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DEVELOPMENT OF VALIDATED QUANTITATIVE ANALYTICAL METHODS FOR PHARMACEUTICAL FORMULATION OF ELIGLUSTAT

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ABSTRACT :

A simple, accurate ,precise, rapid analytical method for the estimation of Eliglustat. The λ max of Eliglustat in methanol was found to be 282nm. All five methods of UV –spectrophotometry based upon zero order , first order and second order derivative have been established considering amplitude and area under curve of the spectrum. In RP-HPLC method, the condition were optimized to obtain an adequate separation of eluted compounds. 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 contain Methanol : Acetonitrile(75:25) with flow of rate 1.2ml/min. the wavelength for detection was 282 nm at which better response for both the drugs was obtained. The retention time of eliglustat was found to be 4.09. The system suitability tests were carried out on freshly prepared stock solution . The calibration curve was linear in concentration range 80ug/ml to 130ug/ml regression 0.999.The method was checked by doing validation according to ICH guidelines. Hence the method is validated it is used for the routine analysis of eliglustat in its pharmaceutical dosage form by RP-HPLC

KEYWORDS: Eliglustat , Acetonitrile, Methanol, UV-Visible spectroscopy.

INTRODUCTION

ELIGLUSTAT is chemically N-[(1R ,2R)-2 –(2,3 dihydro-1,4-benzodioxin-6-yl)-2-2-hydroxy-1-(1-plrrolidinyl methyl)ethyl]-octanamide,2R,3R-dihydroxy butanediode. Its molecular formula is $C_{23}H_{36}N_2O_4$ and the molecular formula is 404.55 . Eliglustat marketed by Genzyme as CARDELG, is a glucosylceramide synthase inhibitor indicated for the long term treatment of type 1 Gaucher's disease. Patients selected for treatment with eliglustat undergoes an FDA approved genotype test to establish if they are CYP2D6 EM (extensive metabolizers), IM(intermediate metabolizers), or PM (poor metabolizers), as the result of this test dictate the dosage of Eliglustat recommended. Eliglustat is indicated for the long-term treatment of type 1Gaucher disease in patients who are CYP2D6 extensive metabolizers (EM) ,Intermediate metabolizers(IM), or poor metabolizer (PM) in treatment – native and treatment – experienced adult patients.

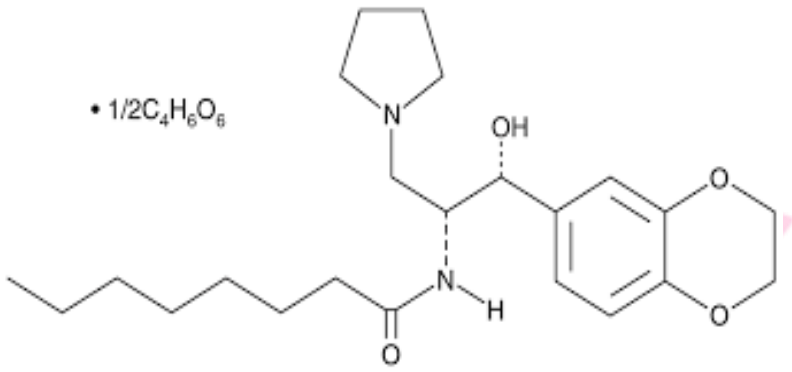


Figure 1: chemical structure of Eliglustat

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

Materials

ELIGLUSTAT was provided by Dr Reddys Lab , Hydrabad (India) and received under the brand name of CERDELGA, manufactured by genzyme corporation . Methanol, water, acetonitrile were used which were of HPLC grade.

METHOD

UV- Visible Spectrophotometer

The wavelength of Eliglustat was found to be 282.0 nm by using UV-Visible Spectrophotometer Jasco V-630 and Shimadzu-UV-1700.

