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Stability-Indicating Method Development and Validation for Estimation of Eliglustat in Formulation by High Performance Liquid Chromatography.

Abhishek Lawsare[1], Shyam Rangari[1], Vaishnavi Umretkar[1], Milind Umekar[1], Radheshyam Lohiya[1]*.

Affiliation:

1) Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra, India.

Corresponding author (*):

Mr. Radheshyam Lohiya

Associate Professor,

Department of Pharmaceutical Chemistry,

Smt. Kishoritai Bhoyar, College of Pharmacy,

Kamptee, Nagpur, Maharashtra.

ABSTRACT

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. To separate the title components, a variety of mobile phase compositions were initially used. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The mobile phase containing mixture of Methanol:Acetonitrile (75:25v/v)with a flow rate of 1.0 ml/minis quite robust. The optimum wavelength for detection was 282 nm at which better detector response for both the drugs was obtained. The retention time of Eliglustat was found to be 4.014 min. System appropriateness studies on freshly manufactured stock solutions were done to gauge its efficacy. The calibration was linear in concentration range of 20 to 140 µg/ ml, with regression 0.9997. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found above 100.53 % for the drug. By performing validation in accordance with ICH guidelines, the Method was examined. Eliglustat in its pharmaceutical dosage form is routinely analyzed using the method by RP-HPLC as a result of its validation.

Key words: Eliglustat, RP-HPLC, Gaucher Disease, Pharmaceutical dosage form

INTRODUCTION:

Gaucher disease is the most common sphingolipidosis. Philippe Gaucher originally identified it in a patient with significant splenomegaly without leukemia in 1882[1]. The GBA1 gene, which is found on chromosome 1, is the site of mutations that lead to the rare, autosomal,recessive condition known as GD [2]. This leads to a markedly decreased activity of the lysosomal enzyme, glucocerebrosidase (GCase, also called glucosylceramides or acid β-glucoside), which hydrolyzes glucosylceramide (GlcCer) into ceramide and glucosidase) [3]. The GBA1 gene has more than 300 GBA mutations that have been documented[4]. Rarely, a lack of the GCase activator saposin C might also result in GD [5]. Although the phenotypic is diverse, three clinical forms have been identified: type 1 is the most prevalent and normally hasno negative effects on the nervous system, whereas types 2 and 3 are marked by neurological dysfunction [6]. However, these distinctions are not absolute, and it is increasingly recognized that neuropathic GD represents a phenotypic continuum, ranging from for treatment with Eliglustat undergo an FDA approved genotype test to establish if they are CYP2D6 EM (extensive metabolizers), IM (intermediate metabolizers), or PM (poor extrapyramidal syndrome in type 1at the mild end, to hydrops fetalis at the severe end of type 2 [7-8] .

Eliglustat, marketed by Genzyme as CERDELGA, is a glucosylceramide synthase inhibitor indicated for the long-term treatment of Type 1 “Gaucher disease” [9]. Patients selectedmetabolizers), as the results of this test dictate the dosage of Eliglustat recommended [10]. Eliglustat was approved for use by the FDA in August2014[11]. Eliglustat is approved for the long-term treatment of type 1 Gaucher disease in adult patients who have either never received medication or have received treatment in the past and are CYP2D6 extensive metabolizers (EMs), intermediate metabolizers (IMs), or poor metabolizers (PMs) [12].

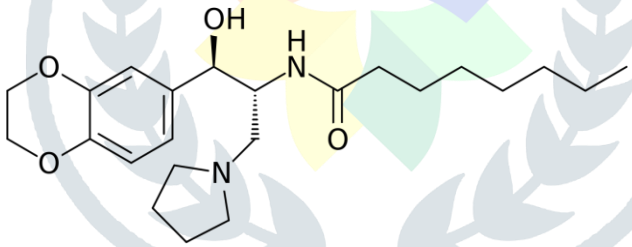


Fig: structure of eliglustat

As there were no HPLC methods for the estimation of Eliglustat, the present work describes the development of a simple, precise, accurate, and reproducible HPLC method for the simultaneousestimation of Eliglustat in dosage form [13-14]. The developed method was validated in accordance with ICH Guidelines [1&18]and successfully employed for the assay of Eliglustat dosage form [15].

