Development and validation of Genotoxic Impurities Chloromethane, Chloroethane and 2-Chloropropane, in Pregabalin drug substance using Head-Space Gas Chromatography

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ABSTRACT

A simple and reliable head space gas chromatographic method has been developed for the determination of residual Chloromethane, Chloroethane and 2-Chloropropane in Pregabalin drug substance. The proposed method is based on flame ionization detection technique with DB-624 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 Chloromethane, Chloroethane and 2-Chloropropane was in the range of 41.37 and 125.18 µg/mL , 41.61 and 126.09 µg/mL and 41.55 and 125.78 µg/mL. The correlation coefficient value of the linearity experiment was 0.9999, 0.9999 and 0.9999. The average recoveries for the accuracy were in the range of 92.8-99.9%. The results proved that the method is suitable for the determination of Chloromethane, Chloroethane and 2-Chloropropane content in Pregabalin drug substance.

Key Words: Chloromethane, Chloroethane and 2-Chloropropane, Genotoxic impurities, Pregabalin Drug Substance, ICH guidelines, Validation.

INTRODUCTION

Pregabalin is an anticonvulsant drug used to treat neuropathic pain conditions and fibromyalgia, and for the treatment of partial onset seizures in combination with other anticonvulsants. Pregabalin is structurally similar to gamma-aminobutyric acid (GABA)-an inhibitory neurotransmitter. It may be used to manage neuropathic pain, postherpetic neuralgia, and fibromyalgia among other conditions. Although as per the FDA Label the mechanism of action has not been definitively defined, there is evidence that Pregabalin exerts its effects by binding to the α2δ subunit of voltage-dependent calcium channels [1-19].

Literature survey reveals that several analytical methods has been reported concerning the analysis of PGB in its dosage form and biological matrices using high-performance liquid chromatography (HPLC) [20-23], spectrophotometry [24], spectroflourimetry [25], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [26-28], gas chromatography (GC) with flame ionization detector (FID) [29], ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [30], and gas chromatography- mass spectrophotometry (GC–MS) in human urine after ethyl chloroformate derivatization [31].

However, no method was reported for the determination of residual Chloromethane, Chloroethane and 2-Chloropropane in Pregabalin Drug substance. Finally, a sensitive GC-HS method was reported for the determination of Chloromethane, Chloroethane and 2-Chloropropane impurities in Pregabalin drug substance.

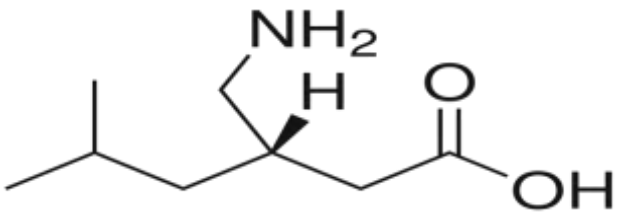

Figure 1. Chemical structure of Pregabalin

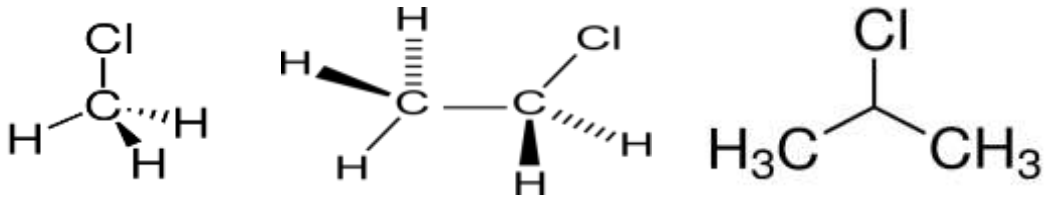

Figure 2. Chemical structures of Chloromethane, Chloroethane and 2-Chloropropane

Materials and Methods

Chemicals and reagents

Chloromethane, Chloroethane, 2-Chloropropane, Dimethylformamide were obtained from Sigma-Aldrich and pure samples of Pregabalin were obtained from synthetic division of Nuray Chemicals Private Limited. (R&D), Thiruvallur and Tamilnadu India.

