ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Suppl. 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

STABILITY-INDICATING REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ESTIMATION OF DARUNAVIR AND RITONAVIR

SINDU PRIYA D*, GOWRI SANKAR D, MASTHANAMMA SK

Department of Pharmaceutical Analysis and Quality Assurance, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India. Email: sindupriya87@gmail.com

Received: 02 March 2016, Revised and Accepted: 12 May 2016

ABSTRACT

Objective: The main objective of the proposed study was to develop and validate a new stability indicating reverse phase high performance liquid chromatography method for the simultaneous estimation of darunavir (DRV) and ritonavir (RTV).

Methods: The method was optimized using Atlantis C18 column (50 mm×4.6 mm, 5 µm, Waters Corporation, Milford, USA). Acetonitrile and water were used as mobile phase in the proportion of 60:40. The flow rate was 0.8 ml/minutes and the effluent was monitored at 230 nm.

Results: The retention time of DRV and RTV was 3.15 minutes and 4.59 minutes, respectively. The method was precise as it showed a % relative standard deviation of <2%. The percentage recoveries of both the drugs DRV and RTV were 99.8-100.01% and 99.5-99.97%, respectively. The linearity of DRV and RTV was in the range of 40-120 and 4-20 µg/ml, respectively. Calibration curve showed good linearity and range. The correlation coefficient of DRV and RTV was 0.999 each. Moreover, the results obtained for limit of quantification, limit of detection, robustness, and ruggedness were well within the acceptance criteria.

Conclusion: The proposed method was found to be simple, rapid, accurate, precise, and stability indicating. It was found to be economical and suitable for simultaneous determination of DRV and RTV which is can also be applied for pharmaceutical dosage form.

Keywords: Darunavir, Ritonavir, Reversed-phase-high performance liquid chromatography, Simultaneous estimation, Forced degradation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9s2.11494

INTRODUCTION [1,2]

Darunavir ethanolate (DRV) is an antiviral drug and inhibitor of the human immunodeficiency virus (HIV) protease. Chemically, it is [(1S,2R)-3-[[(4-aminophenyl) sulfonyl] (2methylpropyl) amino]-2-hydroxy-1(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR) hexahydrofuro [2,3-b] furan-3-yl ester monoethanolate. DRV selectively inhibits the cleavage of HIV-1 encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles. DRV is co-administered with ritonavir (RTV) and with other antiretroviral agents, is indicated for the treatment of HIV-1 infection (Fig. 1a).

RTV is (5S, 8S, 10S, 11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-(2-[1-methylethyl]-4-thiazolyl]-3, 6-dioxo-8, 11-bis (phenyl methyl)-2, 4, 7, 12-tetraazatridecan-13-oic acid 5-thiazolyl methyl ester. RTV is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS (Fig. 1b).

There are very few reported methods for the analysis of drugs DRV and RTV individually and a few methods in combination with other drugs [3-5].

METHODS

Instrumentation

An agilent - 1200 series high performance liquid chromatography (HPLC) consisting of a binary pump, column (Atlantis C18 column (50 mm×4.6 mm, 5 µm) compartment, manual injector, and diode array detector (M/s. Agilent technologies, USA).

Reagents and chemicals

HPLC grade solvents acetonitrile (ACN) and water were obtained from Merck Specialities Pvt., Ltd., India. Water was deionized and further purified using Milli-Q plus water purification system, Millipore Ltd. USA.

Stock solution preparation

A amount of 100 mg of each standard (DRV and RTV) was weighed into different volumetric standard flasks of 100 ml capacity. The concentration in the flask was diluted using diluent. Further, working standard was prepared by diluting further to get 80 µg/ml of DRV and 10 µg/ml RTV.

Method development

Initially, trials were conducted. Each trial mixture of known components was injected and observed for resolution and tailing factor of the peaks. Various proportions of water and ACN were tried as mobile phase and a ratio of ACN and water as 60:40 gave improved peak symmetry and resolution. Different flow rates of the mobile phase were tried for good resolution. Finally, the chromatographic conditions were optimized at a flow rate of 0.8 ml/minutes, the injection volume of 10 µL, run time of 7 minutes. The retention time for DRV and RTV was found to be 3.15 minutes and 4.59 minutes, respectively. An absorption maximum was found to be 230 nm and peaks shape was good. The method was further validated under the optimized chromatographic conditions.

