PHARMACIA

Pharmacia 70(1): 203–207
DOI 10.3897/pharmacia.70.e97339

Research Article

Development and validation of determination of genotoxic impurity Bromoethane in Vigabatrin drug substance using head space gas chromatographic method [HS-GC]

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Received 10 November 2022 ♦ **Accepted** 8 December 2022 ♦ **Published** 14 March 2023

Citation: Narapereddy KP, Alladi DS (2023) Development and validation of determination of genotoxic impurity Bromoethane in Vigabatrin drug substance using head space gas chromatographic method [HS-GC]. Pharmacia 70(1): 203–207. https://doi.org/10.3897/pharmacia.70.e97339

Abstract

A specific HS-GC method has been developed, optimized, and validated for the determination of genotoxic impurity Bromoethane in Vigabatrin (VGB) drug substance. Chromatographic separation of genotoxic Bromoethane impurity was achieved on DB-1 column (30 m × 0.53 mm, 5.0 µm), consists of 100% dimethyl polysiloxane as stationary phase and passing nitrogen carrier gas. The performance of the method was assessed by evaluating the specificity, linearity, sensitivity, precision, and accuracy experiments. The established limit of detection and limit of quantification values for the genotoxic impurity was in the range of 3.57–10.80 µg/mL. The correlation coefficient value of the linearity experiment was 0.9880. The average recoveries for the accuracy were in the range of 95.3–106.8%. The results proved that the method is suitable for the determination of Bromoethane content in Vigabatrin.

Keywords

Bromoethane, Genotoxic impurity, Vigabatrin drug substance, ICH guidelines, Validation

Introduction

Vigabatrin (g-vinyl-aminobutyric acid) is one of the newer generations of anti-epileptic drugs introduced into the UK in 1989. Vigabatrin was specially designed to inhibit the enzyme g-aminobutyrate-a-ketoglutarate aminotransferase (GABA-transaminase). The drug binds covalently to the active site of the enzyme, consuming both enzyme and inhibitor in an irreversible reaction (Grant and Heel 1991). In this way, Vigabatrin increases the concentrations of the inhibitory neurotransmitter g-aminobutyric acid (GABA) in the brain in a dose-dependent manner, thereby increasing seizure control in resistant epilepsy (Jacqz-Aigrain et al. 1997; Chadwick 1999).

Vigabatrin is supplied as a racemic mixture but only the S(+) isomer is active (Fig. 1). The drug is rapidly absorbed, with maximal concentrations occurring within 1 to 2 h, and an elimination half-life of around 7 to 8 h in young adults (Rey et al. 1992).

In the synthesis process of Vigabatrin drug substance, Bromoethane was used as a key raw material. The brominated impurity was generally toxic, cancer suspect agents and come under genotoxic category (Fig. 2).

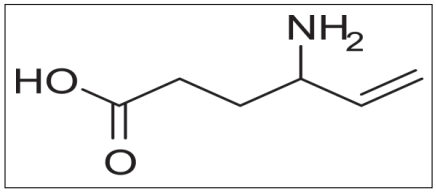

Figure 1. Chemical structure of Vigabatrin.

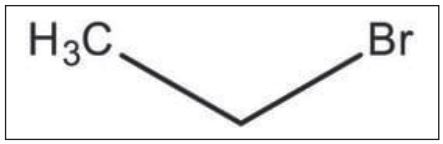

Figure 2. Chemical structure of Bromoethane.

The European Agency for the Evaluation of Medicinal products (EMEA), United States Food and Drug Administration (USFDA) and ICH Q3A/B issued the guidelines and draft guidance on the limitation of genotoxic impurities in pharmaceutical ingredients (European Medicines Evaluation Agency 2006; International Conference on Harmonisation 2006; Genotoxic and carcinogenic impurities in drug substances and Products 2008).

In the available literature, few analytical methods had been reported for the quantification of alkyl bromide impurities. A simple GC/ECD method was reported for the determination of methyl bromide in the ambient air (Hughes et al. 1999) and a rapid HS-GC/MS method was developed for fast selective determination of residues of methyl bromide in food products (Quaglia et al. 1990). An accurate GC/ECD (Wu and Sun 2014) method was reported for determination of residual bromoethane in teneligliptin hydrobromide and a simple GC method (Wesolowski et al. 2013; Tao and Song 2006) was established for the determination of ethyl bromide in the workplace air. Another GC method was reported for the determination of ethyl bromide, ethylene chloride and methyl chloride in the air (Krynska and Posniak 1978). A GC/FID method (Kifune and Shirai 1979) was reported for the simultaneous determination of methyl bromide and ethyl bromide in the air samples. Further, a GC/TCD method was published for the determination of diethyl ether and ethyl bromide in the reaction mixture of ethyl bromide production (Skakun and Timofeeva 1978). A simple GC/FID method was reported for the determination of 1-Bromopropane in the workplace air (Wu et al. 2013; Ling et al. 2014), and in human urine (Zhou et al. 2015). A GC/MS method was reported for the determination of 1-Bromopropane in leather and textiles (Li et al. 2014). Another GC method with ECD detector was reported for the quantification of 1-Bromopropane and 2-Bromopropane in human urine (B'Hymer and Cheever 2005).