Instruments/Equipments

Head space GC analysis was conducted using a Agilent GC-HSS 7890B series equipped with

7697A Headspace Sampler. DB-624 column (0.32 mm × 30 m, 1.8 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

Mixed accurately Dimethylformamide and water in the ratio of 50:50 volume/volume, used as a diluent

Preparation of Chloromethane stock solution

Accurately weighed 5.14 mg of the Chloromethane standard into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Preparation of Chloroethane stock solution

Accurately weighed 5.21 mg of the Chloroethane standard into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Preparation of 2-Chloropropane stock solution

Accurately weighed 5.34 mg of the 2-Chloropropane standard into a 100 mL volumetric flask containing about 50 mL of diluent and make up to volume with diluent.

Preparation of standard solution

Transferred 1.0 mL of each stock solution into 20 mL volumetric flask containing about 10 mL of diluent and make up to volume with diluent.

Preparation of sample solution

Accurately weighed 5.12 mg of the test sample into a 20 mL head space vial added 1.0 mL of diluent and seal the vial immediately.

Preparation of sample spiked solution

Accurately weighed 5.31 mg of the test sample into a 20 mL head space vial added 1.0 mL of standard solution and seal the vial immediately.

Instrumentation

Agilent J&W DB-624, (30m×0.32mm, 1.8 μm) column consists of 6% cyanopropyl/phenyl, 94% polydimethyl siloxane material as a stationary phase. High purity nitrogen gas was used as the carrier gas with the column flow 1.0 mL/min. The initial column oven temperature of 50°C was maintained for 8 min and then increased to 150°C at the rate of 15°C/min, followed by holding at 260°C for 15 min at the rate of 25°C/min. The run time was 33.2 min. The injection volume was 1.0 mL with a split ratio of 1:5. The injector temperature was 220°C, detector temperature was 260°C.

Method Development

The objective of the general method is to determination of Chloromethane, Chloroethane, 2-Chloropropane at low level with selectivity in Pregabalin 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 Chloromethane, Chloroethane, 2-Chloropropane in Pregabalin drug substance. The capillary GC column DB-624 column has reported as suitable for the analysis of a wide range of common ICH residual solvents including Chloromethane, Chloroethane, 2-Chloropropane in pharmaceutical products and thus was selected for method development. The sample diluent, temperature program, split ratio, head space oven temperature, and other head space and GC parameters were investigated and optimised using Chloromethane, Chloroethane, 2-Chloropropane standard solution or Chloromethane, Chloroethane, 2-Chloropropane 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 Pregabalin , 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 were studied. Under different head space oven temperature at 90°C, 100°C and 110°C. Under these GC headspace conditions, the reproducibility of Chloromethane, Chloroethane, 2-Chloropropane spiked Pregabalin was evaluated and the results demonstrated that the DMA: water 1:1 v/v sample diluent at a headspace temperature of 100°C showed the best sensitivity and selectivity.

One of the key objectives of the method development was to achieve adequate sensitivity for low level Chloromethane, Chloroethane, 2-Chloropropane analysis. The Chloromethane, Chloroethane, 2-Chloropropane 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 Chloromethane, Chloroethane, 2-Chloropropane standard solution. Several GC injection split ratios including 5:1, 10:1 and 30:1 were 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 100°C, Loop temperature 105°C, transfer-line temperature 110°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 220°C; detector temperature 260°C; temperature program was set to 50°C for hold time 8 minutes, then raised to 150°C with rate 15°C per minute hold time zero minutes and then maintained at 260°C for with rate 25°C per minute hold time 15 minutes and carrier gas was used nitrogen.

