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# Validation of residual solvents using gas chromatography with head space in active pharmaceutical ingredient

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## ABSTRACT

A new, simple, specific, accurate and precise GC-HS method was developed for determination of residual solvents in zonisamide (anti-epileptic active pharmaceutical ingredient): Methanol, Diethyl ether, Isopropyl Alcohol, Ethylacetate, 1,1,2-Trichloroethene and toluene are the residual solvents present in zonisamide. Agilent DB-624 fused silica capillary column 30m × 0.53mm I.D with a phase thickness of 3.0µ. The split/split less injector was maintained at 140°C with a split injection 1:5 ratio and temperature of the FID was set to 250°C. The carrier gas was nitrogen at a constant flow rate of 5 ml/min. the column oven temperature program involved an initial temperature of 40°C for 20 min; this was increased at 10°C/min to 240°C and hold for 20 min. HS sampling was performed with a AOC 5000 headspace sampler (SHIMADZU). The method was validated for precision, ruggedness, linearity, Limit of detection and limit of quantification and recovery according to the International Conference on Harmonization (ICH) guidelines.



## KEYWORDS

Residual solvent;
ICH;
Validation;
Zonisamide.

## INTRODUCTION

Zonisamide (1,2-benzisoxazole-3-methanesulfonamide) was developed as a new type of anti-epileptic active pharmaceutical ingredient[1] and used as an anticonvulsant in patients with epileptic disorders[2-5] being effective in the treatment of partial and generalized seizures[6-8]. The objective of this method validation is to acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. The solvents are less toxic and describe levels considered to be toxicologically acceptable for some residual solvents. The detection and quantization of residual solvents in drug substances or drug products is an important measure for pharmaceutical quality assurance/quality control (QA/QC)[9-11], because the residual solvents that were not totally removed by practical manufacturing techniques always have potential risk to human health from the toxicity. Based on the guideline Q3C issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) in 1998, solvents has been divided into three classes. They were evaluated for their possible risk to human health and placed into one of three classes as follows: Class 1 solvents: Solvents to be avoided Known human carcinogens, strongly suspected human carcinogens and environmental hazards. Class 2 solvents: Solvents to be limited Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratoge-

nicity. Solvents suspected of other significant but reversible toxicities. Class 3 solvents: Solvents with low toxic potential Solvents with low toxic potential to man; no health-based exposure limit is needed. The limit content for each solvent also was given in terms of their level of hazard to humans and the environment, and their permitted daily exposure (PDE)[12]. Static headspace (SH) injection is the most commonly used sampling techniques for residual solvents testing in pharmaceuticals with gas chromatography (GC).

**TABLE 1 : Chemical name and structures of zonisamide**

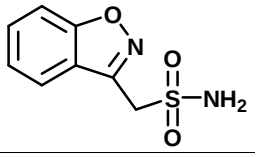
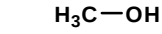
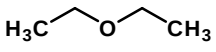
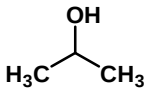
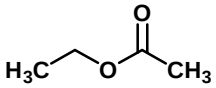
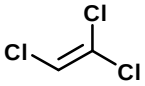
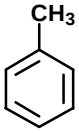
| Abbreviation | Chemical name | Structure |
|---|---|---|
| ZONISAMIDE | 1,2-Benzisoxazole-3-methane sulfonamide |  |

**TABLE 2 : List of solvents in zonisamide and its ICH limits**

| Abbreviation | Chemical name | Structure | Solvent Class (As per ICH) | ICH limit (ppm) |
|---|---|---|---|---|
| MeOH | Methanol |  | 2 | 3000 |
| DEE | Diethyl ether |  | 3 | 5000 |
| IPA | Isopropyl alcohol |  | 3 | 5000 |
| EA | Ethyl acetate |  | 3 | 5000 |
| TCE | 1,1,2-Trichloroethene |  | 2 | 80 |
| TOL | Toluene |  | 2 | 890 |