Materials

DRUG; Eliglustat was acquired under the CERDELGA trade name, which is a product of theGenzyme Corporation.

CHEMICALS; Methanol, acetonitrile , orthophosphoric acid, potassium dihydrogenorthophosphate ,water ,and hydrochloric acid were used which were of HPLC grade.

INSTRUMENT; The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system, in-linedegasser (Waters, model AF), Ultraviolet detector (Water, 2487 model).

EXPERIMENTALWORK:

Chromatographic conditions

The HPLC system Isocratic elution of the mobile phase methanol and Acetonitrile in the ratio of 75:25 v/v with the flow rate of 1.0 ml/min. Separation was performed on a Waters C_{18} (250 x 4.6 mm I.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Lc-Solution software to determine the peak area. Before usage, the mobile phase's contentswere sonicated to remove any gas and filtered using a 0.45 m membrane filter. As diluents, mobile phase was used.

The mobile phase's flow rate was calibrated to 1.0 ml/min, resulting in a column back pressure of 2500–2800 PS. The column temperature was kept at 25°C and the run time was set to 8 minutes. The injection volume was 20 l, and the column was pre-equilibrated with the mobile phase for 30 to 40 minutes before the analyte was injected. At 282 nm, the eluents werediscovered. According to ICH criteria, the developed technique was validated for the assay of eliglustat in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), intra-day and inter-day precision, and robustness.

Preparation of Standard Solution:

1) Standard stock solution A :- 10mg of Eliglustat tartrate drug sample was weighed accuratelyand transferred to 10mL volumetric flask and diluted up to the mark with methanol (1000µg/ml).

2) Standard working solution :- From stock A 8ml was pipette out and was diluted up to 10mlwith methanol in 10ml volumetric flask (80µg/ml).

RESULTS AND DISCUSSION:

Method Development:

Number of mobile phase and their different proportions were tried and finally was selected as Methanol and Acetonitrile in the ratio of 75:25 v/v appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results of system suitability parameters were shown in table 2. The chromatogram of working standard solution is shown in Fig3. Table 1 provided a summary of the chromatographic conditions.

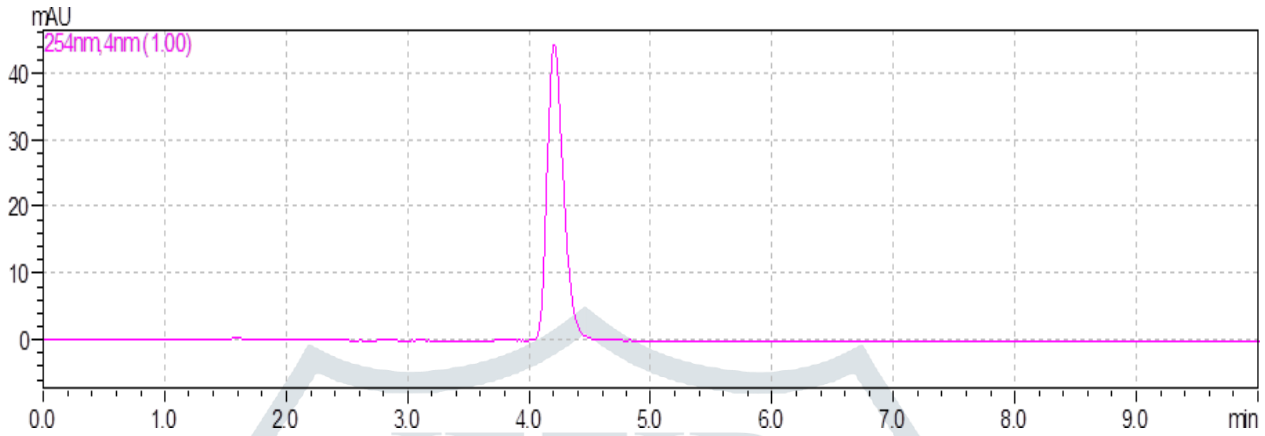


Figure2: Typical Chromatogram of EliglustatTable.1: Typical Chromatogram of Eliglustat

Sr.no	Parameter	Description /value
1.	Stationary phase	Inertsil C_{18}column 5µm
2.	Mobile phase	Methanol: Acetonitrile (75.25v/v)
3.	Flow rate	1.0ml/min
4.	Detection wavelength	282 nm
5.	Detector	Ultraviolet detector
6.	Injection	Manual
7.	Retention time	Eliglustat 4.00 min
8.	Injection volume	20µm
9.	Column temperature	Ambient (25^0 c)
10.	Run time	8 mins
11.	Diluent	Mobile phase

