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Development and Validation of new UPLC method for the simultaneous estimation of darunavir, dolutegravir and ritonavir in combined tablet dosage form

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ABSTRACT

A rapid, simple, sensitive and selective analytical method was developed by using reverse phase Ultra performance liquid chromatographic technique for the simultaneous estimation of darunavir, dolutegravir and ritonavir in combined tablet dosage form. The developed method is superior in technology to conventional high performance liquid chromatography with respect to speed, resolution, solvent consumption, time, and cost of analysis. Elution time for the separation was 3 min and ultra violet detection was carried out at 225 nm. Efficient separation was achieved on BEH C18 (50 x 2.1mm, 1.7) UPLC column using phosphate buffer and acetonitrile as organic solvent in a linear isocratic program. Resolution between darunavir, dolutegravir and ritonavir was found to be more than 3.5. The active pharmaceutical ingredient was extracted from tablet dosage from using a mixture of water and acetonitrile used as diluent. The calibration graphs were linear for darunavir, dolutegravir and ritonavir in the range of 100-600 g/ml, 12.5-75 g/ml and 6.25-37.5 g/ml respectively. The mean percentage recoveries for darunavir, dolutegravir and ritonavir were found to be in the range of 99.13 and 99.59 and 99.72 % respectively. Developed UPLC method was validated as per International Conference on Harmonization specifications for method validation. This method can be successfully employed for simultaneous estimation of darunavir, dolutegravir and ritonavir in bulk drugs and formulations.

INTRODUCTION

Darunavir[1] (TMC114) is an inhibitor of the human immunodeficiency virus (HIV) protease. Darunavir binds tightly to HIV protease with a dissociation constant (K_d) of 4.4 X 10^{-12} M and is highly active against wild-type and resistant strains of the virus inhibiting dimerization and catalytic activity of HIV-1 protease. Dolutegravir[2] (DTG) is a new molecular entity in the integrase strand transfer inhibitor class, indicated for the treatment of HIV-1. Ritonavir[3] is 1 of the 4 potent synthetic HIV protease inhibitors, that have revolutionised HIV therapy. Combination treatment of ritonavir with saquinavir and indinavir results in potent and sustained clinical activity.

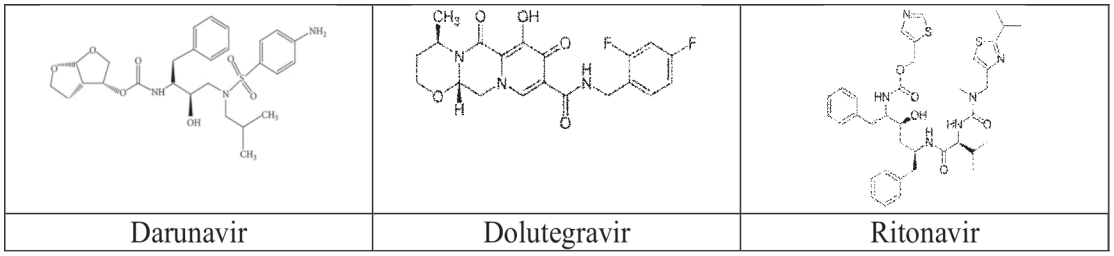
Literature survey revealed that few analytical methods are reported for analysis of three drugs, either individually or in combination by using chromatography [4-25]. Till date there are no published reports for simultaneous estimation of darunavir, dolutegravir and ritonavir by RP-UPLC in their combined pharmaceutical dosage forms. Hence, an attempt has been made to develop a new UPLC method for simultaneous estimation of darunavir, dolutegravir and ritonavir in accordance with the International Conference on Harmonization (ICH) guidelines [26].

MATERIALS AND METHODS

The reference samples of darunavir, dolutegravir and ritonavir were obtained from M/s. mylan labs, Hyderabad, India. UPLC grade methanol, acetonitrile and analytical grade dihydrogen orthophosphate and orthophosphoric acid were obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the study.

Chromatographic conditions:

The analysis was carried out on BEH C18 column (50 x 2.1



Darunavir	Dolutegravir	Ritonavir

Fig. 1 : Molecular structures of drugs

mm, 1.7). The mobile phase composition was 55% phosphate buffer and 45% acetonitrile used as organic solvent isocratic system with a linear time program at a flow rate of 0.3 ml/min, detection was monitored at 225 nm, and chromatographic run time of 3.0 min. The injection volume was 0.5 µl.

Preparation of diluent and stock solutions:

A mixture of water and acetonitrile (50:50 v/v) was used as diluent. Accurately weighed and transferred 100 mg of darunavir, 12.5 mg of dolutegravir and 6.25 mg of ritonavir working standards into 25 ml clean dry volumetric flasks containing 10 ml of diluent, sonicated for 10 minutes and made the final volume with diluents (4000 µg/ml darunavir, 500 µg/ml dolutegravir and 250 µg/ml ritonvir).

