

Development and Validation of RP-HPLC Method for Estimation of Vigabatrin Using Derivatization with 9-Fluorenylmethyloxycarbonyl Chloride

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Abstract

A Simple, efficient and reproducible method for the estimation of Vigabatrin (VGB) from bulk and pharmaceutical formulation has been developed using reversed phase high performance liquid chromatography. The method involves derivatization of the primary amine group of VGB with 9-fluorenylmethyloxycarbonyl chloride (FMOC) followed by isocratic separation using a mobile phase consisting of acetonitrile: water (97.5 : 2.5% V/V). Column used was Finpak SIL C₁₈ (250 X 4.6 mm internal diameter) 5 μ with flow rate of 1 mL/min. The detection wavelength used was 265 nm. The retention time of VGB-FMOC complex was found to be 3.89 min. Linearity of drug was 5-30 μg/mL. The performance of analysis was studied and the validated method showed excellent performance in terms of selectivity, specificity, sensitivity, precision and accuracy. No interferences were found from excipients and other impurities.

Keywords: Vigabatrin, FMOC, RP-HPLC, Derivatization, Validation

1. INTRODUCTION

Vigabatrin (VGB) (4-amino-hex-5-enoic acid) (Fig. 1) is a Gamma-aminobutyric acid (GABA) transaminase inhibitor used in the treatment of infantile spasm and refractory complex partial seizures. It is used as a first line treatment for infantile spasm and the drug of choice for infantile spasm with tuberous sclerosis complex syndrome [1]. VGB exhibits a very low absorption in the UV/Vis region. Thus, derivatization of the drug is necessary if measurement of VGB is intended by UV/Vis or spectrofluorimetric detection to overcome poor sensitivity [2]. Vigabatrin has been determined in different matrices, including dosage forms and biological samples by gas chromatography (GC) [3-5], high-performance liquid chromatography (HPLC) [6-11], spectrofluorimetry [12-14] and capillary electrophoresis (CE) using derivatization of the drug with fluorescence tags [15,16], *o*-Phthaldialdehyde (OPA), dansyl chloride and 4-chloro-7-nitrobenzofurazan (NBD-Cl) have been commonly used as derivatizing agents for fluorescence detection [15]. However, these currently available methods have been found to have low detection limits, long run times, complex derivatization procedures and unstable derivatized products. 9-fluorenylmethyloxycarbonyl chloride (FMOC) is one of the reagents which is used for derivatizing amino acids for HPLC analysis [17].

To date, there is no reported analytical technique for estimation of VGB by pre-column derivatization with FMOC in bulk and marketed formulation. In this study FMOC derivatization method was used to increase sensitivity and stability of derivatized product. In present study, a simple, cost effective, highly sensitive and reproducible RP-HPLC method was developed and validated for estimation VGB in bulk and marketed formulation.

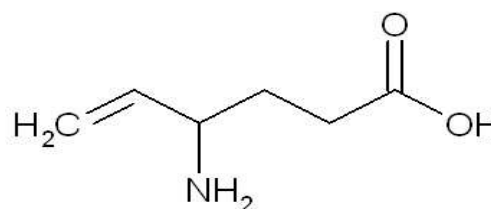


Fig. 1. Structure of Vigabatrin

2. MATERIALS AND METHODS

2.1. Equipments

The chromatographic system (Jasco, LC 2010C HT, Kyoto, Japan) consisted of an Jasco PU 2080 Plus pump and Jasco UV 20705 Plus detector. The separation was performed on Finpak SIL C₁₈ T- 5 column (250 mm X 4.6 mm, 5 μm particle size) with a mixture of water-acetonitrile (97.5 : 2.5, v/v) as mobile phase. The mobile phase was filtered through a 0.22 μm pore size membrane filter and degassed before use. The column was maintained at ambient temperature and the flow rate was 1 mL/min in isocratic mode. Injection volume was 10 μL and the UV detection wavelength was set at 265 nm. The digital pH meter (Equiptronics-EQ-614), electronic balance (Contech CA series) and Mdi 0.2 μm membrane were used.