METHOD VALIDATION:

System suitability parameter

The CDER (Center for Drug Evaluation and Research) recommendations should be followed when determining the System Suitability Testing limitations. The USP (United States Pharmacopeia) and ICH (The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) are further resources for information on the System Suitability Testing. One of the ICH recommendations in particular has a whole section devoted to system suitability testing. System Suitability Testing can be used to test certain parameters, including:

1.Retention time 2.Tailing 3.Theoretical plates4.Resoltion factor 5.Similarity factor

Table.2 system suitability parameter

Sr.no	Parameter	Result (Eliglustat)
1.	Retention time	4.114 min
2.	Tailing	1.41
3.	Theoretical plates (n)	3967.73
4.	Resolution factor	-------
5.	Similarity factor	1.05

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed methodwas accurate.

Table.3 result of accuracy of eliglustat

Sr. No.	Weight oftab taken equivalent to(mg)	Amt of Pure Drug added (mg)	AUC (µV) Std	AUC (µV) Sample	Total Amount Recovered (mg)	% Recovery
1	23.81	8.0	340608	610808	7.93	99.12
2	23.82	10.0		681273	10.01	100.01
3	23.8	12.0		755371	12.17	101.41
Mean						**100.18**
±SD						**1.15**
%RSD						**1.15**

The Recovery of 6 replicates:**mean % recovery of 3 replicates

Precision:

The intraday and interday precision of the proposed method was determined by analyzing mixedstandard solution of Eliglustat at concentration 282 µg/mL, 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation**Table 4. result of intermediate precision (intraday and interday)**

Observation and results of intraday study

Time	Weight of Tablet taken equivalent to (mg)	AUC (µV)	% Label Claim
0 hr.	23.8	337782	99.17
3 hr.		340778	100.04
6 hr.		339128	99.56
Mean			**99.59**
SD			**0.44**
%RSD			**0.44**

Observation and results of interday study

Days	Weight of Tablet taken equivalent to (mg)	AUC (µV)	%Label Claim
Day1	23.8	336512	98.79
Day3		338121	99.26
Day7		337532	99.06
Mean			**99.03**
±SD			**0.24**
%RSD			**0.24**

Linearity

In accordance with ICH recommendations, calibration graphs were created by graphing peak area vs. concentration of Eliglustat and the regression equations were calculated. The calibrationgraphs were plotted over 5 different linear concentrations in the range of80µg/ml-120 µg/ml forEliglustat. Aliquots (20 µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates(n=5)]. In figures 4 and 5, the linearity graphswere displayed.