4 RESULTS AND DISCUSSION

4.1 Preparation of standard stock solution

An accurately weighted (10mg) quantity of Eliglustat was transferred in a 100ml volumetric flask , dissolved in Methanol to prepare a standard stock solution having concentration 500ug/ml of ELIGLUSTAT

4.2 Working standard solution

A 20 mL of above standard solution was diluted up to 50mL methanol. (concentration : 100ug/mL of of eliglustat)

Selection of wavelength :

The working standard solution of ELIGLUSTAT (100ug/ml) was scanned in the range of 400-200 nm in 1.0 cm cell against solvent blank (methanol) and spectrum was recorded. the study of spectra shows peak maxima for eliglustat282nm

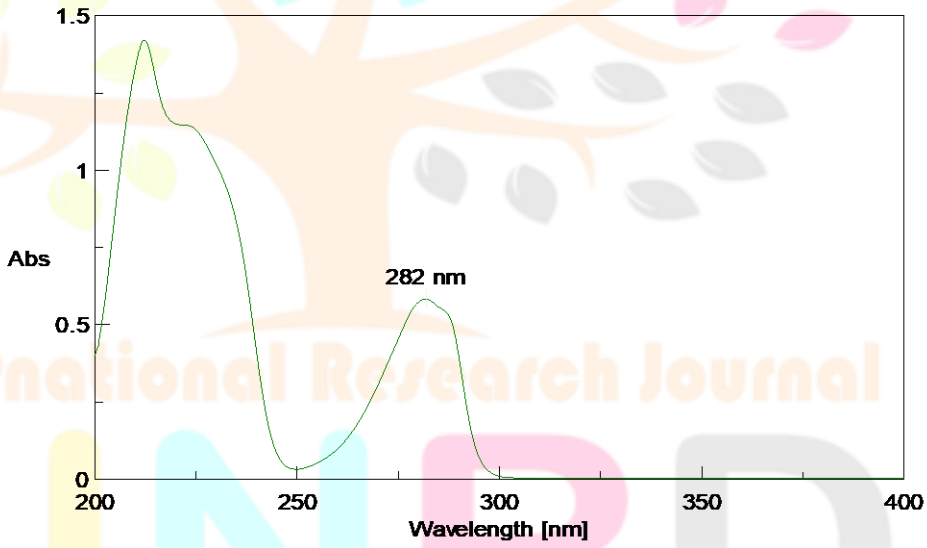


Fig no . 2 Zero order spectra of ELIGLUSTAT

Study of Beer-Lambert Law :

Aliquots of standard stock solution were diluted with Methanol to get a concentration of drug in the sequential series of 80, 90,100, 110, 120, 130 µg/ml Absorbance of each solution was measured at λmax i.e , 282 nm. Result are shown in table no.1 while calibration curve was plotted as absorbance VS concentration and shown in fig

Table no. 1 : observation for study of Beer-Lambert Law of ELIGLUSTAT

Sr.no	concentration	absorbance
1	80	0.4869
2	90	0.5485
3	100	0.6284
4	110	0.6884
5	120	0.7348
6	130	0.8012

Standard absorptivity values [A(1%, 1cm)]:

Standard solution used for Beer-Lamberts law study and absorbance of Eliglustat

Were used to calculate (1% cm) value using formula as gives below below. The results are show

A (1%, 1cm) = $\frac{Absorbance}{Concentration\,(\frac{g}{100}mL)}$----

No2: Absorbance Value [A (1%,1 cm) Method]:

Sr. No	Absorbance	A (1%, 1 cm)
1	0.4869	608.62
2	0.5485	609.44
3	0.6284	628.4
4	0.6884	625.81
5	0.7349	612.41
6	0.8012	616.30

Method-II: first order derivative method

The working standard solution of ELIGLUSTAT(100µg/ml) was scanned in the range of 200-400nm and first order derivative spectra was recorded shows in Fig.No.6.1. from the spectra 282nm was selected for further study

