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# Head Space Gas Chromatography Analysis of Residual Solvents in Temozolomide by Using Zb-624 Column

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**Abstract:** *Residual solvents are organic volatile chemicals that are used in the manufacture of active pharmaceutical ingredients, excipients and drug products. As there is no therapeutic benefit from these residual solvents and also affect the quality and stability of not only the drugs but also drug product, they should be removed to the extent possible to meet product specifications and other quality based requirements. These residual solvents cannot be removed completely; hence they should be within the acceptance limits as per the regulatory guidelines such as ICH guidelines (Q3C). GCHS is the most commonly used technique used for the analysis of volatile solvents. So the aim of the present work is to develop a simple, specific GC-HS method for the determination of residual solvents in temozolomide using nitrogen as the carrier gas at the rate of 4.16ml/min with ZB-624 (30mx0.53mm, 5µ) as column using FID as detector. The developed method was validated as per ICH guidelines and all the parameters are found to be within the limits.*

**Keywords:** *Temozolomide, ZB-624 and Flame Ionization detector.*

## 1. INTRODUCTION

Temozolomide [1] (Fig.1) is an oral chemotherapy drug and chemically it is 4-methyl-5-oxo-2, 3, 4, 6, 8-pentazabicyclonona-2, 7, 9-triene-9-carboxamide. It is an alkylating agent used in the treatment of Grade IV astrocytoma-an aggressive brain tumor as well as melanoma-a form of skin cancer. It undergoes rapid chemical conversion in the systemic circulation at physiological PH to the active compound, 3-methyl-(trizen-1-yl) imidazole-4-carboxamide. Temozolomide [2] exhibits schedule dependant antineopalstic activity by interfering with DNA replication.

From the scheme of synthesis of temozolomide the volatile solvents [3] used at various steps are methanol (class II), acetone (class III), MDC (class II) and DMF (class II). Literature survey revealed very few analytical methods for the estimation of temozolomide by RP-HPLC [4] and UV Spectroscopy [5] method but there is no single GC-HS method for the determination of the residual solvents in temozolomide. Hence the main objective of the present study is to develop a simple GC-HS method for identification and quantification of residual solvents in temozolomide.

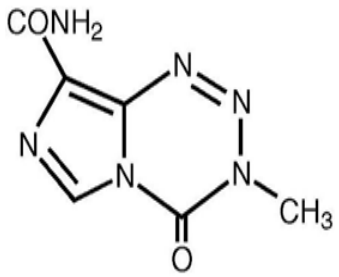


**Fig1.** *Chemical Structure of Temozolomide*

## 2. LITERATURE REVIEW

There are various analytical techniques available in the literature for quantitative determination of temozolomide but there is no single method for the determination of residual solvents in temozolomide. So the author made an attempt to develop a new GC-HS method for the determination of residual solvents.

## 3. MATERIALS AND METHOD

### 3.1. Instrumentation and Chromatographic Conditions

*3.1.1. Head space gas chromatography* [6]

**"**Headspace" is the gas space present above the sample in a chromatography vial. Volatile sample components diffuse into the gas phase, forming headspace gas. Hence it is the analysis of the components present in that gas.

Chromatography analysis was carried out by using GC-HS instrument whose make is shimadzu, model: GC – 2010 and 2010 plus with a TELEDYNE TEKMAR headspace sampler. Gas chromatograph was equipped with standard oven for temperature ramping, split injection port and flame ionization detector. The analyte of interest were separated on a ZB-624 (6%-Cyano propyl phenyl & 94%- Dimethyl poly siloxane) capillary column with nitrogen as carrier gas in split mode by head space injection. Volume of 1ml of standard and sample solution was injected into gas chromatograph injection port using headspace sampler. The injector port temperature was maintained at $220^0$c and split ratio 1:5, with Nitrogen as carrier gas. The column flow was maintained at 4.16ml/min with constant mode. The detector temperature was maintained at $250^0$c. The optimized chromatographic conditions are presented in Table 1.