However, no method was reported for the determination of residual Bromoethane in Vigabatrin Finally, a sensitive GC-HS method was reported for the determination of Bromoethane impurity in Vigabatrin drug substance and the method was validated for specificity, linearity, accuracy, and precision experiments (Raju et al. 2016).

Experimental

Materials and reagents

Bromoethane, Dimethyl acetamide were obtained from Sigma-Aldrich and pure samples of Vigabatrin were obtained from synthetic division of Nuray Chemicals Private Limited, (R&D), Thiruvallur and Tamilnadu India.

Instruments and equipment

Head space GC analysis was conducted using an Agilent GC-HSS 7890B series equipped with 7697A Headspace Sampler. DB-1 column (0.53 mm × 30 m, 5.0 µm; J&W Scientific Inc.) was used for analysis. A Mettler Toledo AT261 Semi-Micro Balance was also used for sample preparation.

Preparation of solutions

Preparation of diluent

Prepared a mixture of Dimethyl acetamide and water in the ratio of 1:1 (%volume/volume) mixed well and sonicated to degas.

Preparation of standard solution

Transferred 35.0 µL of Bromoethane standard into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Further transferred 1.0 mL of the above solution into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Preparation of sample solution

Accurately weighed 500 mg of the test sample into a 20 mL head space vial added 5.0 mL of diluent and seal the vial immediately.

Preparation of sample spiked solution

Accurately weighed 500 mg of the test sample into a 20 mL head space vial added 5.0 mL of standard solution and seal the vial immediately.

Instrumentation

Agilent J&W DB-1, (30 m × 0.53 mm, 5.0 µm) column consists of 100% dimethyl polysiloxane material as a stationary phase. High purity nitrogen gas was used as the carrier gas with the column flow 2.5 mL/min. The initial column oven temperature of 40 °C was maintained for 8 min and then increased to 150 °C at the rate of 10 °C/min, followed by holding at 230 °C for 11 min at the rate of 25 °C/min. The run time was 33.5 min. The injection volume was 1.0 mL with a split ratio of 1:5. The injector temperature was 200 °C, detector temperature was 230 °C.

Method development

The objective of the general method is to determine Bromoethane at low level with selectivity in Vigabatrin drug substance, HS-GC method has intrinsic superior selectivity because this analytical technique only analyses compounds that are evaporated into the sample solution head space. Therefore, a HS-GC method was further explored for the determination of residual Bromoethane in Vigabatrin drug substance. The capillary GC column DB-1 column has reported as suitable for the analysis of a wide range of common ICH residual solvents including Bromoethane in pharmaceutical products and thus was selected for methods development. The sample diluent, temperature program, split ratio, head space oven temperature, and other head space and GC parameters were investigated and optimised using Bromoethane standard solution or Bromoethane standard spiked to sample solutions.

Diluent for sample and standard preparation

The sample diluent is important for a static Headspace GC method as it affects the sensitivity and accuracy by influencing the equilibrium between the analyte in the liquid phase and the analyte in the headspace. Several sample diluents were evaluated including dimethyl sulfoxide, dimethyl acetamide, dimethyl formamide and water alone and in combination. The use of water alone as the sample diluent led to irreproducible results, water solubility of drug (55.0 mg/mL). Overall solubility of the drug Vigabatrin, was also determined in DMA-water and DMSO-water system and the values were found to be 56.23 mg/mL and 50.03 mg/mL respectively. A combination of water with the organic diluents 1:1 ratio was studied. Under different head space oven temperature at 80 °C, 90 °C and 100 °C. Under these GC headspace conditions, the reproducibility of Bromoethane spiked Vigabatrin was evaluated, and the results demonstrated that the DMA: water 1:1 v/v sample diluent at a headspace temperature of 90 °C showed the best sensitivity and selectivity.

