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# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR RESIDUAL SOLVENT DICHLOROMETHANE IN VILAZODONE BY GAS CHROMATOGRAPHY TECHNIQUE

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## ABSTRACT

This paper describes the analytical method for development and validation for residual solvent Dichloromethane in 3-(4-Chlorobutyl)-1H-Indole-5-Carbonitrile by Gas chromatography technique. Proposed method is proven to be sensitive, simple and accurate for quantification of residual Dichloromethane in Vilazodone Hydrochloride. Retention time of Dichloromethane was found to be 6.3 minutes with septum purge flowrate of 3 mL/min by using Flame Ionization detector.The test method is linear for Dichloromethane over the range of LOQ to 150% of target concentration for the analyte response with an acceptable correlation coefficient values at 100% drug. The test method is accurate over the range of LOQ to 150% of target concentration for the estimation of residual solvent in 3-(chlorobutyl)-1H-indole-5-carbonitrile.The test method is rugged for day to day and analyst to analyst variability.

**KEYWORDS:** Dichloromethane; Residual; Vilazofone Hydrochloride; GC method.

## 1. INTRODUCTION

Vilazofone is a seratogenic antidepressant[1,2], In some ways, its activity can be conceptualized as a combination of an SSRI and Buspirone. Structure of Vilazodone is shown in the Fig 1. Literature survey reveals that GC method validation has never been reported for the estimation of residual solvent Dichloromethane in 3-(-4 chlorobutyl) - 1H- Indole- 5-carbonitrile. Only few HPLC, LC-MS methods were developed for estimation of 3-(-4 chlorobutyl) - 1H- Indole- 5- carbonitrile in bulk, formulation and biological samples[4,5,6,7,8].

So here an attempt has been made to develop GC method validation for the estimation of residual solvent Dichloromethane in 3-(-4 chlorobutyl) - 1H- Indole- 5- carbonitrile. The validation study for the parameters namely system suitability, specificity , system precision, method precision, limit of detection, limit of quantitation, linearity, accuracy and range.

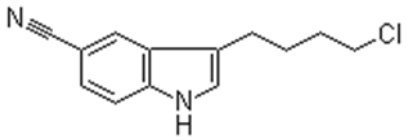


**Fig 1: Structure of 3-(-4 chlorobutyl) - 1H- Indole- 5- carbonitrile.**

## 2. MATERIALS AND METHODS

### 2.1: Reagents and reference materials

All the reagents and chemicals used were of analytical grade

### 2.2: Apparatus and equipment

**Table 1: Apparatus andEquipment.**

| Gas chromatography | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Make** | **Model number** | | **Instrument ID. number** |
| 1. | Shimadzu | 2014 | | TBL/QC/INS/34 |
| 2. | Shimadzu | 2010 Plus | | TBL/QC/INS/50 |
| **Analytical balance :** | | | | |
| **Sr. No.** | **Make** | **Model number** | | **Equipment ID. number** |
| 1. | Sartorius | CPA225D | | TBL/QC/INS/45 |
| **Capillary column :** | | | | |
| **Sr. No.** | **Make** | **Stationary phase** | **Column dimensions** | **Column ID.** |
| 1. | AT-624 | G-43 | 30mX0.53mmX3.0µm | GC/12 |

### 2.4. Procedures

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9µl of Dichloromethane, into a 10mL volumetric flask containing about 5mL of diluent and dilute to volume with diluent.

**Preparation of standard solution**

Transfer 300µl of the standard stock solution into a 10mL volumetric flask containing 5mL of diluents and make up to volume with the diluent. The above solution contains about 600ppm of dichloromethane with respect to 60mg/mL test concentration.

**Preparation of test solution**

Weigh accurately about 600mg of the test sample transferred into a 10mL volumetric flask, dissolve and make up to the volume with diluent.

**Procedure**

After equilibrating the column, inject the blank solution and apply blank correction in case of any interference from the solution used a diluent. Separately inject standard solution for six times and calculate the % RSD and record the chromatograms.

**System Suitability acceptance criteria:** The % RSD for dichloromethane calculated from the area of 6 injections of standard solution is NMT 15.0.

