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Development and Validation of Chiral RP-HPLC Method for Quantification of Optical Isomers in Dolutegravir Sodium

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ABSTRACT

There is rare discussion available on quantitative separation of optical isomers of Dolutegravir, Dolutegravir is most recent FDA approved antiretroviral drug. The author has described simple, sensitive, precise, and specific RP-HPLC method for the separation of (R,R)-diastereomer, (S,S)-diastereomer and (S,R) enantiomer of Dolutegravir from its Process related and degradant impurities using Chiralpak IF-3, HPLC column. The method is validated as per ICH Q2(R1) guideline and can be used in routine analysis.

Keywords: Dolutegravir sodium, RP-HPLC, Optical isomers, Stability indicating.

INTRODUCTION

Dolutegravir sodium

Chemically it is sodium; (4R,12aS)-9-[(2,4-difluorophenyl)methylcarbamoyl]-4-methyl-6,8-dioxo-3,4,12,12a-tetrahydro-2H-pyrido[5,6]pyrazino[2,6-b][1,3]oxazin-7-olate.

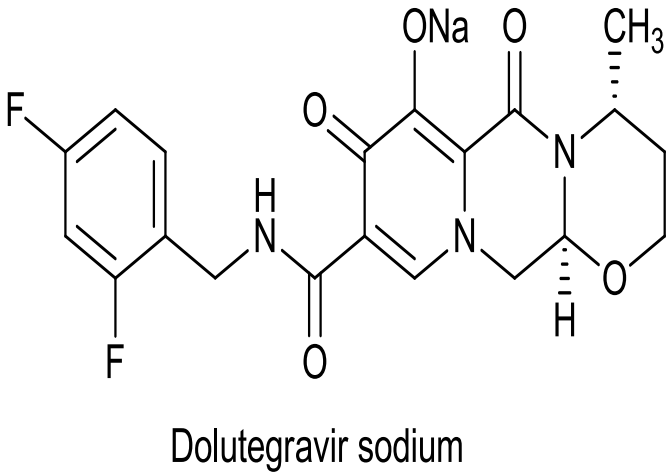

Figure 1: Molecular structure of Dolutegravir sodium.

Molecular mass: 441.36

Molecular formula: $C_{20}H_{18}F_2N_3NaO_5$

Dolutegravir, is a second-generation INSTI (integrase strand transfer inhibitors), is the most recent FDA-approved antiretroviral drug that has demonstrated impressive antiviral efficacy based on randomized controlled trials compared with other first-line regimens recommended by the Department of Health and Human Services HIV treatment guidelines for adults and adolescents. HIV-1 integrase is a viral protein composed of three domains that performs the role of cutting and joining viral DNA into the host genome [1-2]. Upon review of available literature, it is found that very few methods are available for Dolutegravir analysis as individual or combination with drugs, mostly for assay content but not much reference is available which gives a specific, accurate and precise method for routine and stability analysis of its stereoisomers in Dolutegravir drug substance [3-6]. Chandrashekar reddy et al. [17] have separated (R,R)-diastereomer and (S,R) enantiomer from Dolutegravir peak. Since Dolutegrvir has two chiral centres (R,R)-diastereomer, (S,S)-diastereomer and (S,R) enantiomer are undesired optical isomers, which can be present as a chiral impurity. So it is essential to find a effective way to analyze the optical isomers of Dolutegravir (Figure 1).

Dolutegravir, enantiommeric impurity analytical method is reported by reverse phase liquid chromatography using modified amylose as chiral stationary phases. The aim of this work was to optimize the chromatographic conditions in terms of

temperature and mobile phase composition in order to separate, identify and quantify the optical isomers of Dolutegravir. The developed chiral HPLC method was reproducible and accurate for the quantitative determination of optical isomers in Dolutegravir.

EXPERIMENTAL METHOD

Material and reagents

Dolutegravir sodium and optical isomers were synthesized by Department of Research and Development, Macleods Pharmaceutical Ltd. Dolutegravir sodium and impurities were characterized using proton nuclear magnetic resonance and high resolution mass spectrometry. HPLC grade acetonitrile, methanol was procured from J T Baker, tertiary butyl methyle ether, triethyl amine obtained from Rankem. Analytical grade potassium dihydrogen ortho phosphate and orthophosphoric acid obtained from Merck chemicals. HPLC grade water obtained from Millipore system (Millipore Inc., USA) was used throughout the analysis.

