WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 1, 1112-1140. Research Article ISSN 2277– 7105

"STABILITY INDICATING SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF VIGABATRIN USING ICH GUIDELINES"

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Article Received on
23 October 2020,

Revised on 13 Nov. 2020,
Accepted on 03 Dec. 2020

DOI: 10.20959/wjpr20211-19403

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ABSTRACT

Vigabatrin is Anticonvulsant class of drug and works by decreasing abnormal electrical activity in the brain. High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. In AUC method Water was used as solvent and detection was done at 240nm-280nm. The stability indicating HPLC method was developed and validated for estimation of Vigabatrin. The mobile phase was consisting of ACN: methanol (95:05) pH3. Detection was done at 285 nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD for all parameters was found less than 2. The result showed that the proposed method was suitable for the accurate, precise and rapid determination of in Vigabatrin its bulk form.

KEYWORDS:- Vigabatrin, HPLC, Validation, Method development.

INTRODUCTION

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. Absorption spectroscopy is the measurement of the absorption of electromagnetic radiation from definite and narrow wavelength range by molecules, ions and atoms of chemical substance. Techniques most commonly employed in analytical field ultraviolet, visible, infrared and atomic absorption spectroscopy. The term 'Chromatography' covers those processes aimed at the separation of

the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase. High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.[1-25]

Vigabatrin is drug sold under brand name sabril and having chemical formula $C_6H_{11}NO_2$.

Vigabatrin is freely soluble in water and in aqueous solvents; slightly soluble in methanol; very slightly in ethanol and chloroform; and practically insoluble in toluene and n-hexane.

Vigabatrin is an irreversible mechanism-based inhibitor of gamma-amino butyric acid aminotransferase (GABA-AT), the enzyme responsible for the catabolism of GABA. Inhibition of GABA-AT results in increased levels of GABA in the brain. Vigabatrin is a racemic compound, and its [S]-enantiomer is pharmacologically active. Vigabatrin powder is used to control infantile spasms (a type of seizure that babies and children can have) in babies 1 month to 2 years of age. Vigabatrin is in a class of medications called anticonvulsants. It works by decreasing abnormal electrical activity in the brain.[25-50]

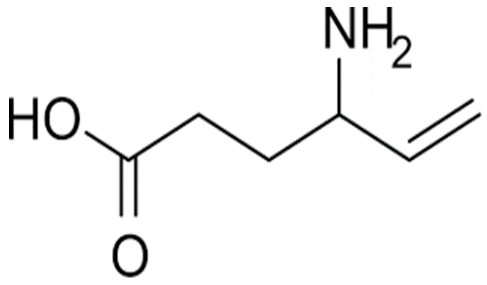

Fig. no. 01: Structure of vigabatrin.

MATERIAL AND METHOD

Identification of drug[1-9,23,32,36] Organoleptic properties of drug

The sample of Vigabatrin was checked for organoleptic properties such as colour and odour.

Solubility analysis

Vigabatrin is freely soluble in water and in aqueous solvents; slightly soluble in methanol; very slightly in ethanol and chloroform; and practically insoluble in toluene and n-hexane.

Fourier Transform Infra-red Spectroscopy (FTIR)

The IR study of pure drug was carried out by using Fourier transform infrared spectrophotometer (BRUKER).

UV-visible spectrophotometric method[2,4,28,50] Method - A: Zero order spectrophotometric method Selection of solvent

In order to select suitable solvent for determination of Vigabatrin the solubility and stability was checked. It is found that Vigabatrin was freely soluble in water. All the dilutions were prepared in the same solvent.

Preparation of standard stock solution

Standard stock solution of Vigabatrin was prepared by accurately weighing 100 mg of Vigabatrin to 100 ml volumetric flask with specific volume of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

Selection of analytical wavelength

0.1mL of the standard stock was pipette out and transfers to 10 ml volumetric flask and volume was made up to mark with water. The solution was scanned in the wavelength range of 200-400 nm.

Selection of concentration Range and Preparation of calibration curve

Aliquots portion 0.05, 0.10, 0.15, 0.20, 0.25 ml was pipetted out from the standard stock solution and transferred to series of 10 mL volumetric flask and volume was made with water to get the concentration range from 5-25 µg/ml. The absorbance was measured three times for each concentration. Absorbance of each solution was measured against water as blank at 285 nm.

Method validation linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Accuracy

Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock to the test solution at three different spiking level 50%, 100%, 150% of the target concentration.

Precision

The precision study of an analytical method expresses the closeness of agreement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions**.**

Limit of Detection & Limit of quantitation

Detection limit and Quantitation limit were determined based on the standard derivative of y-intercepts of five calibration curve and average slop of six calibration curves are mentioned.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness

It is the degree of reproducibility of the test results under variety of conditions like different analyst, different instrument.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

First Order Derivative[2,9,12] Preparation of standard stock solution

Standard stock solution of Vigabatrin was prepared by accurately weighing 100 mg of Vigabatrin to 100 ml volumetric flask with specific volume of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

Method validation

According to ICH Q2 (R1) guidelines the developed method was validated to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity, ruggedness, and robustness.

Area under curve (AUC) method[2,12,15,26,49] Preparation of standard stock solution

Standard stock solution of Vigabatrin was prepared by accurately weighing 100 mg of Vigabatrin to 100 ml volumetric flask with specific volume of water. The drug was sonicated and volume was made up to mark with water to get the concentration of 1000 µg/ml.

Selection of analytical wavelength range for AUC

0.1 ml of the standard stock was pipette out and transfers to 10 ml volumetric flask containing specific volume of water then volume was made up to mark with water. The solution was scanned in the wavelength range of 200-400 nm. The absorption spectra show absorbance maxima (λ max) at 285 nm. Estimation of AUC in absorption spectra was selected ranges from 280-290 nm (285 ± 5nm).

Selection of concentration Range and Preparation of calibration curve

Aliquots portion 0.05, 0.10, 0.15, 0.20, 0.25 ml were pipetted out from the standard stock solution and transferred to series of 10 ml volumetric flask and volume was made with water to get the concentration range from 5-25 µg/ml. The spectrum was measured three times for each concentration. Spectrum of each solution was measured against water as blank at 285 nm. The AUC was measured between 280-290 nm.

High performance liquid chromatographic method optimization of detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected.

Selection of chromatographic conditions

The selection of HPLC method depends upon the nature of the sample, its molecular weight and solubility. RP-HPLC method was selected for the initial separations because of its simplicity and suitability. The chromatographic variables such as mobile phase ratio and flow rate were studied. The condition that gave the best resolution, symmetry and selectivity was selected.

Optimization of chromatographic parameters

Optimizations in HPLC is the process of finding a set of conditions that sufficiently enable the quantification of the analyte with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Preparation of standard stock solutions

Accurately 10.0 mg weighed quantity of Vigabatrin was transferred to 10.0 mL volumetric flask. That was dissolved by adding 5.0 mL mobile phase and then the drug solution was

diluted up to the mark with mobile phase to get the stock solution of 1000 µg/mL of Vigabatrin. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Optimization of mobile phase strength

Based on drug solubility, stability and suitability of drug in different solvents, various mobile phases and compositions were tried to get a good resolution and sharp peak. The standard solution containing drugs were run in different mobile phases.

Mobile phase preparation

Prepare mobile phase by taking methanol and water in various proportion ACN: methanol (95:05). Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

Selection of mobile phase

Pure drug solutions of Vigabatrin (15µg/mL) were injected into the RP-HPLC system and run in different solvent systems. Different mobile phases systems like ACN and methanol were initially tried in the isocratic mode in order to determine the best conditions.