**Calculation**

Calculate the content of the dichloromethane in ppm, using the following equation:

$$\frac{(\text{Area of solvent peak} - \text{Area of the blank peak})}{(\text{Average area of solvent} - \text{Area of the blank peak})} \times \frac{(\text{solvent taken in } \mu\text{L} \times \text{density})}{10\text{mL}} \times \frac{0.3\text{ mL}}{10\text{mL}} \times \frac{\text{sample dilution}}{\text{Wt. of sample}} \times 10^6$$

## 3. RESULTS AND DISCUSSION

### 3.1: Method development

The method developed for the estimation of residual solvent Dichloromethane in 3-(4-CHLOROBUTYL)-1H- INDOLE-5-CARBONITRILE BY GC based on its physicochemical properties. As MDC polar molecule and, therefore, a polar solvent dimethylsulfoxide (DMSO) was used as the diluents because of its high boiling point. The capillary column coated with 6-Cyanopropyl, l% Phenyl, 94% Dimethylpolysiloxane is a good choice for separation of this analyte since they elute as symmetrical peaks at a wide range of concentrations. The GC-FID parameters used in the method development were based on the boiling point. The injection port and detector temperature were set to 140 ºC and 260 ºC respectively. Different temperature programs were investigated for GC oven. The end of this investigation, the best temperature program was selected for a good resolution. The temperature programs of the GC oven with a run time of 39 min was as follows: initial temperature 45ºC, held for 5 min, increased to 150ºC min at a rate of-10º C held for 5 min, and finally to 250º C at a rate of 40ºC and held for 15 min. The head pressure was set to

ensure a nitrogen flow of 2.5 psi. The split mode was chosen. The solvent, column and acquisition parameters were chosen to be a starting point for the 3-(CHLOROBUTYL)-1H-INDOLE-5-CARBONITRILE by GC and the retention time of 6.3 min with good peak shape was found. No further optimization of the method was required. Additionally, preliminary precision studies performed during the development of the method injection volume were adjusted to 1µl showed the reproducible and the peak response was significant at the analytical concentration chosen.

The detailed Chromatographic conditions described in the Table.2.

**Table 2: Chromatographic Conditions.**

| **Column** | **AT-624** |
|---|---|
| Type | 30mx0.53mmx3.0µ |
| Carrier gas | Nitrogen |
| Injector temperature | 140°C |
| Pressure | 2.5psi |
| Septum Purge flow | 3mL/min |
| Split | On |
| Split ratio | 1:5 |
| Injection volume | 1.0µL |
| Oven temperature-1 | 45°C |
| Hold time-1 | 5min |
| Ramp-1 | 10°C per min |
| Oven temperature-2 | 150°C |
| Hold time-2 | 5 min |
| Ramp-2 | 40°C per min |
| Oven temperature-3 | 250°C |
| Hold time-3 | 15 min |
| Type | Flame Ionization Detector |
| Hydrogen flow | 50KPa |
| Zero Air flow | 50KPa |
| Make up flow | 25mL/min |
| Detector temperature | 260°C |

### 3.2: Method validation parameters

### Method Validation[3,9]

The following parameters were used to validate the method for the estimation of residual solvent Dichloromethane in 3-(-4 chlorobutyl) - 1H- Indole- 5- carbonitrile.

**System suitability**

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane, into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about, 600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**System Suitability acceptance criteria:** The % RSD for dichloromethane calculated from the area of 6 injections of standard solution is NMT 15.0.