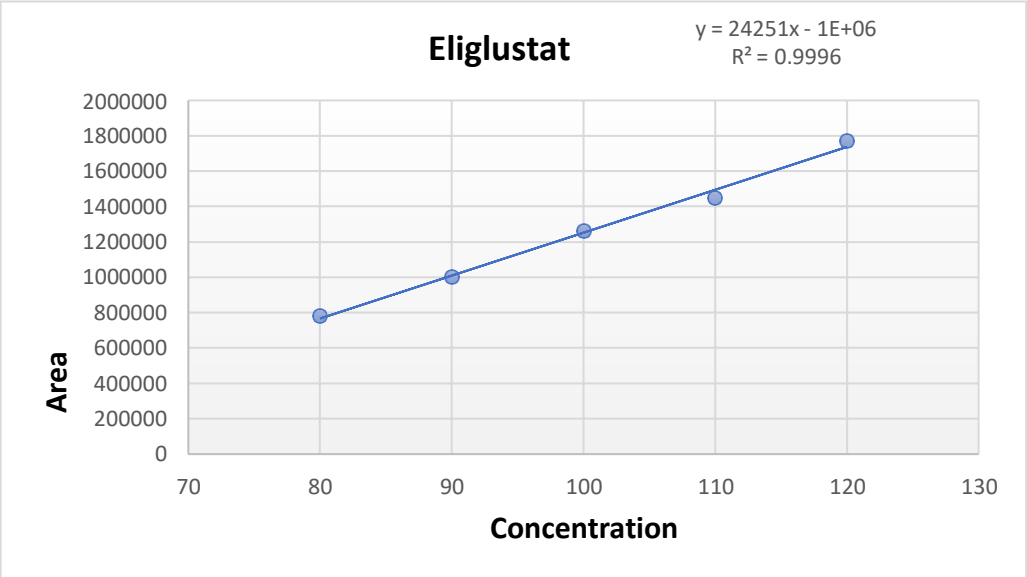


Figure 3: linearity of eliglustat

Limit of Detection(LOD)and Limit of Quantitation(LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of Eliglustat was determined bycalculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD values for Eliglustat was foundto be4.85 and LOQ values for the same was found tobe14.70.

Assay of the tablet dosage form

Eliglustat in pharmaceutical dose form was effectively determined using the suggested validated method. The result obtained for Eliglustat was comparable with correspondinglabeled amounts. The results were tabulated in table 4.

DEGRADATION STUDIES

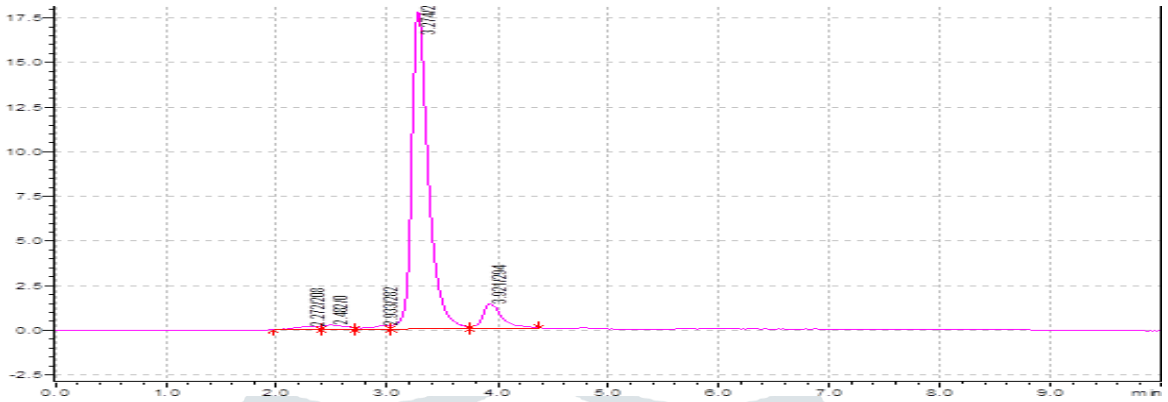
Preparation of stock:

Accurately weigh and transfer 8.0 mg of Eliglustat working standard into a 10 ml clean dry volumetric flask add about 8 mL of Diluent and sonicate to dissolve it completely and use the same solvent (Stock solution) to increase the volume to the desired level.

6.2 Degradation Study

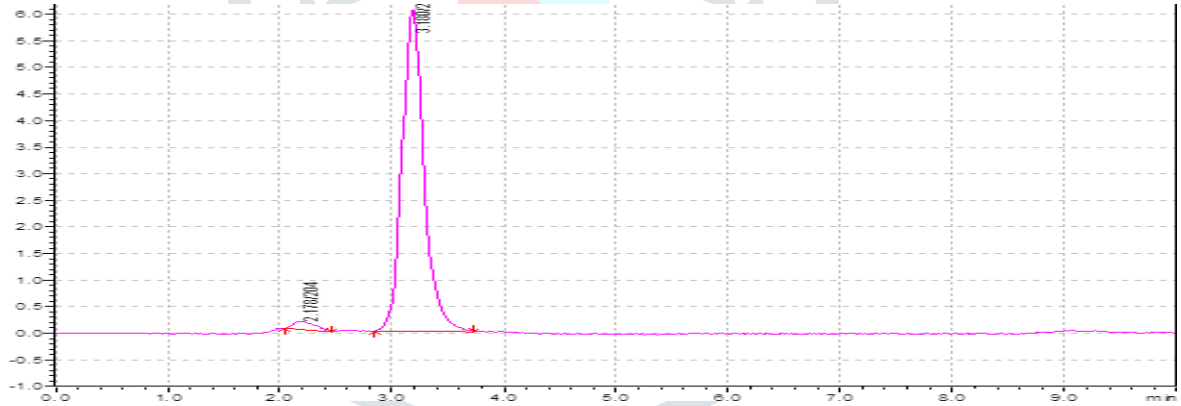
6.2.1 Acidic Degradation Condition

Acid decomposition studies were performed by Transferring 8 ml of stock solution in to 10 ml of volumetric flask. 2 ml of 0.5 N HCl solutions was added and mixed well and put for 4 hrs at 40^0C. Then solution was neutralized with 2ml of 0.5N NaOH and the volume was adjusted withdiluent to get 80µg/ml for eliglustat.