2.2. Materials

Working Standards of pharmaceutical grade VGB was received as a gift sample from Dr. Reddy's Labs Ltd. Hyderabad and FMOC was purchased from Sisco Research Laboratories, Mumbai, India. Oral powder formulation, Sabril (VGB 500 mg/packet) oral powder of Sanofi Aventis was bought from a local pharmacy. All the chemicals and reagents used were of HPLC grade and purchased from Merck, India.

2.3. Solutions

The derivatization reagent consisted of 1.6 g/L FMOC in acetonitrile. Borate buffer solution was prepared by

dissolving 3.1g boric acid in 100 mL water and then adjusted the pH 7.7 with 0.1 N sodium hydroxide solution. Vigabatrin was dissolved in ultrapure water as stock standard solution (2000 μ g/mL). This standard stock solution was then used for derivatization.

2.4. Precolumn derivatization

Standard stock solution (1.0 ml) was mixed 2.0 ml of borate buffer plus 3.0 ml of FMOC in a separating funnel and allowed to stand for 5 min. Ethyl acetate (3.0 ml) was transferred into the separating funnel. The solution was shaken for few seconds and allowed to stand for 5 min to separate layers. Lower layer was used within 8 hrs of preparation [18].

2.5. Analysis of Pharmaceutical Formulation

Sabril oral powder (400 mg) equivalent to 200 mg of VGB was accurately weighed and transferred into 100 ml volumetric flask and dissolved in sufficient volume of distilled water and then volume was made up to the mark to get VGB sample solution (2000 μ g/mL). This sample solution was then used for derivatization as described for standard VGB solution. A volume of 10 μ L of the reaction mixture was injected into the HPLC system.

2.6. Method validation

2.6.1. Accuracy (Recovery Studies)

Accuracy is represented and determined by recovery experiments. In this process, it was tested at three different levels i.e. 50,100 and 150% of the label claim and analyzing chromatograms.

2.6.2. Assay specificity

The specificity of the method was assessed by injecting the placebo solution (without VGB) and VGB solution after derivatization into the HPLC system

2.6.3. Precision

System and method precision was verified by repeatability and intermediate precision studies with the oral powder formulation. Repeatability studies were performed by using analysis of six replicates of standard and sample solution containing 15 μ g/mL of the VGB. The intraday and interday precision studies for VGB was carried out by estimating the corresponding responses three times on the same day and on three different days for the three level of linearity concentrations (5 μ g/ml, 15 μ g/ml and 25 μ g/ml).

2.6.4. Linearity

It was demonstrated by preparing and analyzing standard stock solutions after derivatization corresponding to 5.0-30 μ g/mL of VGB in five replicates. Calibration curve was obtained by plotting the peak area on the abscissa and the respective drug concentrations of standard VGB on the ordinate. The unknown samples concentrations were calculated from the linear regression equation.

2.6.5. System suitability study

System suitability tests were carried out on freshly prepared 5 replicates of working standard solution (15 μ g/ml) of VGB after derivatization to scrutinize the various optimized parameters such as plate count, resolution and tailing factor.

2.6.6. Limit of detection (LOD) & limit of quantification (LOQ)

LOD and LOQ for VGB were estimated by injecting a series of dilute solutions with known concentration. The

parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation.

2.6.7. Robustness of Method

For the determination of method's robustness, flow rate was varied within a realistic range and the quantitative influence of the variables was determined. Robustness of the method was evaluated at a concentration level 15 μ g/mL of drug (n=3).

3. RESULTS AND DISCUSSION

3.1. Optimization of the reaction conditions

The derivatization of VGB with FMOC requires a buffered alkaline pH. The complex formation took place in borate buffer at pH of 7.7 among the other buffer systems tested i.e. acetate and phosphate buffer. Precolumn derivatization with FMOC was fast (5 min), and the VGB derivative was stable up to 8 hr. The chemical reaction between VGB and FMOC is depicted in Fig. 2.