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: Blank**

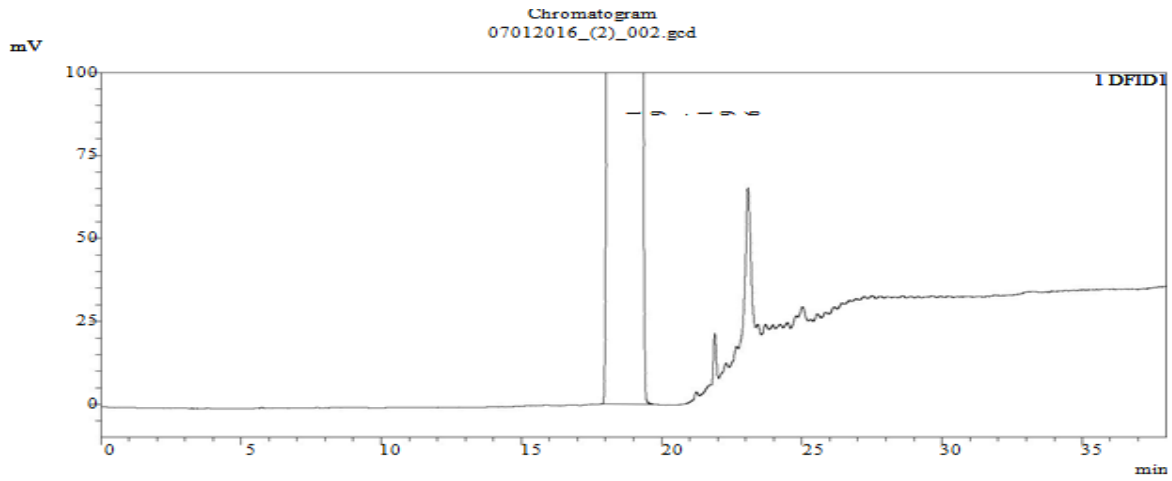


**Fig.2: Chromatogram (Blank)**

**Table 3: Peak data of blank.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | DMSO | 19.196 | 544122656 | 100 |

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: standard solution 1**

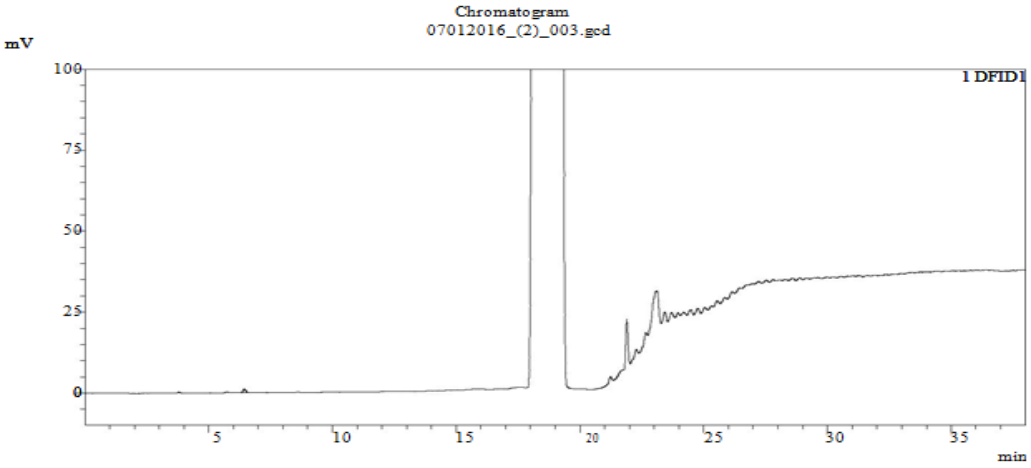


**Fig 3: Chromatogram (Standard).**

**Table 3: Peak data of standard.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.43 | 8700 | 100 |

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: sample**

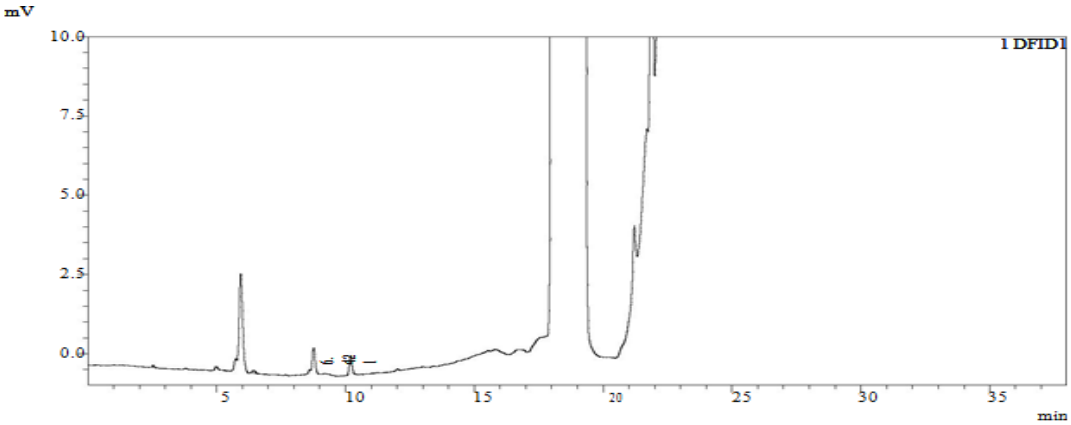


**Fig.4: Chromatogram (Sample).**

**Table 3: Peak data of standard.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.421 | 788 | 100 |

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: sample spiked solution**

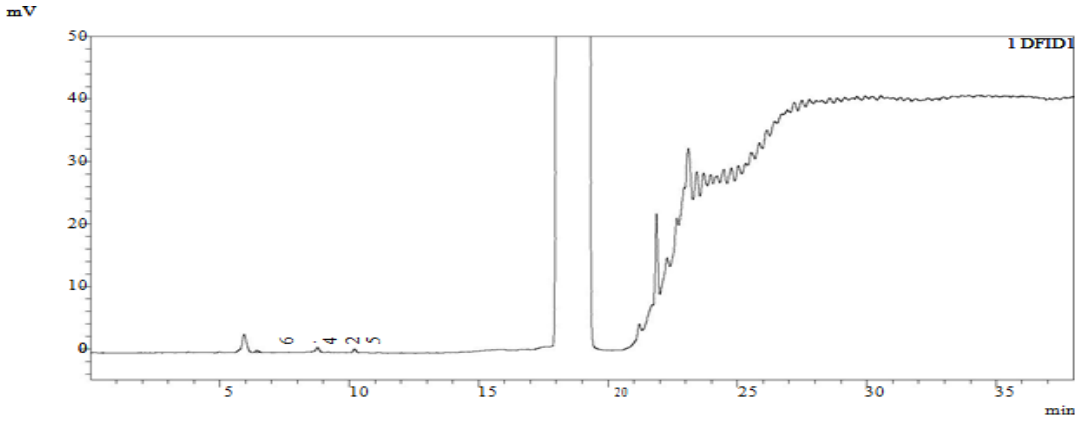


**Fig 5: Chromatogram of spiked sample.**

**Table 3: Peak data of standard.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.425 | 2780 | 100 |

**Specificity**

**Definition:** Specificity is the ability of the method to measure accurately the analyte in presence of components which are expected to be present. Prepare the Standard and Sample solutions as mentioned below.

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane, into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about, 600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of test solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, dissolve and make up to the volume with diluent.

**Preparation of Spiked solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, add 5 mL of diluents and 0.075 mL of dichloromethane stock solution, dissolve and make up to the volume with diluent.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. There should be no interference due to blank at the retention time of residual solvent.

**Precision**

Precision is a measure of degree of reproducibility and of repeatability of the analytical method and is usually expressed as the relative standard deviation.

**System precision**

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane, into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution:** Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about,600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of test solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, dissolve and make up to the volume with diluent.