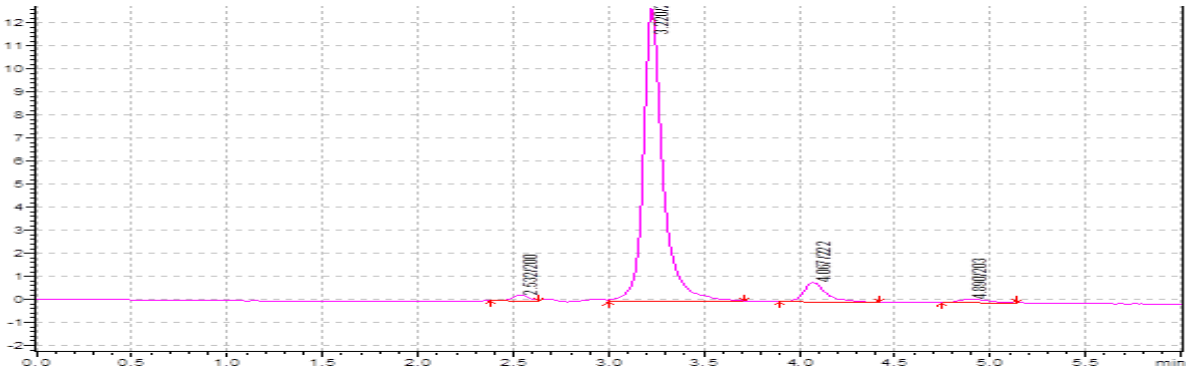
6.2.2 Basic Degradation Condition.

Basic decomposition studies were performed by transferring One ml of stock solution in to 10 ml of volumetric flask. 2 ml of 0.5 N NaOH solutions was added and mixed well and put for 4 hrs 40^0C.Then solution was neutralize with 2ml 0.5N HCL. After time period the volume was adjustedwith diluents to get 80µg/ml for eliglustat.



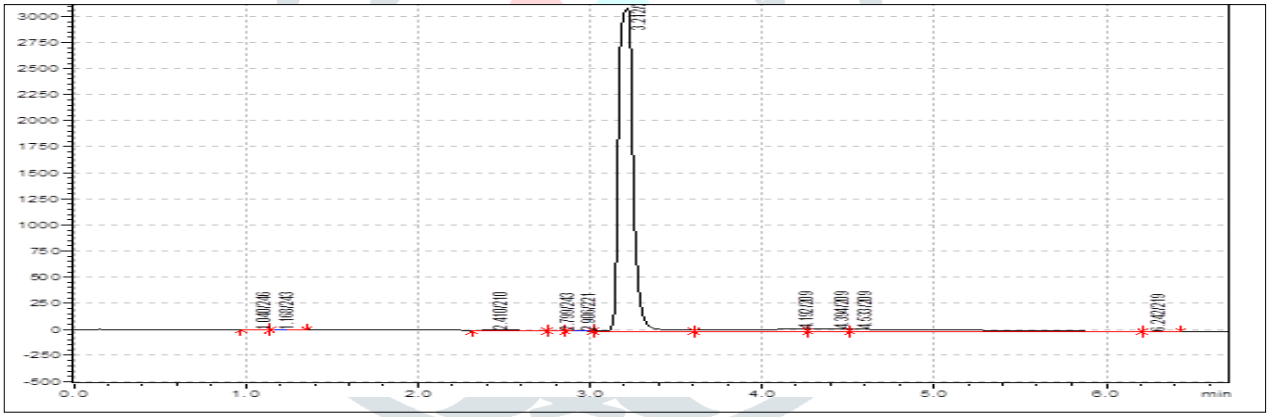
6.2.3 Oxidative Degradation Condition

Oxidation decomposition studies were performed by transferring One ml of stock solution in to 10 ml of volumetric flask. 2 ml of 10% H2O2 solutions was added and mixed well and put for 4hrs. After time period the volume was adjusted with diluents to get 80µg/ml for Eliglustat.