Method validation [6]

Once chromatographic conditions were established; the method was validated in compliance with ICH guidelines. The following parameters like system suitability along with specificity, linearity, precision, accuracy, limits of detection (LOD), and limit of quantification (LOQ) were performed for validation.

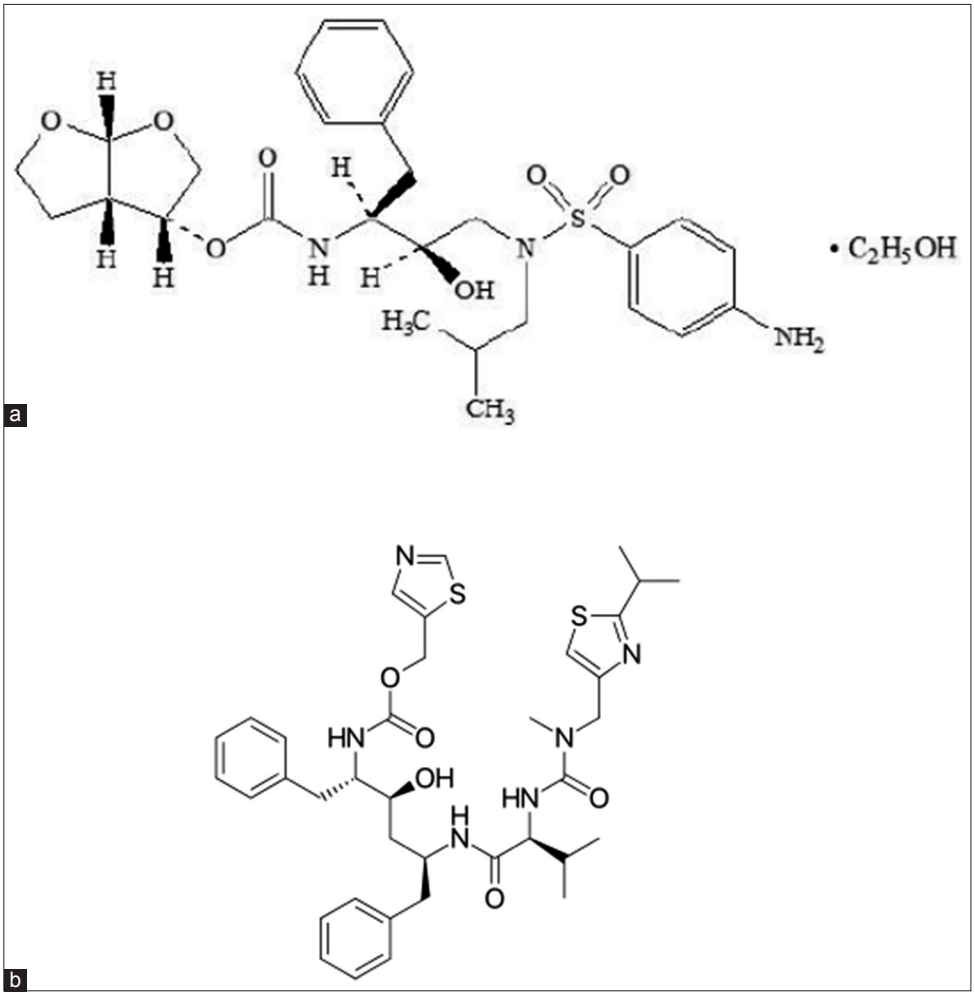

Fig. 1: (a) Darunavir ethanolate. (b) Ritonavir

System suitability

The standard solution was prepared using working standard as per the method. Moreover, six replicates are injected into the system.

Specificity

The specificity of the method was determined by comparing chromatograms obtained from standard, blank and that of forced degradation studies.

Linearity

The linearity of detector response was established by plotting graph between concentrations versus average area counts of the analytes.

Precision

System precision

Six replicate injections of the standard solution were injected into the HPLC system.

Method precision

The precision of test method was evaluated by injecting six individual samples prepared from the same batch.

Accuracy

A study of accuracy (recovery) was performed on known amount of placebo by spiking active pharmaceutical ingredient. Samples were prepared as per the proposed method at 50-150% of the sample concentration.