RESULTS AND DISCUSSION

Preliminary analysis

The sample of Vigabatrin was observed for its colour and texture. It is white powder and odourless.

Solubility study

The Vigabatrin is freely soluble in water, methanol and acetonitrile. It is soluble in DMSO.

Stability study

After observation it was found that there was no degradation of sample with solvent Water, ACN and Methanol.

Method development and validation by UV spectrophotometry

Zero order spectrophotometric method

UV spectrophotometric method was developed for estimation of Vigabatrin in bulk form. The wavelength maxima were found to be 285 nm.

Linearity

The method was found to be linear in concentration range of 5-25 µg/ml with correlation coefficient (r^2) 0.9995.

Table No. 01: Data for calibration curve of vigabatrin by ZOD.

Sr. No.	Conc. (µg/mL)	Absorbance
1	5	0.1289
2	10	0.3026
3	15	0.4469
4	20	0.5879
5	25	0.7395

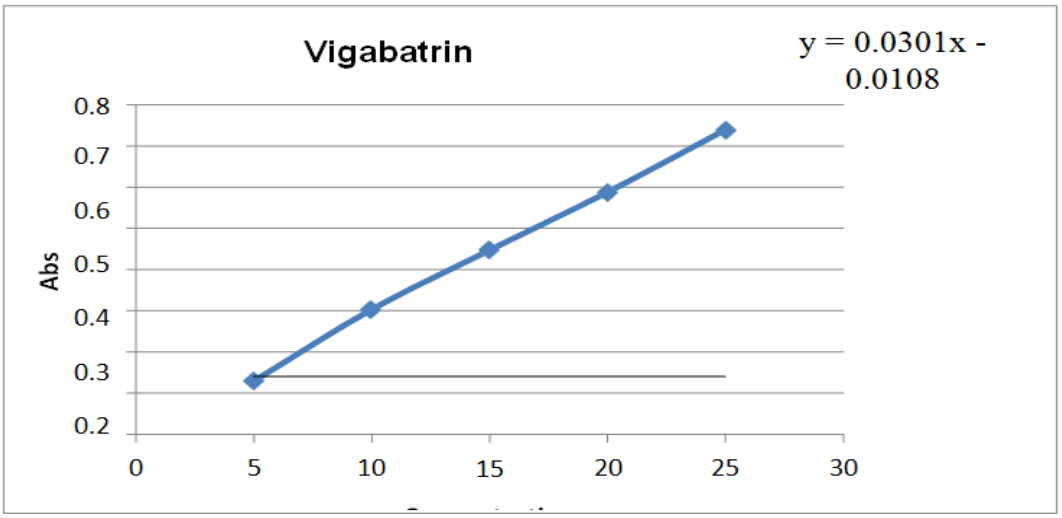

Fig. No. 2: Calibration curve for Vigabatrin by zero order spectrophotometric Method.

Optical characteristics

Table No.02: Optical characteristics for vigabatrin by ZOD.

Sr. No	Parameters	Zero Order spectrophotometric method
1	λmax (nm)	285
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 0.0301x - 0.0108
4	Slope[m]	0.0301
5	Intercept [c]	-0.0108
6	Correlation coefficient [r2]	0.9986
7	Limit of detection (LOD) (µg/mL)	0.0548
8	Limit of quantitation (LOQ) (µg/mL)	0.1661

Accuracy

Table No. 03: Data for recovery study of vigabatrin by zod.

Level of addition	Standard added(µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Abs obtained*	Std Abs	Drug recovered (µg/ml)	%Recovery
50%	5	10	15	0.4521	0.4469	15.174536	101.163571
	5	10	15	0.4451		14.939584	99.5972253
	5	10	15	0.4398		14.761692	98.4112777
100%	10	10	20	0.5863	0.5879	19.945569	99.7278449
	10	10	20	0.5912		20.112264	100.56132
	10	10	20	0.5812		19.77207	98.8603504
150%	15	10	25	0.7361	0.7395	24.885057	99.5402299
	15	10	25	0.7385		24.966193	99.8647735
	15	10	25	0.7402		25.023665	100.094659

Table No. 04: Statistical validation of vigabatrin by zod.

Level of addition	% Mean recovery*	SD	% RSD
50%	99.72	1.3805	1.384342
100%	99.72	0.855	0.85296
150%	99.83	0.2786	0.279023

*****Average of three determination

Precision

Intraday and Interday precision assures the repeatability of test results. The % RSD found was < 2. Result of intraday and Interday precision.

Table No. 05: Data for intraday precision of vigabatrin by zod.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1279	0.12873333	0.00085	0.66066
2	5	0.1296			
3	5	0.1287			
4	15	0.4463	0.44576667	0.001102	0.247106
5	15	0.4458			
6	15	0.4452			
7	25	0.7426	0.73733333	0.004903	0.665018
8	25	0.7365			
9	25	0.7329			

Table No. 06: Data for interday precision of vigabatrin by zod.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1284	0.1274	0.001	0.78492936
2	5	0.1264			
3	5	0.1274			
4	15	0.4465	0.44603333	0.00056862	0.12748466
5	15	0.4454			
6	15	0.4462			
7	25	0.7402	0.73933333	0.00090185	0.12198151
8	25	0.7394			
9	25	0.7384			

Robustness

Table No. 07: Data for robustness study of vigabatrin by zod.

Parameters	Change In Wavelength(±2 nm)			
	Wavelength (243nm)		Wavelength (247nm)	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7394	0.4459	0.7386
SD	0.0007	0.0006	0.0001	0.0016
% RSD	0.1917	0.0988	0.4818	0.2634

Table No. 08: Data for robustness study of vigabatrin by zod.

Parameters	Change in solvent			
	Water		0.1N NaOH	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7364	0.4487	0.7365
SD	0.00079	0.0015	0.0006	0.0008
% RSD	0.290	0.3614	0.2086	0.2091

Ruggedness

Table No. 09: Data for ruggedness study of vigabatrin by zod.

Parameters	Change in analyst			
	Analyst I		Analyst II	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4465	0.7368	0.4485	0.7402
SD	0.0006	0.0008	0.0011	0.0006
% RSD	0.2807	0.2076	0.5467	0.1460

Specificity

Table No. 10: Data for specificity study of vigabatrin by zod.

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Abs	Mean	SD	%RSD
5	10	15	0.1264			
5	10	15	0.1294	0.12746667	0.0016773	1.31587297
5	10	15	0.1266			
10	10	20	0.3026			
10	10	20	0.3024	0.30326667	0.00133167	0.43910715
10	10	20	0.3048			
15	10	25	0.4454			
15	10	25	0.4489	0.44793333	0.00221435	0.49434748
15	10	25	0.4495			

First order derivative spectroscopy

Linearity

The method was found to be linear in concentration range of 5-25 µg/ml.

Table No. 11: Data for calibration curve of vigabatrin by first order spectrophotometric method.