For Chiral HPLC methods

Optimization experiments

In the process of developing HPLC method for chiral separation normal phase and reversed phase both type of work was performed. In normal phase HPLC three key parameters were studied which influence the selectivity such as chemistry of stationery phase, and organic modifiers. 0.1% triethyl amine and 0.1% trifluoric acetic acid in hexane with ethayl acetate, ethanol and iso propylalcohol in different combination (two or more than two solvents) were tried. HPLC columns used for development of method were Chiralpack IA, Chiralpak IB, Chiralpak IC and Chiralpak IF. The impurities spiked solution in Dolutegravir sodium; were studied but no proper separation could be achieved in normal phase method. Hence all above columns tried with aquous phosphate buffer in different combination with organic solvents acetonitrile, methanol and tertiary butyl methyl ether**.** Finally the specific method was optimised using Chiralpak IF column. The impurities spiked solution in Dolutegravir sodium was injected and studied at different temperature and different pH and data recorded. Method was optimized with satisfactory resolutions among all impurities with mobile phase of potassium dihydrogen phosphate, pH 2.0 ± 0.05 adjusted with dilute ortho phosphoric acid solution and solvent mixture of acetonitrile and tertiary butyl methyl ether at 35°C.

Instrumentation and chromatographic conditions

Agilent HPLC 1200 (Agilent Technologies, Germany) equipped with photodiode array detector was used for method development, forced degradation studies and method validation. Chiralpak IF-3, 3µm (250 mm x 4.6 mm) HPLC column thermostat at 35˚C was used for the separation. 0.01mol potassium dihydrogen orthophosphate aqueous solution was adjusted to pH 2.0 with orthophosphoric acid used as buffer. Solvent mixture was prepared by mixing tertiary butyl methyl ether and acetonitrile in the ratio of 10:35 v/v. The mobile phase was prepared by mixing buffer and solvent mixture in the ratio of 63:37 v/v. The flow rate and injection volumes were 1.0 ml/min and 10 µl respectively. The analysis was carried out in isocratic condition. The column temperature was kept 35°C. The data was acquired at 260 nm for 40 min and processed by using chemstation and chromline HPLC software Photodiode array detector was used to determine the peak purity of all impurities spike solution.

Preparation of solutions and analytical procedure

Mobile phase was used as diluent in the preparation of analytical solutions. The test sample solution having concentration of 500 $\mu g.ml^{-1}$ was prepared for the determination of enantiomeric purity and quantification of isomers. The system suitability solution was prepared by dissolving (S,R) enantiomer, (S,S)-diasteromer, 2,4-didesfluorodolutegravir impurity, (R,R)-diastereomer and 2-desfluorodolutegravir impurity and spiked in dolutegravir sodium 500 $\mu g.ml^{-1}$ at 0.15% level. Sensitivity solution was prepared by dissolving dolutegravir sodium standard at 0.25 $\mu g.ml^{-1}$. The blank, sensitivity solution, system suitability solution and sample solution of 500 $\mu g.ml^{-1}$, were injected separately and chromatographed under the optimized chromatographic conditions. The resolution NLT 1.1, between 2,4-didesfluorodolutegravirimpurity + 4-desfluoro dolutegravirimpurity and (R,R) diastereomer peak, also resolution between R,R diastereomer and 2-Desflurorodolutegravir impurity peak should not more than 1.5 were set as system suitability criteria. All impurities were quantified by % area normalisation method applying the derived relative response factor (RRF). The relative retention time with respect to Dolutegravir peak and RRF of all impurities and optical isomers are as shown in Table 1 (Figures 2 and 3).

Table 1: Impurities Relative retention time and relative response factor of optical isomers with respect to Dolutegravir peak.

