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A Novel RP HPLC Method Development and Validation of Lopinavir and Ritonavir in Bulk and Pharmaceutical Dosage Forms

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Abstract: *The literature review reveals the few HPLC methods for the estimation of Lopinavir and Ritonavir and in combination with other drugs. Few methods are also reported for estimation of both drugs from formulation .we intends to develop a RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity and faster elution. Present study is to Develope of a HPLC method for analysis of both the drugs and Validation of the method using formulations High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Lopinavir and Ritonavir was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} 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.*

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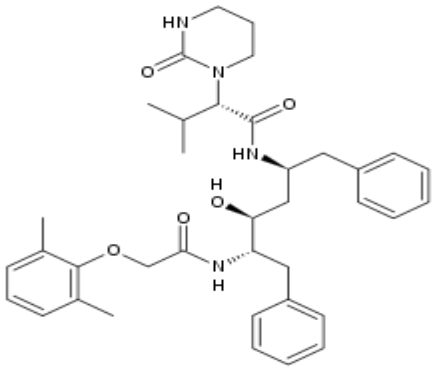
I. Literature Review

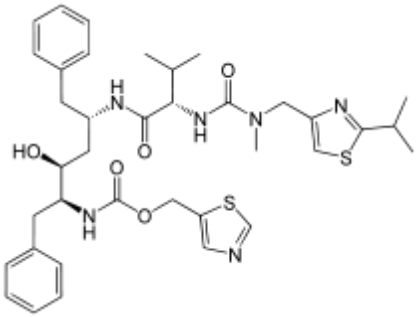
A.SUNEETHA et al,

An accurate, sensitive, precise and robust reverse phase high performance liquid chromatographic method for the simultaneous estimation of lopinavir and ritonavir in combined dosage form ha s been developed and validated. Chromatographic separation was conducted on Phenomenex Gemini C18 (250 mm×4.6 mm, 5µ) column at room temperature using Potassium hydrogen phosphate buffer (pH adjusted to 6.0 ± 0.1 with diluted potassium hydroxide solution), acetonitrile and methanol in the ratio of 50:35:15v/v and at a flow rate of 1.0 ml / min, while UV detection was performed at 254 nm. The retention time for lopinavir and rit onavir was found to be 6.0±0.2 and 3.7±0.1 min, respectively. The method was found to be linear in the range o f 400-600µg/ml for lopinavir and 100-150 µg/ml for ritonavir. The developed method was validated in terms of accuracy, precision, LOD, LOQ, robustness and solution stability. The proposed method can be successfully use d for the estimation of lopinavir and ritonavir in bulk and combined dosage forms.

Drug Profile:
LOPINAVIR



IUPAC Name **:** (2S)-N-[(2S, 4S, 5S)-5-[2-(2, 6-dimethylphenoxy) acetamido]-4-hydroxy-1, 6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1, 3-diazinan-1-yl) butanamide
Chemical formula **:** $C_{37}H_{48}N_4O_5$
RITONAVIR

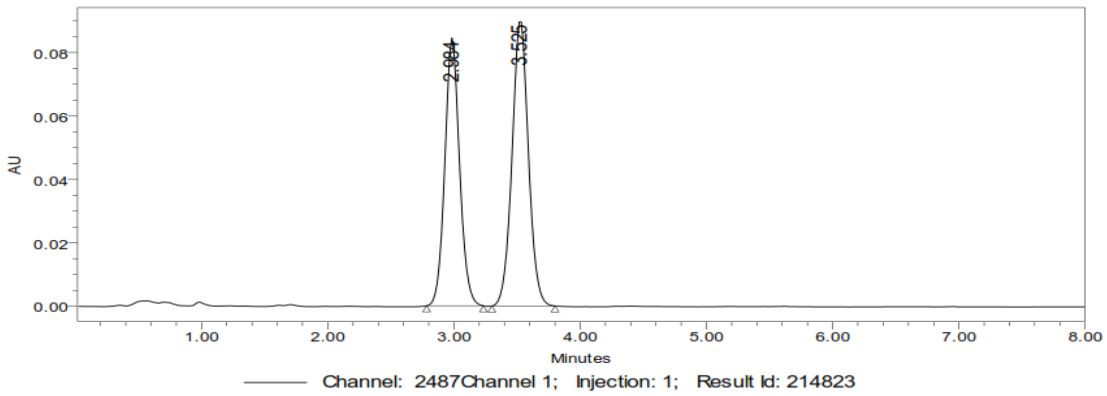


IUPAC Name **:** 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4yl]methyl})carbamoyl]amino} butanamido]-1,6-diphenylhexan-2-yl]carbamate
Chemical formula **:** C37H48N6O5S2