One of the key objectives of the method development was to achieve adequate sensitivity for low level Bromoethane analysis. The Bromoethane method sensitivity was further optimized by the evaluating the effect of split ratio on the noise level and S/N value of a 50 ppm Bromoethane standard solution. Several GC injection split ratios including 5:1, 10:1 and 30:1 was studied. The optimal noise level and adequate signal was observed using the 5:1 spilt ratio injection parameter.

Chromatographic and headspace parameters conditions

For head space GC, vial equilibration temperature 90 °C, Loop temperature 95 °C, transfer-line temperature 100 °C, equilibration time 10 min, pressurization time 0.2 min and injection time of sample 1.0 min. GC cycle time 40 minutes. The flame ionization detector (FID) detector was used split ratio 5:1, injection port temperature 200 °C; detector temperature 230 °C; temperature program was set to 40 °C for hold time 8 minutes, then raised to 150 °C with rate 10 °C per minute hold time zero minutes and then maintained at 230 °C for with rate 25 °C per minute hold time 11 minutes and carrier gas was used nitrogen.

Method validation (ICH 2005)

Specificity

The method specificity was validated for potential interference from blank, standard, sample, and spiked sample solution. There are no detectable peaks in the chromatograms of blank, standard, sample, and spiked sample. The Bromoethane peak in the chromatogram of 50 ppm Bromoethane spiked sample solution is sufficiently resolved from all other peaks before and after Bromoethane peak. The retention time of Bromoethane in the chromatogram of 50 ppm Bromoethane spiked sample solution matches well with that from 50 ppm Bromoethane standard solution (Figs 3, 4, 5, 6).

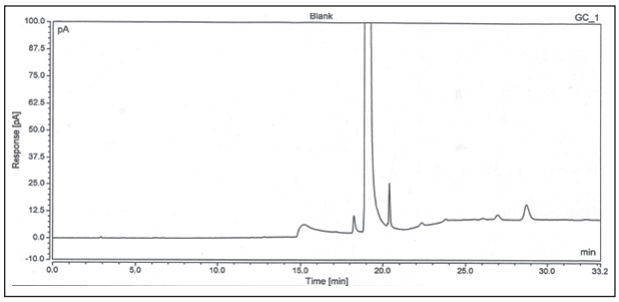

Figure 3. Typical chromatogram of blank.

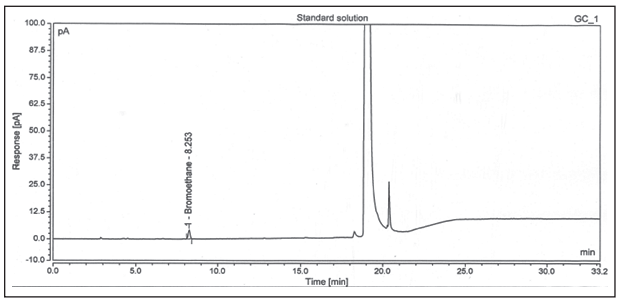

Figure 4. Typical chromatogram of standard.

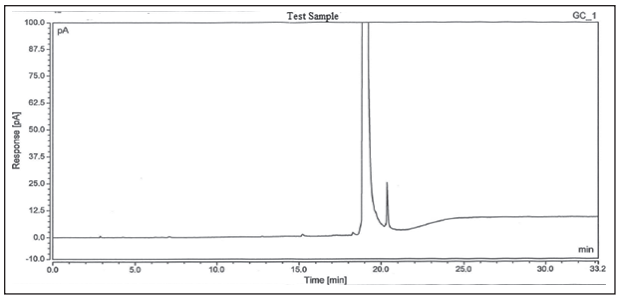

Figure 5. Typical chromatogram of sample.

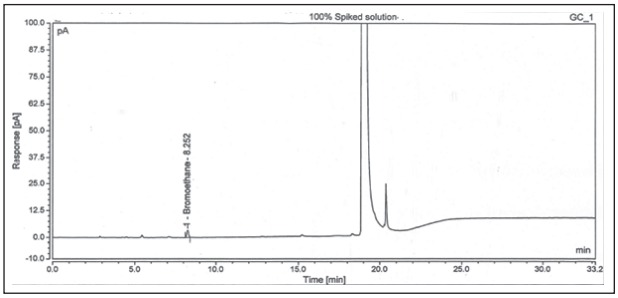

Figure 6. Typical chromatogram of spiked sample.