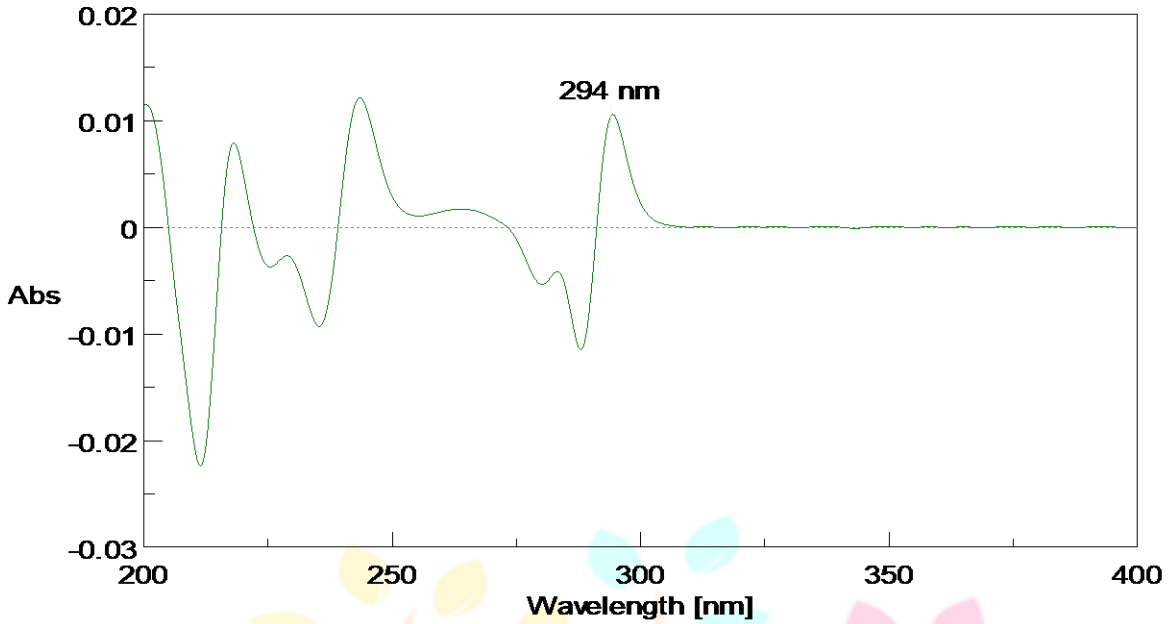


Fig.No2 : First order derivative Spectra of ELIGLUSTAT

The results are shown in table no. and calibration curve plotted curve as absorbance vs concentration which is shown in fig no

Table No. 3: Observation of Eliglustat for Method II

Sr.no.	concentration	Absorbance
1	80ug/ml	0.09025
2	90ug/ml	0.0966
3	100ug/ml	0.09978
4	110ug/ml	0.1078
5	120ug/ml	0.1135
6	130ug/ml	0.1183
	Coefficient of correlation	0.992

