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Method Development for the Control of Potential Genotoxic Impurities in Vigabatrin Using Gas Chromatography Techniques and Mass Spectroscopy Detector

D. Srikanth[1], S. Ganapaty[1*], P. Manik Reddy[2], K. Sunitha[1] and G. Sowjanya[1]

[1]GITAM Institute of Pharmacy, GITAM Deemed to be University, Visakhapatnam-530045, India.
[2]SIONC Pharmaceuticals, Paravada, Visakhapatnam-531021, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DS, PMR and SG designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors KS and GS involved in editing the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To develop a sensitive headspace GC-MS method for the determination of potential genotoxic impurities in Vigabatrin.
Place and Duration of Study: The study was performed in SIONC Pharmaceuticals, Visakhapatnam from June 2020 to March 2021.
Methodology: The impurities were determined by selected ion monitoring mode using VF -WAXms (30 mts length, 0.25 mm internal diameter, 1.0 µ film thickness) column. Helium gas was used as carrier gas with a column flow of 1.0 mL/min. and injector temperature maintained at 220 ^{0}C. Oven Temperature, loop temperature and transfer line temperature were maintained in the head space at 70^{o}C, 90^{o}C and 100^{o}C respectively.
Results: The linearity of the method was proposed in the range of LOQ to 150 % for the genotoxic impurities by subjecting the data obtained to statistical analysis using linear regression model (r^2 > 0.99). The method also gave acceptable recovery of all the four impurities at each level and was

**Corresponding author: E-mail: ganapatyseru@gmail.com;*

found to be accurate. The % RSD obtained in the method precision and intermediate precision were less than 11% depicting the precision of the method. The LOD and LOQ values were calculated based on the signal to noise ratio and are indicating the sensitivity of the method. The specificity of the method was checked for blank interference at the retention time of respective impurities.

Conclusion: The results proved that the proposed headspace GC-MS method for the study of potential genotoxic impurities of Vigabatrin was sensitive, precise and accurate and could be routinely used in the quality control testing of the active pharmaceutical ingredient.

Keywords: Vigabatrin; potential genotoxic impurities; GC-MS; ICH guidelines.

ABBREVIATIONS

1	*USFDA*	*United states Food Drug Administration*
2	*CDER*	*Centre for Drug Evaluation and Research*
3	*CBER*	*Centre for Biologics Evaluation and Research*
4	*ICH*	*International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*
5	*GABA*	*Gama aminobutyric acid*
6	*TTC*	*Threshold of Toxicological Concern*
7	*GC-MS*	*Gas chromatography- Mass spectroscopy*
8	*LOD*	*Limit of Detection*
9	*LOQ*	*Limit of Quantitation*
10	*ppm*	*Parts per million*
11	*RSD*	*Relative standard deviation*
12	*µg*	*Microgram*

1. INTRODUCTION

Vigabatrin is an antiepileptic drug rationally designed to have a specific effect on brain chemistry by inhibiting the GABA-degrading enzyme, GABA transaminase, resulting in increase in GABA concentrations in the brain. The increase in GABA functions as a brake on the excitatory processes that can initiate seizure activity [1]. It is approved as adjunctive therapy for adult patients with refractory complex partial seizures and as monotherapy for pediatric patients aged 30 days to 2 years with infantile spasms [2]. Vigabatrin carries an USFDA black box warning about the potential for causing loss of peripheral vision [3,4]. It is available as a generic medication since 2019 by USFDA.

Control strategies of Impurities with genotoxic or carcinogenic potential in drug substance and drug products is a current regulatory requirement as per the guidance from FDA (CDER/CBER), EMEA and ICH. These guidance addresses synthetic impurities, degradants, starting materials, raw materials, reagents, primary packing materials and other anticipated reaction products as source of impurities with genotoxic or carcinogenic potential and are required to be controlled in the drug substances. FDA allows a maximum daily exposure target of 1.5 µg per day, which is referred as Threshold of Toxicological Concern (TTC) by EMEA for a lifetime of daily exposure of the drugs for human use. According to the guidance of drug regulations, it is crucial to regulate the level of genotoxic impurities in the drug substances based on the daily dose [5,6]. The control strategy of the impurities in the drug substance with respect to raw materials or packing materials, facility controls, imbedded design of the manufacturing process, inprocess controls and controls on drug substance (i.e., testing) are required [7]. Virtual prediction of potential genotoxic activity based on the structural alerts is now well established and is based on the published data available [8]. The higher TTC limits can be justified based on the drug exposure for human use in clinical trials. The acceptable limits for daily intake are 5, 10, 20, and 60 µg/day for durations of exposure of 6-12 months, 3-6 months, 1-3 months, and less than 1 month, respectively.