**Preparation of Spiked solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, add 5 mL of diluents and 0.075 mL of dichloromethane stock solution, dissolve and make up to the volume with diluent.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. The % RSD for dichloromethane from the area of 6 injections of spiked solution is NMT 15.0
3. The % RSD for dichloromethane content (in ppm) from 6 injections of spiked solution is NMT 15.0

**Method precision**

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane, into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about, 600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of test solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, dissolve and make up to the volume with diluent.

**Preparation of Spiked solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, add 5 mL of diluents and 0.075 mL of dichloromethane stock solution, dissolve and make up to the volume with diluent.

Prepare the spiked solution in six different times and inject them individually.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. The % RSD for dichloromethane from the area of 6 preparations of spiked solution is NMT 15.0
3. The % RSD for dichloromethane content (in ppm) from 6 preparations of spiked solution is NMT 15.0

**LOD & LOQ**

**Limit of detection**

**Approach**

The approach adopted for the determination of detection limit is based on signal-to-noise ratio.

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane, into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about, 600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of LOD solution**

Based on the signal to noise ratio obtained from the Standard Solution, prepare LOD Solution for dichloromethane to obtain the signal to noise ratio about 3:1.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. Signal to Noise ratio should be established to about 3.

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: LOD solution injection**

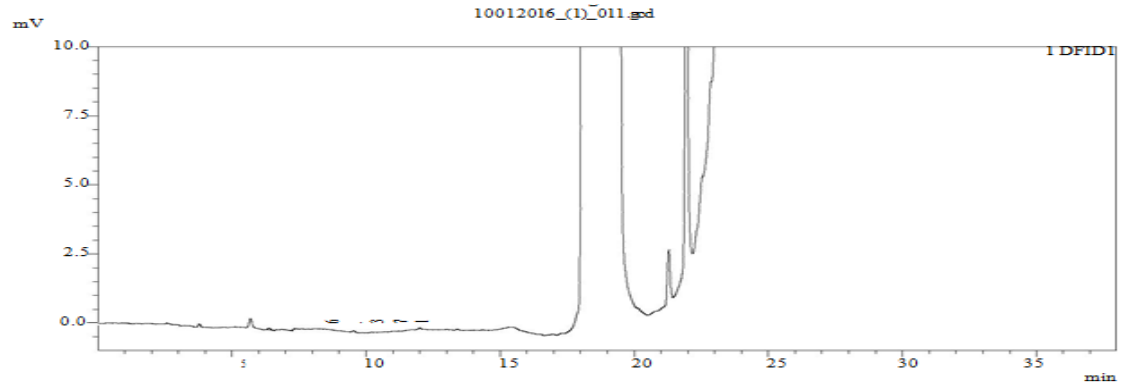


**Fig 6: Chromatogram of LOD solution.**

**Table 3: Peak data of standard.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.371 | 492 | 100 |

**Limit of quantitation**

**Approach**

The approach adopted for the determination of Limit of Quantitation is based on signal-to-noise ratio.