### 3.2. Residual Solvents Used

Methanol, acetone, dichloromethane (MDC) and dimethyl formamide (DMF) were obtained from Merck – Mumbai and used as such.

### 3.3. Preparation of Blank

5ml of N-methyl-2-pyrrolidone was transferred using a pipette into a headspace vial and sealed.

### 3.4. Preparation of Mixed Standard Solution

A standard stock solution was prepared such that the final conc. Contains 3.8µl of methanol, 6.3µl of acetone, 0.5µl of MDC and 0.9µl of DMF by using N-methyl-2-pyrrolidone as diluents. From this 5ml of solution was transferred into headspace vial and sealed.

Standard solution was prepared according to the ICHQ (2) rules. By this method, the quantity of each solvent was calculated by using the following formula.

$$\frac{\text{Solvent ICH limit} \times \text{sample weight}}{\text{Sample dilution}} = A$$

$$\frac{\text{Density of solvent}}{\text{Standard dilution}} \times 10^6 = B$$

$$\frac{\text{A}}{\text{B}} = \mu \text{ ml}$$

### 3.5. Preparation of Sample

100mg of temozolomide drug sample was weighed and transferred into a headspace vial, dissolved in 5ml of N-methyl-2-pyrrolidone and sealed.

## 4. METHOD DEVELOPMENT

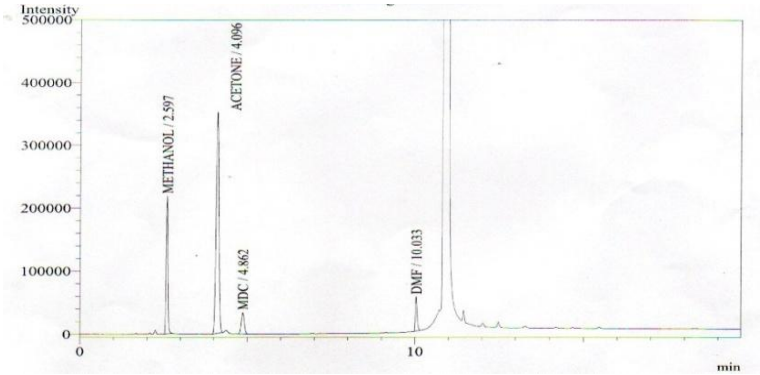


**Fig2.** *Chromatogram of mixed standard solution*

**Table1.** *Optimised chromatographic conditions*

| S.No. | Parameters | Values |
|---|---|---|
| 1. | Column | ZB-624 |
| 2. | Dimension | 30mx0.53mm |
| 3. | Detector | Flame ionization detector |
| 4. | Detector temperature | $250^0$ C |
| 5. | Injector temperature | $220^0$ C |
| 6. | Injector volume | 1ml vapor |
| 7. | Column Programming | $40^0$C hold for 5 min, rise at $30^0$C/min to $240^0$C hold for 8 min. |
| 8. | Run time | 19.67 min. |
| 9. | Split ratio | 1:5 |
| 10. | Carrier gas | 4.16ml/min(Nitrogen) |
| 11. | Makeup gas | 25ml/min(Nitrogen) |
| 12. | Vial equilibrium temperature | $90^0$C |
| 13. | Loop temperature | $100^0$C |
| 14. | Transfer line temperature | $110^0$C |
| 15. | Vial equilibration time | 30 min. |
| 16. | GC cycle time | 22 min. |
| 17. | Loop fill time | 0.15 min. |
| 18. | Loop equilibration time | 0.2 min. |
| 19. | Injection time | 1.0 min. |

A method was developed by performing several trials with diluents like Dimethyl acetamide, dimethyl sulfoxide and N-methyl-2-pyrrolidone (NMP) but the final trail using NMP was selected. All the parameters were optimized based on the acceptance limits of ICH (Table1). Blank, standard and sample each of 5ml was pipette out into the headspace and their chromatograms were recorded (fig.2).