Robustness

Robustness of the method was investigated by varying the instrumental conditions such as flow rate (±10%), wavelength of detection (±5 nm), and organic content in mobile phase (±2%) and results were shown in Table 1 standard solution was prepared and analysed as per the test procedure monitored the system suitability results.

LOD and LOQ limits

The LOD and LOQ values were calculated from the calibration curves as k*SD/b where k=3 for LOD and 10 for LOQ. SD is the standard deviation of the response of the minimum detectable drug concentration, and b is the slope of the calibration curve.

Forced degradation studies [7]

Acid degradation studies

To 5 ml of working standard containing DRV and RTV, 2.5 ml of 0.1N HCl was added and refluxed at 60°C for 30 minutes and neutralized with 0.1N NaOH. The resultant solutions were injected into system and chromatograms were recorded to assess the stability of the sample.

Alkali degradation studies

To 5 ml of working standard containing DRV and RTV, 2.5 ml of 0.1N NaOH was added and refluxed at 60°C for 30 minutes and neutralized with 0.1N HCl. The resultant solutions were injected into system and chromatograms were recorded to assess the stability of the sample.

Table 1: Accuracy data (triplicate values at 50, 100 and 150% levels) of DRV and RTV

Concentration of spiked level	Amount added	Amount found	Recovery %	Mean recovery %	RSD %
RTV (%)					
50	5.02	4.98	99.20	99.7	1.11
	5.08	5.03	99.15		
	4.93	4.98	101.42		
100	10.02	9.99	99.06	99.5	0.38
	9.98	9.89	99.82		
	10.07	10.05	99.80		
150	14.97	14.85	99.19	99.97	0.7
	14.82	14.9	100.53		
	15.01	15.04	100.19		
DRV (%)					
50	40.09	40.2	100.27	100.01	0.44
	40.12	40.23	100.22		
	40.3	40.1	99.52		
100	79.82	79.86	100.01	99.82	0.02
	80.09	79.84	99.68		
	80.22	80.01	99.73		
150	120.42	120.21	99.82	99.89	0.08!
	120.17	119.96	99.82		
	120.05	120.01	99.96		

DRV: Darunavir, RTV: Ritonavir, RSD: Relative standard deviation

Dry heat degradation

To 5 ml of working standard containing DRV and RTV was transferred into a 10 ml vial and placed in a hot air oven at 105°C for 6 hrs and the vial was reconstituted to 10 ml. The resultant solutions were injected into system and chromatograms were recorded to assess the stability of the sample.

Photostability studies

Photostability of the drug was studied by exposing 5 ml of working standard containing DRV and RTV to UV light by keeping the beaker in UV Chamber for 7 days or 200 Watt hrs/m^2 in photostability chamber. For HPLC study, from the resultant solution, 10 µl was injected into the system and chromatograms were recorded to assess the stability of the sample.

Oxidative degradation

To 5 ml of working standard containing DRV and RTV, 5 ml of 3% H_2O_2 was added, kept at room temperature for 30 minutes at 600°C. The resultant solutions were injected into system and chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

For six replicate injections, system suitability parameters like number of theoretical plates and tailing factor were found to be within specified limits. System suitability results were given in Table 2. Respective chromatograms of standard drug, blank and degradation studies were represented from Figs. 2a-h. There was no interference of any peak at the retention time of analyte peaks from blank and peak purity of all forced degradation treated samples were passed. From this study, it has been concluded that the proposed method is specific and stability indicating for the estimation of DRV and RTV. The data for forced degradation are tabulated in Table 3. Linearity data were shown in Table 4 and represented graphically in Figs. 3a and b. Indicate that the response is linear over the specified range. The % relative standard deviation for system precision and method precision was found to be in the limits, i.e., <2 as given in Table 5. Accuracy data are shown in Table 1 indicate that the method has an acceptable level of percentage recoveries for both the drugs DRV and RTV of 99.8-100.01% and 99.5-99.97%, respectively. LOD and LOQ were found to be 2.724 and 9.080 µg/ml for DRV and 0.549 and 1.830 µg/ml for RTV. The method was found to be robust as the method resisted the deliberate changes in the optimized conditions. The results of robustness are given in Table 6. The sample solutions of DRV and RTV were stable up to 24 hrs at 25°C temperature and did not show any appreciable change in the sample area.