1,4-Dichlorobut-2-ene (Trans isomer) is the key starting material for the synthesis of Vigabatrin, whereas chloroprene, 3,4-dichlorobut-1-ene and 1,4-Dichlorobut-2-ene (Cis isomer) are its potential related impurities. Chloroprene, 3,4-

Dichlorobut-1-ene, 1,4-Dichlorobut-2-ene (Cis isomer), 1,4-Dichlorobut-2-ene (Trans isomer) are considered as the potential genotoxic impurities in the Vigabatrin, based on the structure and a control strategy is required in the drug substance. The structures of Vigabatrin and potential genotoxic impurities are shown in Table 1.

In the purview of control strategies to be adopted for genotoxic impurities in pharmaceuticals, an attempt was made to develop a sensitive GC-MS method using head space technique to determine chloroprene, 3,4-Dichlorobut-1-ene, 1,4-Dichlorobut-2-ene (Cis isomer) and 1,4-Dichlorobut-2-ene (Trans isomer) with a specified limit of 0.5 ppm in Vigabatrin as there were no previous reports available, particularly regarding the analysis of the specified genotoxic impurities in Vigabatrin drug substance. It was proposed to develop and validate the method using gas chromatography with head space techniques with mass spectrometry detector, since all the impurities appear to be liquids and would the most preferred method to detect the impurities at much lower concentrations than the specification level of 0.5 ppm.

1.1 Threshold of Toxicological Calculation

Maximum daily dose of Vigabatrin is 3000 mg/day. Vigabatrin is an antiepileptic drug indicated for refractory complex partial seizures and considering the usage of the drug for more than 10 years, the Threshold of Toxicological Concern (TTC) was considered as 1.5 µg/day. Genotoxic impurity limit = TTC (µg/day) / Dose (g/day) = 1.5/3.0 = 0.5 ppm.

2. MATERIALS AND METHODS

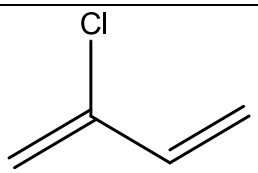
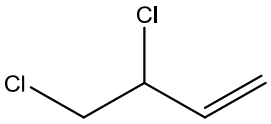
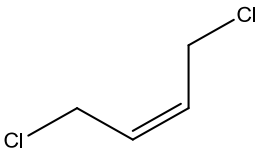
2.1 Instrumentation

An integrated head space gas chromatography system with a mass spectrometry detector (Agilent make) using computer-based chromatography software (open lab CDS and mass hunter) was used for the work. VF-WAXms (30 mts length, 0.25 mm internal diameter, 1.0 µ film thickness) column was used in the study.

2.2 Chemicals and Reagents

Vigabatrin was synthesized at Sionc Pharmaceuticals Private Limited, Visakhapatnam (India). GC grade N-Methyl-2-pyrrolidine and methanol were obtained from Spectrochem Pvt Ltd and Merck respectively. Sodium sulphate anhydrous (GR grade) was obtained from Merck. High purity water was prepared using Milli-Q purification system (Millipore) and filtered through 0.45 µm filter paper. Chloroprene (2000 µg/mL in methanol) was obtained from Sigma Aldrich, 3,4-Dichlorobut-1-ene was obtained from Tokyo Chemical Industry Co., Ltd, 1,4-Dichlorobut-2-ene (Cis-isomer) & 1,4-Dichlorobut-2-ene (Trans-isomer) was obtained from Changzhou Lanming Science and Technology Co., Ltd.

Table 1. Potential genotoxic impurities of Vigabatrin

S. No	Name of the Impurity	Structure
1.	Chloroprene	
2.	3,4-Dichlorobut-1-ene	
3.	1,4-Dichlorobut-2-ene (Cis Isomer)	
4	1,4-Dichlorobut-2-ene (Trans Isomer)	

2.3 Chromatographic Conditions

The headspace GC-MS method was optimized with a view to quantitate genotoxic impurities in the drug substance, Vigabatrin. A well-defined symmetrical peak was obtained with good resolution between the impurities under the optimized conditions after experimental trials were summarized. Injector temperature was optimised to 220°C with split ratio of 1:5. Helium gas with a column flow of 1.0 mL/min. and a total run time of 30 min. GC oven program is given in Table 2. Oven temperature, loop temperature and transfer line temperature were maintained in the head space at 70°C, 90°C and 100°C respectively. Source temperature, quad temperature and mass spectroscopy detector transfer line temperature were maintained at 230°C, 150°C and 250°C respectively. Selective ion monitoring mode with positive ion electrospray ionization method was employed and dwell time of 300 ms with gain factor of 20 were optimized for the study. The analyte, chloroprene, and the isomers of dichlorobutene were monitored with its molecular ion (m/z) at 88 and 75 respectively.