## 5. METHOD VALIDATION

All the parameters are validated as per ICH guidelines.

### 5.1. System Suitability

Accurately 3.8µl of Methanol, 6.3µlof Acetone, 0.5µlof Dichloromethane, 0.9µlof Dimethyl formamide was transferred using a micro syringe into a 50ml volumetric flask containing 35 ml ofN-methyl-2-pyrrolidone and was mixed well. The volume was made up to the level withN-methyl-2-pyrrolidone (NMP).From this 5ml of solution was pipetted into 6 head space vial fitted with septum and sealed with the sealer to study system suitability parameters. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates and resolution between the peaks were determined. The results obtained are presented in table 2.

**Table2.** *Results for the chromatograms for system suitability*

| Solvent Name | Retention Time n=6 | Avg Area n=6 | Resolution | Tailing Factor n=6 | Theoretical Plates n=6 |
|---|---|---|---|---|---|
| METHANOL | 2.653 | 287487 | 0 | 1.566 | 12708.205 |
| ACETONE | 4.084 | 1432005 | 11.22 | 1.326 | 10218.342 |
| MDC | 4.853 | 33929 | 4.235 | 0.897 | 92171.014 |
| DMF | 10.946 | 37328 | 36.423 | 1.658 | 107855 |

### 5.2. Specificity

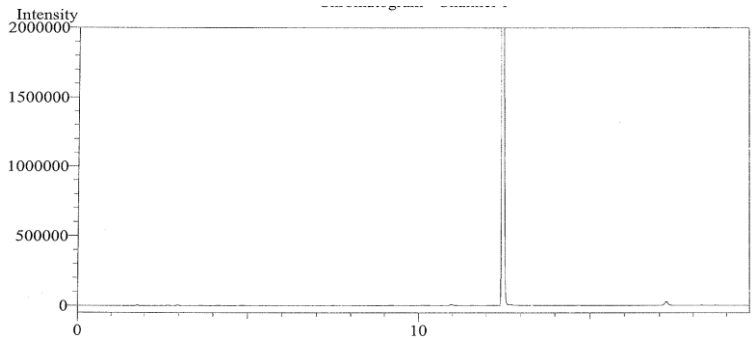


**Fig3.** *Chromatogram of blank for Specificity*

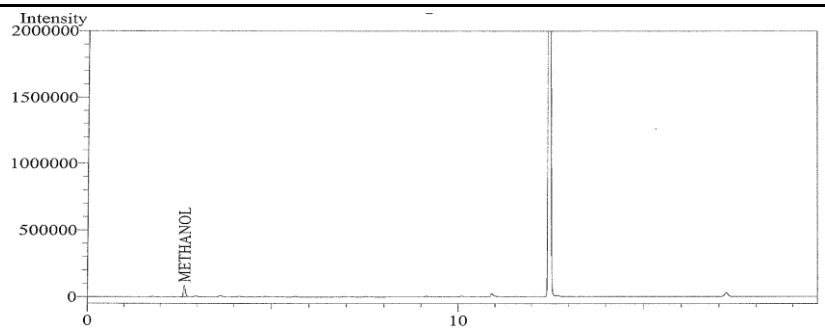


**Fig4.** *Chromatogram of individual Standard solution of Methanol*

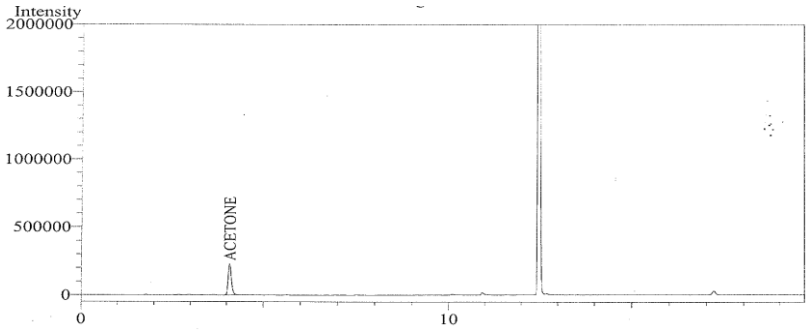


**Fig5.** *Chromatogram of individual Standard solution of Acetone*

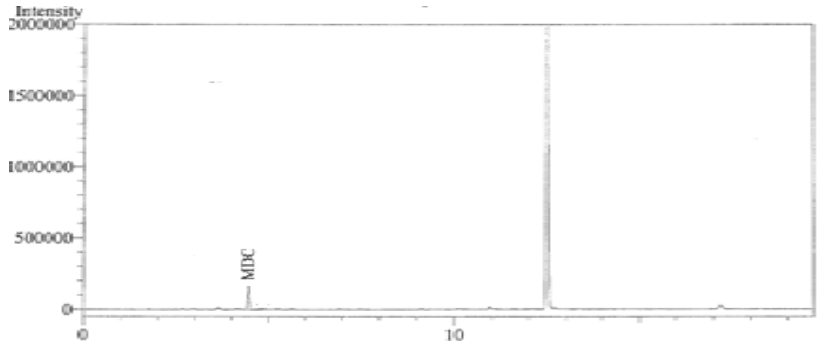


**Fig6.** *Chromatogram of individual Standard solution of MDC*

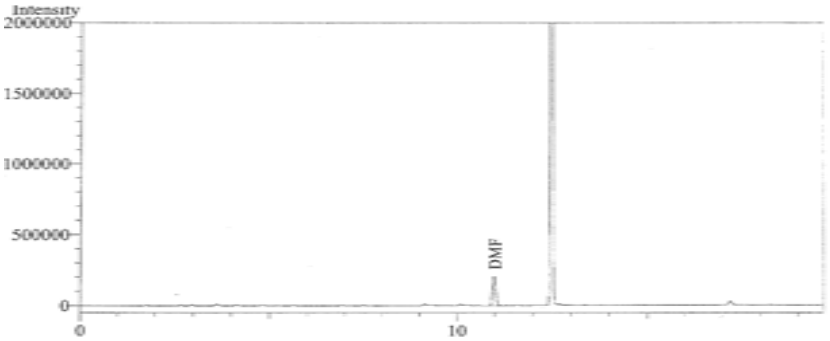


**Fig7.** *Chromatogram of individual Standard solution of DMF*

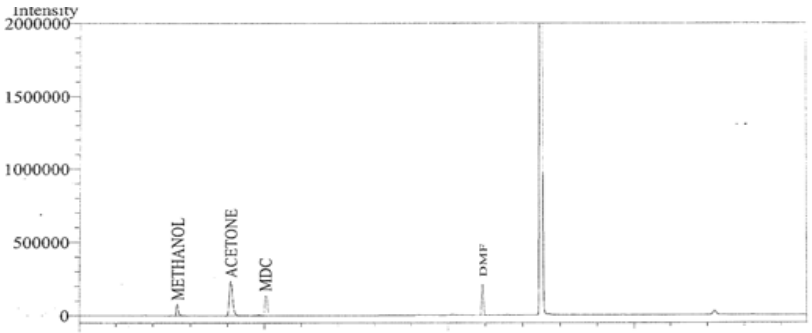


**Fig8.** *Chromatogram of mixed Standard solution*

Specificity study of the method was carried out by injecting a blank i.e.; Diluent (N-methyl-2-pyrrolidine), a mixed standard solutions, the pure drug sample solution and individual solvents such as methanol, acetone, MDC and DMF into Gas chromatography and their retention times were obtained from the respective chromatograms (Fig.3 to 8represents individual solvent chromatograms and mixed standard solution).The Rt of individual drugs are shown in the Table 3.