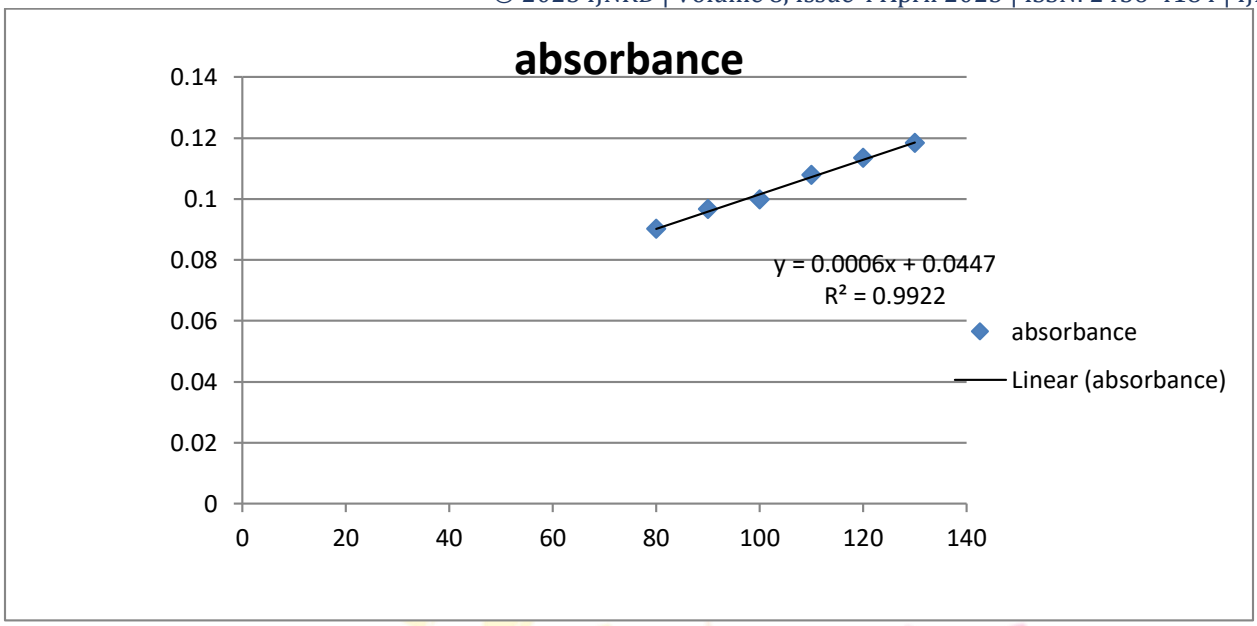


Fig no 3 calibration curve for method II

II (Second order derivative method)

The working standard solution of ELIGLUSTAT(100µ/mL) was scanned in range of 200-400 