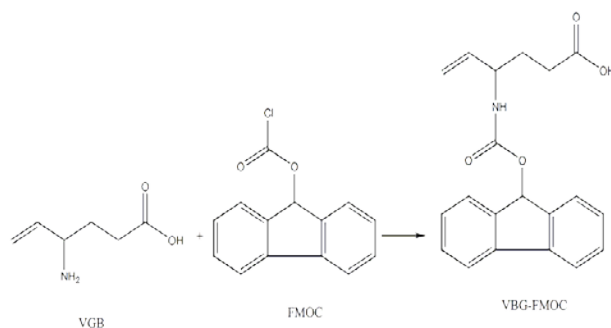


Fig. 2. Reaction between VGB and FMOC

3.2. Optimization of chromatographic technique

The HPLC method was optimized with a goal of developing a well-defined symmetrical peaks to quantify VGB from pharmaceutical dosage form. The initial optimization trials were carried out using acetonitrile with methanol, water and buffer in a various ratios as mobile phases. However, satisfactory results were obtained with acetonitrile : water (97.5 : 2.5 v/v) in isocratic elution mode. A representative chromatogram of standard vigabatrin obtained with optimized chromatographic condition is shown in Fig. 3.

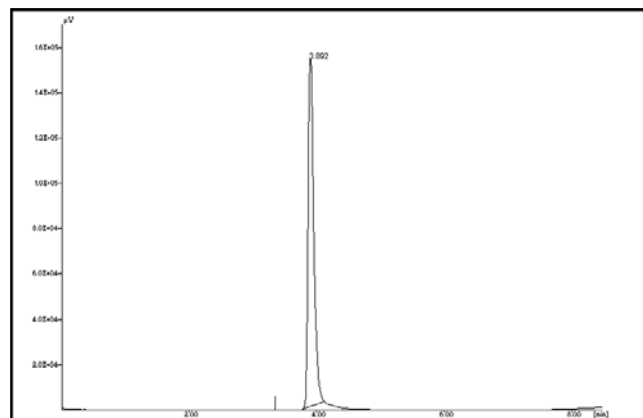


Fig. 3. A representative chromatogram of standard vigabatrin obtained with optimized chromatographic conditions

3.3. Analysis of formulation

Sabril oral powder containing 500 mg of VGB per packet was analyzed by this developed method and percentage of the assay were calculated. The percentage mean assay was found to be 100.8% w/w (% RSD=0.2494) which is within ICH limits.

3.4. Validation of chromatographic method

Validation of the method for the standard drug and marketed formulation was carried out with respect to different parameters as mentioned in ICH guidelines for method validation [19]. VGB-FMOC complex showed good correlation coefficient in concentration range of 5-30 μ g/mL ($R^2 = 0.9992$). The linear regression data is presented in Table 1. The linearity of calibration graph and adherence of the system to Beer's law was validated by determination of correlation coefficient. These values were found to be well within the accepted limit. The regression plot is shown in Fig. 4.

Table 1. Linear regression data for calibration curve (n=3)

Parameters	Result
Linearity range	5-30 μ g/mL
R^2	0.999
Slope	35830x
Intercept	11702
$Y=mX+C$	35816x + 11982

