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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF DARUNAVIR AND ELVITEGRAVIR IN API

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ABSTRACT

A simple and accurate method to determine Darunavir, and Elvitegravir in binary mixture, was developed and validated using High performance liquid chromatography (HPLC). The HPLC separation was achieved on Zodiac C18 150 × 4.6 mm in the isocratic mode using Acetonitrile : water (80: 20, v/v), at a flow rate of 0.8 ml/min. The retention times were about 2.30 and 3.88 min for DRV and EVG, respectively. Quantification was achieved with PDA- detector at 290 nm over the concentration range of 10-50µg/ml and 20-100 µg/ml for DRV and EVG respectively, with mean recoveries of 99.13 and 98.96 for DRV and EVG, respectively. The method was validated, and was found to be simple, specific, accurate, precise and robust. The method was successfully applied for the determination of DRV and EVG in binary mixture without any interference from common excipients.

INTRODUCTION:

Darunavir is an antiretroviral drug from the protease inhibitor class used to treat HIV infection
and AIDS. Nomenclature:- [(1S,2R)-3-[[(4-Amino phenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy -1-(phenyl methyl) propyl] carbamic acid (3R,3aS,6aR)- hexahydrofuro[2,3-b]furan-3-yl ester. Molecular formula is C27H37N3O7S. Molecular weight is 547.66. Melting point of drug is 74 ^{0}C.2 It is a amorphous white, solid, freely soluble in methanol, acetonitrile and soluble in ethanol. Darunavir contains a bis-tetrahydro-furanyl (bis-THF) moiety and sulphonamide isostere; the drug is administered as its ethanolate salt. Elvitegravir (EVG, formerly GS-9137) is an integrase inhibitor used to treat HIV infection. Nomenclature:- 6-(3-Chloro-2-fluorobenzyl)-1-[(2S)-1hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo- 1,4-dihydroquinoline-3-carboxylic acid..

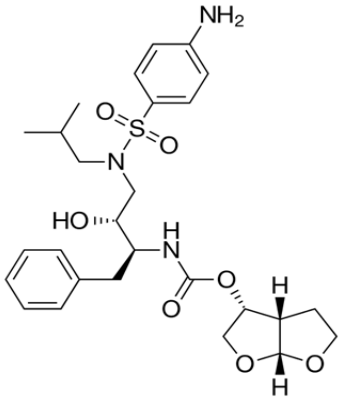

Figure 1: Chemical structure of Darunavir

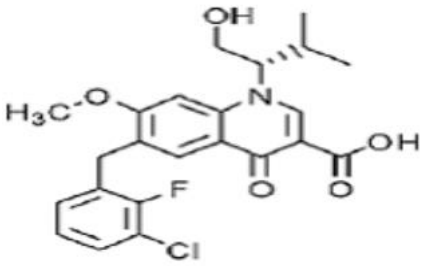

Figure 2: Chemical Structure of Elvitegravir

Literature survey reveals that Darunavir was estimated independently and in combination with other drugs by several chromatographic, spectrometric and flourimetric methods in pharmaceutical formulations and in biological samples. Similarly Elvitegravir was estimated by HPLC. And no analytical method was found for simultaneous estimation of Darunavir and Elvitegravir in combination[4-.15] Hence we have explored in developing and validating a new accurate, precise, linear and cost effective reverse phase HPLC method for the simultaneous estimation of Darunavir and Elvitegravir in API.

MATERIALS AND METHODS

Chemicals and materials: Darunavir and Elvitegravir were received as a gift samples from Mylan Drugs Ltd., Hyderabad. Acetonitrile, Methanol of HPLC grade was obtained Millipore Water.

Instrumentation: Quantitative HPLC was performed on Schimadzu UFLC LC-20AD with a PDA detector equipped with manual injector with injection volume 20 µl. A symmetry C18 column (150 × 4.6 mm) Make: Zodiac was used.

Method development and optimization

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of Darunavir and Elvitegravir in combination in API. Mobile phase tried for this purpose were Acetonitrile: Water (65 :35), Acetonitrile : Water (70 : 30) , Acetonitrile : Water (80 : 20), with various flow rates. The condition that gave best resolution and symmetry was selected.