Results and Discussion

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 Chloromethane, Chloroethane, 2-Chloropropane peaks in the chromatogram of 500 ppm Chloromethane, Chloroethane, 2-Chloropropane spiked sample solution is sufficiently resolved from all other peaks before and after Chloromethane, Chloroethane, 2-Chloropropane peaks. The retention time of Chloromethane, Chloroethane, 2-Chloropropane in the chromatogram of 500 ppm Chloromethane, Chloroethane, 2-Chloropropane spiked sample solution matches well with that from 500 ppm Chloromethane, Chloroethane, 2-Chloropropane standard solution.

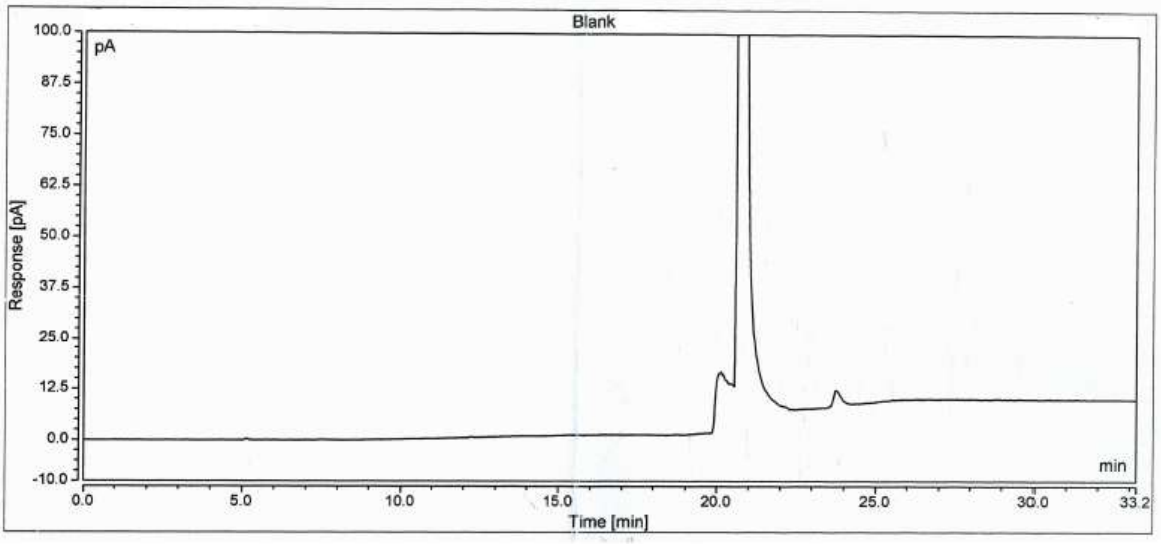

Figure 3. Typical chromatogram of blank

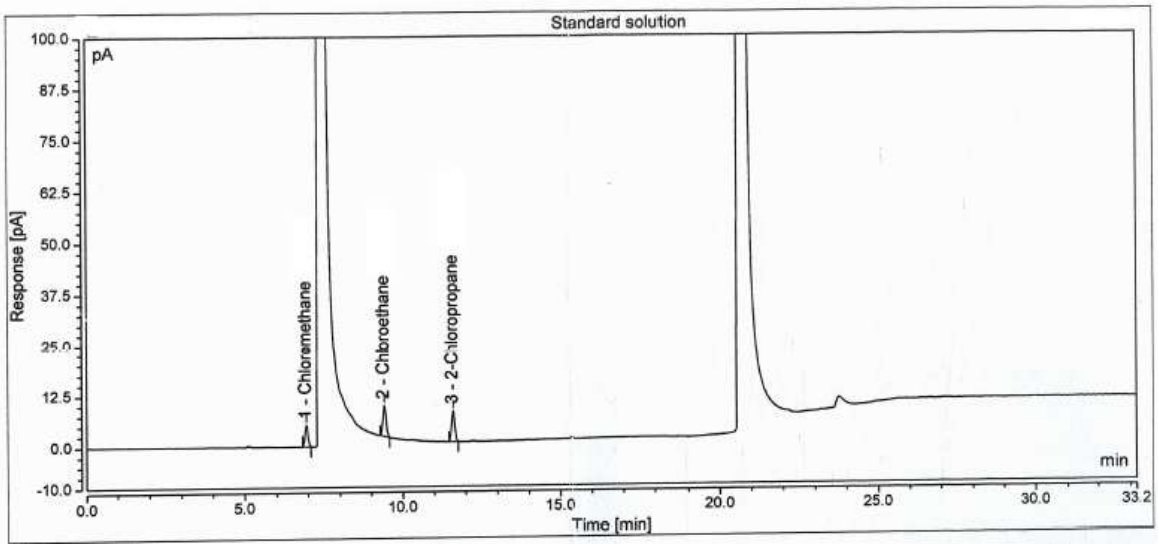

Figure 4. Typical chromatogram of standard

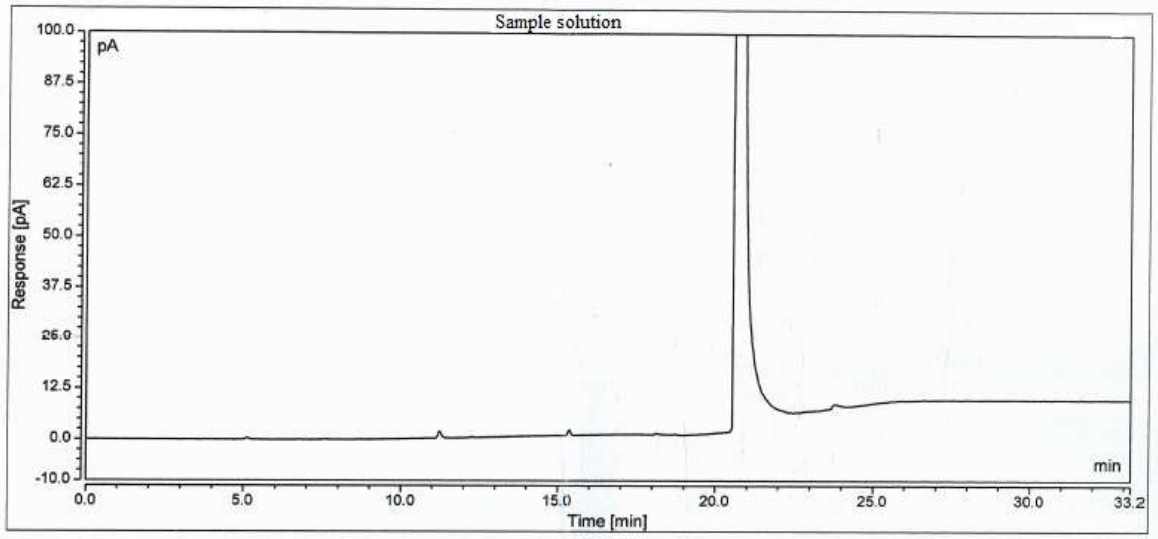

Figure 5. Typical chromatogram of sample

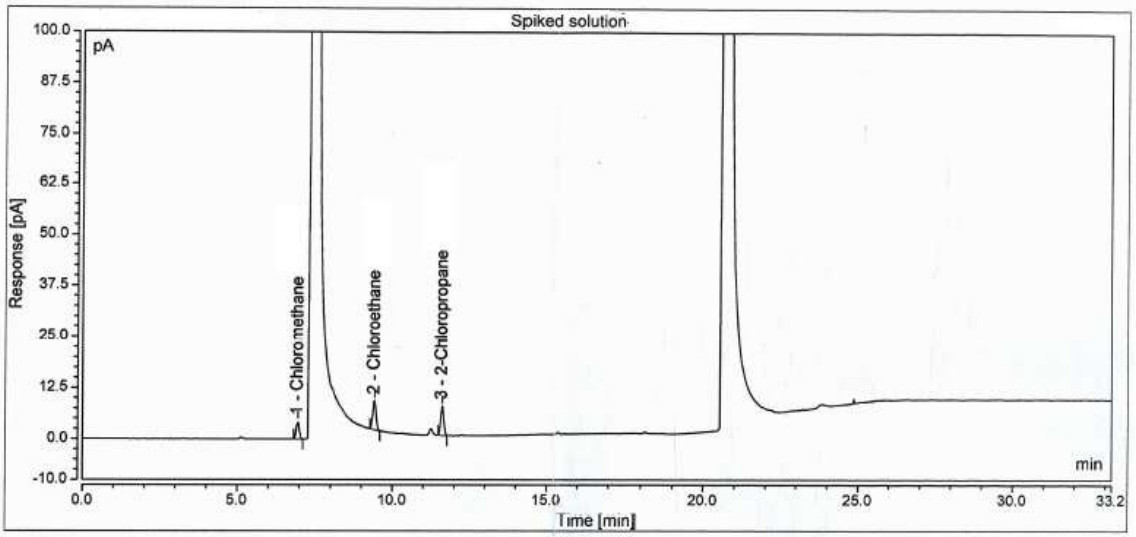

Figure 6. Typical chromatogram of spiked sample

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.

Method precision

Precision (repeatability) was evaluated from the recovery data. Recovery data was determined by prepared and injecting six control sample solutions and six spiked sample solutions spiked chloromethane, Chloroethane and 2-chloropropane 500 ppm at specification level. The samples were prepared as per the method.