Table 2: System suitability

Serial number	Drug	Tailing factor	Theoretical plates*
1	DRV	0.96	7518
2	RTV	1.02	6382

*Mean of six determinations, DRV: Darunavir, RTV: Ritonavir

Table 3: Forced degradation data

Treatment	Label claim %	Degradation %	Peak purity		Pass/ fail
			Purity angle	Purity threshold	
DRV					
Control	100	0	1.349	4.256	Pass
Acid	84.9	15	1.832	4.129	Pass
Alkali	83.1	16.8	1.724	4.953	Pass
Peroxide	82.8	16.9	1.426	4.092	Pass
Thermal	80.5	19.3	1.37	4.772	Pass
Photolytic	85.3	14.6	1.448	4.821	Pass
Hydrolysis	85.6	14.2	1.312	4.637	Pass
RTV					
Control	100	0	1.634	4.397	Pass
Acid	82.9	16.9	1.562	4.132	Pass
Alkali	83.6	16.3	1.643	4.982	Pass
Peroxide	82.8	17.1	1.248	4.756	Pass
Thermal	79.8	20	1.534	4.343	Pass
Photolytic	82.3	17.5	1.562	4.621	Pass
Hydrolysis	82.1	17.7	1.234	4.213	Pass

DRV: Darunavir, RTV: Ritonavir

Table 4: Linearity of the proposed method

RTV		DRV	
Concentration µg/ml	Peak area*	Concentration µg/ml	Peak area*
4	68933	40	240296
6	103849	50	290369
8	140466	60	355443
12	207699	80	480591
16	276932	100	590738
20	356166	120	720886
Y-intercept	−3035	Y-intercept	−5350
Slope	17761	Slope	6023
R^2	0.999	R^2	0.999