nm and second order derivative spectra was recorded shown in fig .no. from the spectra 282 nm was selected for further study

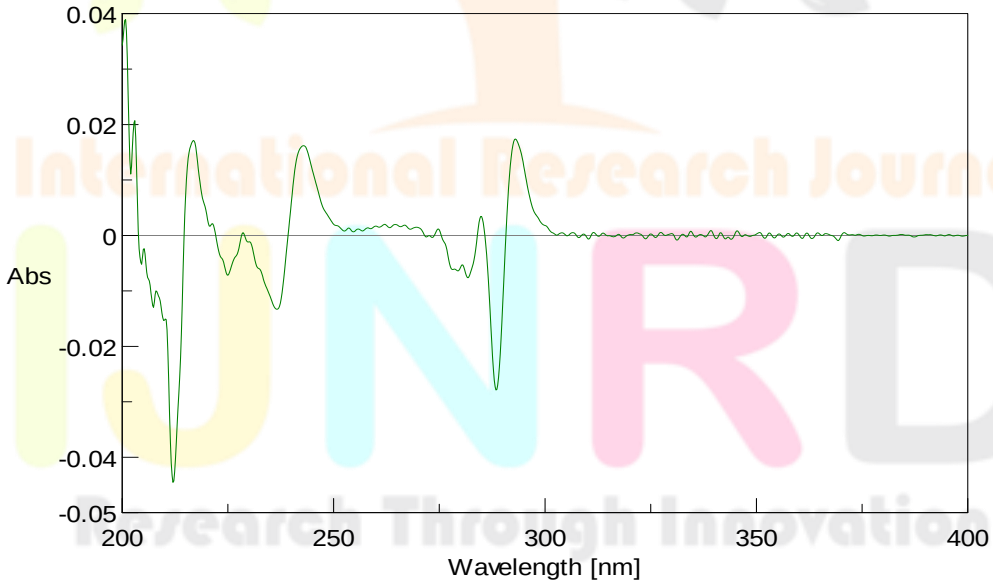
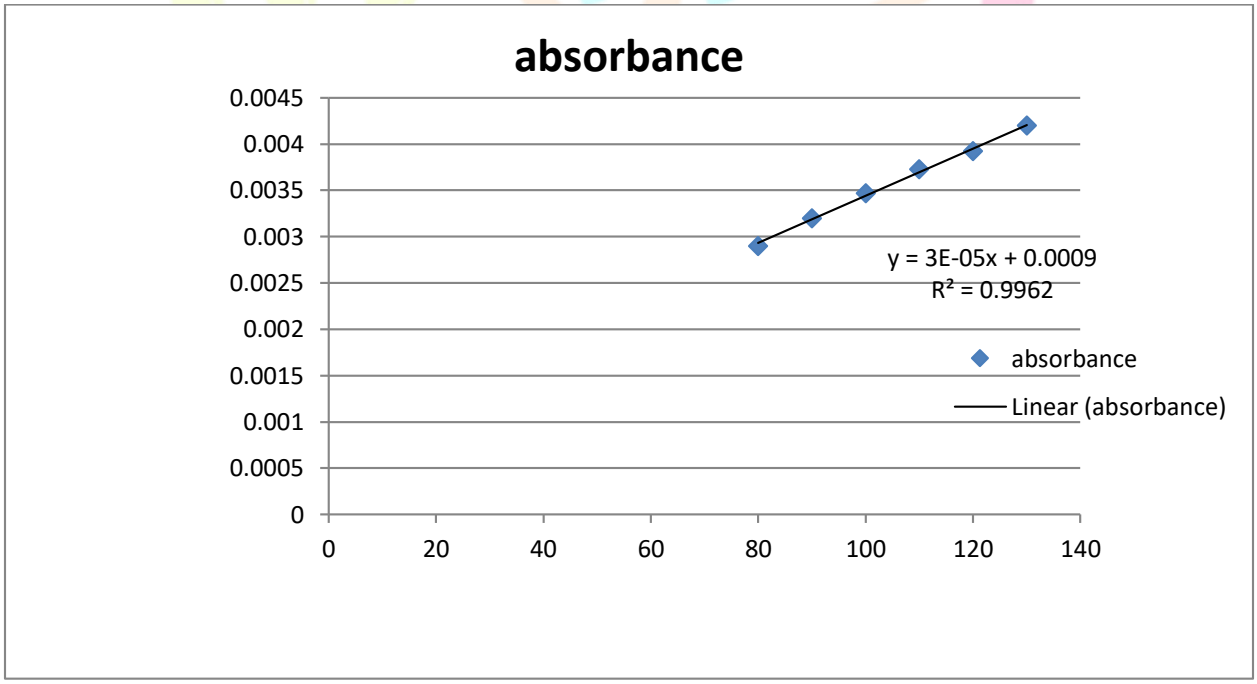