S. No.	Name	RRT	RRF
1	(S,R)-Enantiomer	~0.66	0.95
2	(S,S)-diastereomer	~0.71	0.86
*3	2,4-Didesfluorodolutegravirimmpurity + 4-desfluorodolutegravirimpurity	~0.76	---
4	(R,R)-diastereomer	~0.81	0.88
*5	2-Desfluorodolutegravirimpurity	~0.85	---
6	Dolutegravir	1	1

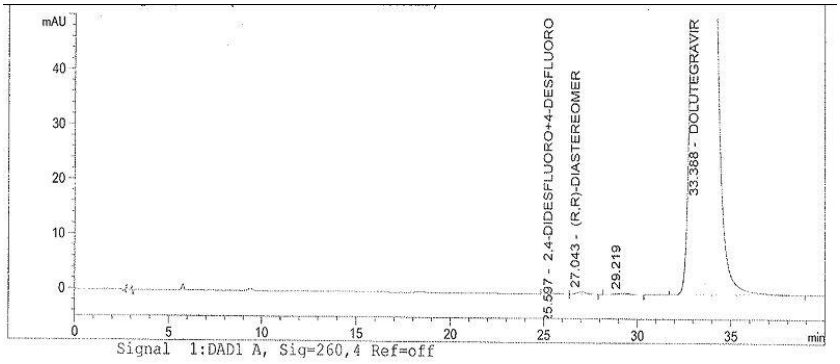

Figure 2: Sample chromatogram.

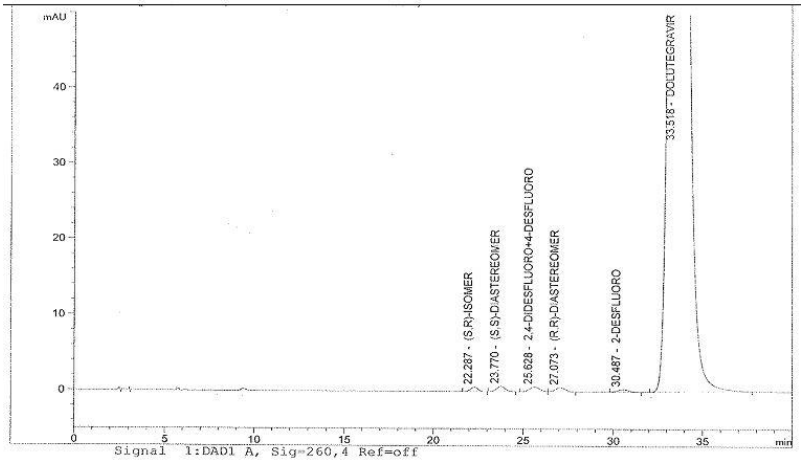

Figure 3: Impurities spiked at 0.15% level in Dolutegravir sodium.

Validation

Specificity (Selectivity)

The blank, system suitability solution, sensitivity solution and sample solution were prepared as described in the methodology. The impurity solutions and spiked sample solution were prepared as per specification limit and injected into the HPLC system. The retention time of all peaks observed in the resulting chromatograms were recorded. Based on the obtained result it is concluded that no interference observed due to blank at the same retention time of any of the impurities and main peak. All the known impurities peaks are well resolved from each other and spectrally pure.

Analytical solution stability

The system suitability solution and sample solution were prepared as described in the methodology and stored at room temperature (25°C). The stored solutions were injected at initial and intermediate intervals specified in the following Table 2. The % impurities in the sample solution were calculated at each time interval. The obtained results for system suitability solution and sample solution are presented as follows.

Resolution between (2,4-didesfluoro impurity + 4-desfluoro impurity and R,R diastereomer peak) and (R,R diastereomer and 2-Desfluroro impurity peak) was within the acceptance criteria not be less than 1.1 when stored at 25°C after 35 hours. The absolute difference of impurity results in the sample solution after 28 hours with respect to initial not more than 10% of specification limit. When stored at (25°C). Based on the above data it is concluded that the system suitability solution and sample solution can be used up to 24 hours when stored at (25°C).

Limits of Detection and Quantification (LOD and LOQ)

According to ICH Q2 (R1) recommendations the limits of detection (LOD) and the limit of quantification (LOQ) for Dolutegravir and optical isomers were estimated by calibration curve method [standard deviation of the response (σ) and the slope (S)], by injecting the series of dilute solutions of known concentration. The values of LOQ and LOD for optical isomers were found as depicted in Table 2.

Table 2: LOQ and LOD values for Dolutegravir optical isomers.