2.4 Preparation of Solutions

2.4.1 Preparation of blank

Transferred accurately 1 mL of diluent and 1 mL of water into a head space vial containing 500.0 mg of sodium sulphate and crimped the cap with septum.

2.4.2 Preparation of chloroprene standard stock solution

Transferred accurately 1 mL chloroprene solution **(**2000 µg/mL in methanol) into a 20 mL volumetric flask and made up to the mark with methanol.

2.4.3 Preparation of 3,4-dichlorobut-1-ene standard solution

Accurately weighed about 50.0 mg of 3,4-Dichlorobut-1-ene into a 50 mL volumetric flask and made up to volume with the diluent.

2.4.4 Preparation of 1,4-dichlorobut-2-ene (Cis isomer) standard solution

Accurately weighed about 50.0 mg of 1,4-Dichlorobut-2-ene (Cis isomer) into a 50 mL volumetric flask and made up to volume with the diluent.

2.4.5 Preparation of 1,4-dichlorobut-2-ene (Trans isomer) standard solution

Accurately weighed about 50.0 mg of 1,4-Dichlorobut-2-ene (Trans isomer) into a 50 mL volumetric flask and made up to volume with the diluent.

2.4.6 Preparation of impurities standard stock solution

Accurately transferred 1 mL each of 3,4-Dichlorobut-1-ene standard stock solution, 1,4-Dichlorobut-2-ene (Cis isomer) standard stock solution, 1,4-Dichlorobut-2-ene (Trans isomer) standard stock solution into a 100 ml volumetric flask and made up to the mark with diluent.

2.4.7 Preparation of impurities standard solution

Transferred about 2 mL impurities standard stock solution and 0.2 mL of chloroprene standard stock solution into a 100 ml volumetric flask and made up to the mark with diluent. Transferred about 1 mL of standard solution and 1 mL of water into a head space vial containing 500.0 mg of sodium sulphate and crimped the cap with septum.

2.4.8 Preparation of test solution

Weigh and transfer 400.0 mg of test sample into a headspace vial containing 500.0 mg of sodium sulphate, add 1ml diluent and 1 ml of water, crimp the cap with septum.

2.5 Validation of Method

The newly developed head space GC-MS method's validation was performed according to the ICH guidelines [9,10] in relation to the analytical parameters such as specificity, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and robustness to demonstrate the feasibility of the method.

2.5.1 System suitability

The system suitability solution prepared above was injected in replicates at the beginning of each study and the data is given in Table 3.

Table 2. GC Oven program

Ramp	Rate (ºC/min.)	Value (ºC)	Hold time (min.)	Run time (min.)
Initial	------	50	0	0
Ramp 1	10	140	8	17
Ramp 2	20	220	9	30

2.5.2 Specificity

Specificity is the ability of the method to measure the presence of process related impurities of Vigabatrin. The specificity of the developed head space GC-MS method was carried out to check the interference from blank at retention time of each impurity by injecting blank solution and process-related impurities separately. The chromatogram was compared with the blank chromatogram, to verify the blank interference.

2.5.3 Linearity, LOD and LOQ

Standard solutions at different concentration levels ranging from LOQ to 150% of specification limit were injected into the chromatographic system and from the response obtained calibration curves were constructed and the impurities were analyzed.

2.5.4 Accuracy, precision and robustness

The accuracy of the developed method was established in terms of the amount of impurities recovered in the spiked drug substance at four different levels (LOQ, 50%, 100 % and 150%). The percentage recovery was calculated to establish accuracy of the method. The precision of the method was established from the % RSD values (n=6) obtained in method precision and intermediate precision study. The effect of small deliberate variations in the optimized chromatographic conditions such as flow rate (± 0.1 mL/min.) and oven temperature (± 5ºC) were studied to establish the robustness of the method.