Sr. No.	Conc. (µg/mL)	Absorbance
1	5	0.1165
2	10	0.2812
3	15	0.4312
4	20	0.5715
5	25	0.7264

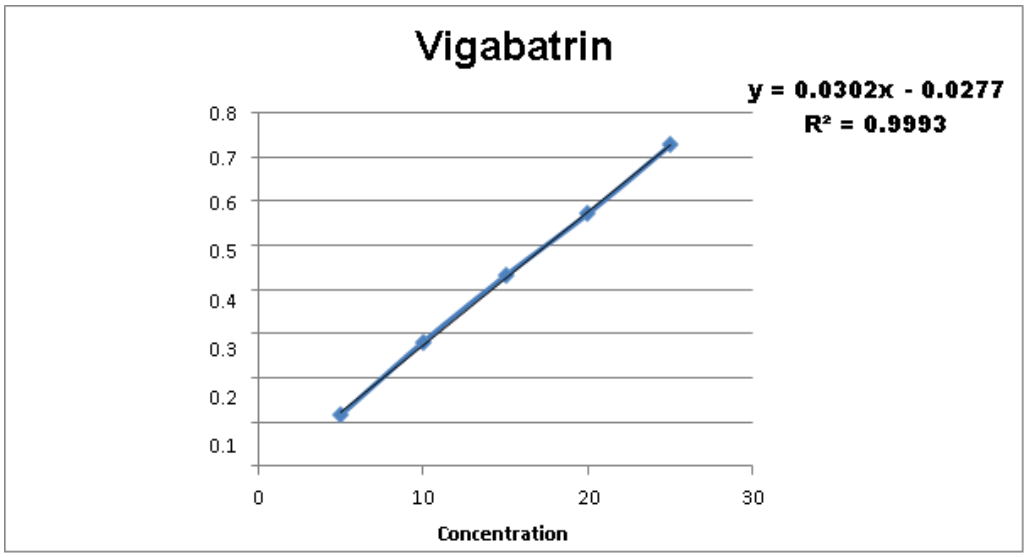

Fig. No. 3: Calibration curve for vigabatrin by first order spectrophotometric method.

Optical characteristics

Table No. 12: Optical characteristics for vigabatrin.

Sr. No	Parameters	First Order spectrophotometric method
1	λmax (nm)	280
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 0.0302x - 0.0277
4	Slope[m]	0.0302
5	Intercept [c]	0.0277
6	Correlation coefficient [r2]	0.9993
7	Limit of detection (LOD) (µg/mL)	0.01639
8	Limit of quantitation (LOQ) (µg/mL)	0.0496

Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit.

Table No. 13: Data for recovery study of vigabatrin by fod.

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Abs obtained*	Std Abs	Drug recovered (µg/ml)	%Recovery
50%	5	10	15	0.4386	0.4469	14.721414	98.1427612
	5	10	15	0.4385		14.718058	98.1203849
	5	10	15	0.4498		15.097337	100.648915
100%	10	10	20	0.5715	0.5879	19.442082	97.2104099
	10	10	20	0.5714		19.43868	97.1934002
	10	10	20	0.5792		19.704031	98.5201565
150%	15	10	25	0.7265	0.7395	24.560514	98.2420554
	15	10	25	0.7295		24.661934	98.647735
	15	10	25	0.7369		24.912103	99.6484111

Table No. 14: Statistical validation of vigabatrin by fod.

Level of addition	% Mean recovery*	SD	% RSD
50%	98.97	1.4534	1.468547
100%	97.64	0.7611	0.779527
150%	98.85	0.7239	0.732302

***Average of three determination

Precision

Intraday & interday precision assures the repeatability of test results. The % RSD found was less than 2.

Table No. 15: Data for intraday precision of vigabatrin by fod.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1165	0.11573333	0.001079	0.931952
2	5	0.1145			
3	5	0.1162			
4	15	0.4736	0.47566667	0.003921	0.824292
5	15	0.4759			
6	15	0.4775			
7	25	0.7256	0.7272	0.002042	0.280811
8	25	0.7265			
9	25	0.7295			

Table No. 16: Data for interday precision of vigabatrin by fod.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1186	0.11893333	0.00075719	0.63664892
2	5	0.1198			
3	5	0.1184			
4	15	0.4362	0.43886667	0.00496924	1.13228894
5	15	0.4358			
6	15	0.4446			
7	25	0.7265	0.7266	0.00185203	0.25488934
8	25	0.7248			
9	25	0.7285			

Robustness

Table No. 17: Data for robustness study of vigabatrin by fod.

Arameters	Change In Wavelength (±2 nm)			
	Wavelength (278nm)		Wavelength (282nm)	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4326	0.7265	0.4384	0.4335
SD	0.0015	0.0008	0.0015	0.0021
% RSD	0.5351	0.2368	0.6862	0.6132

Table No. 18: Data for robustness study of vigabatrin by fod.

	Change In Solvent			
	Water		0.1N NaOH	
	15ppm	25ppm	15ppm	25 ppm
Mean (n=3)	0.4384	0.7246	0.4376	0.7268
SD	0.0004	0.0015	0.00041	0.0004
% RSD	0.3500	0.6512	0.3269	0.1742

Ruggedness

Table No. 19: Data for ruggedness study of vigabatrin by fod.

Parameters	Change in Analyst			
	Analyst I		Analyst II	
	15ppm	25ppm	15ppm	25ppm
Mean(n=5)	0.4368	0.7236	0.4398	0.7284
SD	0.0006	0.0006	0.0008	0.0015
% RSD	0.3050	0.2812	0.4756	0.6563

Specificity

Table No. 20: Data for specificity study of vigabatrin by fod.

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Abs	Mean	SD	%RSD
5	10	15	0.1145	0.11516667	0.0011547	1.00263433
5	10	15	0.1165			
5	10	15	0.1145			
10	10	20	0.2894	0.28846667	0.00090185	0.31263576
10	10	20	0.2884			
10	10	20	0.2876			
15	10	25	0.4365	0.43633333	0.00056862	0.13031873
15	10	25	0.4357			
15	10	25	0.4368			

Area under curve method

UV spectrophotometric method was developed for determination of Vigabatrin in bulk form. The absorption spectra show the absorbance maxima 285 at nm and area under curve in absorption spectra was measured between 280-290 nm.

Linearity

The linear plot was observed in the concentration range of 5-25 µg/ml.

Table No. 21: Data for calibration curve of vigabatrin by auc.

Sr. No.	Conc. (µg/ml)	Area
1	5	0.1465
2	10	0.3198
3	15	0.4568
4	20	0.6046
5	25	0.7569

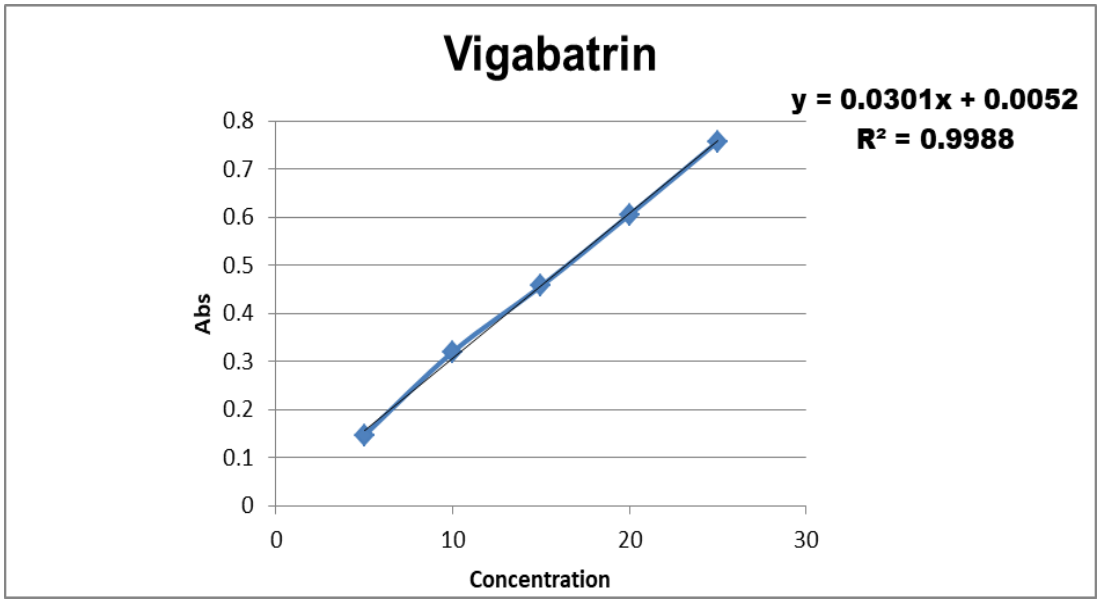

Figure No. 4: Calibration curve for vigabatrin by AUC method.