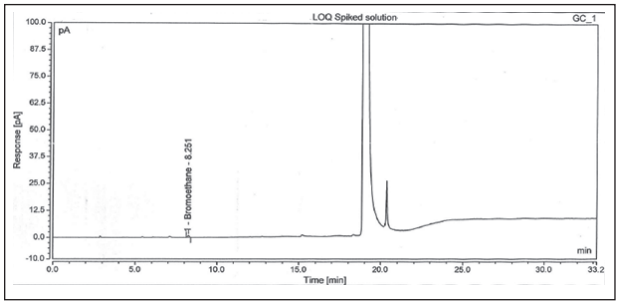

Figure 7. Typical chromatogram of LOQ.

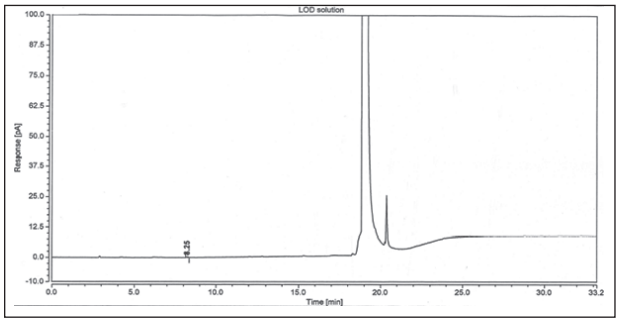

Figure 8. Typical chromatogram of LOD.

System precision

System precision was demonstrated by preparing standard solution as per method and chromatographed the same into GC system in six replicated injections of standard solution. The peak areas of analyte were recorded for these standard injections. The system precision was evaluated by computing the % Relative standard deviation for the peak area of these standard injections (see Table 1).

Table1. Validation data of Vigabatrin for the determination of Bromoethane.

Parameter	Bromoethane
LOD (ppm)	3.57
LOQ (ppm)	10.80
Precision at LOQ level (RSD, %)	4.6
System Precision at sixth level (RSD, %)	2.8
Method Precision at sixth level (RSD, %)	1.6
Linearity range (µg/mL)	LOQ-150
Correlation coefficient	0.9880
Slope	0.57
Intercept	-1.06
% of y-intercept	-4.2
Accuracy at LOQ (mean recovery, %)	96.8
Accuracy at 50 (mean recovery, %)	95.3
Accuracy at 100 (mean recovery, %)	106.8
Accuracy at 150 (mean recovery, %)	98.6

Detection limit (LOD), Quantitation limit (LOQ)

A solution containing 10.80 ppm of Bromoethane standard was injected six times. The RSD of areas and S/N ratios for each standard were calculated. A solution containing 3.57 ppm of Bromoethane standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections (Figs 7, 8).

Therefore, the quantitation limit (QL) and the detection limit (DL) was thus set at 10.80 ppm and 3.57 ppm, respectively (see Table 1). These S/N ratios are much greater than ICH recommended S/N value for DL (S/N~3) and QL (S/N~10).

Method precision

Precision (repeatability) was evaluated from the recovery data. Recovery data was determined by injecting six sample solutions spiked Bromoethane 50 ppm at specification level (see Table 1). The samples were prepared as per the analytical method.

Linearity and range

The linearity of Bromoethane was evaluated from 10.80 ppm to 77.18 ppm (six levels with duplicate preparations at each level). The peak areas were plotted against the corresponding concentrations and the linear regression was performed. The range 10.80–77.18 ppm was established by meeting the acceptable criteria of linearity, accuracy, and precision for the entire concentration interval study (see Table 1).

Accuracy

Accuracy was determined by analyzing the triplicate preparation of Bromoethane standard at low 10.80 ppm, 25.0 ppm, 50.0 ppm and 75.0 ppm levels in the presence of Vigabatrin drug substance, as per the analytical method. The accuracy as % recovery was calculated from the experimental concentrations of Bromoethane standards by the theoretical concentrations. The recovery of ranged from 95.3% to 106.8% were obtained for the three concentrations levels (see Table 1).

Solution stability

The stability of standard, sample and spiked sample solutions were prepared in duplicate and stored at ambient laboratory conditions (25±5 °C), refrigeration (2–8 °C), respectively. Therefore, the standard solution, sample solution and spiked sample solution were stable for 24 hrs at both room and refrigerated temperature conditions.

Conclusion

The Head space Gas chromatographic presented in this report, successfully achieved the main objective of method development, which was to obtain a method that can be used as general method to determine residual bromoethane in various pharmaceutical drug substances. Therefore, this new method can be used as a general method to determine residual bromoethane because it has a good potential to work either as-is or with minor modifications for other liquid pharmaceutical drug substances.

Acknowledgment

The authors are grateful to Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India, for providing facilities to carry this research work.

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