SHORT COMMUNICATIONS

STABILITY INDICATING HPLC METHOD FOR ESTIMATION OF ELIGLUSTAT TARTRATE

ABSTRACT

For determination of eliglustat tartrate, a new, specific, simple, accurate (recovery) and precise stability-indicating HPLC method has been developed and validated according to ICH Q2(R1) guidelines. The HPLC method was developed using acetonitrile: 0.1 % orthophosphoric acid 40:60 (V/V) as mobile phase, with pH of mobile phase adjusted to 3.0. The column used was HiQSil C-18 (250 mm x 4.6 mm, 5 µm) as stationary phase at a flow rate of 0.7 mL min^{-1}. Retention time for eliglustat tartarate was found to be 5.9 min. The eluted compounds were detected using a PDA detector. The drug was subjected to stress testing as per ICH Q1A (R2) guideline. The degradation products did not interfere with the retention time of eliglustat. The calibration curve was found to be linear over a concentration range of 10-50 µg mL^{-1}. For the calibration plots, R^2 was found as 0.9995, indicating good linear relationship. The accuracy of the developed method was in the range of 99.87-100.62 %. The limit of detection of eliglustat was found to be 0.23 µg mL^{-1} and limit of quantification was 0.68 µg mL^{-1}.

Keywords: Eliglustat tartrate, Stability indicating, Validation, ICH guidelines, RP-HPLC, Forced degradation

INTRODUCTION

Eliglustat tartrate is described chemically as 2*R*, 3*R*-2,3-dihydroxybutanedioic acid; bis (*N*-[(1*R*,2*R*)-1-(2,3-dihydro-1,4-benzodioxin-6-yl)-1-hydroxy-3-(pyrrolidin-1-yl)propan-2yl octanamide (Fig. 1a). Eliglustat is a drug used for treatment of type-1 Gaucher's disease, commonly used as the tartrate salt. It works by inhibition of glucosylceramide synthase[1-2].

Literature review revealed that there is one bioanalytical method reported for eliglustat tartarate[3], however no stability indicating RP-HPLC method has been reported for this drug.

METHODS

Development of optimum mobile phase

The main aim of the present research work was to quantitate eliglustat tartarate (obtained as a gift sample from Dr. Reddy's Laboratories Ltd) and separate the drug from its degradation products. Jasco HPLC system consisting of a quaternary gradient pump having PDA detector and manual injection facility was used. During the optimization process on C-18 column, several conditions with various mobile phases like methanol/water and acetonitrile/water in presence of ortho phosphoric acid in different proportionalities were tried in an isocratic mode. To detect drug and degradation products with sufficient peak intensity, the wavelength at 281 nm (λ max) was chosen. Finally, a mobile phase consisting of acetonitrile and 0.1% orthophosphoric acid (pH 3.0) (40:60 V/V) at a flow rate of 0.7 mL min^{-1} and PDA detection at 281nm (Fig. 1b), in an isocratic mode gave good separation of drug and its degradation products. The retention time for the drug was found to be 5.9 min ± 0.2 min (Fig. 1c).

Validation of analytical method

The method validation was in accordance with ICH guidelines Q2(R1)[4] (Table I).

Specificity

Eliglustat peak was indicated by peak purity values of eliglustat, which was found to be 0.999.

Linearity and range

Linearity of the method was studied by injecting 10-50 µg mL^{-1}concentrations of eliglustat prepared in the mobile phase.

Precision

In the intra-day and inter-day studies for precision, three replicates of three different concentrations (10, 30, 50 µg mL^{-1}) were studied and the RSD values studies were found to be less than 2%. This low value of RSD indicates that proposed method is precise.