Preparation of mobile phase: 80 ml of acetonitrile was mixed with 20 ml of water. The solution was degassed in an ultrasonic Water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent preparation: Mobile phase was used as Diluent

Preparation of Standard solution

Weigh accurately 100 mg of Darunavir and 100 mg of Elvitegravir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 µg/ml of Darunavir and 100 µg/ml of Elvitegravir is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

RESULTS AND DISCUSSION

Method Development: Repeated trials employing different mobile phases and different compositions of mobile phase as per literature survey were tried and tested. The Ideal mobile phase was finally found to be that of Acetonitrile and Water in the ratio of 80 : 20 respectively. The resulting mobile phase gave satisfactory elution results with good resolution for both Darunavir and Elvitegravir. The elution time for both the drugs i.e Darunavir and Elvitegravir was found to be 2.3 and 3.8 mins respectively, using a Zodiac C18 column (150 × 4.6 mm).The Chromatographic device was maintained at a flow rate of 0.8 ml/ min .The injection Volume was 20 µl.

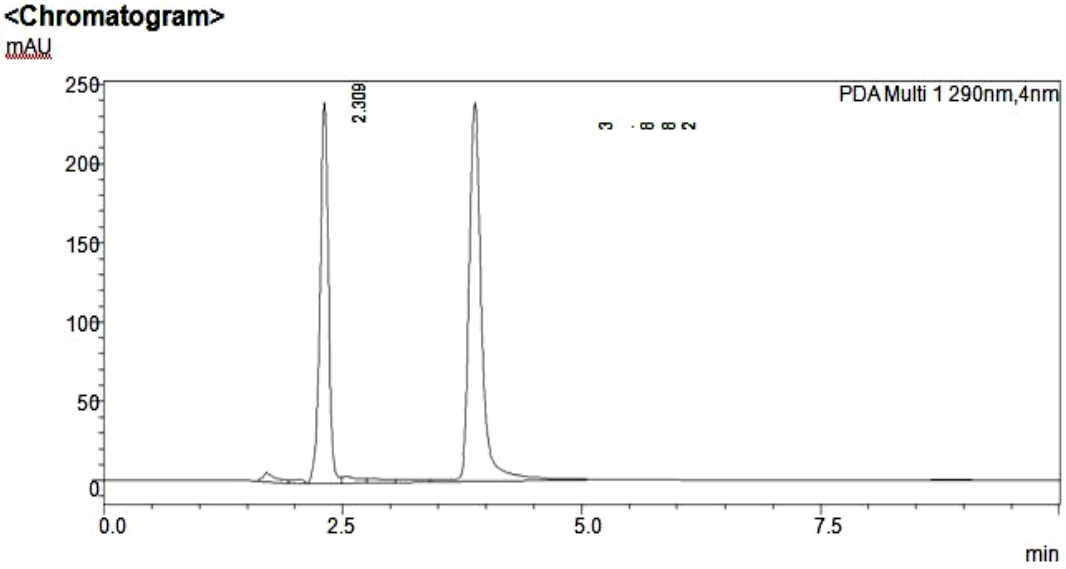

Figure 3: Chromatographic representation of Darunavir and Elvitegravir

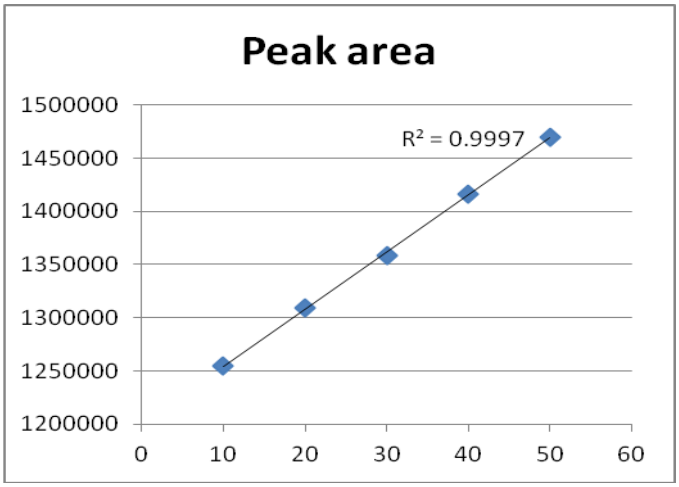

Figure 4: Linearity of Darunavir

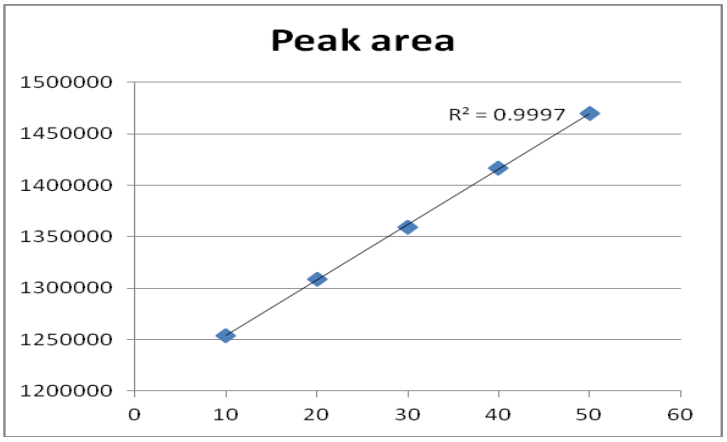

Figure 5: Linearity of Elvitegravir

Table no 1 : System Suitability Parameters for Darunavir

Injection	Retention Time	Peak Response	Theoretical Plates	Tailing Factor
1	2.297	1412456	2745	0.903
2	2.298	1423295	2786	0.889
3	2.302	1425415	2898	0.893
4	2.305	1432868	2865	0.916
5	2.308	1447285	2846	0.927
6	2.308	1458658	2795	0.932
Mean	2.303	1433329		
STDEV	0.004	16944.23		
%RSD	0.209	1.18		

Table no 2: System Suitability Parameters for Elvitegravir

Injection	Retention Time	Peak Response	Theoretical Plates	Tailing Factor
1	3.845	2165362	4298	1.296
2	3.856	2174396	4369	1.289
3	3.862	2186549	4357	1.302
4	3.884	2217654	4390	1.310
5	3.868	2226745	4415	1.314
6	3.878	2238655	4368	1.298
Mean	3.865	2201560		
STDEV	0.014	30142.48		
%RSD	0.371	1.36		

Table no 3: Summary of Validation Parameters

S.No	Validation Parameter	Darunavir	Elvitegravir
1	Accuracy (% recovery)	99.78,98.96,98.67	98.86,98.39,99.64
	Precision (%RSD)		
2	Intraday	0.745	1.51
3	Interday	0.836	1.15
4	Linearity (r^2)	0.9997	0.9969
5	LOD	0.9	0.76
6	LOQ	1.34	1.56
7	Robustness	Robust	Robust