**Preparation of LOQ solution**

Consider the reference solution as LOD reference solution.

Based on the signal to noise ratio obtained from the Standard Solution, prepare LOQ Solution for dichloromethane to obtain the signal to noise ratio about 10:1.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. Signal to Noise ratio should be established to about 10.

**LOQ Precision**

**Preparation of solutions**

Prepare and inject the LOQ solution as obtained in the LOQ parameter for six times.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Acceptance criteria**

1. System suitability criteria should meet the requirements.
2. %RSD for precision at LOQ level for dichloromethane should be NMT 15.0

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: LOQ solution injection**

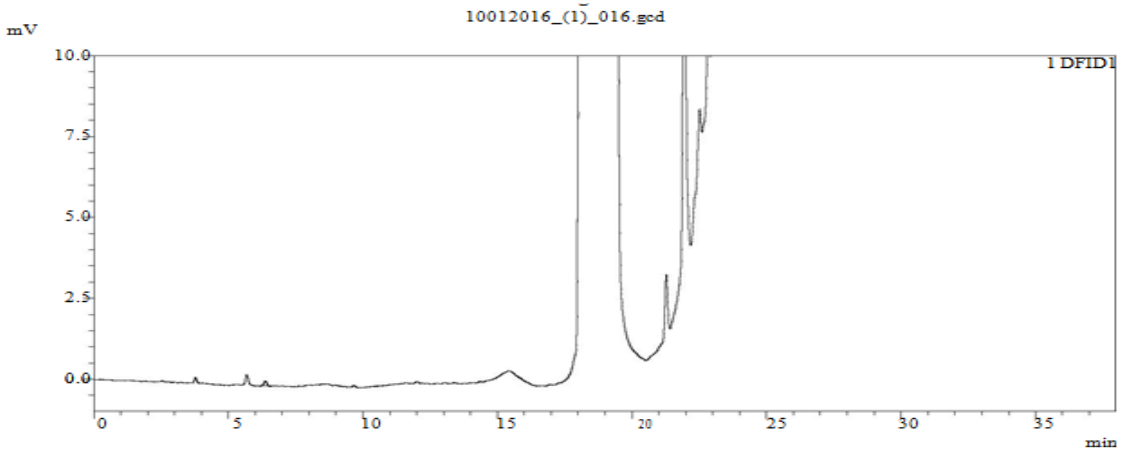


**Fig 7: Chromatogram of LOQ solution.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.365 | 1107 | 100 |

**Linearity**

Linearity is the ability of the method to elicit test contents that is directly proportional to analyte concentration within a given range.

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about, 600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of Linearity Solutions**

**Preparation of Linearity solution-1: [LOQ level]**

Prepare the LOQ solution as mentioned in LOQ parameter.

**Preparation of Linearity solution-2: [25% level]**

Transfer 0.019 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Preparation of Linearity solution-3: [50% level]**

Transfer 0.038 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Preparation of Linearity solution-4: [75% level]**

Transfer 0.057 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Preparation of Linearity solution-5: [100% level]**

Transfer 0.075 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Preparation of Linearity solution-6: [125% level]**

Transfer 0.094 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Preparation of Linearity solution-7: [150% level]**

Transfer 0.113 mL of dichloromethane standard stock into a 10.0 mL volumetric flask and make up to the mark with diluent and mix well.

**Note**

Solutions preparations can be scaled up or down based on the solution quantity requirement.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

**Calculate the % Y-intercept for each component.**

$$\% \text{ Y-intercept} = \frac{\text{Y-intercept value}}{\text{Average area at 100\% level}} \text{ X } 100$$

**Acceptance criteria**

1. System suitability criteria should meet.
2. The regression coefficient for each component should not be less than 0.99.
3. %Y-intercept should be in between ± 5.
4. Precision at higher level for dichloromethane peak area should be NMT 15.0

