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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ANTI-VIRAL DRUGS (ATAZANAVIR AND RITONAVIR) IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC METHOD

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ABSTRACT

In the research analysis a rapid, accurate and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated by selecting chromatographic parameters for simultaneous estimation of Atazanavir and Ritonavir in pharmaceutical dosage forms. The HPLC method was developed using reverse phase Phenomenex Luna C18 (4.6mm×150mm, 5µm) column with mobile phases containing Methanol: Tri Ethyl Amine Buffer (35:65% v/v) as mobile phase. The flow rate was 1ml / min with PDA detection at λ max 261nm and the injection volume was set at 10 µl with 10 min run time. This method has been validated by the use of different validation parameters such as accuracy, precision, linearity, lod and loq. Such findings showed that the system could find practical use in its tablet dosage forms as a quality assurance tool for evaluating the drug in pharmaceutical industries.

INTRODUCTION

Atazanavir is an antiretroviral protease inhibitor that is used in the therapy and prevention of human immunodeficiency virus (HIV-1) infection and the acquired immunodeficiency syndrome (AIDS). Atazanavir is a heavily substituted carbohydrazide that is an antiretroviral drug of the protease inhibitor (PI) class used to treat infection of human immunodeficiency virus (HIV). It has a role as an antiviral drug and a HIV protease inhibitor. Ritonavir is an antiretroviral protease inhibitor that is widely used in combination with other protease inhibitors in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS).

Ritonavir is a peptidomimetic agent that inhibits both HIV-1 and HIV-2 proteases. Ritonavir is highly inhibited by serum proteins but boosts the effect of other HIV proteases by blocking their degradation by cytochrome P450.

HPLC method development

Preparation of standard solution

10 mg of Atazanavir and Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 1ml of the above Atazanavir and 3ml of Ritonavir stock solutions into a 10ml

volumetric flask and dilute up to the mark with Methanol.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA Detector
Temperature : 38ºC
Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size
Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)
Flow rate : 1ml/min
Wavelength : 261 nm
Injection volume : 10 µl
Run time : 10 min

METHOD VALIDATION

System suitability

10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of Atazanavir and 3ml of Ritonavir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Specificity

Planning of Standard Solution

Precisely gauge and move 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flask and make volume sufficient with a similar dissolvable. (Stock arrangement). Further pipette 1ml of Atazanavir and 3ml of Ritonavir from the above stock arrangements into a 10ml volumetric jar and weaken sufficient with diluents.

Planning of Sample Solution

weight 10 mg proportionate load of Atazanavir and Ritonavir test into a 10mL clean dry volumetric cup and include about 7mL of Diluent and sonicate to break up it totally and make volume sufficient with a similar dissolvable. Further pipette 1ml of Atazanavir and 3ml Ritonavir above stock arrangement into a 10ml volumetric flagon and weaken sufficient with diluent.

Precision

50% Standard stock arrangement

Precisely gauge and move 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flask and make volume sufficient with a similar dissolvable. (Stock arrangement) Further pipette 0.5ml of Atazanavir and 1.5ml of Ritonavir from the above stock arrangements into a 10ml volumetric jar.

100% Standard stock arrangement

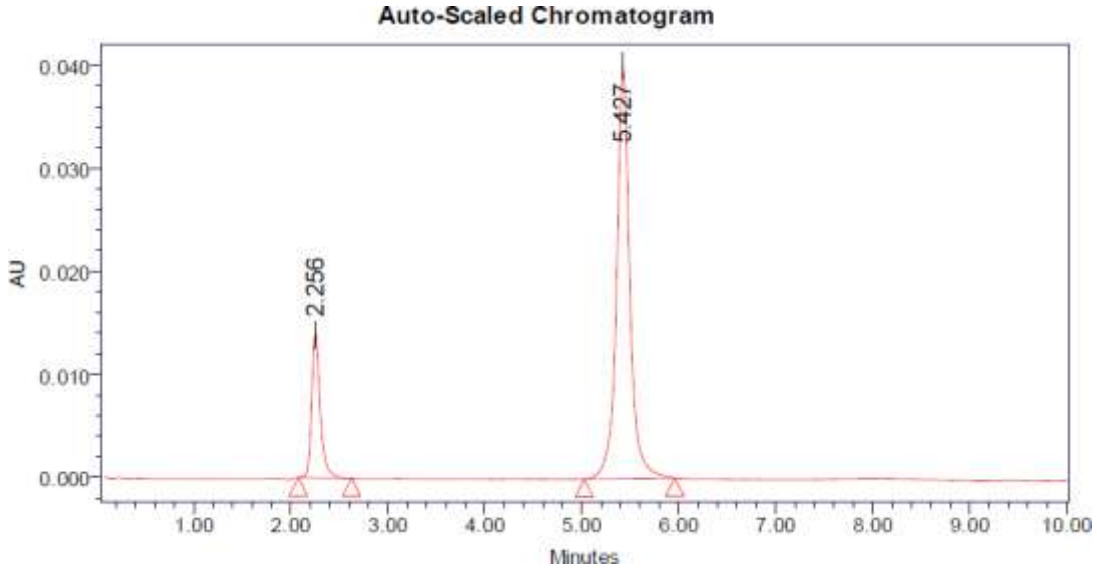
Precisely gauge and move 10mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flask and make volume sufficient with a similar dissolvable. (Stock arrangement) Further pipette 1ml of Atazanavir and 3ml of Ritonavir from the above stock arrangements into a 10ml volumetric flask.