**Table3.** *Data of specificity study*

| Solvent name | Area | Retention time |
|---|---|---|
| METHANOL | 299302 | 2.653 |
| ACETONE | 1469995 | 4.084 |
| MDC | 35750 | 4.853 |
| DMF | 56097 | 10.946 |

## 5.3. Method Precision

Method Precision was carried out by injecting one batch of sample at 100% concentration 6 times into the headspace sampler and % RSD values were calculated and tabulated in Table. 4.

**Table4.** *Results of precision study*

| Solvent Name | Average Area n=6 | SD n=6 | % RSD |
|---|---|---|---|
| METHANOL | 31449 | 939 | 2.9 |
| ACETONE | 18449 | 599 | 3.2 |
| MDC | 2261 | 18.05 | 0.81 |
| DMF | 3903 | 19 | 0.48 |

*SD-Standard deviation, %RSD –Percent relative standard deviation*

## 5.4. Linearity

### *5.4.1. Preparation of 150% Solution*

Accurately 5.7 µl of methanol, 9.45 µl of acetone, 0.75 µl of MDC, 1.35 µl of DMF was transferred using a micro syringe into a 50 ml volumetric flask containing 35 ml of N-methyl-2-pyrrolidine (diluents) and was mixed well. The volume was made up to level with N-methyl-2-pyrrolidone. From this 5ml of solution was pipette into 3 headspace vials fitted with septum and sealed with sealer.

### *5.4.2. Preparation of 125% Solution*

Accurately 41.6 ml of above 150% solution was transferred into a 50 ml volumetric flask and diluted up to the mark with diluent (N-methyl-2-pyrrolidone). From this 5ml of solution was pipetted into 3 headspace vial fitted with septum and sealed.

### *5.4.3. Preparation of 100% Solution*

Accurately 33.3ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluents (N-methyl-2-pyrrolidine). From this 5ml of solution was pipetted into 3 headspace vials fitted with a septum and sealed.

### *5.4.4. Preparation of 75% Solution*

Accurately 25.0 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluents (N-methyl-2-pyrrolidone). From this 5ml of solution was pipetted into 3 headspace vials fitted with septum and sealed.

### *5.4.5. Preparation of 50% Solution*

Accurately 16.6 ml of above 150% solution was transferred into a 50 ml volumetric flask and was diluted up to the mark with diluents (N-methyl-2-pyrrolidone). From this 5 ml of solution was pipetted into 3 headspace vials fitted with septum and sealed.

### *5.4.6. Procedure*

Linearity study of the method was carried out by injecting each 50%, 75%, 100%, 125% and 150% standard solution in triplicate into the headspace. Five point calibration curves were plotted by taking average peak areas of solvents on y-axis and corresponding concentration on the x-axis (Fig.9). Linearity has been confirmed by statistical analysis and respective correlation coefficients and regression equations were calculated and the values are given in Table 5.