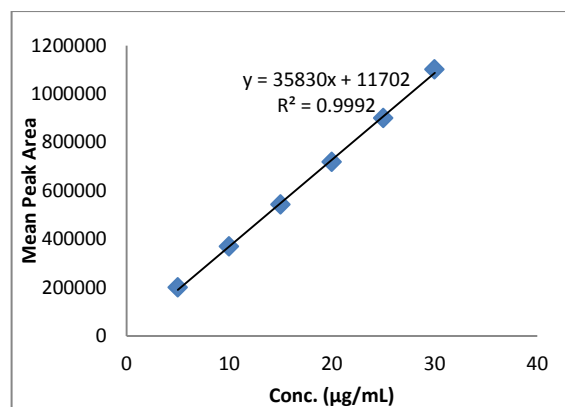


Fig. 4. Regression plot of VGB

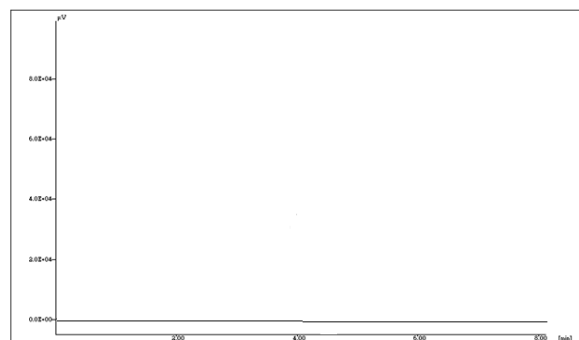


Fig. 5. Specificity chromatogram of Placebo

Table 2: Recovery determination of drug by HPLC (n=3)

Sr. No.	Recovery level %	Standard added (mg)	Area of std + placebo (500mg)	Amount Recovered (mg)	% Recovery
1.	50% set- 1	400	545432	396	98.99
2.	50% set- 2	410	546897	401	99.98
3.	50% set- 3	399	549870	398	99.96
4.	100% set- 1	501	555488	500	99.98
5.	100% set- 2	500	555698	498	99.97
6.	100% set- 3	499	555809	495	98.98
7.	150% set- 1	600	559098	599	99.75
8.	150% set- 2	601	567845	500	99.65
9.	150% set- 3	600	560987	597	98.91
Mean					99.96
SD (\pm)					0.37011
% RSD (\pm)					0.37096

Table 3. Precision study (n=3)

Precision	Conc. (μ g/ml)	Mean Peak Area	% RSD
System Precision	15	556530	0.24370
Method Precision (Repeatability)	15	556528	0.24471
Intraday precision	5	200037	0.00822
	15	543712	0.10160
	25	900571	0.01663
Interday precision	5	210483	0.00523
	15	560096	0.00981
	25	910132	0.00552

Table 4. System suitability test of VGB (n=3)

	Area	RT(min)	USP Plate count	USP Tailing
Mean	556528	3.85	6532	1.022
SD	1361.9	0.02549	19.28	0.008367
%RSD	0.24471	0.6622	0.2952	0.8186

Table 5. Robustness study (n=3)

Flow rate (mL/min)	Mean Area (% RSD)	Mean Tailing Factor (% RSD)	Mean t _R in min (% RSD)
0.5	562280 (0.0784)	1.3 (0.7692)	3.89 (1.3180)
1	564370.3 (0.03015)	1.10 (1.0434)	3.83 (1.2328)
1.5	562240 (0.01081)	1.33 (0.8660)	3.71 (0.5852)

Recovery study was conducted at three different levels and mean recovery value was found to be 99.96%. The results are shown in Table 2. Repeatability and intermediate precision of the analytical method was established for both system and method. The relative standard deviation was found less than 2. The results are displayed in Table 3. The specificity of the method was confirmed from the chromatogram of the working placebo solution not showing any interference at the retention time of the VGB-FMOC complex at the working wavelength of 265 nm (Fig. 5). The limit of detection and limit of quantification of VGB were found to be 1.32 µg/mL and 4.01 µg/mL respectively which indicate that the method was extremely rapid and sensitive. System suitability was achieved by checking various parameters and found within the ICH limit (Table 4). Insignificant differences in the peak areas of drug were observed when flow rate was varied within a realistic range indicating robustness of the method. Results for robustness are presented in Table 5.

4. CONCLUSION

The RP-HPLC method was successfully used to estimate the amount of VGB in marketed powder formulation containing 500 mg of VGB per packet. Precolumn derivatization of VGB with FMOC was fast, the derivative stable. VGB-FMOC complex showed significant improvement in UV absorption along with bathochromic shift in absorption maxima. The results obtained were comparable with the corresponding labeled amounts, indicating non-interference of excipients in the estimation. By observing validation parameters of the method, it was found to be specific, accurate, precise, repeatable and reproducible. Furthermore the drug estimated within 8 min, hence the present method is cost effective and faster, can be used for the routine analysis of these drug from marketed formulations.

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