## EXPERIMENTAL

### Materials and methods

Zonisamide is synthesized by Synthetic organic Chemistry group and characterized for identity and purity. GC grade Methanol (MeOH), Diethylether (DEE), Isopropyl Alcohol (IPA), Ethyl acetate (EA), 1,1,2-Trichloroethene (TCE) ,Toluene (TOL) and N,N-Dimethyl acetamide (DMA) were obtained from Merck, India. Gas chromatography system used was Shimadzu 17A with flame ionization detecter (Shimadzu Japan). The employed column was an Agilent DB-624 fused silica capillary column 30m×0.53mm I.D (J&W Scientific, USA) with a phase thickness of 3.0µ (6% Polycynopropyl phenyl Siloxane and 94 % polydimethyl siloxane). The split/splitless injector was maintained at 140°C with a split injection 1:5 ratio, and temperature of the FID was set to 250°C. The carrier gas was nitrogen at a constant flow rate of 5 ml/min. the column oven temperature program involved an initial temperature of 40°C for 20 min; this was increased at 10°C/min to 240°C and hold for 20 min. HS sampling was performed with a AOC 5000 headspace sampler (SHIMADZU). The analytes in 20ml GC vial were equilibrated with oven temperature 80°C for 75 min. Each sample, after homogenization, was analyzed in triplicate with a 2ml injection volume. The signal was acquired and processed using GC-solution software.

### Preparation of solutions and chromatographic conditions

#### Diluent

DMA: Water (75:25)

#### Standard solution

#### SS-1

Accurately weigh about 80 mg of Trichloroethene in to 100ml volumetric flask and make up to volume with diluent.

#### Stock standard solution

Accurately weigh about 240mg of Methanol, 400mg of Diethyl ether, 400mg of IPA, 400 mg of Ethyl acetate and 71.2mg of Toluene and 8 ml of TCE solution (SS1) in 200ml volumetric flask and make up to volume with diluent.

#### Working standard solution

Dilute 25 ml of stock standard solution to 100 ml with diluent.

#### Test solution

Weigh accurately about 1000mg of the substance under examination, dissolve and dilute to 10ml with diluent.

#### Procedure

Add 10ml of diluent in to the headspace vial and record the chromatogram.

Discard any peak corresponds to diluent appearing in this chromatogram in all-subsequent chromatograms. Add 10ml of working standard solution in to the headspace vial and record the chromatogram.

**System suitability**

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. The retention time for respective solvents, Methanol is about 2.5, Diethyl ether is about 3.2, IPA is about 4.0, Ethyl acetate is about 7.4, TCE is about 12.50 and Toluene is about 21.6. The Resolution R obtained between Methanol, Diethyl ether, IPA, Ethyl acetate, TCE and Toluene should not be less than 2.0. The theoretical Plates for Methanol, Diethyl ether, IPA, Ethyl acetate, TCE and Toluene should not be less than 2000. The tailing factor for

Methanol, Diethyl ether, IPA, Ethyl acetate, TCE and Toluene should not more than 2.0. Add 10 ml of working standard in five different headspace vials and record the chromatogram. Calculate % RSD of peak area for each solvent. The system is suitable when the % RSD is not more than 15 %.

**Precision and ruggedness**

Precision of the method was determined by injecting six different test preparations and determining the system suitability parameters as well % RSD of residual solvents values. Ruggedness of the method was determined by performing quantification of residual solvents on two different GC systems and columns by two analysts.

**Linearity in absence of sample matrix**

Linearity response for all residual solvents MeOH, DEE, IPA, EA, TCE and TOL were determined in the range of 20% to 200% of the limit concentration MeOH 3000ppm, DEE 5000ppm, IPA 5000ppm, EA 5000ppm, TCE 80ppm and TOL 890ppm with respect to test concentration 100mg/ml. The % RSD for linearity solution is not more than 15.0%.There should be no non-linear trend at the ends of the plotted fitted line. Correlation coefficient ($R^2$) is NLT 0.99.

**Limit of detection (LOD) and limit of quantification (LOQ)**

LOD and LOQ of all residual solvents MeOH, DEE, IPA, EA, TCE and TOL were determined by Signal-to-Noise method. LOD and LOQ Solution were prepared in the range of 0.03% and 0.1% with respect to test respectively and injected in six times. The signal-to-noise ratio (S/N) in LOQ solution should be about 10:1 for all residual solvents. The signal-to-noise ratio (S/N) in LOD solution should be about 3:1 for all residual solvents.

**Linearity in presence of sample matrix (Recovery)**

Accuracy of the method was determined by recovery studies. MeOH, DEE, IPA, EA, TCE and TOL were spiked in pre-analyzed test of zonisamide and its percent recovery was determined. The data of Recovery solution (Matrix solution) done as part of Accuracy experiment is used to establish the linearity in presence of sample matrix. Each Recovery solution (20% to 200%) is spiked in pre-analyzed test of zonisamide analyzed. The corrected concentration and the corresponding peak area are used to estimate the linearity. The mean values of the determinations for the six solvents studied were should be between 80% to 120%.

