Research Article

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Method and Development and Validation for Simultaneous Estimation of Ritonavir and Darunavir in Its API and Pharmaceutical Dosage Form by Using RP HPLC Method

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ABSTRACT

A short selective, precise, accurate and sensitive for the estimation of Ritonavir and Darunavir was done by RP-HPLC. The chromatographic separation was clear at the flow rate of 1 ml/min, at UV detection of 244 nm. The assay for ritonavir and darunavir were found to be 100.10 and 100.53 respectively. Which shows that the method is useful for routine analysis. The linearity of Ritonavir and Darunavir was found to be direct with a relationship coefficient of 0.999 and 0.999, which appears that the strategy is competent of creating great affectability the acknowledgment criteria for LOD and LOQ are 3.07 and 10. The LOD and LOQ for Ritonavir was found to be 3.00 and 10.02 and LOD and LOQ for Darunavir was found to be 3.02 and 10.07. The vigor restrain for versatile stage variety and stream rate variety are well inside the constrain, the % debasement comes about are in limits. Which appears that the strategy is having great framework reasonableness and accuracy beneath given set of conditions. From the recovery and the stability studies, showing acceptable limits, it can be concluded that this can be employed for estimation of ritonavir and darunavir in tablet dosage forms.

Keywords: Ritonavir, Darunavir, Validation, stability indicating method, degradation products.

INTRODUCTION

Ritonavir is an antiretroviral medication used along with other medications to treat HIV/AIDS.[1] It is an HIV protease inhibitor that interferes with the reproductive cycle of HIV.[2,3] 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.[4,5] It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules.[6] Ritonavic inhibits the HIV viral proteinase enzyme that normally cleaves the structural and replicative proteins that arise from major HIV genes, such as gag and pol. Ritonavir (Fig. 1) prevents the cleavage of the gag-pol polyprotein, which results in noninfectious, immature viral particles.[7,8] It is a type II ligand that perfectly fits into the CYP3A4 active site cavity and irreversibly binds to the heme iron via the thiazole nitrogen, which decreases the redox

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potential of the protein and precludes its reduction with the redox partner, cytochrome P450 reductase.[9-11] Ritonavir is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of ritonavir in ethanol is approximately 5 mg/ml and approximately 15 mg/ml in DMSO and DMF. [12-15] Ritonavir is sparingly soluble in aqueous buffers. Darunavir (Fig. 1)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.[16,17] It is generally recommended for use with other antiretrovirals. It is often used with low doses of ritonavir or cobicistat to increase darunavir levels.[18] It may be used for prevention after a needlestick injury or other potential exposure.[19,20] It is taken by mouth once to twice a day. Darunavir is a nonpeptidic inhibitor of protease (PR) that lodges itself in the active site of PR through a number of hydrogen bonds. It was developed to increase interactions with HIV-1 protease and to be more resistant against HIV-1 protease mutations. Darunavir is known to bind to two distinct sites on the enzyme: the active site cavity and the surface of one of the flexible flaps in the protease dimer.[21-25] Darunavir ethanolate is a white to off-white powder with a solubility of approximately 0.15 mg/mL in water at 20°C. It is soluble in organic solvents such as DMSO and dimethyl formamide (DMF) which should be purged with an inert gas. It is sparingly soluble in aqueous buffers.[25,26] Only few methods were reported for the simultaneous estimation of ritonavir and darunavir by HPLC.[27-29] Hence we had made an attempt to develop a simple, accurate and precise RP-HPLC method for the simultaneous estimation of ritonavir and darunavir.

METHODOLOGY

Gift samples of ritonavir and darunavir were received from pharmatrain lab, Hyderabad, whereas water, methanol for HPLC, acetonitrilefor HPLC and phosphoric acid were purchased from lichrosolv, Merck.

Instrumentation

Waters HPLC (2695 separation module) was used for the separation of ritonavir and darunavir. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Preparation of buffer

Pipette 1ml of orthophosphoric corrosive broken up in 1000 ml of HPLC water Ph was balanced up to 3.0. Last arrangement was sifted through 0.44 µm Film channel and sonicate it for 10 mins.

Preparation of mobile phase

Precisely measured 300 ml (30%) of over buffer, 600 ml (60%) of Methanol HPLC and 100 ml (10%) of Acetonitrile HPLC were blended and degassed in an ultrasonic water shower for 10 minutes and after that sifted through 0.45 µ channel beneath vacuum filtration and used as the diluent.

Standard Solution Preparation

Precisely weigh and exchange 10 mg of Ritonavir and 60 mg of Darunavir working standard into a 100 ml clean dry volumetric jar include around 7 mL of Diluent and sonicate to break up it totally and make volume up to the stamp with the same dissolvable. (Stock solution) Further pipette 3 ml of the over stock arrangements into a 10ml volumetric jar and weaken up to the stamp with diluent.