The above optimized method was validated as per standard ICH and USP guidelines for routine analysis and quality control.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to the pre-analyzed sample solution at three different levels 80%, 100%, 120%. The percentage recoveries were calculated, results of which are summarized in table no 2 and 3**.**

Precision

Precision of the method was fixed by the repeated analysis of test sample six times. The % RSD values were found to be satisfactory, hence the method was precise. Intraday and Interday Precision of the method was also performed by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. The peaks were recorded and % RSD was calculated for both the analytes under study. The % RSD of Intraday and Inter day precision was calculated and the validation results are summarized in table no 2 and 3 .

Linearity

Calibration Curves were obtained from a graphical plot between peak area and concentration of the drug by subjecting to regression analysis and correlation coefficients. Table no 3 represents the linearity of the proposed method. Linearity

curves of DRV and EVG were shown in figures 4 and 5.

LOD and LOQ

LOD and LOQ were calculated using the equation 3.3 σ/S and 10 σ/S respectively where "σ" is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD value for both Darunavir and Elvitegravir was determined to be 0.9 µg/ml and 0.76 µg/ml respectively. The LOQ was found to be 1.34 µg/ml and 1.56 µg/ml for Darunavir and Elvitegravir respectively.

Robustness

As defined by ICH, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters in order to establish an indication of its reliability during routine usage of the method for quality control. Robustness was performed by injecting sample solutions in the prescribed chromatographic conditions with minute variations in the volume of mobile phase composition and flow rate in the range of ± 2%. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method was robust in nature. Results are presented in Table no 2 and 3.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

CONCLUSION

A method was developed on trial & error basis by changing the variables wherever required. As there was no interference due to mobile phase, the method was found to be specific. The method was robust as observed from insignificant variation in the results of analysis by changes in flow rate and wavelength variation separately and analysis being performed by different analysts. The present method is validated and the results are better than previous methods which are performed on these drugs. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Darunavir and Elvitegravir in Active Pharmaceutical Ingredient.

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