Optical characteristics

Table No. 22: Optical characteristics for vigabatrin.

Sr. No	Parameters	Area under curve method
1	λmax (nm)	280-290
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 0.0301x + 0.0052
4	Slope[m]	0.0301
5	Intercept [c]	0.0052
6	Correlation coefficient [r2]	0.9988
7	Limit of detection (LOD) (µg/mL)	0.0164
8	Limit of quantitation (LOQ) (µg/mL)	0.045

Accuracy

Table No. 23: Data for recovery study of vigabatrin by auc.

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Abs obtained*	Std Abs	Drug recovered (µg/ml)	%Recovery
50%	5	10	15	0.457	0.4568	15.006567	100.043783
	5	10	15	0.4556		14.960595	99.737303
	5	10	15	0.4549		14.937609	99.584063
100%	10	10	20	0.6056	0.6049	20.023144	100.115722
	10	10	20	0.6087		20.125641	100.628203
	10	10	20	0.6032		19.943792	99.7189618
150%	15	10	25	0.7589	0.7569	25.066059	100.264236
	15	10	25	0.7532		24.877791	99.511164
	15	10	25	0.7598		25.095785	100.383142

Table No. 24: Statistical validation of vigabatrin by auc.

Level of addition	% Mean recovery*	SD	% RSD
50%	99.79	0.2341	0.234574
100%	100.2	0.4558	0.455144
150%	100.1	0.4729	0.472614

***Average of three determination

Precision

Table No. 25: Data for intraday precision of vigabatrin by auc.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1465	0.14766667	0.00185	1.252974
2	5	0.1467			
3	5	0.1498			
4	15	0.4532	0.4538	0.00394	0.868123
5	15	0.456			
6	15	0.4522			
7	25	0.7595	0.75733333	0.002434	0.321363
8	25	0.7547			
9	25	0.7578			

Table No. 26: Data for interday precision of vigabatrin by auc.

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	5	0.1498	0.1462	0.00375899	2.57112806
2	5	0.1465			
3	5	0.1423			
4	15	0.4568	0.4564	0.00272213	0.59643548
5	15	0.4589			
6	15	0.4535			
7	25	0.7559	0.75566667	0.00077675	0.10278942
8	25	0.7563			
9	25	0.7548			

Robustness

Table No. 27: Data for robustness study of vigabatrin by auc.

Parameters	Change in Solvent			
	Water		0.1 N NaOH	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4516	0.7546	0.4591	0.7544
SD	0.0015	0.0023	0.0006	0.0007
% RSD	0.5162	0.6026	0.3265	0.1653

Ruggedness

Table No. 28: Data for ruggedness study of vigabatrin by auc.

Parameters	Change in Analyst			
	Analyst I		Analyst II	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4565	0.7548	0.4538	0.7596
SD	0.0006	0.0014	0.0012	0.0014
% RSD	0.4563	0.7563	0.9569	0.6126

Specificity

Table No. 29: Data for specificity study of vigabatrin by auc.

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Abs	Mean	SD	%RSD
5	10	15	0.1498			
5	10	15	0.1446	0.14696667	0.00263122	1.79035345
5	10	15	0.1465			
10	10	20	0.3154			
10	10	20	0.3165	0.31676667	0.00151767	0.47911407
10	10	20	0.3184			
15	10	25	0.7598			
15	10	25	0.7584	0.76106667	0.00347755	0.45693067
15	10	25	0.765			

Development of HPLC method for Vigabatrin

High performance liquid chromatographic method was developed and validated for determination of Vigabatrin in bulk form. Mobile phase consists of ACN: methanol (95:05) pH3.

(1)3.8157

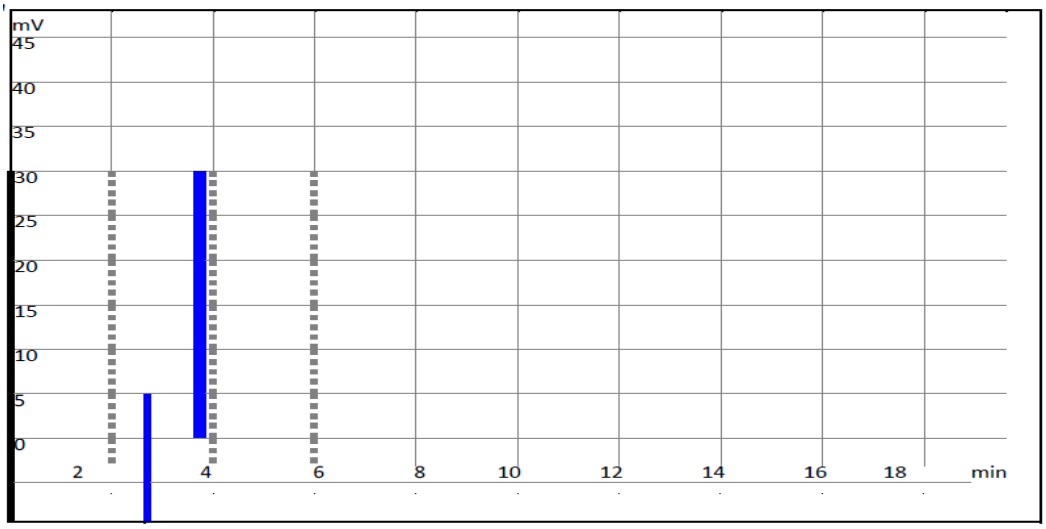

Fig. No. 4: Typical chromatogram of vigabatrin.

Linearity

Table No. 30: Data of calibration curve of vigabatrin by HPLC method.

Sr. No.	Conc. (µg/ml)	Area
1	5	2015565
2	10	4232656
3	15	6012665
4	20	8165656
5	25	10165658

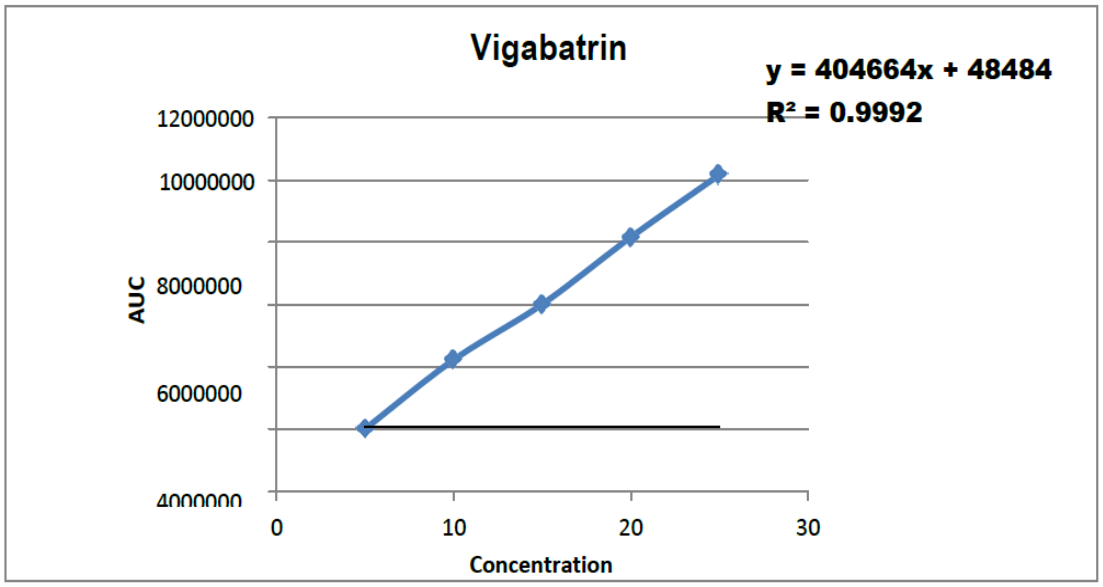

Fig. No.05: Calibration curve for vigabatrin.