*Mean of three determinations, DRV: Darunavir, RTV: Ritonavir

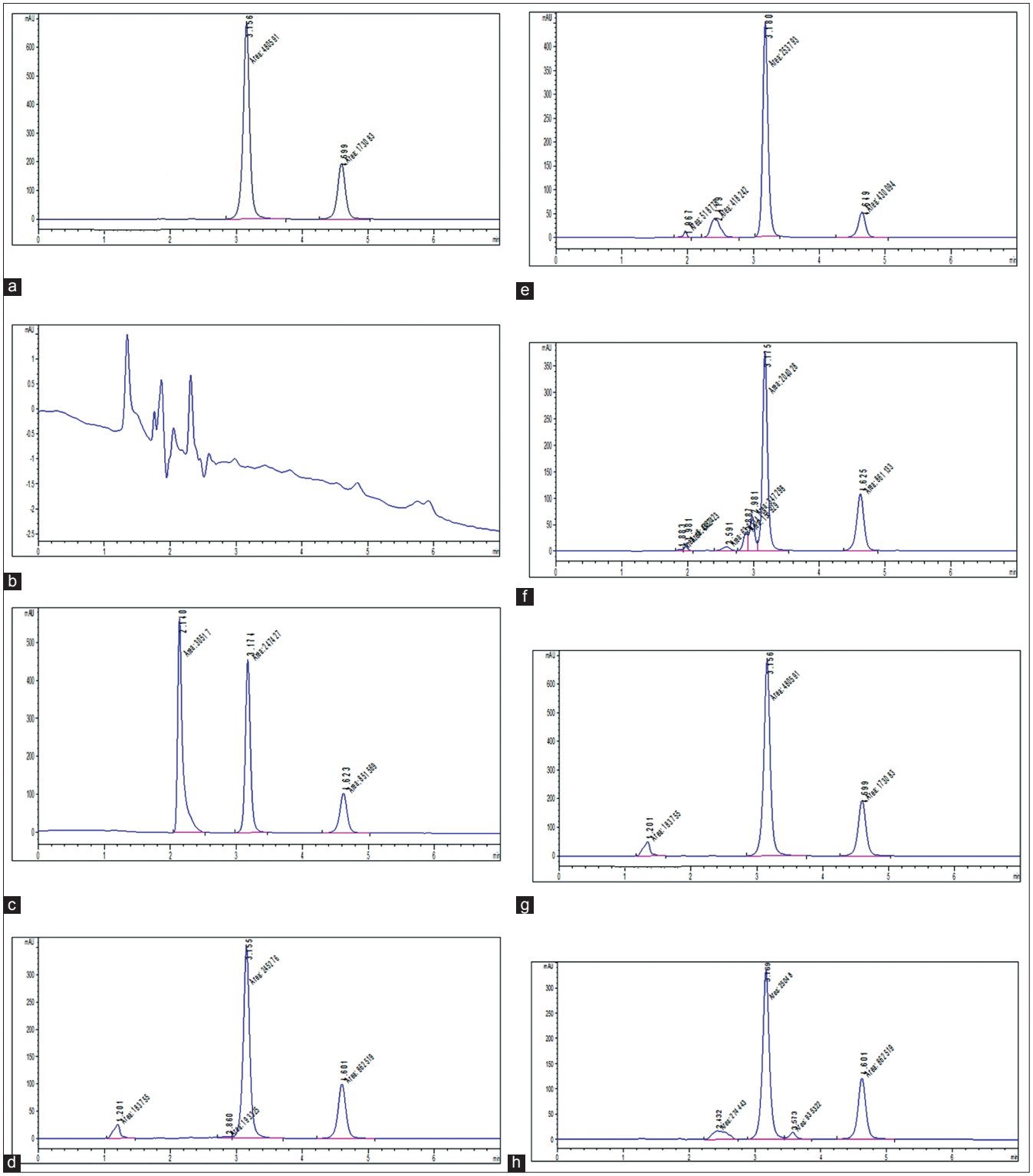

Fig. 2: (a) Chromatogram of standard. (b) Chromatogram of blank. (c) Chromatogram of acid degradation. (d) Chromatogram of alkali degradation. (e) Chromatogram of peroxide degradation. (f) Chromatogram of photolytic degradation. (g) Chromatogram of hydrolysis degradation. (h) Chromatogram of thermal degradation

CONCLUSION

This intended study can be concluded as the proposed method is simple, highly fast, economical, sensitive, and reliable and is found to be more precise, accurate, specific, stability indicating, rugged, and robust. Hence, it can be employed for routine estimation of tablets containing DRV and RTV. Conventional reported chromatographic methods may be replaced by the proposed stability indicating HPLC method because of its superiority in cost effectiveness, short analysis time per sample, and better detection. For faster samples testing routinely in QC lab, the validated method may be used.