Fig .no. 4 : second order derivative spectra of ELIGLUSTAT

Table no.4 Observation table for Eliglustat method III

Sr no.	concentration	absorbance
1	80µg/ml	0.002896
2	90µg/ml	0.00320
3	100µg/ml	0.00347
4	110µg/ml	0.003729
5	120µg/ml	0.003921
6	130µg/ml	0.004199
	Coefficient of correlation	0.996

The result are shown in the table no.3 and calibration curve plotted as absorbance vs concentration which is shown in Fig No 4



Calibration curve for method III

6.1.14. Area under curve method:

For the method wavelength selected was 277nm (λ_2) and 267 nm (λ_1). The area under curve for solution concentration range 80-130 µg/Ml of ELIGLUSTAT was noted in the range 277nm(λ_2) and 267 nm (λ_1). The AUC (area under curve) involves the calculation of integrated valve of

absorbance with respect to the wavelength λ1 and λ2 . The area calculation processing item calculation the area bound by the curve the horizontal axis. The horizontal axis was selected by entering the wavelength range over which area has to be calculated . the result are shown in table …. The spectra displaying AUC was shown in fig. no. Calibration curve plotted as area under curve against concentration and shown in fig

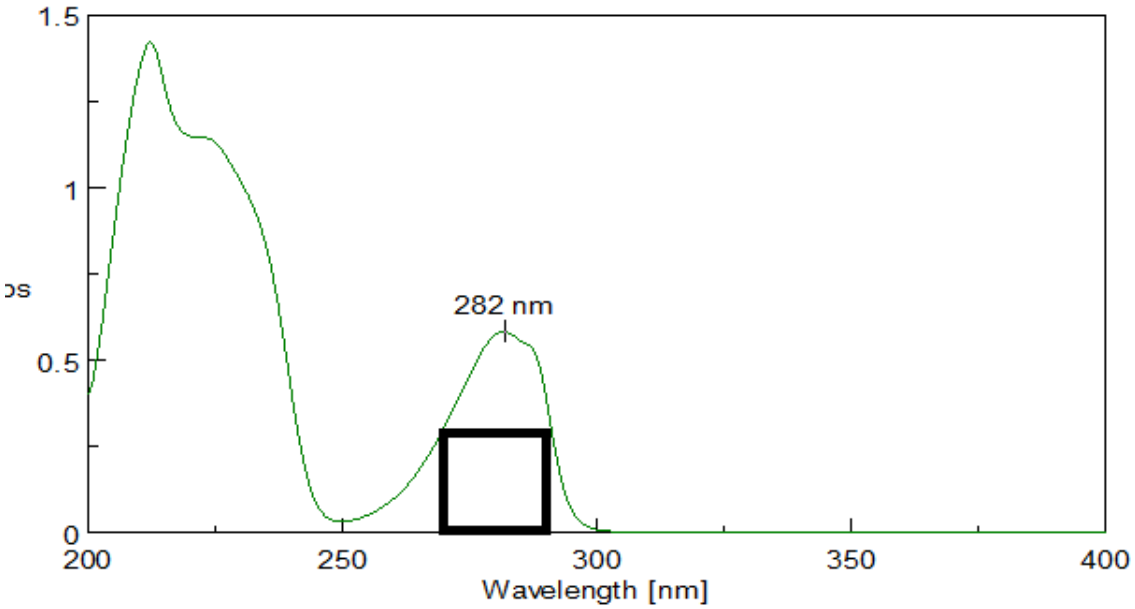


Fig. no.5 : Area under curve

Table No.5 : Observation of Eliglustat for Method-IV

Sr. No.	Concentration(µg/mL)	Area under curve (mV)
1	80 µg/ml	0.865
2	90 µg/ml	0.945
3	100 µg/ml	1.02
4	110 µg/ml	1.101
5	120 µg/ml	1.171
6	130 µg/ml	1.24
	Coefficient of correlation	0.999

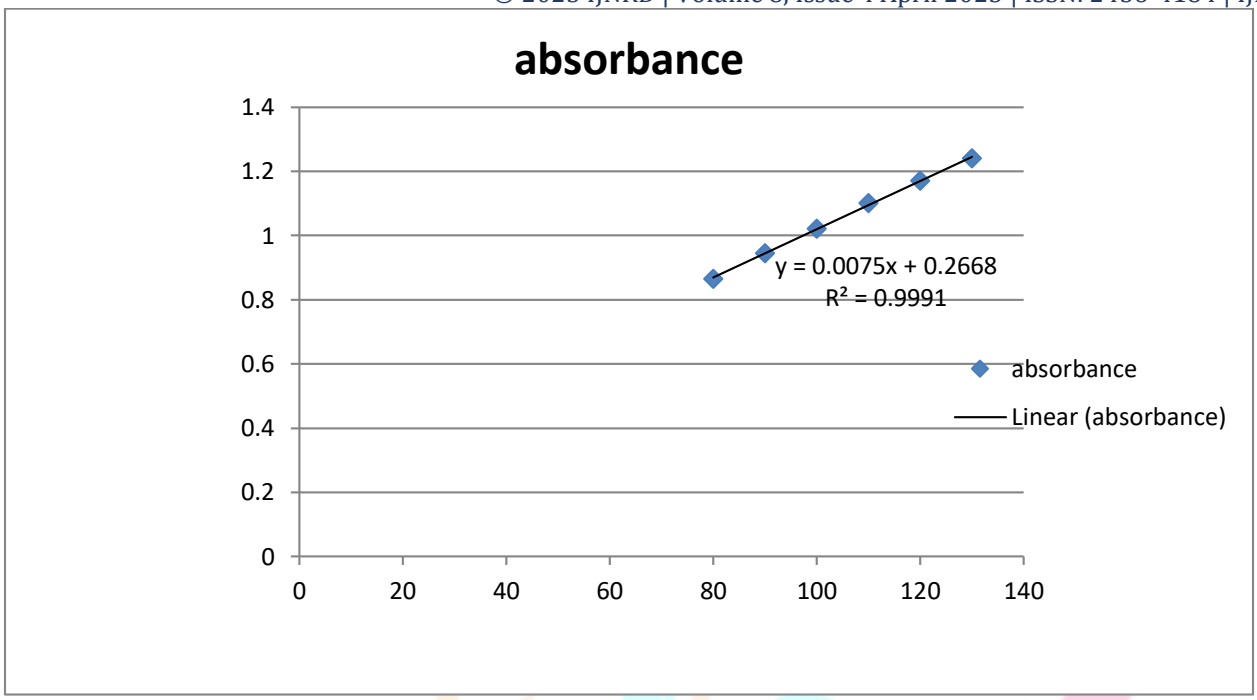


Fig. no.6 calibration curve for method IV

DEVELOPMENT OF RPHPLC METHOD FOR ESTIMATION OF ELIGLUSTAT

METHOD DEVELOPMENT :