Optical characteristics

Table No. 31: Optical characteristics for vigabatrin.

Sr. No	Parameters	High performance liquid chromatography method
1	λmax (nm)	285
2	Beer's law limit (µg/mL)	5-25
3	Regression equation[y]	y = 404664x + 48484
4	Slope[m]	404664
5	Intercept [c]	48484
6	Correlation coefficient [r^2]	0.9992
7	Limit of detection (LOD) (µg/mL)	0.0817
8	Limit of quantitation (LOQ) (µg/mL)	0.2478

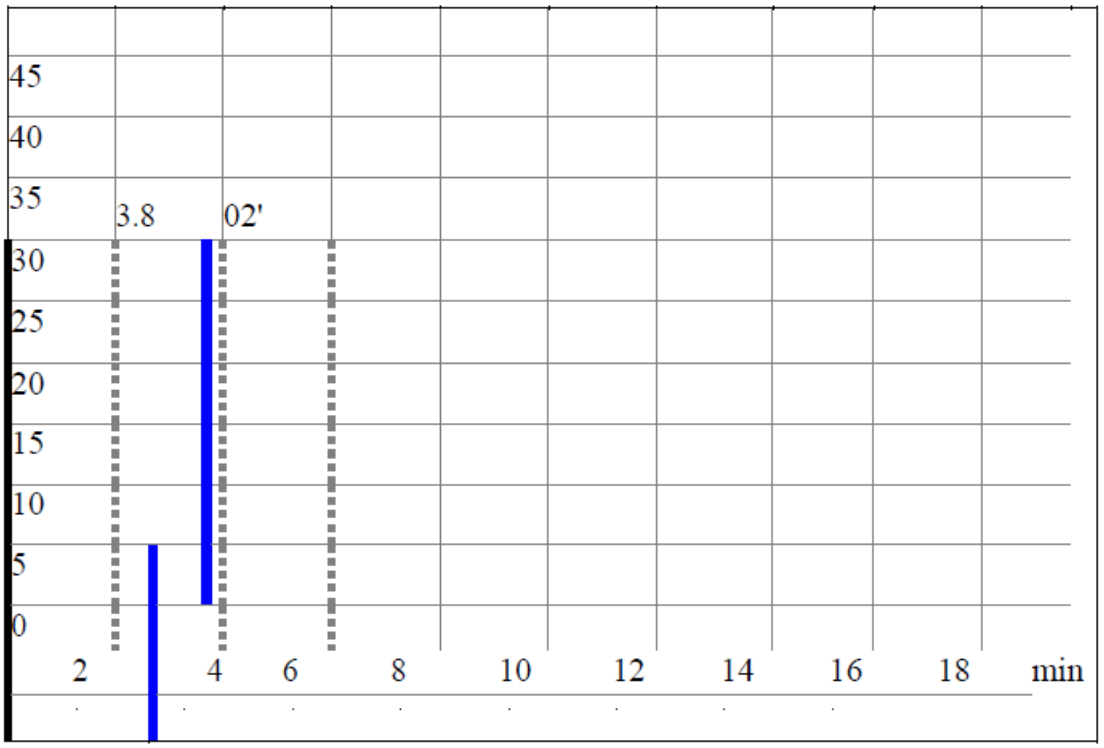

Fig. No. 06: Chromatogram of Linearity 5µg/ml Vigabatrin.

Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit.

Table No. 32: Data for recovery study of vigabatrin by HPLC method.

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	5	10	15	6016555	6012665	15.009705	100.064697
	5	10	15	6016584		15.009777	100.065179
	5	10	15	6017856		15.01295	100.086334
100%	10	10	20	8165918	8165656	20.000642	100.003209
	10	10	20	8163256		19.994122	99.9706086
	10	10	20	8163258		19.994127	99.9706331
150%	15	10	25	10168921	10165658	25.008025	100.032098
	15	10	25	10163956		24.995814	99.9832574
	15	10	25	10169827		25.010253	100.041011

Table No. 33: Statistical validation of Vigabatrin by HPLC method.

Level of addition	% Mean recovery*	SD	% RSD
50%	100.1	0.0124	0.012347
100%	99.98	0.0188	0.018818
150%	100	0.0311	0.031086

*Average of three determination

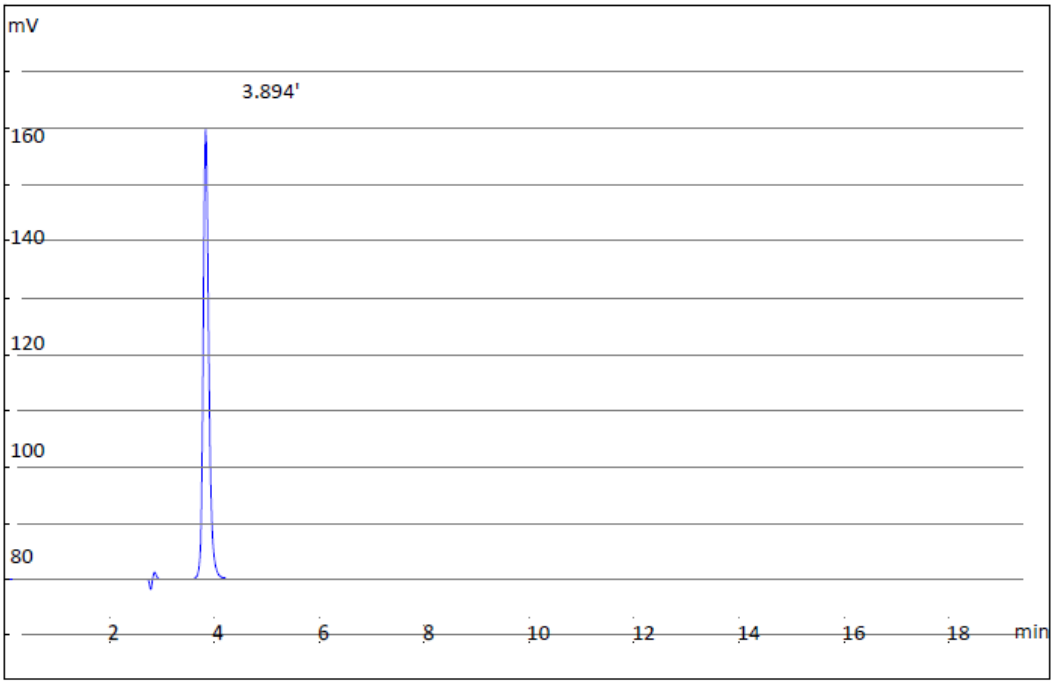

Fig. No. 08: A Chromatogram of % Recovery of 15µg/ml Vigabatrin.