150% Standard stock arrangement

Precisely gauge and move 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flask and make volume sufficient with a similar dissolvable. (Stock arrangement) Further pipette 1.5ml of Atazanavir and 4.5ml of Ritonavir from the above stock arrangements into a 10ml volumetric flask.

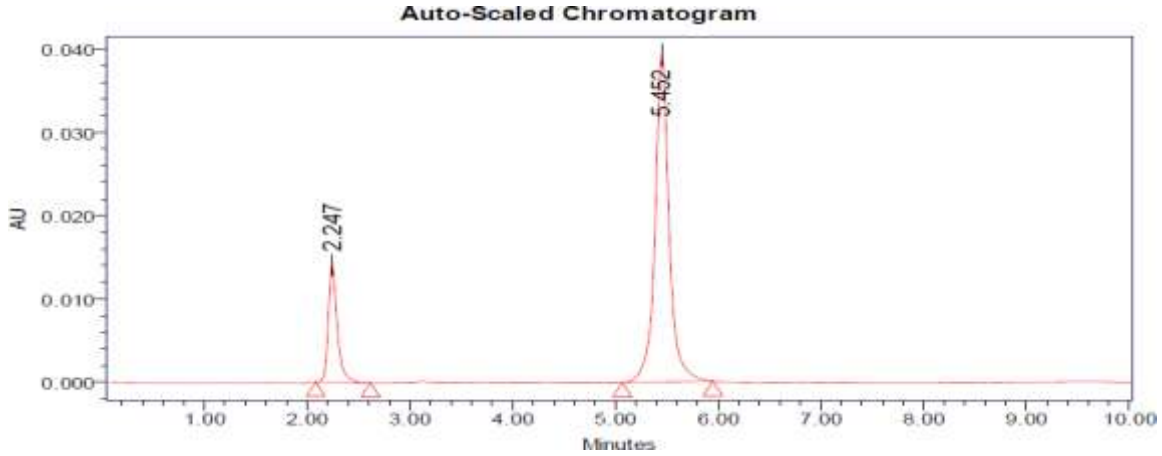
CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Atazanavir and Ritonavir in pharmaceutical dosage forms. Atazanavir was found to be soluble in water, Acetonitrile or Methanol, isopropyl alcohol, ammonium hydroxide, propylene, DMSO, Ethanol and dimethyl formamide (DMF). Methanol: Tri Ethyl Amine Buffer (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within the limit and the method was found to be precise. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Atazanavir and Ritonavir in Pharmaceutical dosage forms



S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Atazanavir	2.256	105265	18564	--	1.08	7589
2	Ritonavir	5.427	1858475	63598	5.85	1.04	6354

Optimized Chromatogram (Sample)



S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Atazanavir	2.247	106532	19865	--	1.09	7698
2	Ritonavir	5.452	1869582	645265	5.89	1.05	6452