3. RESULTS AND DISCUSSION

A simple and sensitive head space GC-MS method was developed and validated as per ICH guidelines for the analysis of genotoxic impurities in Vigabatrin. A lot of trials have been performed in the optimization of various method parameters and finally the present method was proposed. The system suitability data as given in the below table indicates the suitability of the instrument and method parameters. In the specificity study, the chromatograms of impurities and blank were verified, and no peak was observed at the retention time of impurities. Hence, the method was found to be specific for the determination of process related impurities in Vigabatrin. Specificity chromatograms of the blank, chloroprene, 3,4-Dichlorobut-1-ene, 1,4-Dichlorobut-2-ene (Cis isomer) and 1,4-Dichlorobut-2-ene (Trans isomer) were shown in Fig. 1a to 1f. The data obtained in the linearity, LOD and LOQ study for various impurities is summarized in Table 4. Recovery data obtained in accuracy study is given in Table 5 and the % RSD (8.5 - 10.4) values obtained in precision study are given in Table 6. The small variations in flow rate and oven temperature did not show any significant changes in the system suitability parameters and proved the robustness of the method.

Table 3. System suitability data

Name	RT	Reproducibility (% RSD)
Chloroprene	3.260	8.4
3,4-Dichlorobut-1-ene	7.731	10.9
1,4-Dichlorobut-2-ene (Cis Isomer)	11.262	14.5
1,4-Dichlorobut-2-ene (Trans Isomer)	12.179	12.9

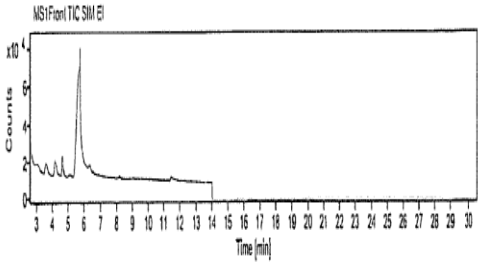


Fig. 1a. Chromatogram of blank

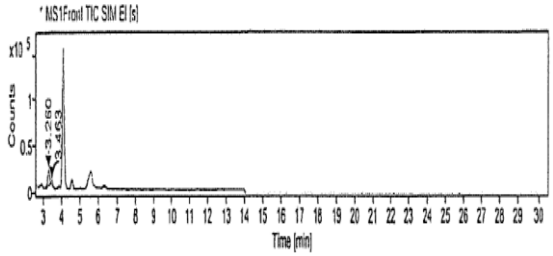


Fig. 1b. Chromatogram of chloroprene standard, RT at 3.26 min

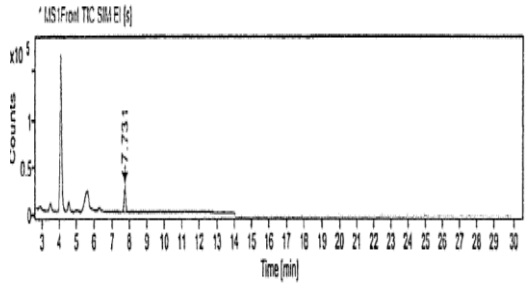


Fig. 1c. Chromatogram of 3,4-Dichlorobut-1-ene standard, RT at 7.731 min

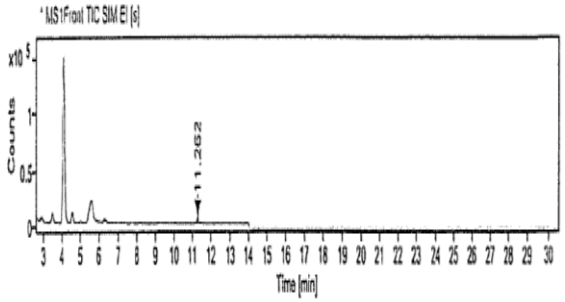


Fig. 1d. Chromatogram of 1,4-Dichlorobut-2-ene (Cis isomer) standard, RT at 11.262 min

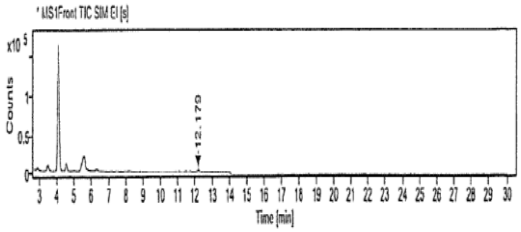


Fig. 1e. Chromatogram of 1,4-Dichlorobut-2-ene (Trans isomer) standard, RT at 12.179 min