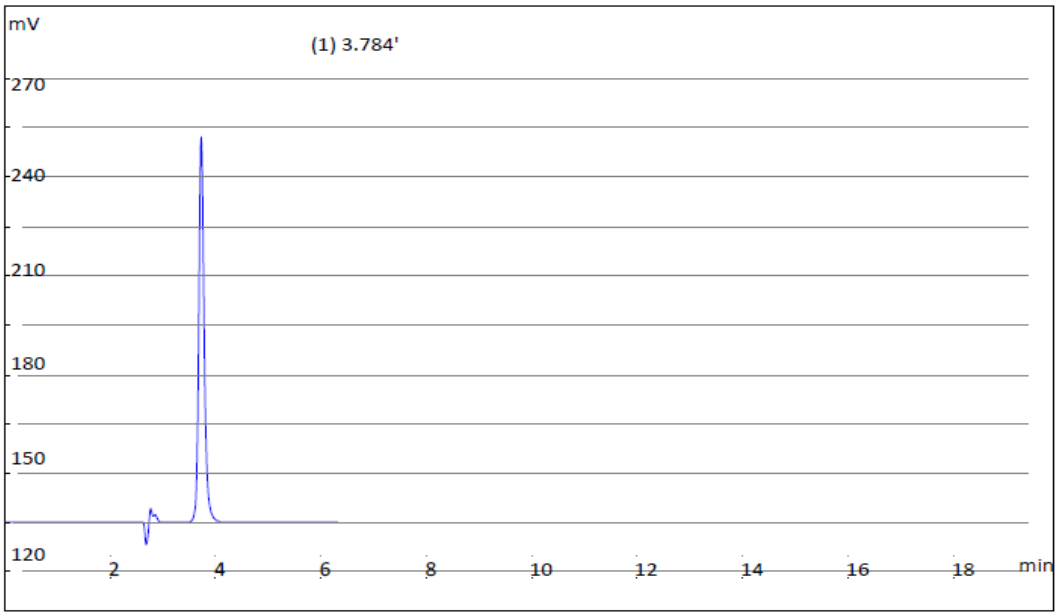

Fig. No. 09: A Chromatogram of % Recovery of 25µg/ml Vigabatrin

Precision

Table No. 34: Data for intraday precision of vigabatrin by HPLC method.

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	2015858	2026114.67	10028.85	0.494979
2	5	2026587			
3	5	2035899			
4	15	6026389	6025413	20808.78	0.34535
5	15	6014555			
6	15	6035295			
7	25	10156559	10174269.7	15992.86	0.157189
8	25	10178595			
9	25	10187655			

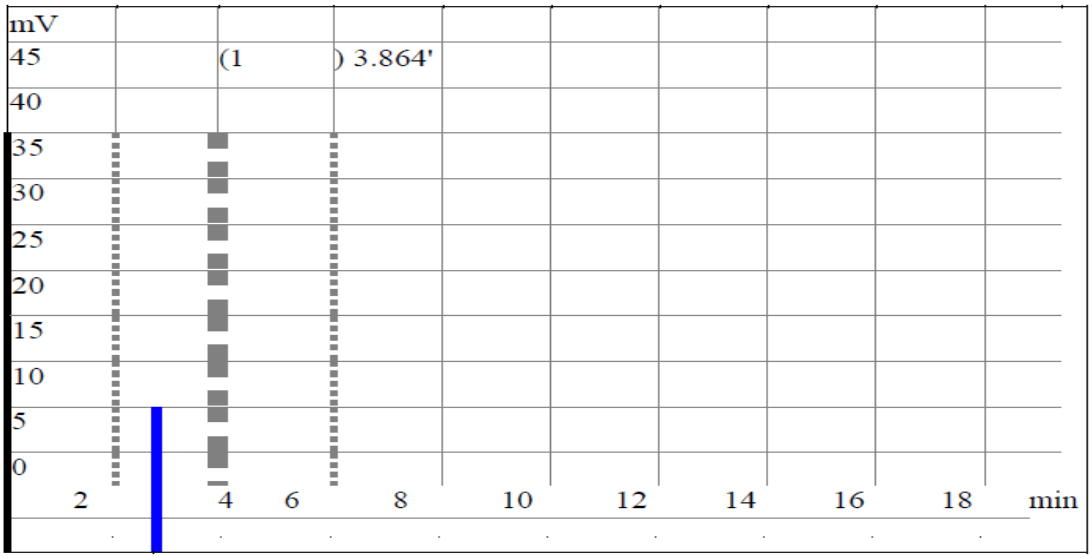

Fig. No. 10: A Chromatogram of Intraday Precision of 5µg/ml Vigabatrin.

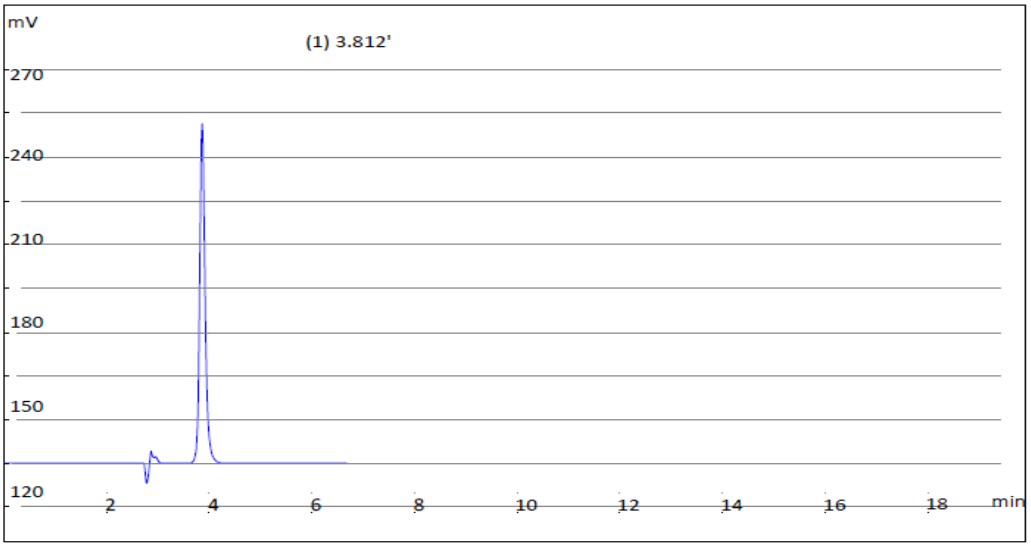

Fig. No. 11: A Chromatogram of Intraday Precision of 25µg/ml Vigabatrin.

Table No. 35: Data for interday precision of vigabatrin by HPLC method.

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	2012548	2035352.33	22226.4982	1.09202214
2	5	2036557			
3	5	2056952			
4	15	6015897	6022649.33	11957.8775	0.19854846
5	15	6036456			
6	15	6015595			
7	25	10154588	10180209	23758.8565	0.2333828
8	25	10184525			
9	25	10201514			