The % RSD for recovery solution is not more than 15.0%. Correlation coefficient ($R^2$) is NLT 0.99.

## CONCLUSIONS

The test method for the Residual Solvents analysis for of zonisamide is specific, precise, accurate, linear and rugged. Hence the method can be used for the estimation of Residual solvent of zonisamide. An excellent resolution (R) obtained for all solvents; between MeOH and DEE is 9.81, DEE and IPA is 5.05, IPA and EA is 18.19, EA and TCE is 16.58, and TCE and TOL is 23.43. Tailing factor (TF) for Methanol 1.32, Diethyl ether 1.12, IPA 1.09, Ethyl acetate 1.07, TCE 1.02 and Toluene 1.01 was obtained and theoretical plate (TP) for Methanol 17539, Diethyl ether 12806, IPA 13279, Ethyl acetate 15633, TCE 16309, Toluene 52334. The described found to be precise %RSD for standard between 3.9% to 5.53% and for test between 1.71% to 3.17% and rugged as % deviation with

precision result did not deviate significantly between 4.91% to 8.90% (TABLE 3, 4).

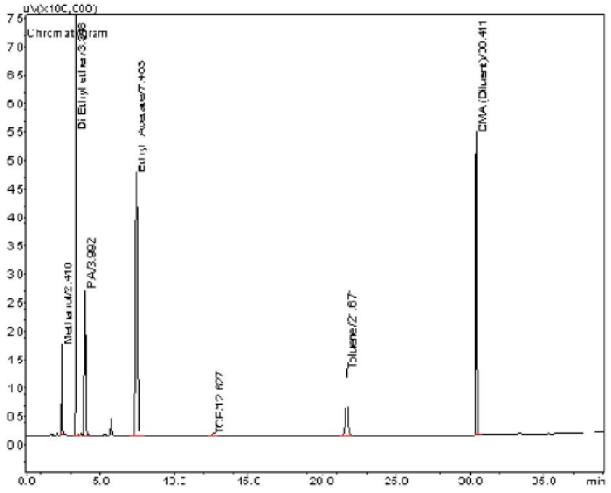



**Figure 1 : GC Chromatogram of working standard solution. Chromatogram with peak retention time for respective solvents, Methanol 2.4, Diethyl ether 3.3, IPA 3.9, Ethyl acetate 7.4, TCE 12.6, Toluene 21.6 and DMA (diluent) 30.4**

**TABLE 3 : System suitability results**

| Parameters | Sub Parameters | Name of the Solvents | | | | | |
|---|---|---|---|---|---|---|---|
| | | MeOH | DEE | IPA | EA | TCE | TOL |
| System Suitability | Retention Time (RT) | 2.41 | 3.34 | 3.99 | 7.39 | 12.62 | 21.66 |
| | Tailing Factor (TF) (NMT 2.0%) | 1.32 | 1.12 | 1.09 | 1.07 | 1.02 | 1.01 |
| | Theoretical plate (TP) (NLT 2000) | 17539 | 12806 | 13279 | 15633 | 16309 | 52334 |
| | Resolution (R) (NLT 2.0 %) | -- | 9.81 | 5.05 | 18.19 | 16.58 | 23.43 |

**Note : RT- Retention Time, TF - Tailing Factor, TP - Theoretical plate, R- Resolution, ND-Not detected, MeOH- Methanol, DEE-Diethyl ether, IPA-Isopropyl alcohol, EA-Ethyl acetate, TCE- 1,1,2-Trichloroethene, TOL-Toluene**

**TABLE 4 : Precision and ruggedness results**

| Parameters | Sub Parameters | Name of the Solvents | | | | | |
|---|---|---|---|---|---|---|---|
| | | MeOH | DEE | IPA | EA | TCE | TOL |
| Precision | % RSD for Standard (NMT 15%) | 3.90 | 5.53 | 4.60 | 5.46 | 5.28 | 5.47 |
| | % RSD for Test (NMT 15%) | 1.71 | ND | 3.17 | ND | ND | 2.17 |
| Ruggedness | % RSD | 1.82 | 0.85 | 1.75 | 0.93 | 0.79 | 0.93 |
| | % D with precision | 4.91 | ND | 5.89 | ND | ND | 8.90 |