Fig. 1a – 1e. Typical chromatograms of blank and impurities

Table 4. Linearity data, LOD and LOQ of impurities

Level	Chloroprene		3,4-Dichlorobut-1-ene e		1,4-dichlorobut-2-ene (Cis Isomer)		1,4-dichlorobut-2-ene (Trans Isomer)	
	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area
LOQ	0.0030	10050	0.0255	1972	0.0074	5570	0.0255	3368
50 %	0.0500	141593	0.0509	3264	0.0495	37487	0.0510	4408
75 %	0.0750	211953	0.0764	5085	0.0743	56311	0.0765	7348
100 %	0.100	296445	01018	6490	0.0990	75525	0.1020	9980
125 %	0.1250	339996	0.1273	8613	0.1238	97266	0.1275	13006
150%	0.1500	400889	0.1527	9781	0.1485	111652	0.1530	15211
Slope	2685360.908		63439.146		764844.893		0.9941	
Intercept	8366.2438		215.0720		-87.2598		98197.1989	
r	0.9973		0.9978		0.9993		0.9941	
LOD (µg/mL)	0.0009		0.0022		0.0076		0.0077	

Table 5. Recovery studies of impurities

Name	Spike level (%)	Concentration spiked (µg/mL)	Concentration recovered (µg/mL)	*Recovery (%)
Chloroprene	LOQ	0.0149	0.0141	94.6
	50	0.2486	0.2183	87.8
	100	0.4983	0.4758	95.5
	150	0.7481	0.6836	91.4
3,4-Dichlorobut-1-ene	LOQ	0.0352	0.0291	82.7
	50	0.2630	0.2410	91.6
	100	0.5272	0.5671	107.5
	150	0.7915	0.6351	80.2
1,4-Dichlorobut-2-ene (Cis isomer)	LOQ	0.1233	0.1205	97.7
	50	0.2459	0.2186	87.7
	100	0.4993	0.5376	107.7
	150	0.7496	0.58053	77.4
1,4-Dichlorobut-2-ene (trans isomer)	LOQ	0.1248	0.1417	113.6
	50	0.2496	0.2263	90.7
	100	0.5003	0.5642	112.8
	150	0.7511	0.6159	82.0

** Mean of three replicates*

Table 6. Precision study of impurities

Name	Method precision	Intermediate precision
	% RSD (n=6)	
Chloroprene	6.7	8.5
3,4- dichlorobut-1-ene	9.3	9.7
1,4-dichlorobut-2-ene (Cis isomer)	7.2	10.4
1,4-dichlorobut-2-ene (Trans isomer)	8.0	9.8

4. CONCLUSION

A new headspace GC-MS method was developed for the separation and determination of potential genotoxic impurities in Vigabatrin and validated as per ICH guidelines. The method was found to be simple, sensitive, precise, robust and accurate as observed from the statistical data. The present method could achieve separation of potential genotoxic impurities and can be concluded as a selective method for the determination of impurities in Vigabatrin. Therefore, this method can be used for routine quality control testing of Vigabatrin drug substance by gas chromatography.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ben-Menachem E. Mechanism of action of vigabatrin: Correcting misperceptions. Acta Neurologica Scandinavica. 2011;124(Suppl. 192):5-15.
2. Rebecca B, Myrsini G, Melissa JM, Catrin TS, Anthony GM. Vigabatrin add-on therapy for drug-resistant focal epilepsy. Cochrane Database of Systematic Reviews. 2020;7(7):CD007302.
   Available:https://doi.org/10.1002/14651858.CD007302.pub3
3. Plant GT, Sergott RC. Understanding and interpreting vision safety issues with vigabatrin therapy. Acta Neurologica Scandinavica. 2011;124(Suppl. 192):57-71.
4. Rod Foroozan. Vigabatrin: Lessons learned from the United States experience. Journal of Neuro-Ophthalmology. 2018;38(4):442-450
5. Muller, et al. Regulatory toxicology and pharmacology. 2006;44:198-211.
6. Brigo A, Müller L. Development of the threshold of toxicological concern concept and its relationship to duration of exposure in genotoxic impurities (Ed. A. Teasdale), John Wiley & Sons, Inc., Hoboken, NJ, USA; 2011.
7. M7(R1) Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk guidance for industry (fda.gov); 2018.
8. David JS. Genotoxic impurities: from structural alerts to qualification: Organic process research & development. 2010;14:960–976.
9. ICH Harmonized Tripartite Guideline; Validation of Analytical Procedures: Text and Methodology, Q2(R1); International conference on Harmonization, IFPMA, Geneva, Switzerland; 2005.
10. ICH Harmonized Tripartite Guideline; Impurities in New Drug Substances Q3A (R2); International conference on Harmonization, IFPMA, Geneva, Switzerland; 2006.

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