Sample Solution Preparation

Precisely weigh 10 tablets pulverize in mortor and pestle and exchange proportionate to 10 mg of Ritonavir and 60 mg Darunavir test into a 100 mL clean dry volumetric jar include almost 7 mL of Diluent and sonicate it up to 15 mins to break up it totally and make volume up to the check with the same dissolvable. At that point it is Sifted through 0.45 micron Infusion channel. (Stock solution) Further pipette 3ml of Ritonavir and Darunavir from the over stock arrangement into a 10ml volumetric jar and weaken up to the stamp with diluent.

Method development and optimisation

Due to the significant difference in the physical and chemical properties of ritonavir and darunavir, several mobile phases and columns were initially trialed in order to have both eluents on the same chromatogram. The suitability of the column and the mobile phase used in the optimized method have been decided based upon the basis of the selectivity, sensitivity as well as acceptable

chromatographic parameters of the produced peaks in terms of peak sharpness, peak symmetry, tailing factor and resolution between the two peaks. (Fig. 2)We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

RESULTS AND DISCUSSION

Method Validation

The optimized method for simultaneous determination of ritonavir and darunavir has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) [26] for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.[30]

Optimized Chromatographic Conditions

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Xterra C18 (4.6*150mm, 5µ) column
Buffer	:	0.1% OPA
pH	:	3.0
Mobile phase	:	30% buffer 60% Methanol 10% Acetonitrile
Flow rate	:	1 ml per min
Wavelength	:	220 nm
Injection volume	:	20 µl
Run time	:	10 min.

System Suitability

Following figure 2 for the crests due to Ritonavir and Darunavir in Standard arrangement ought to not be more than 2.0 Theoretical plates for the Ritonavir and Darunavir crests in Standard arrangement ought to not be less than 2000. Resolution for the Ritonavire and Darunavir crests in standard arrangement ought to not be less than 2(Table 1).

Linearity

The standard stock solution of Darunavir is diluted in the concentration range of (60–300 µg/ml). Triplicates of such concentration range were prepared and plotted on a calibration curve. (Fig.3,4) The standard stock solution of Ritonavire is diluted in the concentration range of (10–50 µg/ml). Triplicates of such concentration range were prepared and plotted on a calibration curve. Slope, intercept and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the analytical method.(Table 2)

Accuracy

Accuracy of the proposed method was confirmed with ritonavir and darunavirseparately at 3 different levels 50%, 100% and 150%, the determinations of these 3 levels have been recorded to obtain the mean and % recovery.(Table 3,4)

LOD and LOQ

The LOD and LOQ arrangements was arranged infused, for three times and measured the region for all three infusions in HPLC. The %RSD for the zone of six reproduce infusions was found to be inside the required limits.(Table 5,6)

Degradation Studies

The ICH entitled solidness testing of modern sedate substances and items requires that stretch testing be carried out to illustrate the characteristic soundness characteristics of the dynamic substance. (Table 7)The point of this work was to perform the stretch debasement considers on the ritonavir and darunavir utilizing the proposed strategy.