Analysis of marketed formulations (Sample Preparation):

Accurately weighed and transferred the sample powder equivalent to 80mg darunavir, 10mg dolutegravir and 5mg ritonvir tovolumetric flask, 25 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered (8000 µg/ml darunavir, 1000 µg/ml dolutegravir and 500 µg/ml ritonvir). 0.5ml of filtered sample solution was transferred to 10 ml volumetric flask and made up with diluent (400 µg/ml darunavir, 50 µg/ml dolutegravir and 25 µg/ml ritonvir).

Method validation:

Method validation was performed according to ICH guidelines with respect to precision, linearity, accuracy, detection limit, quantification limit, specificity and robustness. The precision of the developed method was evaluated by six replicate injections of the above standard mixture. The RSD of three drugs was calculated for peak areas, USP tailing factor and plate count. The intermediate precision of the method was also evaluated on a different column. The solutions for linearity were prepared at six concentration levels. The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined. Standard addition and recovery experiments were conducted to determine accuracy of the method for the quantification of three drugs. The study was carried out at 50, 100 and 150% for three replicate injections of each concentration of the analyte followed by calculation of the percentage recovery. The limit of detection (LOD) and limit of quantitation (LOQ) is calculated by signal to noise ratio method of 3:1 and 10:1 respectively. For robustness evaluation the experimental conditions were deliberately altered and resolution between darunavir, dolutegravir and ritonavir was evaluated. The impact of flow rate on resolution, tailing factor and plate count was studied by the alteration of ±0.1 mL/min. Solution stability of three drugs solution in a tightly capped volumetric flask for 7 days when stored in a refrigerator at 2-8C temperature was studied. The contents of three drugs were determined in 8 h intervals. Mobile phase stability was assessed over a period of 72 h by injecting the freshly prepared sample solutions in 8 h interval as well. The contents of three drugs were determined in the test solutions.

Forced degradation studies:

Forced degradation studies of three drugs were carried out under conditions of acid hydrolysis (2N HCl), base hydrolysis (2N NaOH) and peroxide treatment (20% H_2O_2). Aliquot

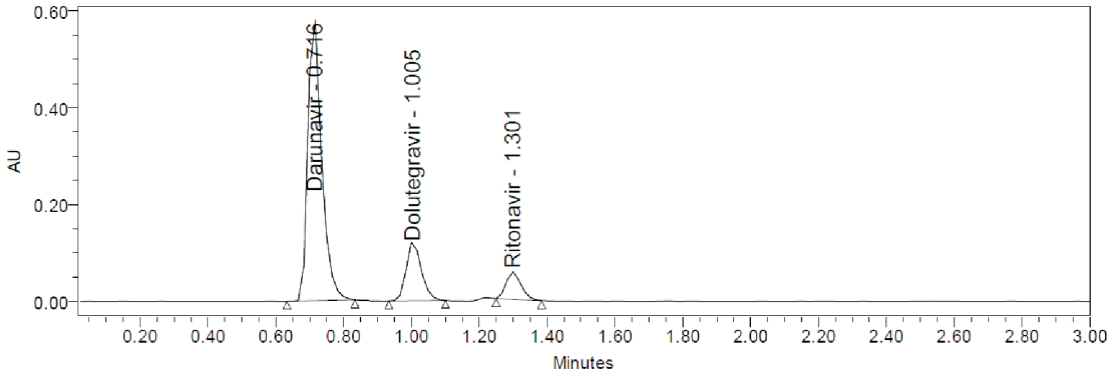


Fig. 2 : Chromatogram of mixture of darunavir, dolutegravir and ritonavir. Darunavir with t_R of 0.716 min, dolutegravir with t_R of 1.005 min and ritonavir with t_R of 1.301 min.

quantities of mixture of three drugs were weighed in different volumetric flasks and added 3.0 ml of 2 N HCl, 3.0 ml of 2 N NaOH, and 1.0 ml of 20% H_2O_2, respectively and diluted to 50 ml with diluent, These solutions were refluxed at 80° for 8 h, cooled to room temperature, made up to final volume 100 ml with diluent and analysed by UPLC.

RESULTS AND DISCUSSION

This work was intended to develop a rapid, precise and reliable method in reverse-phase UPLC separation combined with UV detection for simultaneous estimation in bulk samples and in dosage formulations. Development of a rapid and suitable UPLC method for the separation of darunavir, dolutegravir and ritonavir required a number of trials to be carried out using different mobile phase compositions. As part of the preliminary work, separation was attempted using BEH C18 (2.7 X 50 mm, 1.7 µm) column with 55% phosphate buffer and 45% acetonitrile as organic in different gradient programs at a flow rate of 0.3 ml/min, at a common UV maxima 225 nm. To improve the resolution between three drugs, attempts were made with different percentages of acetonitrile. Trials were also done at different flow rates and different temperatures to optimize the peak shape, sensitivity, tailing factor and resolution among three drugs.