METHOD VALIDATION

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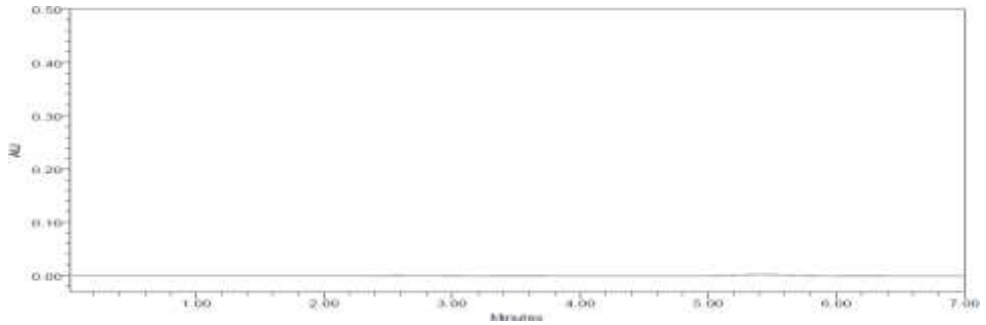
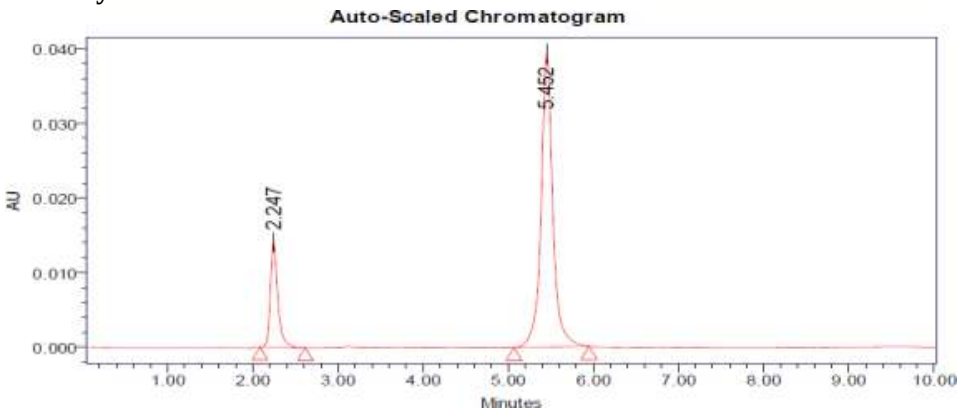
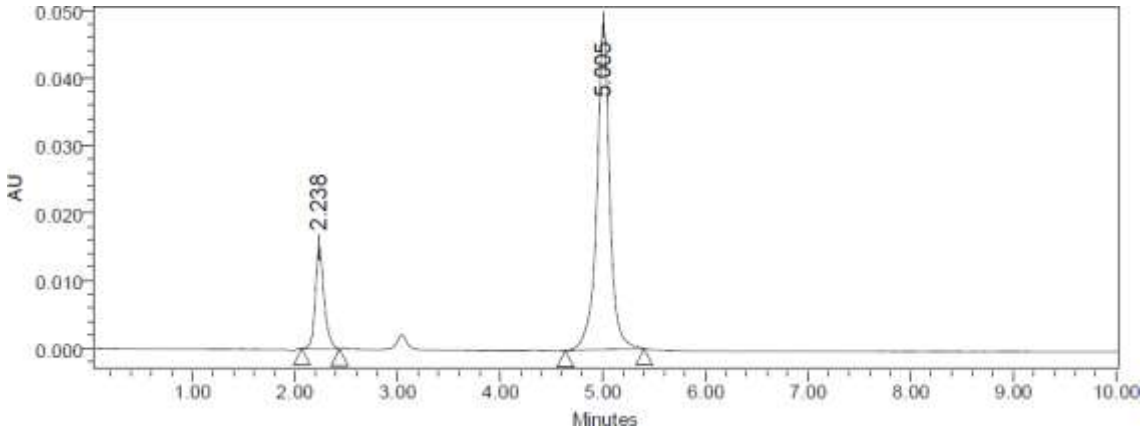


System Suitability:



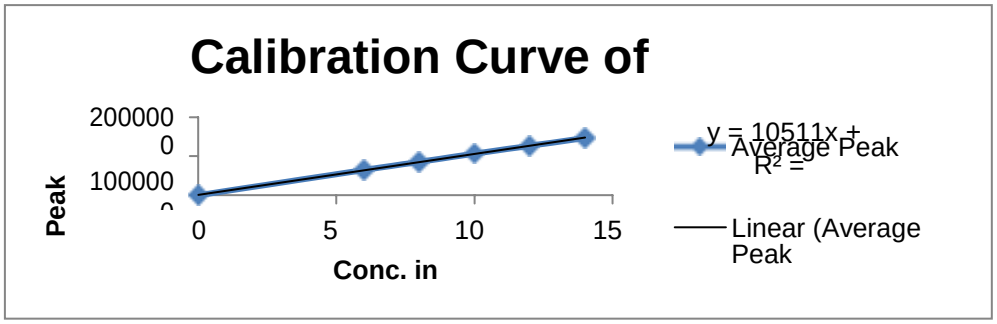
LINEARITY



LINEARITY STUDY

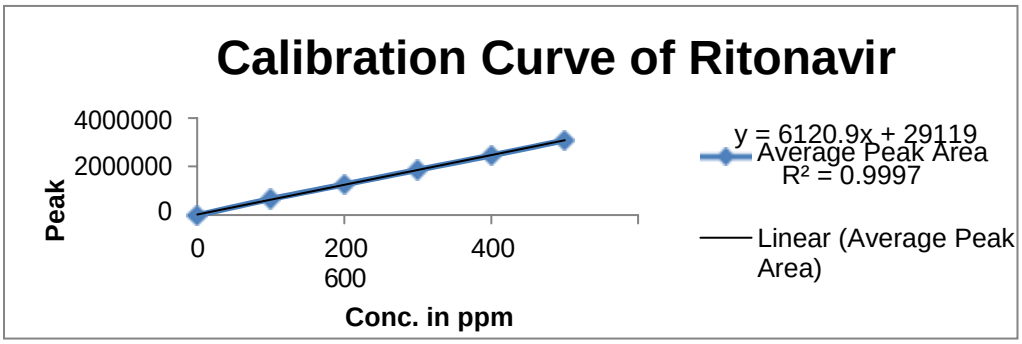
Atazanavir

Concentration µg/ml	Average Peak Area
60	648743
80	856982
100	1068542
120	1268984
140	1469853



Ritonavir

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864



ACCURACY

Accuracy 50%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Atazanavir	2.251	538358	16854	--	0.95	6352	1
2	Ritonavir	5.466	949238	56859	5.06	0.99	5826	1
3	Atazanavir	2.251	539865	16895	--	0.96	6384	2
4	Ritonavir	5.447	949158	56874	5.07	0.99	5896	2
5	Atazanavir	2.252	538987	16859	--	0.95	6395	3
6	Ritonavir	5.425	948985	56485	5.06	1.00	5845	3

Accuracy-100%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Atazanavir	2.261	1063258	18659	--	1.08	7659	1
2	Ritonavir	5.416	1866475	63658	5.86	1.05	6426	1
3	Atazanavir	2.261	1064232	18697	--	1.09	7658	2
4	Ritonavir	5.395	1868343	63698	5.87	1.06	6495	2
5	Atazanavir	2.267	1063243	18659	--	1.09	7624	3
6	Ritonavir	5.382	1868654	63685	5.86	1.05	6439	3

Accuracy-150%

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Atazanavir	2.271	1586986	21542	--	1.16	8256	1
2	Ritonavir	5.368	2784853	78548	6.52	1.12	7698	1
3	Atazanavir	2.272	1587568	21659	--	1.15	8295	2
4	Ritonavir	5.354	2783658	78695	6.53	1.13	7629	2
5	Atazanavir	2.273	1586894	21758	--	1.16	8263	3
6	Ritonavir	5.339	2787452	78564	6.52	1.12	7684	3

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

REFERENCES

1. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWil ey & Sons, Inc., Publication, (2007), P P 15-23.
2. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, IFPMA, Switzerland, (1995), vol 60, PP 1126.
3. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
4. B. Prathap, V. Haribaskar, stability indicating RP-HPLC method for simultaneous estimation of ritonavir and darunavir in bulk and its synthetic Mixture, J. Global Trends Pharm Sci, 2018, 9(2): 5549 – 5560.
5. Disha A Patel, Bhavini N Patel, Chhaganbhai N Patel, Spectrophotometric method for simultaneous estimation of Atazanavir sulfate and ritonavir in tablet dosage form, Year: 2011 Volume 6(1).
6. C.Varaprasad and K.Ramakrishna, Simultaneous Quantification of Atazanavir and Ritonavir in Pharmaceutical Dosage Form by Validated RP-HPLC Method, IJPPR, Human, 2015, Vol. 3 (4): 26-37.
7. C. Divya, A. Ajitha, T. Rama Mohana Reddy, V. Uma maheswara Rao, method development and validation of Atazanavir sulfate by various analytical techniques - a review, Int J Pharm 2014, 5(4): 1293-1296.
8. Dnyaneshwar Sukhadev Pawar, Manjusha dole, Sanjay Sawant, Jyoti M Salunke, Development and validation of RP-HPLC method for the simultaneous estimation of Atazanavir sulphate and Ritonavir in bulk and formulations, Int J Pharm Sci, 2013, vol 5, 905-909.