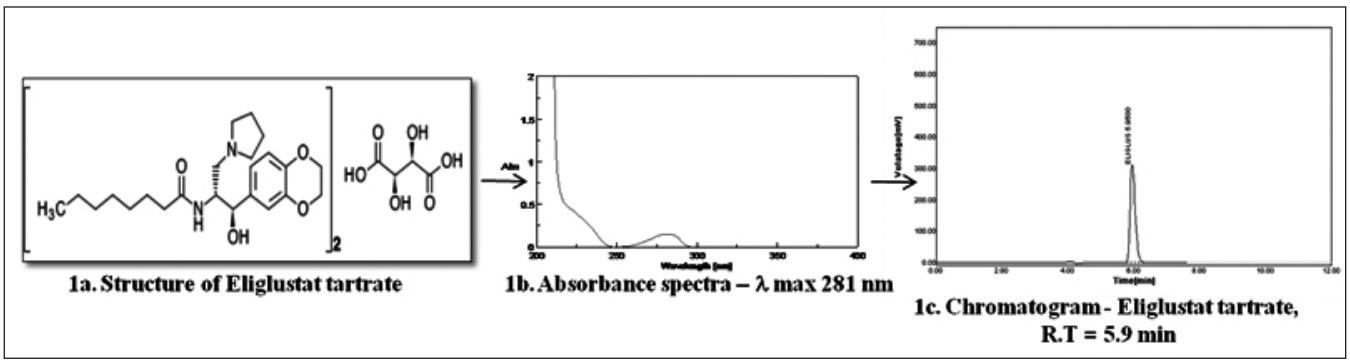

Fig. 1: Optimization of mobile phase

Table I: Summary of validation parameters

Sr. No.	Validation parameter	Results
		Eliglustat tartrate
1.	Linearity equation	y = 154.8x + 239.96
2.	Range	10–50 µg mL^{-1}
3.	Assay (Mean ± % RSD)	98.44 ± 0.7523
4.	Precision	Mean ± % RSD
	Intra-day precision	98.94 ± 0.075
	Inter-day precision	98.96 ± 0.052
5.	Accuracy	Mean ± % RSD
	80 %	100.62 ± 0.163
	100 %	99.95 ± 0.119
	120 %	99.87 ± 0.111
6.	LOD	0.23 µg mL^{-1}
7.	LOQ	0.68 µg mL^{-1}
8.	Robustness	Mean ± % RSD
	Flow rate (mL min^{-1})	0.154 ± 0.097
	Wavelength	0.221 ± 0.147
	Change in pH of mobile phase	0.167 ± 0.216

Accuracy

The accuracy of the developed method was checked in different levels, i.e., 80, 100 and 120 %. The concentration of eliglustat sample was 10 µg $mL^{-1}$and to it 8, 10 and 12 µg $mL^{-1}$of standard eliglustat was spiked. Recovery of eliglustat was found to be 99.87%-100.62% with less than 2% of RSD value.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ values were 0.23 µg mL^{-1} and 0.68 µg mL^{-1}, respectively which were calculated from the formulae as mentioned below:

LOD = 3.3 x SD/Slope and LOQ = 10 x SD/Slope

Robustness

Robustness of the method was determined by carrying out the analysis under variations of flow rate, wavelength and pH of mobile phase. The %RSD values were found to be within the limit of 2.

Forced degradation studies[5]

Forced degradation studies were conducted to evaluate the specificity and stability of the method. Degradation products were observed when the drug was subjected to acidic, alkaline, neutral, photolytic and oxidative stress conditions. Eliglustat has shown 12.48 % degradation in alkaline medium, 8.56 % degradation in acidic medium, 9.63 % degradation in hydrogen peroxide, 7.42 % degradation in photo stability study and less than 5 % degradation in neutral study, whereas it is stable showing very low degradation in dry heat.

DISCUSSION

Degradation of eliglustat tartrate was found to occur under alkaline condition, acidic condition, oxidative condition, neutral and photolytic stress. When the drug is exposed to forced degradation, the octanoyl, pyrrolidine, and 1,4 benzodioxane moieties may undergo oxidation upon exposure to oxidative and acidic stress. However, the drug has also been found to degrade under alkaline, neutral and photolytic stress.

CONCLUSION

In the present work, stability indicating RP-HPLC method for the estimation of eliglustat tartrate was developed and validated as per ICH guidelines. The % RSD values are within limit, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Hence, it can be concluded

that the developed method is simple, accurate, precise, reproducible and economic.

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(Received 26 February 2020) (Accepted 13 May 2020)

https://doi.org/10.53879/id.59.04.12344