Number of mobile phase and their different proportions were tried and finally was selected as Methanol and Acetonitrile in the ratio of 70:30 v/v appropriate mobile phase which gave good resolution and acceptable system suitability parameters . The result of system suitability parameter were shown in table 2. The chromatogram of working standard solution is shown in fig 3. The summary of chromatographic condition was given in table 1.

Table no.1 : summary of chromatographic condition

Sr. no.	parameter	Description /value
1	Stationary phase	Inertsil C18 column (250×4.6mm)5µm.
2	Mobile phase	Methanol :Acetonitrile (70:30)
3	Flow rate	1.2ml/min
4	Detection wavelength	282nm
5	Detector	D2W
6	Injection	Auto sampler- waters
7	RT	4.09
8	Injection volume	20µl
9	Column Temperature	25-30° c
10	Run Time	4min
11	Diluent	Mobile phase [acetonitrile:methanol (75:25)]

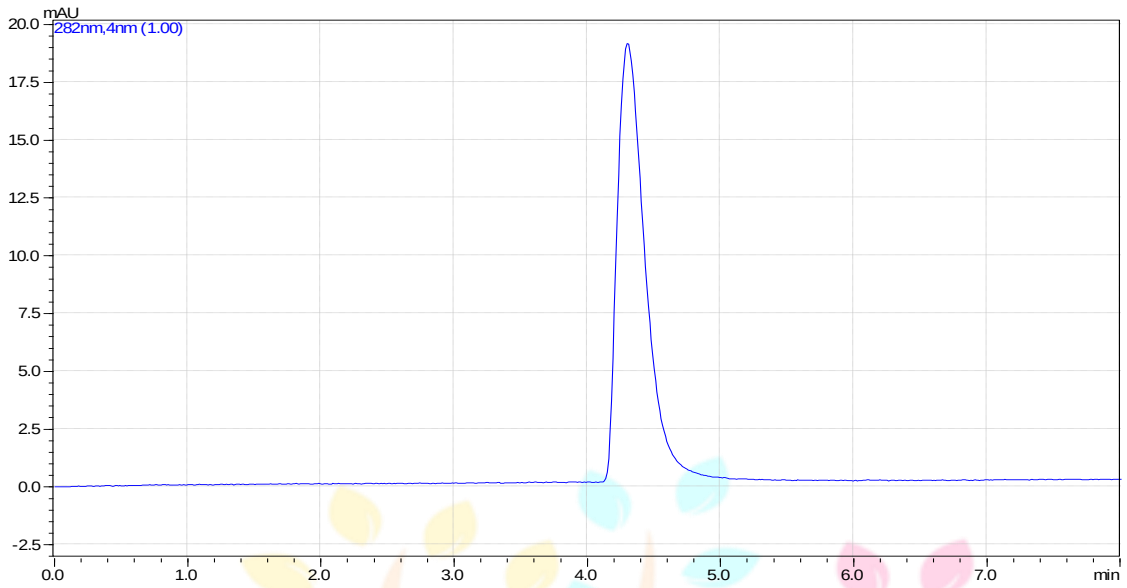


Figure2: Chromatogram of Eliglustat

Table 2: system suitability parameter

Sr .no.	parameter	Result Eliglustat
1	Retention time	4.090
2	Tailing	1.863
3	Theoretical plate(n)	147.67
4	% RSD	1.61

Study of Linearity (calibration curve)

As per ICH guideline, calibration graphs were constructed by plotting peak area vs. concentration of eliglustat and the regression equation were calculated. The calibration graph were plotted indifferent linear concentration in the range of 80µg/ml-130µg/ml for eliglustat .Aliquots (20µl) of each solution were injected under the operating chromatographic condition describe above . the linearity graphs were shown in fig.3

