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RESEARCH ARTICLE

Simultaneous RP-HPLC Method for the Quantification of Durnavir and Ritonavir in bulk and it's Pharmaceutical Dosage form

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ABSTRACT
The aim of present research work to develop and validated RP-HPLC method for the simultaneous estimation Durnavir and Ritonavir in bulk and pharmaceutical dosage form. The chromatographic separation was achieved on Inertsil ODS (4.6 x 100mm, 5µm) column and maintained flow rate was 1.0 ml/min. The injection volume was 20 µl. Detection of absorption maxima was monitored at 220 nm. The optimized mobile phase was consisting of phosphate buffer pH 3.5 and acetonitrile + Methanol in the ratio of 30:70 %v/v. The linearity over to the obtained concentrations from 120-600 ppm for Durnavir and 20-100ppm for Ritonavir with correlation coefficient was found to be not more than 0.999 for both drugs. In precision studies % RSD was found to be less than 2%. The mean percentage recovery was found to be 100.86 % for Durnavir and 99.58% for Ritonavir. All the validation parameters results were found to be within the limit. So the developed method can be suggest. that routine quality control analysis of Durnavir and Ritonavir in analytical laboratories.
Keywords: Durnavir, Ritonavir, RP-HPLC, Mobile phase, Validation

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CONTENTS

1. Introduction

Darunavir is a protease inhibitor used to treat HIV. It acts on the HIV aspartyl protease which the virus needs to cleave the HIV polyprotein into its functional fragments. Darunavir, co-administered with ritonavir, and with other antiretroviral agents, is indicated for the treatment of human immunodeficiency virus (HIV) infection in antiretroviral

treatment-experienced adult patients, such as those with HIV-1 strains resistant to more than one protease inhibitor.

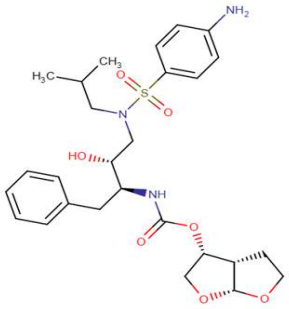

Fig 1: Chemical Structure of Durnavir

Ritonavir is an HIV protease inhibitor that interferes with the reproductive cycle of HIV. Although it was initially developed as an independent antiviral agent, it has been shown to possess advantageous properties in combination regimens with low-dose ritonavir and other protease inhibitors. It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules.

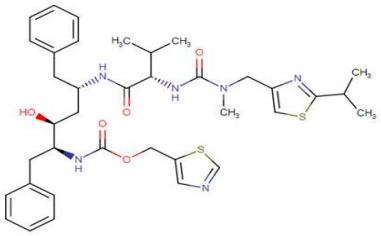

Fig 2: Chemical Structure of Ritonavir

Darunavir and Ritonavir are existing drugs. Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

2. Materials and Methods

Materials:

The following Instruments and Chemicals are used to the simultaneous estimation of Durnavir and Ritonavir.

Table 1: List of Instruments

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV 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	Company Name
1	Ritonavir	PHARMATRAIN
2	Darunavir	PHARMATRAIN
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

HPLC Method Development:

Wave length selection:

UV spectrum of 10 µg/ml Ritonavir and 10 µg/ml Darunavir in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 220 nm. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column Phenomenex column, YMC, and Inertsil ODS column. Inertsil ODS (4.6 x 100mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions:

Instrument used **:** Waters HPLC with auto sampler and UV detector.

Temperature **:**Ambient(25° C)

Mode of separation **:**Isocratic mode

Column **:**Inertsil ODS(4.6 x 100mm, 5µm))

Buffer **:**Phosphate buffer pH 3.5

Mobile phase **:**Phospahte buffer 3.5 pH and Acetonitrile (500ml)+Methanol (100ml) (30:70)

Flow rate **:** 1 ml per min

Wavelength **:** 220 nm

Injection volume **:** 20 µl

Run time **:** 10 min.