**Sample name: 3-(4-chlorobutyl)-1H-indole-5-carbonitrile.**

**Sample ID: Linearity solution-1 [LOQ level]**

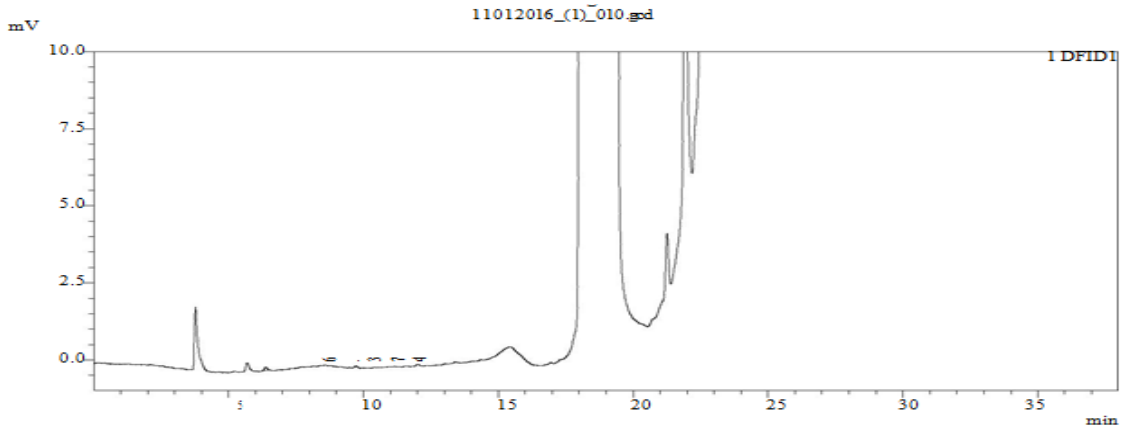


**Fig.8: Chromatogram LOQ solution.**

| Peak | Name | Ret. time | Area | %Area |
|---|---|---|---|---|
| 1 | MDC | 6.374 | 861 | 100 |

**Accuracy [%Recovery study]**

The accuracy of an analytical method expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted value and the value found.

**Preparation of Solutions**

**Preparation of dichloromethane standard stock solution**

Transfer about 9 µL of Dichloromethane into a 10 mL volumetric flask containing about 5 mL of diluent and dilute to volume with diluent.

**Preparation of Standard solution**

Transfer 300 µL of the standard stock solution into a 10 mL volumetric flask containing 5 mL of diluents and make up to volume with the diluent. The above solution contains about,600 ppm of dichloromethane with respect to 60 mg/mL test concentration.

**Preparation of test solution**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, dissolve and make up to the volume with diluent.

**Preparation of Spiked solution at LOQ level**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, dissolve and make up to the volume with LOQ level concentration solution.

Prepare the spiked solution in three different times and inject them individually.

**Preparation of Spiked solution at 100% level**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, add 5 mL of diluents and 0.075 mL of dichloromethane stock solution, dissolve and make up to the volume with diluent.

Prepare the spiked solution in three different times and inject them individually.

**Preparation of Spiked solution at 150% level**

Weigh accurately about 600 mg of the test sample transferred into a 10 mL volumetric flask, add 5 mL of diluents and 0.113 mL of dichloromethane stock solution, dissolve and make up to the volume with diluent.

Prepare the spiked solution in three different times and inject them individually.

**Procedure**

Equilibrate the system for at least 30 min for stabilization.

$$\% \text{ of Recovery} = \frac{\% \text{ of Impurity obtained [Corrected Content]}}{\% \text{ of Impurity spiked}} \times 100$$

**Acceptance criteria**

1. System suitability criteria should meet.
2. % of Recovery for LOQ level solution should be 100 ± 20.
3. % of Recovery for 100% level and 150% level solution should be 100 ± 15.

**Range**

Range is the interval between the upper and lower levels of impurities that has been demonstrated to be determined with precision, Linearity and accuracy using the method.

Establish the range for dichloromethane by Linearity and accuracy studies.

**Ruggedness: [Intermediate Precision]**

Ruggedness is the degree of reproducibility of the contents obtained under variety of conditions, expressed as % of relative standard deviation. These conditions include differences in analysts, instruments, days and columns. If any abnormality observed in the above condition, perform the Ruggedness parameter individually.

**Conditions**

1. System to System variation
2. Column to Column variation
3. Day to Day variation
4. Analyst to analyst variation

Follow the preparation of solutions, injection sequence and system suitability criteria as mentioned in the Method precision and calculate the cumulative %RSD for dichloromethane content (in ppm) obtained from the Ruggedness study and Method precision.

**Acceptance criteria**

1. System suitability criteria should meet.

2. The % RSD for dichloromethane from the area of 6 preparations of spiked solution is NMT 15.0

3. The % RSD for dichloromethane content (in ppm) from 6 preparations of spiked solution is NMT 15.0

4. The cumulative % RSD for dichloromethane in ppm obtained from the method precision and ruggedness should be NMT 15.0.

**Table 3: System suitability.**

| **System suitability** | |
|---|---|
| **Sr. No.** | **Peak area** |
| | **Dichloromethane** |
| 1. | 8700 |
| 2. | 8717 |
| 3. | 8614 |
| 4. | 8986 |
| 5. | 8901 |
| 6. | 8895 |
| **Average** | **8802** |
| **% RSD** | **1.6** |
| **Conclusion:** From the above data it was concluded that the system is suitable for the analysis of residual solvent Dichloromethane in 3-(-chlorobutyl)-1H-indole-5-carbonitrile. | |