S. No.	Name	LOD (%)	LOQ (%)
1	Dolutegravir	0.012	0.04
2	(S,R)-enantiomer	0.002	0.006
3	(S,S)-diastereomer	0.002	0.006
4	(R,R)-diastereomer	0.004	0.01

Precision was studied at the LOQ level in by injecting six in-dividual preparations of Dolutegravir and its impurities, followed by the calculation of % RSD of the peaks areas. The % RSD of LOQ precision was below 10%.

Linearity

A series of solutions were prepared by quantitative dilutions of the stock solution of impurity standard and main drug to obtain solutions at LOQ to 250% of the specification limit. A series of solutions were prepared by quantitative dilutions of the stock solution of main drug to obtain solutions at 80% to 120% of the sample concentration (Figures 4-7).

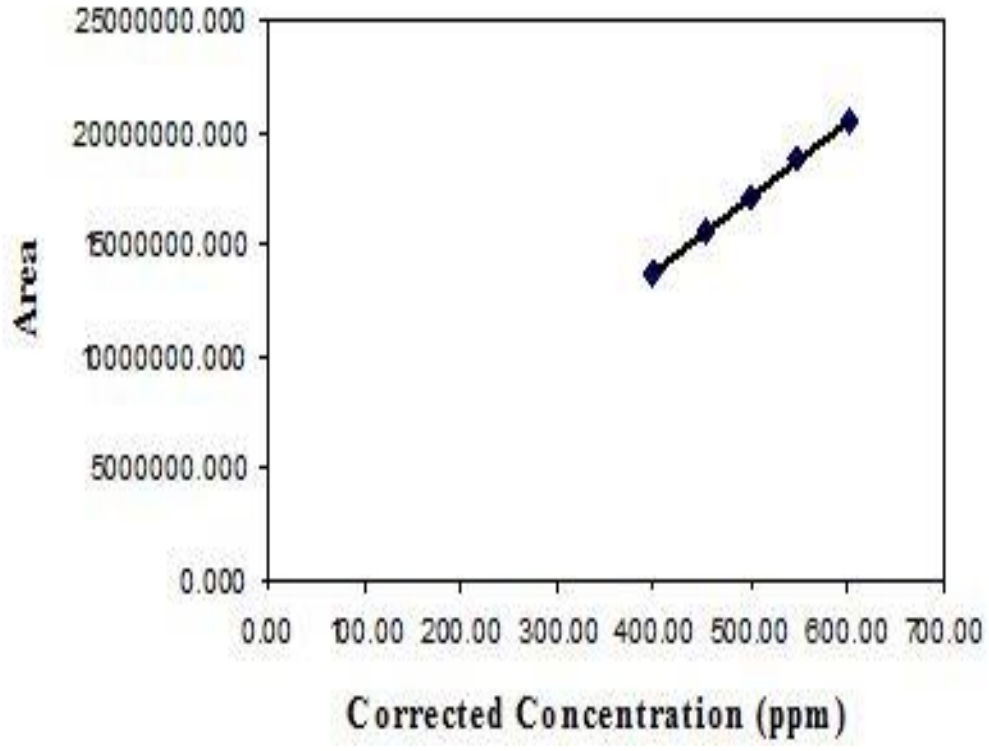

Figure 4: Linearity of Dolutegravir sodium.