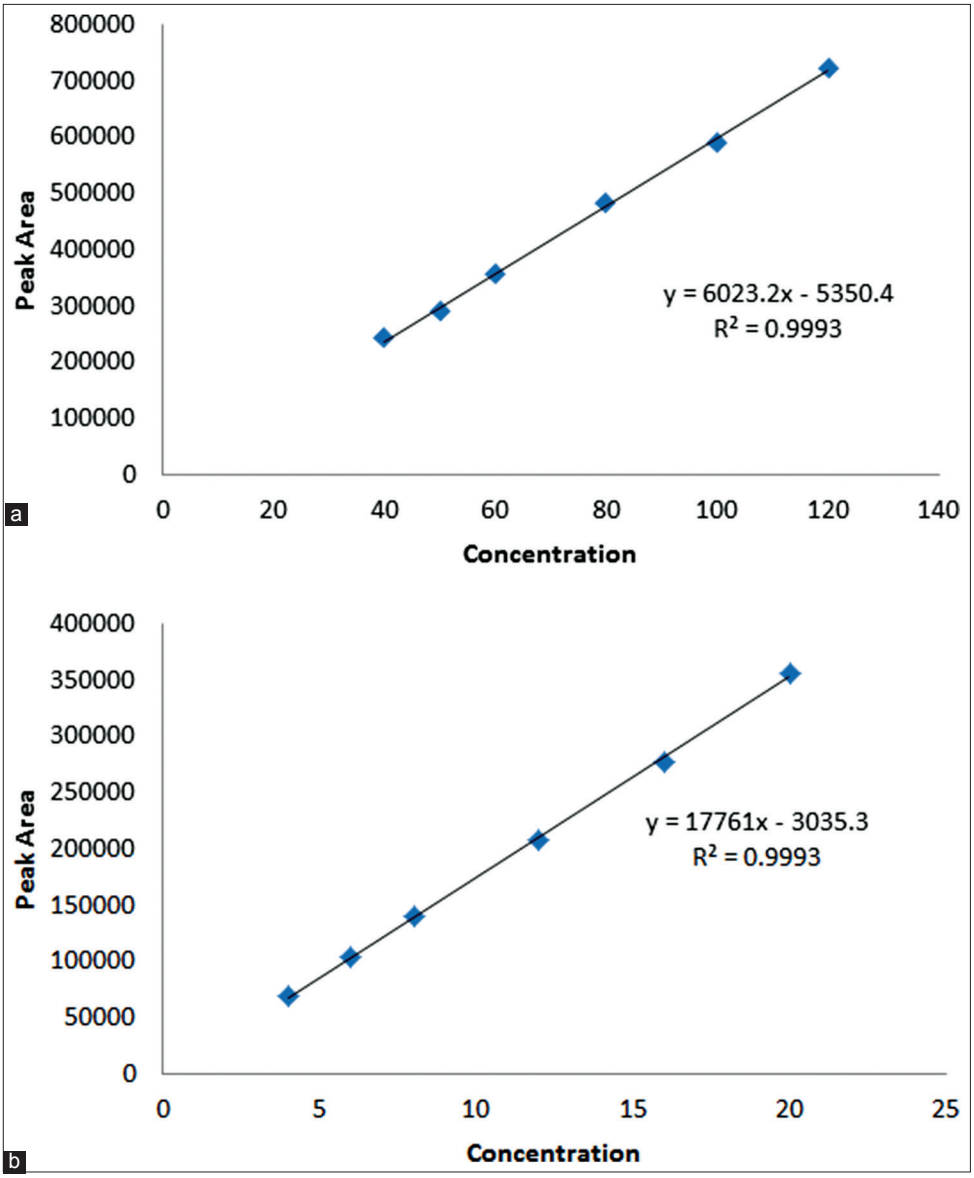

Fig. 3: (a) Linearity curve for darunavir. (b) Linearity curve for ritonavir

Table 5: Precision data of the proposed method

Injection	Peak area			
	DRV		RTV	
	Method precision	System precision	Method precision	System precision
1	480591	483189	171083	173437
2	481329	483291	172912	175902
3	482098	480285	171362	174930
4	479480	485284	172820	178321
5	478128	483021	171415	175320
6	480294	482840	170382	174285
Mean	480320.0	482985.0	171662.33	175365.83
SD	1397.422	1595.80	1792.61	1679.35
RSD	0.29%	0.33%	0.58%	0.96%

DRV: Darunavir, RTV: Ritonavir, SD: Standard deviation, RSD: Relative standard deviation

Table 6: Robustness data of DAR and RTV

Variations	DRV			RTV		
	% assay*	Theoretical plates*	Tailing factor*	% assay*	Theoretical plates*	Tailing factor*
43% of ACN in mobile phase	100.08	7634	0.92	99.92	6898	1.32
47% of ACN in mobile phase	100.04	7645	0.97	99.89	6974	1.2
Flow rate at 0.9 ml/minutes	100.2	7490	1.02	99.96	6875	0.78
Flow rate at 1.1 ml/minutes	100.19	7590	1.05	100.02	6912	0.95
Wavelength at 243 nm	100.01	7490	1.03	100.04	6874	1.02
Wavelength at 247 nm	100.08	7634	0.92	99.92	6898	1.32

*Mean of three determinations, DRV: Darunavir, RTV: Ritonavir

REFERENCES

1. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020945s033lbl.pdf.
2. Available from: http://www.investor.jnj.com/releasedetail.cfm?releaseid=536723.
3. Babu GR, Rao AL, Rao JV. Development and validation of novel HPLC method for estimation of darunavir in pharmaceutical formulations. Int J Res Pharm Chem 2013;3(2):438-43.
4. Salunke JM, Singh S, Malwad S, Chavhan VD, Pawar MS, Patel C*, et al*. A validated RP HPLC method for simultaneous estimation of lopinavir and ritonavir in combined dosage form. Sch Res Libr 2013;5(4):1-6.
5. Reddy BV, Jyothi G, Reddy BS, Raman NV, Chander KS, Rambabu C. Stability-indicating HPLC method for the determination of darunavir ethanolate. J Chromatogr Sci 2013;51(5):471-6.
6. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.
7. Stability Testing: Photostability Testing of New Drug Substances and Products q1b, ICH Harmonised Tripartite Guideline, Current Step 4 Version Dated 6 November; 1996.