**Table 4: System precision.**

| **Acceptance criteria:** | |
|---|---|
| % RSD of peak area of individual solvents peaks from six standard injections of standard solution (system suitability solution) should not be more than 15.0 %. | |
| **System suitability:** | |
| **Sr. No.** | **Peak area** |
| | **Dichloromethane** |
| 1 | 8297 |
| 2 | 8415 |
| 3 | 8208 |
| 4 | 8610 |
| 5 | 8445 |
| 6 | 8397 |
| **Average** | **8395** |
| **%RSD** | **1.6** |
| **Observation:** The %RSD for Dichloromethane calculated from area of 6 injections is 1.4% | |

**Table 5: Method precision.**

| **Acceptance criteria:** | |
|---|---|
| % RSD of peak area of all the solvents peaks from six standard injections of Standard solution should not be more than 15.0 %. | |
| **Method precision set peak areas:** | |
| **Sr. No.** | **Peak area** |
| | **Dichloromethane** |
| 1 | 2030 |
| 2 | 2055 |
| 3 | 2008 |
| 4 | 2041 |
| 5 | 2063 |
| 6 | 2096 |
| **Average** | **2049** |
| **%RSD** | **1.5** |
| **Acceptance criteria:** | |
| % RSD for individual residual solvents of six preparations is not more than 15.0 %. | |
| **Observation:** | |
| % RSD for individual residual solvents of six preparations is less than 15.0 %. | |
| **Conclusion:** | |
| The contents of solvents were found in acceptable % RSD of less than 15.0 %.<br>This implies that the method is precise for quantification of Dichloromethane in 3-(-chlorobutyl)-1H-indole-5-carbonitrile. | |

**Table 6: Limit of Detection.**

| **Limit of Detection:** | | |
|---|---|---|
| **LOD solution** | **S/N ratio** | |
| LOD solution preparation-1 | 4 | |
| LOD solution preparation-2 | 6 | |
| LOD solution preparation-3 | 5 | |
| **Observation:** | | |
| Signal to Noise ratio is established to about 3 | | |
| **Limit of Quantitation & LOQ Precision:** | | |
| **Sr. No.** | **Peak area of Dichloromethane** | **S/N Ratio** |
| LOQ preaparation-1 | 1107 | 10 |
| LOQ preaparation-2 | 1078 | 12 |
| LOQ preaparation-3 | 943 | 10 |
| LOQ preaparation-4 | 1057 | 13 |
| LOQ preaparation-5 | 1191 | 10 |
| LOQ preaparation-6 | 1101 | 10 |
| **Average** | **1080** | |
| **%RSD** | **7.5** | |
| **Observation:** Signal to Noise ratio is established to about 10<br>%RSD for precision at LOQ level for dichloromethane is 7.5% | | |

**Table 7: Linearity study.**

| **Results of Linearity study:** | | |
|---|---|---|
| **Linearity Plot of Dichloromethane:** | | |
| **Linearity level** | **Concentration (ppm)** | **peak area** |
| LOQ% | 59.697 | 936 |
| 50% | 75.616 | 1308 |
| 75% | 113.424 | 1903 |
| 100% | 149.243 | 2401 |
| 125% | 187.051 | 2766 |
| 150% | 224.859 | 3568 |
| | **Correlation coefficient** | **0.995** |
| | **Y-intercept** | **121.84** |