Table no 3observation of linearity of ELIGLUSTAT

Sr no	Wt. of std. eliglustat	Concentration	AUC(mAu)
1	100mg	**80**	**514621**
2		**90**	**767461**
3		**100**	**1029267**
4		**110**	**11257978**
5		**120**	**1493167**
6		**130**	**1768351**
		Correlation coefficient	**0.999**

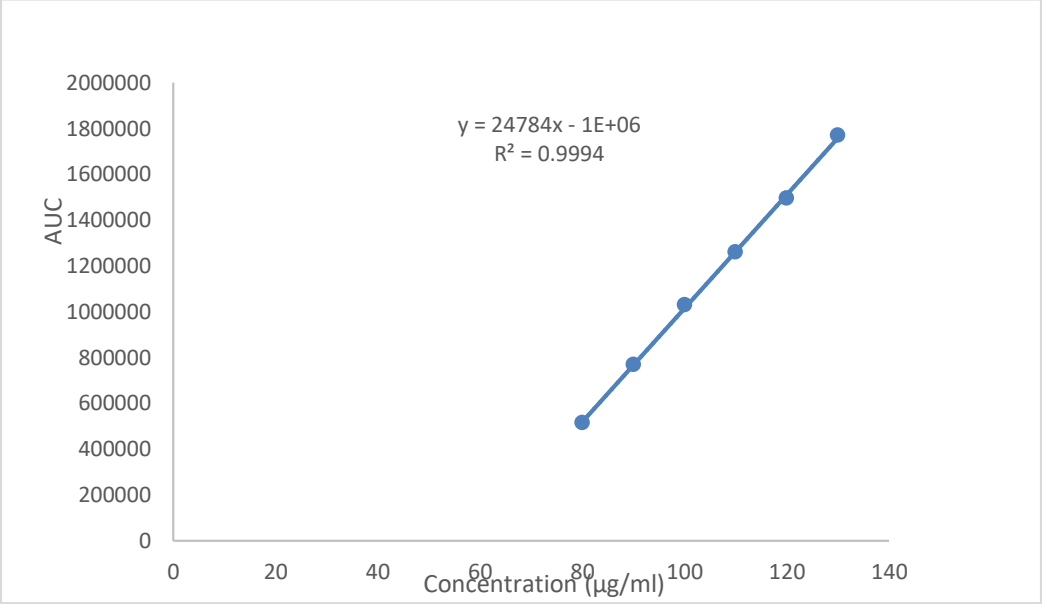


Fig no 4 .Calibration curve of Eliglustat

CONCLUSION :

The proposed method has advantage of simplicity and convenience for the separation and quantization of eliglustat in the combination which can be used for the assay of their dosage form. Also, the low solvent consumption and shorts analytical run time lead to environmentally friendly chromatographic procedure . The method is accurate, precise, rapid and selective for simultaneous estimation of eliglustat in capsule dosage form. Hence it can be conveniently adopted for routine analysis. The future scope of the work it is used for the bioequivalence study and also developed the more hplc methods

REFERENCES :

1. **Tazeen A et al., (2017)** have reported the analytical method development and validation for the estimation of eliglustat using RP-HPLC method in bulk and pharmaceutical dosage form
2. **Yixaun cui et al., (2020)** have reported method for the quantification of one PGI (namely 1,4- benzodioxzne -6-aldehyde) in Eliglustat tartrate (EGT) active pharmaceutical ingredient (API) substances using dispersive liquid-liquid micro extraction (DLLME) as sample preparation to remove matrix effect and detected by HPLC- UV
3. **Guodong sun et al., (2019)** have reported an an Efficient and Scalable Asymmetric Synthesis of Eliglustat via Ruthenium(II)-Catalyzed Asymmetric Transfer Hydrogenation.
4. **Umamaheshwar puppala et al., (2020)** have reported the characterization of degradation products of Eliglustat by liquid chromatography-tandem mass spectrometry(HRMS) and 2D-NMR techniques along with the development and validation of stability indicating ultra performance liquid chromatographic method for determination and quantification of Eliglustat drug in presence of characterized impurities.