopinavir in bulk drug and pharmaceutical formulation in the presence of its potential impurities and degradation products. *Biomedical Chromatography*. **2007**; 21(7):716–723.
9. Sulebhavikar AV, Pawar UD, Mangoankar KV, Prabhunavelkar ND. HPTLC Method for Simultaneous Determination of Lopinavir and Ritonavir in Capsule Dosage Form. *E J Chem* **2008**; 5(4):706-712.
10. Carolina Lupi Dias, Ana Maria Bergold, Pedro Eduardo Froehlich. UV-Derivative Spectrophotometric Determination of Ritonavir Capsules and Comparison with LC Method. *Anal Letrs* **2009**; 42(12):1900-1910.
11. Ponnilavarasan I, Rajasekaran A, Dharuman JG, Kalaiyarasi D and Senthilkumar M. RP – HPLC method for simultaneous estimation of antiretroviral drugs lopinavir and ritonavir in tablet dosage form, *Digest Journal of Nanomaterials and Biostructures*, **2010**; 5(3):771-778.

12. Suneetha A, Kathirvel S and Ramachandrika G. A validated RP -HPLC method for simultaneous estimation of lopinavir and ritonavir in combined dosage form, *International journal of pharmacy and pharmaceutical sciences.* **2011**;3(1):49-51

13. Phechkrajang CM, Thin EE, Sratthaphut L, Nacapricha D and Wilairat P. Quantitative Determination of Lopinavir and Ritonavir in Syrup Preparation by Liquid Chromatography, *Journal of Pharmaceutical Science*. **2009;** 36(4):1-12.

14. Vaishali P. Nagulwar, Kishore P. Bhusari. Simultaneous Estimation of Ritonavir and Lopinavir by Absorption ratio (Q-analysis) UV Spectrophotometric Method in Combined Tablet Dosage Form. *Der Pharmacia Lettre*, **2010**: 2 (1):196-200.

15. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text on Validation of Analytical Procedures Q2A, **1994.**