Preparation of phosphate buffer:

Accurately weigh and dissolve 6.8gms of Potassium dihydrogen ortho phosphate in 1000ml of water and adjust the pH-3.5 with orthophosphoric acid and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of Phospahte Buffer and 700 ml of Acetonitrile+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.

Standard Solution Preparation:

Accurately weigh and transfer 20 mg of Ritonavir and 120 mg of Darunavir working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20 mg of Ritonavir and 120 mg of Darunavir sample(synthetic mixture) into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

The developed method was validated according to ICH guidelines. The validation was performed by different parameters like specificity, linearity, precision, accuracy, quantification limits, robustness and system suitability.

3. Results and Discussion

System Suitability:

System suitability was integral part of many analytical procedures. It was evaluated by three replicates of Durnavir and Ritonavir standard solutions were injected into the chromatographic system and then determine the parameters like tailing, resolution and USP plate count. Resolution between two drugs must be not less than 2. Theoretical plates must be not less than 2000. Tailing factor must be not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

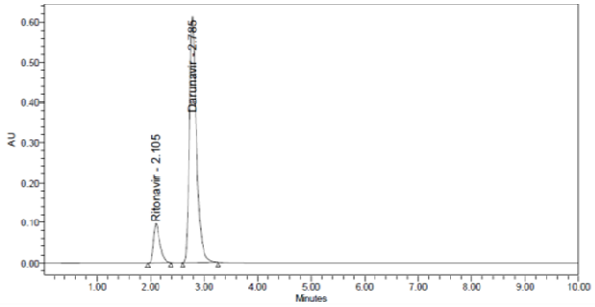

Fig 3: Chromatogram for system suitability

Linearity:

Inject each level into the chromatographic system and measure the peak area.The linearity range was found to lie from 20µg/ml to 100µg/ml of Ritonavir, 120µg/ml to 600µg/ml of Darunavir Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The data was shown in table 4 and fig 4,5.

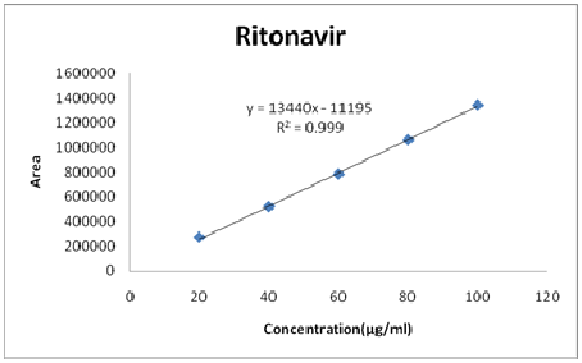

Fig 4: Calibration graph for Ritonavir

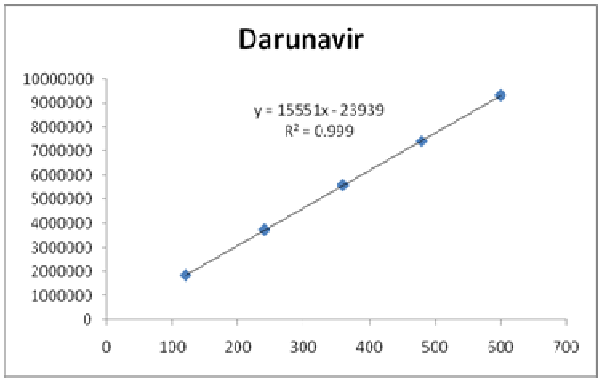

Fig 5: Calibration graph for Darunavir

Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The standard solution was injected for six times and measured the area for all injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The % RSD for the area of six standard injections results should not be more than 2%.

Intermediate Precision:

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. The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The % RSD for the area of six standard injections results should not be more than 2%.

Accuracy

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. Calculate the Amount found and Amount added for Ritonavir & Darunavir and calculate the individual recovery and mean recovery values.