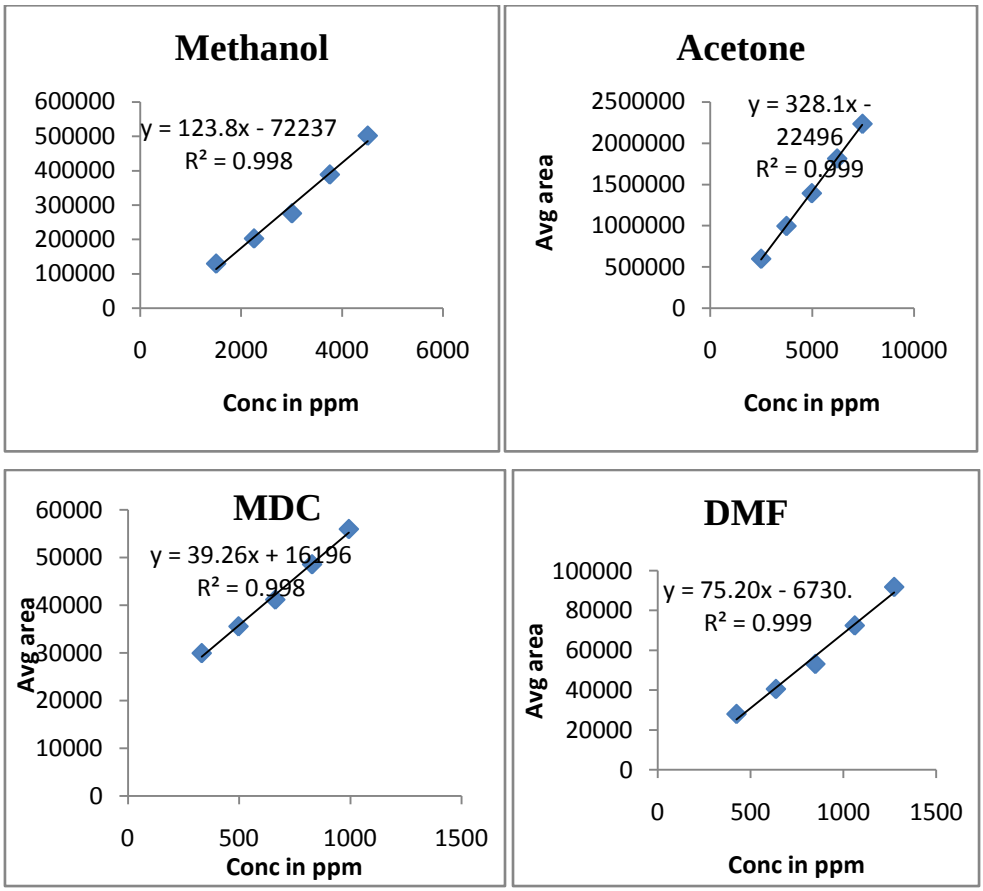


**Fig9.** *Linearity curves of the residual solvents*

**Table5.** *Linearity data*

| Solvent Name | Average Peak Area | | | | | Correlation coefficient | Regression Equation |
|---|---|---|---|---|---|---|---|
| | Concentration at | | | | | | |
| | 50% | 75% | 100% | 125% | 150% | | |
| METHANOL | 129877 | 202999 | 276121 | 389151 | 502182 | 0.998 | y = 123.8x - 72237 |
| ACETONE | 600796 | 997489 | 1394182 | 1814005 | 2233829 | 0.999 | y = 328.1x - 22496 |
| MDC | 26915 | 35530 | 41145 | 48538 | 55931 | 0.998 | y = 39.26x + 16196 |
| DMF | 27954 | 40536 | 53118 | 72500 | 91882 | 0.999 | y = 75.20x - 6730 |

### 5.5. Limit of Detection and Limit of Quantification

LOD and LOQ values were determined by signal-to-noise ratio (S/N) method by injecting each standard solution 6 times at its DL and QL concentration level. The results obtained are presented in table 6 and 7.

**Table6.** *Results of Limit of detection data*

| Solvent Name | Level S/N | Detection Limit | Avg Area (n=6) | SD (n=6) | %RSD |
|---|---|---|---|---|---|
| METHANOL | 1049.6 | 0.001887 | 1321 | 149 | 11.25 |
| ACETONE | 3023.6 | 0.000779 | 3476 | 354 | 10.18 |
| MDC | 153.921 | 0.019349 | 2160 | 195 | 9.04 |
| DMF | 82.5 | 0.011030 | 822 | 80 | 9.76 |

**Table7.** *Results of Limit of quantification data*

| Solvent Name | Level S/N | Quantification Limit | Avg Area (n=6) | SD (n=6) | %RSD |
|---|---|---|---|---|---|
| METHANOL | 1049.6 | 0.0062271 | 3761 | 293 | 7.80 |
| ACETONE | 3023.6 | 0.0025707 | 11094 | 1205 | 10.87 |
| MDC | 153.921 | 0.0638517 | 7016 | 226 | 3.22 |
| DMF | 82.5 | 0.0330921 | 2665 | 146 | 5.50 |

### 5.6. Robustness

This study was performed by making small but deliberate variations in the method parameters and observing the changes. The effects of variation were $\pm 5^0$C change in the column oven temperature and ±2ml/min in the column flow. A blank (N-methyl-2-pyrrolidone), mixed standards and a pure drug sample solution was introduced into the headspace sampler (n=6) and concentration of each solvent was calculated.