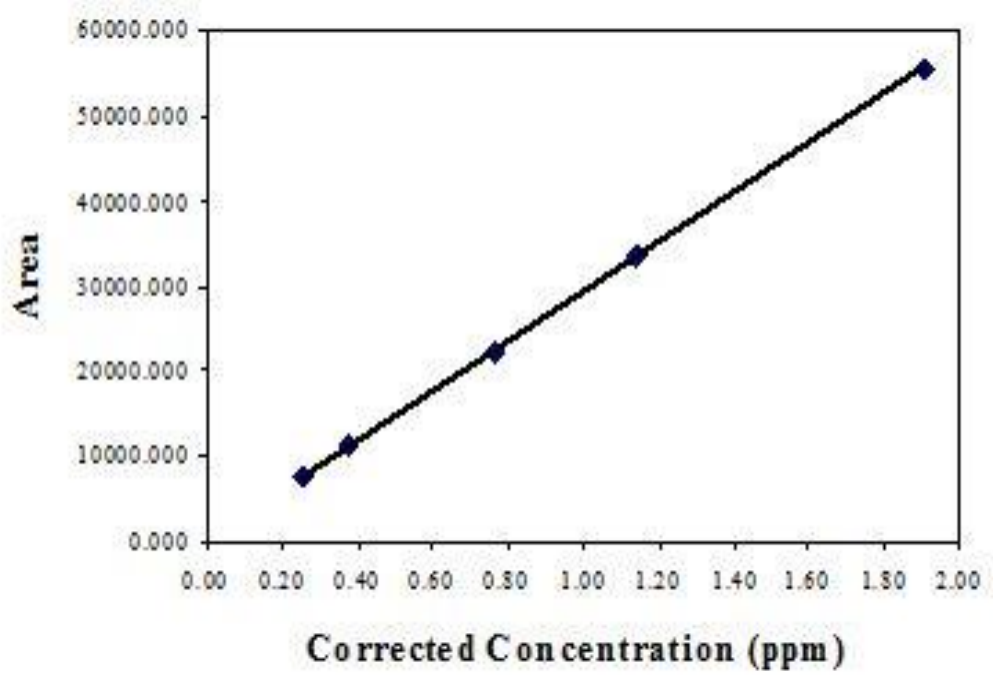

Figure 5: Linearity of SS diastereomer.

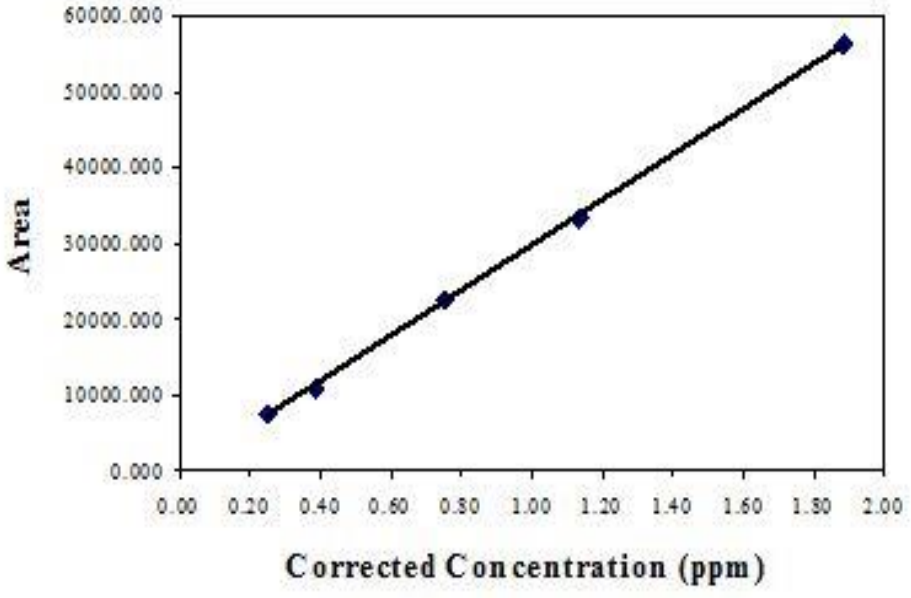

Figure 6: Linearity of RR diastereomer.