Detection limit (LOD), Quantitation limit (LOQ)

A solution containing 125.18 ppm, 126.09 ppm and 125.78 ppm of chloromethane, Chloroethane and 2-chloropropane standard was injected six times. The %RSD of areas and S/N ratios for each standard were calculated. A solution containing 41.37 ppm, 41.61 ppm and 41.55 ppm of chloromethane, Chloroethane and 2-chloropropane standard was injected three times.

Linearity

The linearity of chloromethane, Chloroethane and 2-chloropropane was evaluated from 125.18 ppm to 750.10 ppm, 126.09-750.27 ppm and 125.78-750.19 ppm (six levels with duplicate preparations at each level. The peak areas were plotted against the corresponding concentrations and the linear regression was performed.

Accuracy

Accuracy was determined by analyzing the triplicate preparation of chloromethane, Chloroethane and 2-chloropropane standard at LOQ-150% levels in the presence of Pregabalin drug substance, as per the analytical method. The accuracy as % recovery was calculated from the experimental concentrations of chloromethane, Chloroethane and 2-chloropropane standards by the theoretical concentrations.

Solution stability

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

Table 1. Validation data of Pregabalin for the determination of Chloromethane, Chloroethane and 2-chloropropane

Parameter	Chloromethane	Chloroethane	2-Chloropropane
LOD (ppm)	41.37	41.61	41.55
LOQ (ppm)	125.18	126.09	125.78
Precision at LOQ level (RSD, %)	1.66	1.59	2.58
System Precision (RSD, %)	1.57	1.68	0.86
Method Precision (RSD, %)	2.00	1.65	2.00
Linearity range (µg/mL)	125.18-771	126.09-781.5	125.78-801
Correlation coefficient	0.9999	0.9999	0.9999
Slope	79.1447	127.1228	109.9840
Intercept	398.3605	98.1974	564.2751
% of y-intercept	0.97	0.15	0.95
Accuracy at LOQ (mean recovery, %)	95.3	98.8	99.6
Accuracy at 50 (mean recovery, %)	94.2	94.5	98.3
Accuracy at 100 (mean recovery, %)	97.7	98.1	97.8
Accuracy at 150 (mean recovery, %)	92.8	99.7	98.9

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 Chloromethane, Chloroethane and 2-Chloropropane in various pharmaceutical drug substances. Therefore, this new method can be used as a general method to determine residual Chloromethane, Chloroethane and 2-Chloropropane because it has a good potential to work either as-is or with minor modifications for other liquid pharmaceutical drug substances.

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CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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