### 5.7. Ruggedness

Ruggedness study of the material was carried out by injecting mixed standard solution by two different analysts on two different days and concentrations of four solvents were calculated.

### 5.8. Batch Analysis

Batch analysis was carried out by injecting a pure drug sample solutions and a marketed formulation sample solution into the headspace.

*5.8.1. Preparation of Marketed Formulation*

A weighed quantity equivalent to 100 mg of temozolomide marketed formulation was transferred into 20 ml headspace vial and 5ml of N-methyl-2-pyrrolidone (diluent) was added to the same vial fitted with septum and sealed and the chromatogram was recorded (Fig.10). The results are presented in the table 8 and 9.

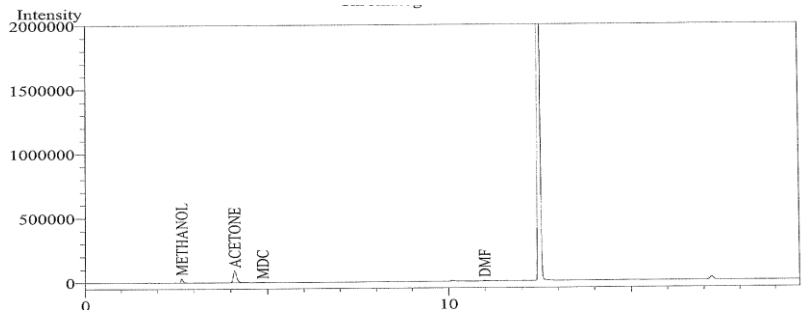


**Fig10.** *Chromatogram of formulation of temozolamide*

**Table8.** *Results obtained from chromatogram of pure drug sampleand marketed formulation*

| **Solvent Name** | **Avg. Area** | | **Avg. ppm** | | **Actual ppm** (limit) |
|---|---|---|---|---|---|
| | **Pure drug** | **Marketed form** | **Pure drug** | **Marketed form** | |
| METHANOL | 2799 | 26989 | 290 | 275 | 3000 |
| ACETONE | 18083 | 17063 | 63 | 52 | 5000 |
| MDC | 2262 | 2154 | 44 | 35 | 600 |
| DMF | 3908 | 3814 | 90.3 | 83.7 | 880 |

### 5.9. Accuracy

A recovery study was carried out by standard addition method at three different levels i.e.; 50%, 100% and 150%. The percentage recoveries of methanol, acetone, MDC and DMF in the sample mixture was determined by statistical evaluation and given in Table 10.

**Table9.** *Accuracy of the proposed method*

| **Solvents** | **Level** | **Avg. peak area** | | | **%Recovery** | **Mean % Recovery** |
|---|---|---|---|---|---|---|
| | | **Spiked solution** | **Non spiked solution** | **Standard solution** | | |
| Methanol | 50% | 10250 | 119627 | 129877 | 85 | 86.73 |
| | 100% | 20500 | 255621 | 276121 | 86.5 | |
| | 150% | 30750 | 471432 | 502182 | 88.7 | |
| Acetone | 50% | 8981 | 591815 | 600796 | 97 | 97.2 |
| | 100% | 17962 | 1376220 | 1394182 | 97.4 | |
| | 150% | 26943 | 2206886 | 2233829 | 97.5 | |
| MDC | 50% | 1131 | 28784 | 29915 | 92 | 89.5 |
| | 100% | 2262 | 38883 | 41145 | 89 | |
| | 150% | 3393 | 52538 | 55931 | 87.5 | |
| DMF | 50% | 1954 | 26000 | 27954 | 86 | 86.16 |
| | 100% | 3908 | 49213 | 53118 | 85 | |
| | 150% | 5862 | 86020 | 91882 | 87.5 | |