6.2.4 Thermal Degradation Condition

A84 mg of Eliglustat were taken in same petridish, petridish was put in oven for 6hrs at 105^0C temperature, than after petridish was removed and cool at room temperature, than this combined powder was transferred to 100ml volumetric flask and volume was made up with mobile phase, 1ml of this solution was transferred in 10ml volumetric and volume was made up with mobile phase to make 80µg/ml for Eliglustat.



6.2.5 Photolytic Degradation Condition

In order to conduct photo degradation investigations, 1 ml of the stock solution was transferred into a 10 ml volumetric flask.. The volumetric flask was kept under UV light in UV Chamber for 24hrs. Then the volume was adjusted with diluent to get 80µg/ml for Eliglustat.

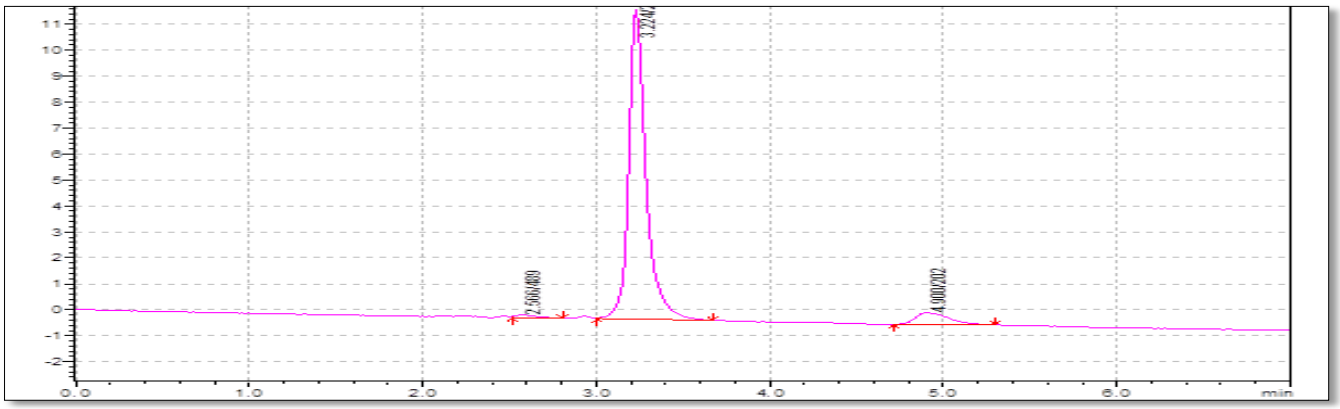


Table 4. Result of degradation Study

Mode of degradation	condition	% Assay	% Degradation control
Control sample	Untreated	99.98	0.02
Acid	0.1N HCL	95.18	4.99
Base	0.1N NAOH	94.18	5.82
Oxidative	3% H_2O_2	90.76	9.24
Neutral	H_2O	98.78	1.42
Photo (UV)	282 nm	91.75	8.25

Significance of Forced degradation studies:

According to ICH recommendations, forced degradation studies are helpful in:

1. Stability indicating methods.
2. Knowledge of the drug substance's breakdown processes and byproducts.
3. Understanding the chemical behavior of the molecule.
4. Conducting studies on degradation mechanisms etc.

CONCLUSION

The HPLC method developed for the analysis of eliglustat in their pharmaceutical preparations issimple, rapid and economic with less run time. The method has been validated, and it has been demonstrated that it is robust with modest fluctuations in chromatographic parameters as well as dependable, linear, accurate, and exact. Therefore, it can be applied for both routine analyticaland quality control assay and it could be a very powerful tool to investigate stability of Eliglustat.

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this research project.

ABBREVIATIONS

RP-HPLC: reversed phase-high performance pressure liquid chromatographyICH :International Conference of Harmonization

IM :Intermediate MetabolizerPM :Poor Metabolizer

Ems :Extensive Metabolizer LOD :Limit of Detection LOQ :Limit of Quantification

CONFLICT OF INTEREST: There is no conflict of interest.

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