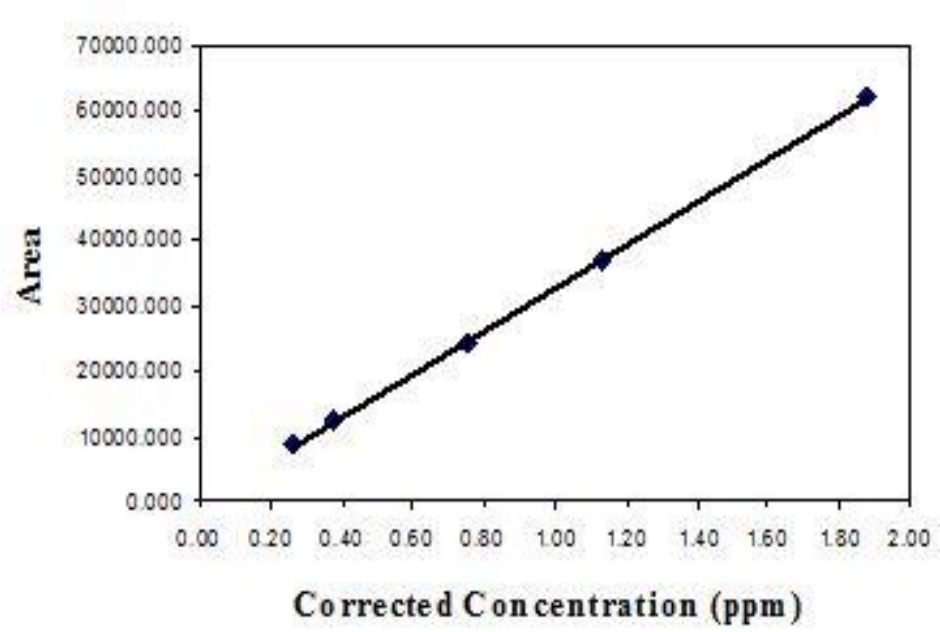

Figure 7: Linearity of SR enantiomer.

Each solution was injected and peak area was recorded. The slope, Y-intercept, correlation coefficient of the regression line and residual sum of squares were calculated. Correlation coefficient for optical isomers found above 0.99. Linearity plot of Dolutegravir and its stereoisomers are given below.

Precision

The precision of method is degree of agreement between the results. Precision of the method was studied for method precision and intermediate precision. Six separate test sample solutions of Dolutegravir were prepared by spiking the optical isomers in method at specification level for measuring the method precision. The % RSD (n = 6) for each related impurity was evaluated and found in between 0% to 2.91%. The similar procedure of method precision was carried out by a different analyst, using different mobile phase and diluent preparations and instrument on a different day with different lot of same brand column for intermediate precision study. The % RSD of results for intermediate precision study was calculated and compared with the method precision results and it is concluded that method for determination of % impurities is rugged.

Accuracy (Recovery)

Accuracy of the method for optical isomers was determined by analyzing Dolutegravir sample solutions spiked with all the impurities at four different concentration levels of LOQ, 50%, 1005, 150% and 250% of each in triplicate at the specified limit. The recovery of all these impurities were found to be in-between the predefined acceptance criterion of 80.0% to 120.0%. Hence it is concluded that method for determination of % impurity is accurate (Table 3).

Table 3: % Recovery observed at different level.

Level	(RR) Diastereomer	(SS) Diastereomer	(SR) Enantiomer
	% Recovery		
LOQ-1	96.6	100.7	106.1
LOQ-2	97.6	100.4	110.5
LOQ-3	96.7	98.9	106.4
50%-1	95.7	95.4	104.2
50%-2	94.7	96.7	104.1
50%-3	95.8	96.9	106.4
100%-1	95.2	97.3	105.7
100%-2	94.4	96.9	104.4
100%-3	92.9	96.1	102.2
150%-1	96.4	99.2	100.1
150%-2	94.7	97.6	100
150%-3	95.6	98.5	100.7
250%-1	93.6	97.1	100.8
250%-2	94.9	97.6	100.3
250%-3	94	97.2	100.2
Average% Recovery	95.5	97.8	103.5

Robustness

The chromatographic conditions were deliberately altered to evaluate the robustness of developed method. The resolution between closely eluting peaks was evaluated on altered chromatographic condition. Following parameters were changed one at a time keeping other parameters constant. First the effect of column temperature on resolution was studied at 30˚C and 40˚C instead of 35˚C. Then changing the composition of mobile phase (61:39 v/v, 65:35 v/v) and effect on resolution of all close eluting impurities were studied and found well separated. The system suitability parameter like Resolution and Signal to noise ratio are not significantly changed with altered conditions for column temperature and composition of mobile phase.

CONCLUSION

A new chiral RP-HPLC method was developed for the separation of three optical isomers of dolutegravir sodium. This is the first robust and accurate RP-HPLC method has been developed and successfully validated for the monitoring of optical isomers in dolutegravir sodium drug substance. The results of the HPLC validation tests indicated that the method was accurate, precise, robust, and reproducible. Hence, the proposed HPLC method is suitable for routine analysis of dolutegravir sodium.

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