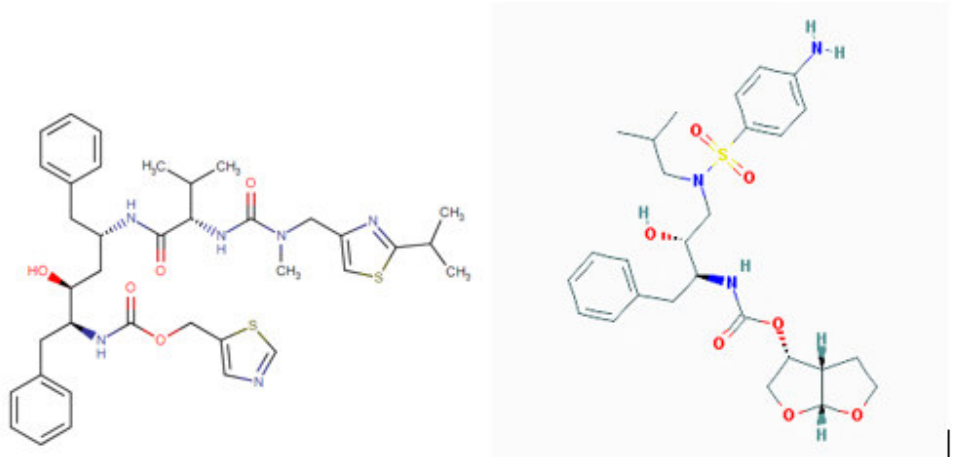

Fig.1: Structure of Ritonavir and Darunavir

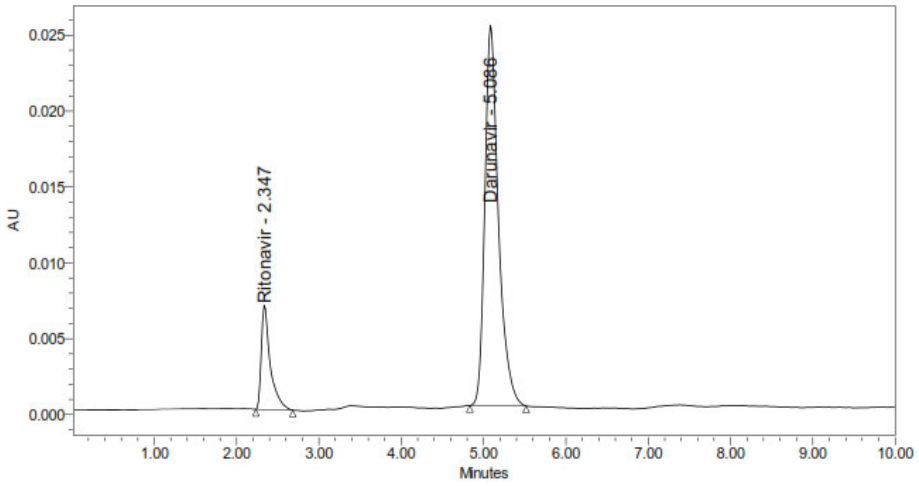

Fig.2: Standard Chromatogram ofRitonavir and Darunavir

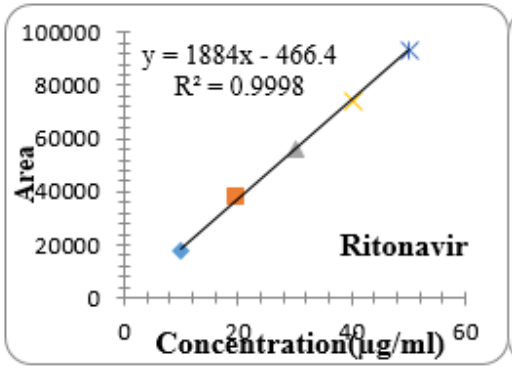

Fig 3: Calibration graph for Ritonavir

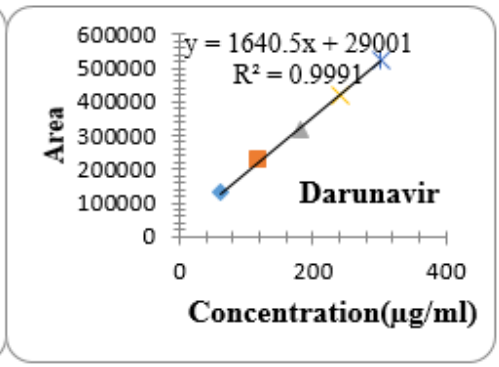

Fig 4: Calibration graph for Darunavir

Table 1: Results of system suitability parameters

S .No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Ritonavir	2.347	56445	6857		1.41	2593.29
2	Darunavir	5.086	320903	25250	11.53	1.18	4843.11

Table 2: Results of Linearity of Ritonavir and Darunavir

S. No.	Ritonavir Concentration (µg/ml)	Area	Darunavir Concentration (µg/ml)	Area
1	10	17896	60	132359
2	20	37780	120	223105
3	30	56233	180	320315
4	40	74754	240	419173
5	50	93611	300	526461

Table 3: Accuracy (recovery) data for Ritonavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	28244.7	5	5.01	100.26	
100%	56457.3	10	10.02	100.20	100.36
150%	85035.3	15	15.09	100.61	

*Average of three determinations

Table 4: Accuracy (recovery) data for Darunavir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	161058.3	30	30.12	100.39	
100%	323719.3	60	60.54	100.89	100.64
150%	484374.0	90	90.58	100.64	

*Average of three determinations

Table 5: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ritonavir	43	129	3.00
Darunavir	43	130	3.02

Table 6: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Ritonavir	43	431	10.02
Darunavir	43	433	10.07

Table 7: Results for Stability of Ritonavir and Darunavir

Sample Name	Ritonavir		Darunavir	
	Area	% Degraded	Area	% Degraded
Standard	56232.7		320211.3	
Acid	54275	3.48	295636	7.67
Base	52453	6.72	302783	5.44
Peroxide	53967	4.03	289767	9.51
Thermal	51867	7.76	316254	1.24
Photo	50162	10.80	286735	10.45

CONCLUSION

The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific and linear. It can be used for routine analysis of ritonavir and darunavir in combination products.

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