## 6. RESULTS AND DISCUSSION

### 6.1. Method Development

*Column selection:* The importance of column selection was to resolve i.e. separate and quantify the four volatile solvents (methanol, acetone, MDC and DMF) used in the manufacture of temozolomide. Several trails were made finally ZB-624 column (30 m length, 0.53 mm internal diameter and 5µ particle size with stationary phase of 6% cyano propyl phenyl & 94% Dimethyl poly siloxane) was found to be the best one for separation of all 4 solvents in less time.

*Thermal programming:* Trails were performed by changing linear thermal gradients, among them an initial temperature of $40^0$C hold for 5min and linear thermal gradient to $240^0$C at $30^0$C/min was found to elute all the solvents and showing the resolution more than 2.

### 6.2. Method Validation

*System suitability:* System suitability parameters like asymmetry and resolution were calculated to evaluate the chromatographic parameters. The number of theoretical plates for the six replicate injections of mixed standard solution was found to be more than 3000, tailing factor was found to be less than 2 and the resolution between any two adjacent peaks were more than 2.0. The system suitability parameters were found to be in the acceptable range, which indicates suitability of system for the quantification of these solvents by this method.

*Specificity:* It was observed that the blank chromatogram did not show any interference with the solvents peaks. Rt of individual sovents are compared with Rt of solvents peaks of sample and Rt values for methanol, acetone, MDC, DMF were found to be 2.653min, 4.084min, 4.853min and 10.946min respectively.

*Method precision:* It was done by injecting one batch of sample at 100% concentration for six times. For each solvent, from chromatogram peak areas % relative standard deviation was calculated. % RSD for four solvents was found to be less than 15%. Hence the method is precise.

*Linearity:* Linearity is performed from 50-150% and graphs obtained were found to be linear showing correlation coefficient $R2 \geq 0.998\%$.

*Detection (DL) and Quantification (QL) Limit:* The DL and QL for all solvents was determined by signal-to-noise ratio (S/N) method. The minimum concentration (ppm) at 3:1 S/N (for DL) and the quantification concentration are at 10:1 S/N (for QL).

*Robustness:* It was performed by making small variations in optimized parameters such as flow was done 3.9 and 4.4 ml/min, initial column oven temperature was changed to $35^0$C and $45^0$c, headspace vial equilibration time was changed from 30min to 25min and 35 min and vial equilibration temperature of headspace study was performed by changing from $90^0$C to $85^0$C and $95^0$C. There was no marked change in %RSD, hence the method is said to be robust.

*Ruggedness:* It was found that the method was accurate as it was producing same results even though the analyst and day of performing the experiment were changed. The % RSD value was less than 15%.

*Batch analysis:* Batch analysis was performed by injecting two test samples and a formulated product of a batch and whose results were found to be within the limits and the values for methanol, acetone, MDC, DMF were found to be 290ppm, 63ppm, 44ppm and 90.3ppm where as the acceptable limit is 3000ppm, 5000ppm, 600ppm and 880ppm.

*Accuracy:* Accuracy of the method was done by recovery experiments by spiking known amount of each solvent at quantification limit 50%, 100% and 150% of 5000ppm to the test solution. Each preparation was analyzed in triplicate (n=3) and percent recovery was calculated. The recovery was found to be between 86.16 and 97.2.

## 7. CONCLUSION

Excellent results were obtained, with an global validation reference values, particularly low taking into accounts the low concentration levels investigated. The test method was validated and had good reproducibility and linearity for the solvents used in the manufacture process.

Hence it was concluded that the suggested method is simple, rapid, precise, accurate and cost effective that can be effectively applied for the routine analysis in research institutions, quality control departments and clinical pharmacokinetic studies for the determination of residual solvents in pure drug and marketed formulation.

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