II. Methodology:

Chromatogram for Lopinavir and Ritonavir

Column	:	Inertsil C18 (4.6 x 250mm, 5µm)
Buffer pH	:	3.0.
Mobile phase	:	30% buffer 70% Methanol
Flow rate	:	1.0ml per min
Wavelength	:	260 nm
Temperature	:	ambient.
Run time	:	0.8 min.



Peaks of both the drugs are well separated and no peak splits are observed so this is considered as final

VALIDATION PARAMETERS

PRECISION:

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

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

INTERMEDIATE PRECESSION (RUGGEDNESS): There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Name: Lopinavir

	Name	RT	Area
1	Lopinavir	3.524	813507
2	Lopinavir	3.533	817673
3	Lopinavir	3.533	815189
4	Lopinavir	3.517	815816
5	Lopinavir	3.530	815356
Mean			815508
Std. Dev.			1492.7
% RSD			0.18

Name: Ritanovir

	Name	RT	Area
1	Ritanovir	3.001	673725
2	Ritanovir	3.009	672535
3	Ritanovir	3.010	676216
4	Ritanovir	2.997	679037
5	Ritanovir	3.007	677101
Mean			675723
Std. Dev.			2611.5
% RSD			0.39

ACCURACY:
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%	

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%	

Acceptance Criteria:

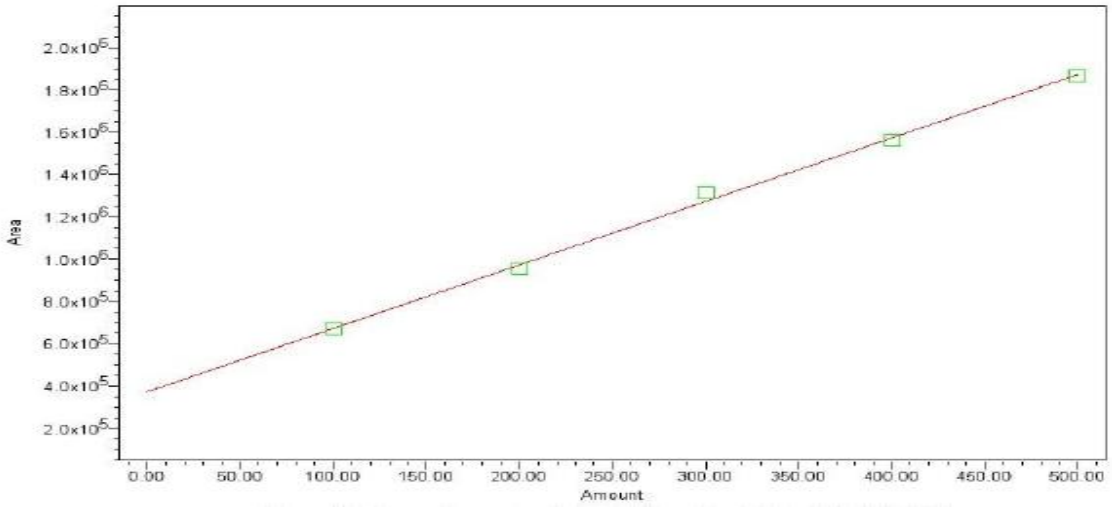
- The % Recovery for each level should be between 98.0 to 102.0%.
- The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate

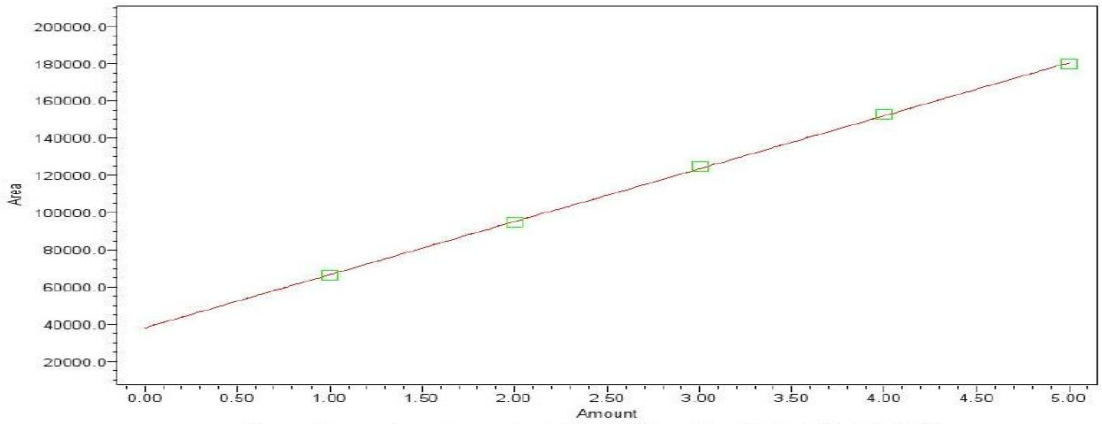
LINEARITY: The linearity range was found to lie from 100µg/ml to 500µg/ml of Lopinavir, 10µg/ml to 50µg/ml 0f Ritonavir

Parameters	Lopinavir	Ritonavir
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

calibration graph for Lopinavir at 260 nm



calibration graph for Ritonavir at 260 nm



LIMIT OF DETECTION FOR LOPINAVIR AND RITONAVIR The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

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.

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

ROBUSTNESS:

Flow Rate (ml/min) data for Lopinavir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	2716	0.9
2	0.8	3521	1.0
3	1.0	2685	0.9

flow rate (ml/min) data for Ritonavir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2090	0.9
2	1.0	3115	1.1
3	1.2	2503	0.9

Change in Organic Composition in the Mobile Phase for Lopinavir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3107	1.0
2	*Actual	3546	1.0
3	10% more	3001	1.0

Change in Organic Composition in the Mobile Phase for Ritonavir

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2818	1.1
2	*Actual	3115	1.1
3	10% more	2707	1.1

III. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Lopinavir and Ritonavir was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} 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.

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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.

References

[1]. Beckett A.H and Stenlake J.B;text book of pharmaceutical chemistry 4th Edn,-part 2 CBS publishers and Distriburots,New Delhi,1998:278,307
[2]. Douglas Skoog A., James Hollar F. and Timothy Nieman,.A Principles of Instrumental Analysis. 5thed., Thomson Learning Inc. Singapore, 1998;110 ,300
[3]. Sethi, P.D.,Quantitative Analysis of Drugs in Pharamceutical Formulation,3rded.,CBS Publishers and Distributors, 1997; 1-29 ,50-64
[4]. Mendham, R.C., Denny, J.D., Barnis , M. and Thomas, J.K., Vogel"s Text Book of Quantitative Chemical Analysis, 6thed., Pearson Education,2003; 1, 676
[5]. Sharma,B.K., Instrumental method of Chemical Analysis, 24th ed., GOEL Publishing House, Meerut,2005; 46, 68.
[6]. Chatwal G.R and Anand K.S;instrumental methods of chemical analysis,5th Edn Himalaya publishing House,mumbai,2002,2-149
[7]. Munson J.W:Modern Methods of Pharmaceutical Analysis,Medical book distributors,Mumbai,2001,17-54.
[8]. Willard H.H,Merritt L.L,Dean J.A. and settle F.A:Instrumental Methods of analysis,7th Edn,CBS Publishers and Distributors, New Delhi 1988,436-439.
[9]. Synder K.L,Kriklad J.J and Glajch J.L:Practical HPLC Method Development 2nd Edn,Wiley-Interscience Publication, USA, 1983,1-10.
[10]. Bently and Drivers:text book of pharmaceutical chemistry,8th Edn,O'Brein,oxford university press,1985,1-3.

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