Linearity of the method was evaluated from triplicate injections of standard solutions of mixtures of analytes prepared in diluents with a concentration ranged from 20% to 200% of MeOH 61.3-613 ppm, DEE 103.3-1033 ppm, IPA 100.6-1006, EA 100.6-1006, TCE 2.28-22.8 ppm and TOL 17.8-178 ppm. The %RSD of the peak area in the range of MeOH 0.15-6.04 %, DEE 0.23-6.89%, IPA 0.21-7.48%, EA 0.09-7.89%, TCE 1.0-7.76%, and Toluene 0.16-7.76% respectively. Linear for all solvents, there is no non-linear trend at the ends of the plotted fitted line, Correlation coefficients ($R^2$) was found to be more than 0.99 was obtained for each individual solvent in this study. LOD and LOQ for all solvents were determined by Signal-to-Noise method. LOD for MeOH is 1.85 ppm, DEE is 3.1 ppm, IPA is 3.02 ppm, EA is 3.02 ppm, TCE is 0.07 ppm, TOL is 0.55 ppm and LOQ for MeOH is 6.13 ppm, DIE is 10.33 ppm, IPA is 10.06 ppm, EA is 10.06 ppm, TCE is 0.228ppm, TOL is 1.85 ppm with respect to zonisamide drug matrix (test concentration) respectively (TABLE 5)

**TABLE 5 : Linearity and LOD and LOQ results**

| Parameters | Sub Parameters | Name of the solvents | | | | | |
|---|---|---|---|---|---|---|---|
| | | MeOH | DEE | IPA | EA | TCE | TOL |
| Linearity | Concentration µg/ml | 61.3 – 613 | 103.0 -1030 | 100.6 – 1006 | 100.6 – 1006 | 2.28 – 22.8 | 17.8 – 178 |
| | % RSD | 0.15 – 6.04 | 0.23 – 6.89 | 0.21 – 7.48 | 0.09 – 7.89 | 1.0 – 7.76 | 0.16 -7.69 |
| | Correlation coefficient ($r^2$) | 0.999 | 0.996 | 0.998 | 0.997 | 0.996 | 0.996 |
| | LOD ppm | 1.85 | 3.10 | 3.02 | 3.02 | 0.07 | 0.55 |
| | LOQ ppm | 6.13 | 10.33 | 10.06 | 10.06 | 0.228 | 1.85 |

Linearity in presence of sample matrix (recovery) of the method was evaluated from triplicate injections of standard solutions of mixtures of analytes prepared in diluents with a corrected concentration with respect to precision test result ranged from 20% to 200% of MeOH 73.59-735.9 ppm, DEE 100.35-1003.5 ppm, IPA 135.77-1357.7, EA 100.59-1005.9, TCE 2.28-22.8 ppm and TOL 19.87-198.7 ppm. The %RSD of the peak area in the range of MeOH 0.46-7.56 %, DEE 0.14-2.37%, IPA 0.29-6.19%, EA 0.39-1.98%, TCE 0.32-3.76%, and Toluene 0.22-1.18% respectively. Linear for all solvents, there is no non-linear trend at the ends of the plotted fitted line, Correlation coefficients ($R^2$) was found to be more than 0.99 was obtained for each individual solvent in this study Accurate, the %Re-

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covery is between 94.0% to 103.3% for all solvents. (TABLE 6).

**TABLE 6 : Linearity In presence of sample matrix (Recovery) results**

| Parameters | Sub Parameters | Name of the solvents | | | | | |
|---|---|---|---|---|---|---|---|
| | | MeOH | DEE | IPA | EA | TCE | TOL |
| Linearity with sample matrix | Concentration in ppm (spiked) | 73.59– 735.9 | 100.35 – 1003.5 | 135.77 – 1357.7 | 100.59 – 1005.9 | 2.28 – 22.8 | 19.87 – 198.7 |
| | % RSD | 0.46 – 7.56 | 0.14 – 2.37 | 0.29 – 6.19 | 0.39 – 1.98 | 0.32 – 3.76 | 0.22 – 1.18 |
| | Correlation coefficient ($r^2$) | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 |
| Accuracy | % Recovery | 96.31 | 92.37 | 103.3 | 94.0 | 97.5 | 94.2 |

This demonstrates that the developed GC-HS method is new, simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be used for the determination of residual solvents in zonisamide.

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