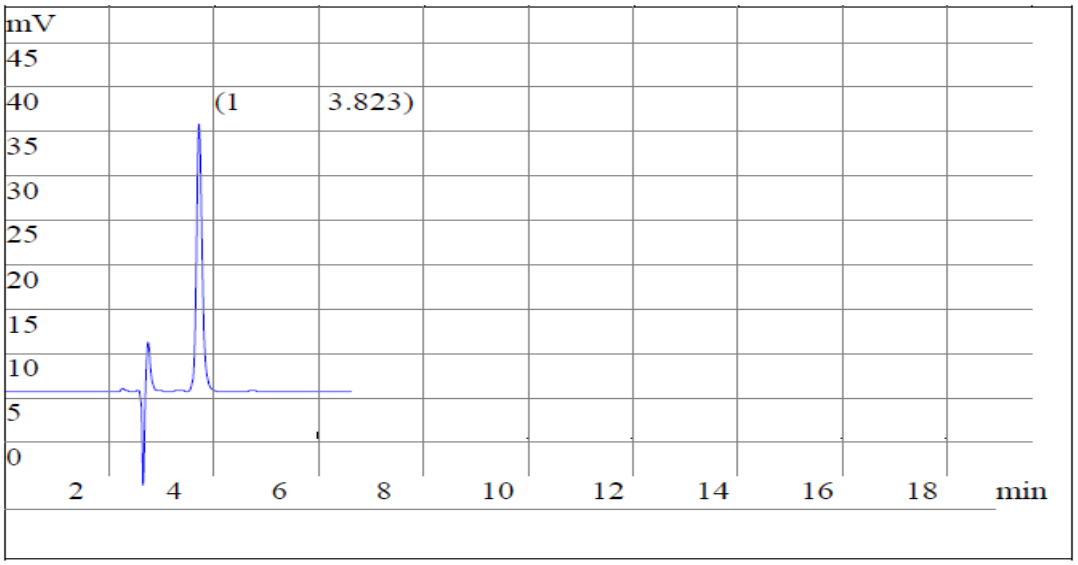

Fig. No. 12: A Chromatogram of interday precision of 5µg/ml vigabatrin.

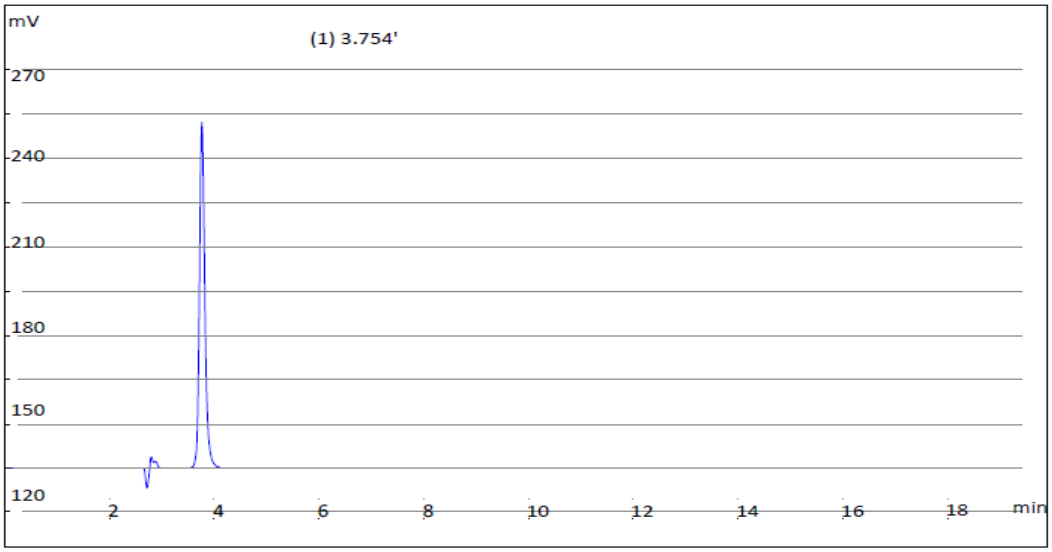

Fig. No. 13: A Chromatogram of Interday precision of 25µg/ml Vigabatrin.

Limit of detection and limit of Quantitation:

Table No. 36: Results of LOD and LOQ values of Vigabatrin.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Vigabatrin	0.0.814	0.2478

Robustness:

Table No. 37: Data for robustness study of vigabatrin by HPLC method.

Sr. No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	6015326	6017787	2200.74	0.03657
2		1	6018469			
3		1.1	6019566			
1	Change in Wavelength (nm)	243	6021526	6021026	7131.64	0.11845
2		285	6027895			
3		247	6013658			

*Average of three determination

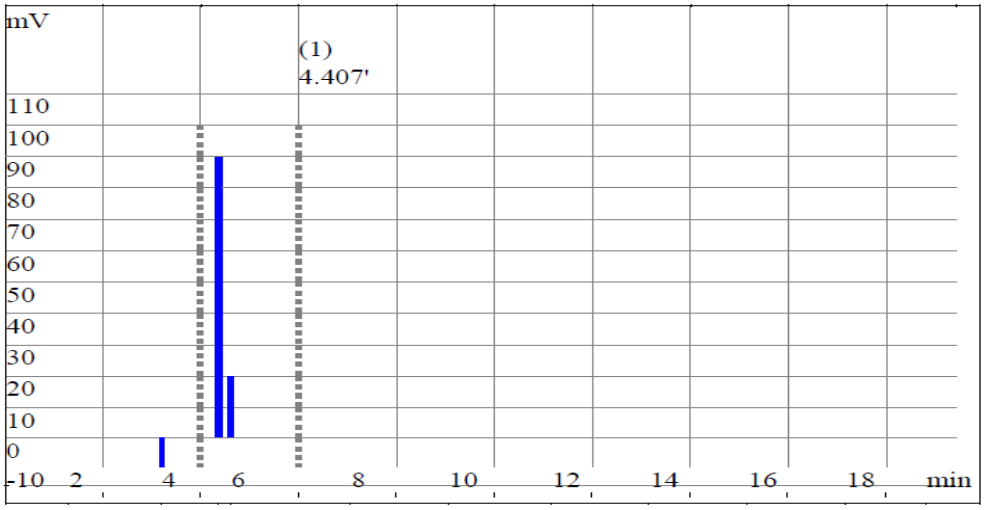

Fig. No. 14: Chromatogram of 15µg/ml Vigabatrin at flow rate 0.9 ml/min.

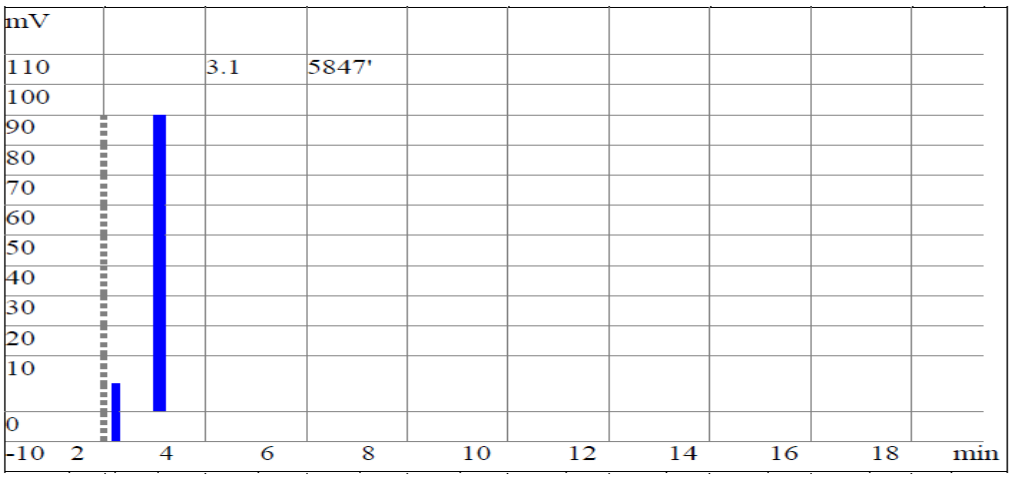

Fig. No. 15: Chromatogram of 15µg/ml Vigabatrin at flow rate 1.1 ml/min.