LOD & LOQ:

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

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

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1 ml/min:

Standard solution 60 ppm of Ritonavir& 360 ppm of Darunavir was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

The Organic composition in the Mobile phase was varied from ±10%:

Standard solution 60 ppm of Ritonavir & 360 ppm of Darunavir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%.Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Ritonavir and Darunavir using the proposed method.

4. Conclusion

The estimation of Ritonavir and Darunavir was done by RP-HPLC. The linearity of Ritonavir and Darunavir was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.4 and 0.5 for Ritonavir and Darunavir which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.3 and 0.3 for Ritonavir and Darunavir which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.58% and 99.86% for Ritonavir and Darunavir. The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits which shows that the method is having good system suitability and precision under given set of conditions.

Table 3: Results of system suitability parameters

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Ritonavir	2.105	784954	96962	3.04	1.45	3568.55
2	Darunavir	2.785	5528694	612232		1.35	5239.73

Table 4: Area of different concentration of Ritonavir and Darunavir

S. No	Ritonavir		Darunavir	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	20	268654	120	1832427
2	40	520739	240	3726834
3	60	783140	360	5582709
4	80	1061084	480	7407799
5	100	1342518	600	9322648

Table 5: Results of Precision for Ritonavir and Darunavir

Injection	Area for Ritonavir	Area for Darunavir
Injection-1	789316	5523508
Injection-2	785334	5528488
Injection-3	780020	5591669
Injection-4	786180	5523942
Injection-5	781227	5539053
Injection-6	782839	5567550
Average	784152.7	5545701.7
Standard Deviation	3450.5	27917.4
%RSD	0.4	0.5

Table 6: Results of Intermediate precision for Ritonavir and Darunavir

Injection	Area for Ritonavir	Area for Darunavir
Injection-1	784589	5550899
Injection-2	787669	5526967
Injection-3	788979	5549869
Injection-4	783607	5543117
Injection-5	786196	5540984
Injection-6	781921	5582718
Average	785493.5	5549092.3
Standard Deviation	2627.3	18577.0
%RSD	0.3	0.3

Table 7: Accuracy (recovery) data for Ritonavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	396812	10	10.08	100.85	99.58
100%	787039	20	20.00	100.01	
150%	1173386.0	30	29.82	99.40	

*Average of three determinations

Table 8: Accuracy (recovery) data for Darunavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2754176	60	59.84	99.73	100.86
100%	5551291.7	120	120.61	100.51	
150%	8229366.3	180	178.79	99.33	

*Average of three determinations

Table 9: Results of LOD & LOQ

Drug name	LOD	LOQ
Ritonavir	2.90	9.90
Darunavir	3.10	10.10

Table 10: Results for variation in flow for Darunavir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3497.58	1.12
2	1	3568.55	1.45
3	1.1	3191.13	1.25

Table 11: Results for variation in flow for Ritonavir

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Plate Count	USP Tailing
1	0.9	2.93	6032.68	1.30
2	1	3.04	5239.73	1.35
3	1.1	2.50	5428.68	1.15

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 12: Results for variation in mobile phase composition for Darunavir

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3631.56	1.10
2	*Actual	6037.41	1.25
3	10% more	3662.04	1.54

Table 13: Results for variation in mobile phase composition for Ritonavir

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Resolution	USP Plate Count	USP Tailing
1	10% less	2.59	5528.47	1.50
2	*Actual	3.94	7679.28	1.08
3	10% more	3.08	5509.65	1.47

*Results for actual Mobile phase composition have been considered from Accuracy standard.

Table 14: Results for Stability of Ritonavir and Darunavir

Sample Name	Ritonavir		Darunavir	
	Area	% Degraded	Area	% Degraded
Standard	785386	-	5512235	
Acid	763563	2.78	5312622	3.62

Base	757893	3.50	5286737	4.09
Peroxide	759376	3.31	5297856	3.89
Thermal	735422	6.36	5215762	5.38
Photo	745353	5.10	5257689	4.62

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