The typical retention times of darunavir, dolutegravir and ritonavir is 0.716, 1.005 and 1.301 min, respectively in a total chromatographic run time of 3 min (fig. 2). The system suitability test results are summarized in Table 1. The repeatability was checked by repeatedly injecting (n=6) solution of darunavir, dolutegravir and ritonavir and the RSD values for peak areas of darunavir, dolutegravir and ritonavir were found to be within 2.0% confirming a suitable precision of the method. The correlation coefficient obtained was greater than 0.99 for tablet dosage form which confirmed the linear relationship between peak areas and concentrations. Slope and Y-Intercept values were 3769.4 and 371.21 for darunavir, 10958 and 1550.3 for

Table 1 : SUMMARY OF SST AND VALIDATION PARAMETERS

S. No.	Parameter (Units)	Darunavir	Dolutegravir	Ritonavir
1	Retention time ± allowable time (min.)	0.716 ± 0.1	1.005±0.1	1.301±0.1
2	Theoretical plates	7580	5323	3818
3	Tailing factor (asymmetry factor)	1.24	1.02	1.19
4	Method Precision (% RSD, n=6)	0.1	0.4	0.3
5	Linearity range (ppm)	100-600	12.5-75	6.25-37.5
6	Correlation coefficient	0.9994	0.9998	0.9997
7	LOD (µg/ml)	0.38	0.15	0.11
8	LOQ (µg/ml)	1.14	0.46	0.34
9	Recovery (%)	99.13	99.59	99.72

Note: SST stands for system suitability test, LOD stands for limit of detection, LOQ stands for limit of quantification.

Table 2 : LINEAR REGRESSION DATA FOR CALIBRATION CURVES

S.No.	Parameter (Units)	Darunavir	Dolutegravir	Ritonavir
1	Linearity range (µg/ml)	100-600	12.5-75	6.25-37.5
2	r2±%RSD	0.999±0.02	0.9998±0.03	0.9997±0.04
3	Slope±%RSD	3769.4±0.3	10958±0.2	12274±0.5
4	Intercept±%RSD	371.21±0.9	1550.3±1.1	1235.9±0.8

Note: r is regression co-efficient, RSD stands for relative standard deviation.

Table 3 : Degradation data for darunavir, dolutegravir and ritonavir.

	darunavir	% degraded	dolutegravir	% degraded	ritonavir	% degraded
Standard	1534358		545558		310660	
Acid	94.43	5.57	95.29	4.71	93.77	6.23
Base	95.46	4.54	96.82	3.18	94.46	5.54
Peroxide	96.18	3.82	96.96	3.04	94.44	5.56
Thermal	97.61	2.39	97.21	2.79	97.11	2.89
UV	98.36	1.64	98.82	1.18	98.15	1.85
Water	99.90	0.10	99.43	0.57	99.63	0.37

dolutegravir, 12274 and 1235.9 for ritonavir.

Linearity was determined for six concentrations of each in three replicate injections. The Linearity test results are summarized in Table 2. The mean percentage recovery obtained for darunavir, dolutegravir and ritonavir are 99.13 %, 99.59 % and 99.72 % respectively. The Accuracy test results are summarized in Table 1. The LOD values for darunavir, dolutegravir and ritonavir were found to be 0.38, 0.15 and 0.11for dosage form, respectively. The LOQ values for darunavir, dolutegravir and ritonavir were found to be 1.14, 0.46 and 0.34 for dosage form, respectively and the results were summarized in Table 1.

Degradation studies:

Degradation study was conducted by subjecting a sample to various stress conditions such as acid hydrolysis (using 2N HCl), base hydrolysis (using 2N NaOH), oxidative hydrolysis (using 20% H_2O_2), thermal degradation (heated at 1100°C for 24 hours) and photolytic degradation (overall illumination of ≥200Wh/m2 at 25°C for 7 days with UV radiation), to evaluate the ability of the proposed method to separate darunavir, dolutegravir and ritonavir from its degradation products. The results are shown in table 3.

CONCLUSION

In the proposed study, Reverse Phase UPLC method with UV detection was described and validated for the simultaneous separation and estimation of darunavir, dolutegravir and ritonavir for tablets. Statistical analysis proved that method was accurate, precise and reproducible. The developed method was found to be simple, rapid, sensitive and selective for analysis of darunavir, dolutegravir and ritonavir for tablet dosage form without any interference from the excipients. The method was successfully used for estimation of drugs in a pharmaceutical formulation. As the chromatographic run time is short and the method is economical, it can be applied in routine and in quality control analysis where there is no need of two different methods. The proposed UPLC method is non-hazardous to human health and also to the environment and is more economic due to the reason that a couple of hundreds of samples can be analyzed in a single day.

CONFLICTS OF INTEREST

No conflicts of interest declared for this work

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