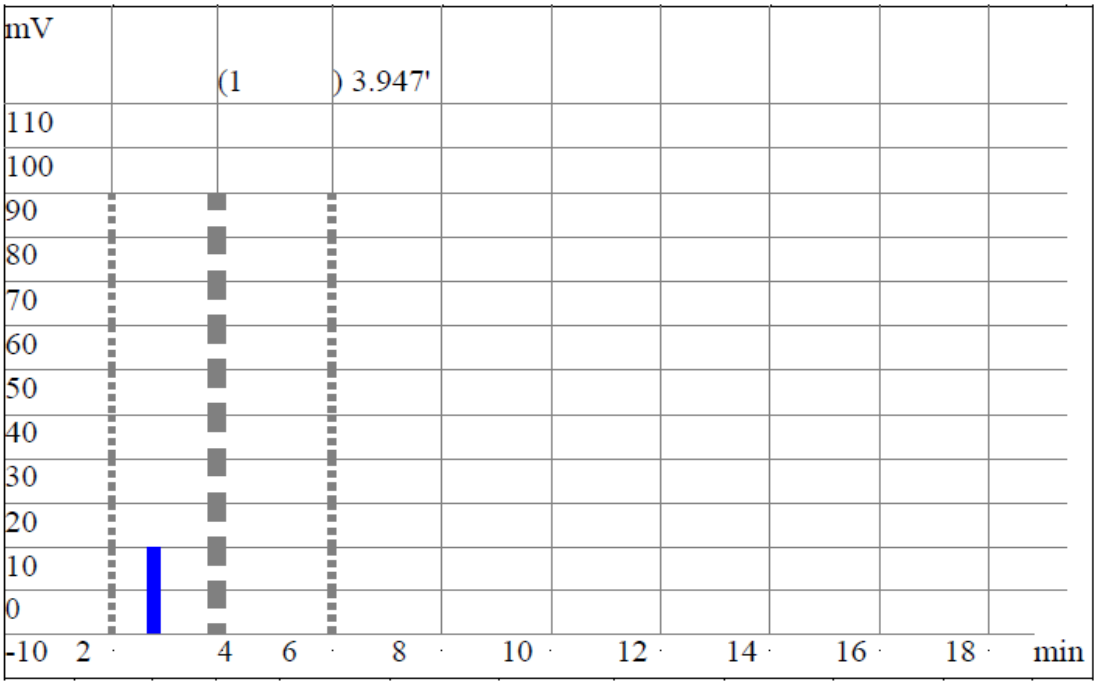

Fig. no. 16: Chromatogram of 15µg/ml Vigabatrin at Wavelength 283nm.

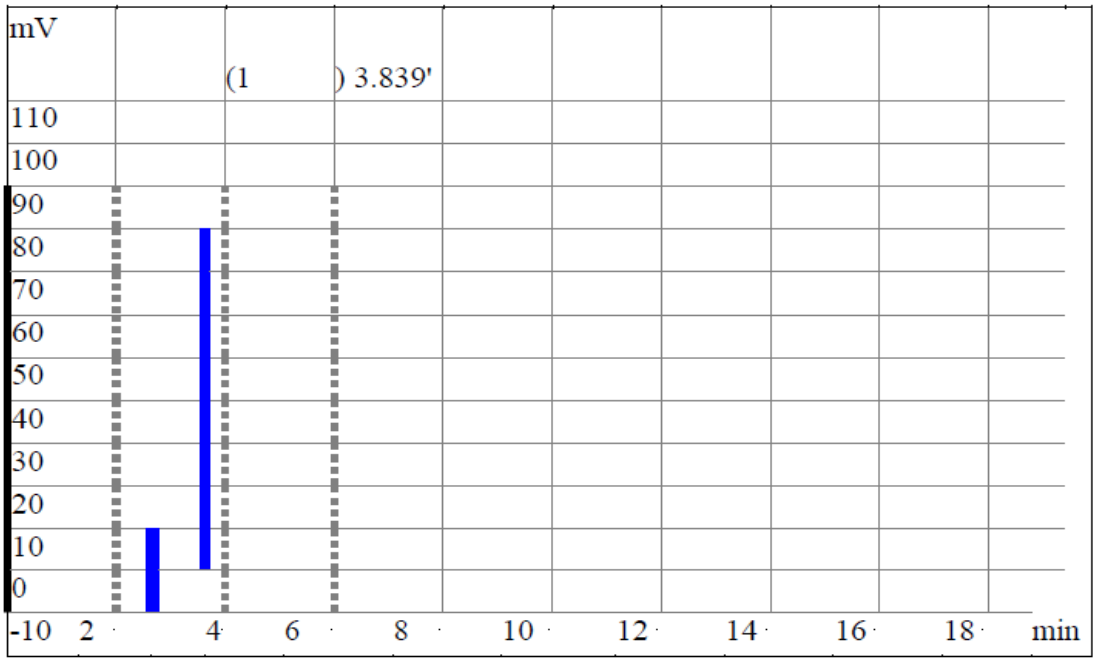

Fig. No. 17: Chromatogram of 15µg/ml Vigabatrin at Wavelength 287nm.

Ruggedness

Ruggedness was studied by different analyst. Results obtained are shown in Table no.51

Table No. 51: Data for ruggedness study of Vigabatrin by HPLC method.

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	15	6018456	6019759	1477.98072	0.02855216
			6019456			
			6021365			
2	Analyst-II	15	6030215	6022570.67	8870.07251	0.14728051
			6024652			
			6012845			

*Average of three determination

Specificity

Table No. 39: Data for specificity study of Vigabatrin by HPLC method.

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
5	10	15	2019565	2017848	3650.10808	0.18089113
5	10	15	2013656			
5	10	15	2020323			
10	10	20	4256322	4255790	40101.6467	0.94228443
10	10	20	4295623			
10	10	20	4215425			
15	10	25	6002152	6015750.33	15452.3792	0.25686537
15	10	25	6012545			
15	10	25	6032554			

System suitability

Table No. 40: Data for System suitability study of Vigabatrin by HPLC Method.

Sr. No.	conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	15	3.8	8487	1.25
2	15	3.7	8552	1.24
3	15	3.8	8462	1.25
4	15	3.7	8359	1.23
5	15	3.9	8252	1.24
6	15	3.8	8539	1.25
Mean		3.783333333	8441.83333	1.2433333
SD		0.075277265	115.691688	0.008165
%RSD		1.989707452	1.37045691	0.6566997

Degradation studies

Stress testing of the drug substance can help to identify the likely degradation products, the stability and specificity of the analytical procedure.

Table No. 41: Results of Forced Degradation Studies for Vigabatrin hemihydrate.

	Acid stress	Alkali stress	Peroxide stress	Thermal stress	Photolytic stress
% Recovered	84.23%	89.64%	80.85%	95.65%	98.15%
% Degradation	15.77%	10.36%	.19.15%	4.35%	1.85%

CONCLUSION

In Zero order derivative method Water was used as solvent and detection was done at 285 nm. The % RSD for all parameters was found less than 2. The result showed that the proposed method was suitable for the accurate, precise and rapid determination of Vigabatrin in its bulk form. In FOD method Water was used as solvent and detection was done at 280 nm. The % RSD for all parameters was found less than 2. The result showed that the proposed method was suitable for the accurate, precise and rapid determination of in Vigabatrin its bulk form. In AUC method Water was used as solvent and detection was done at 240nm-280nm. The % RSD for all parameters was found less than 2. The result showed that the proposed method was suitable for the accurate, precise and rapid determination of in Vigabatrin its bulk form. The stability indicating HPLC method was developed and validated for estimation of Vigabatrin. The mobile phase was consisting of ACN: methanol (95:05). Detection was done at 285 nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit. The result showed that proposed method was suitable for the accurate, precise and rapid determination of in Vigabatrin its bulk form.

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