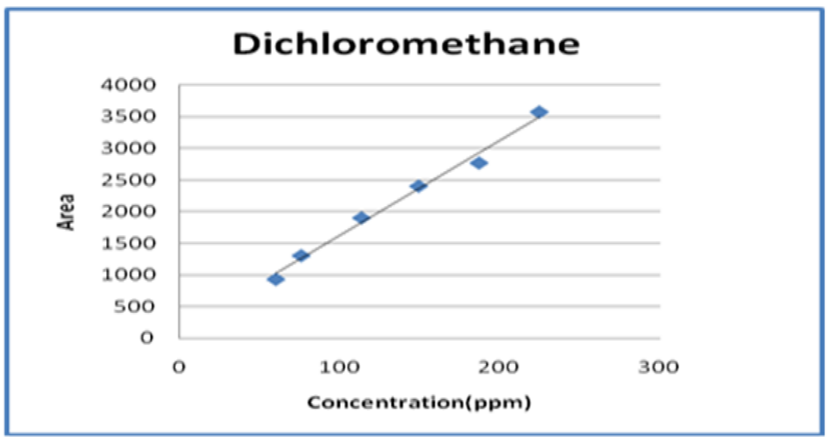


**Fig 8: Linearity.**

**Table 8: Accuracy data.**

| **Control sample peak areas:** | | | |
|---|---|---|---|
| **Control sample** | **Peak area** | | |
| | **Dichloromethane** | | |
| Preparation-1 | Not Detected | | |
| Preparation-2 | Not Detected | | |
| Preparation-3 | Not Detected | | |
| | | | |
| **LOQ level peak areas:** | | | |
| **LOQ level** | **Peak area** | | |
| | **Dichloromethane** | | |
| Preparation-1 | 1278 | | |
| Preparation-2 | 1108 | | |
| Preparation-3 | 1293 | | |
| Average | 1226 | | |
| **100 % level peak areas:** | | | |
| **100 % level** | **Peak area** | | |
| | **Dichloromethane** | | |
| Preparation-1 | 3069 | | |
| Preparation-2 | 3042 | | |
| Preparation-3 | 3119 | | |
| Average | 3077 | | |
| **150 % level peak areas:** | | | |
| **150 %level** | **Peak area** | | |
| | **Dichloromethane** | | |
| Preparation-1 | 4363 | | |
| Preparation-2 | 4537 | | |
| Preparation-3 | 4467 | | |
| Average | 4456 | | |
| | | | |
| **%Recovery results :** | | | |
| **Level** | **LOQ Level** | **100% level** | **150% level** |
| Preparation-1 | 108 | 104 | 98 |
| Preparation-2 | 94 | 103 | 102 |
| Preparation-3 | 110 | 106 | 101 |
| **Average** | 104 | 104 | 100 |
| **SD** | **8.72** | **1.53** | **2.08** |
| **% RSD** | **8.4** | **1.5** | **2.1** |
| **Acceptance criteria:** | | | |
| The recovery for each level should be as per table given below: | | | |
| **Sr. No** | **Level** | **Acceptable % recovery** | |
| 1 | LOQ Level | Between 80.0 and 120.0 | |
| 2 | 100 % Level | Between 85.0 and 115.0 | |
| 3 | 150 % Level | Between 85.0 and 115.0 | |

**Table 9: Method Precision and Intermediate Precision.**

| Method precision and Intermediate Precision Results (ppm) | |
|---|---|
| **Method Precision Sets** | **Dichloromethane** |
| 1 | 144.07 |
| 2 | 145.81 |
| 3 | 142.48 |
| 4 | 144.92 |
| 5 | 146.39 |
| 6 | 148.90 |
| **Intermediate Precision Sets** | **Dichloromethane** |
| 1 | 130.37 |
| 2 | 150.76 |
| 3 | 136.88 |
| 4 | 149.24 |
| 5 | 149.45 |
| 6 | 153.09 |
| **Overall Average** | **145.20** |
| **Overall SD** | **6.33** |
| **Overall %RSD** | **4.4** |
| **Observation:** | |
| % RSD for Dichloromethane from the area of six preparations is less than 15.0 %.<br>% RSD for Dichloromethane content (in ppm) from six preparations is less than 15.0 %.<br>Cumulative %RSD for Dichloromethane in ppm obtained from method precision and ruggedness is less than 15.0% | |

## 5. CONCLUSIONS

Aanalytical method validation study for residual solvent by GC of 3-(-chlorobutyl)-1H-indole-5-carbonitrile drug substance is done as per the requirement of analytical R&D. Precision and system suitability is established as mentioned in the test method. The test method is specific for the estimation of residual solvent in 3-(-chlorobutyl)-1H-indole-5-carbonitrile drug substance. The test method is precise for the estimation of residual solvent in 3-(-chlorobutyl)-1H-indole-5-carbonitrile drug substance, which was indicated from the acceptable %RSD of six residual solvents test results obtained during method precision study. The test method is linear for Dichloromethane over the range of LOQ to 150% of target concentration for the analyte response with an acceptable correlation coefficient values at 100% drug. The test method is accurate over the range of LOQ to 150% of target concentration for the estimation of residual solvent in 3-(-chlorobutyl)-1H-indole-5-carbonitrile.The test method is rugged for day to day and analyst to analyst variability.

## 6. REFERENCES


1. Dawson LA, Watson JM. Vilazodone: A 5HT1a receptor agonist/ serotonin transporter inhibitor for treatment of affective disorders. CNS Neurosci Ther. 2009; 15(2): 107-117.
2. Hughes ZA, Starr KR, Langmead CJ, Hill M, Bartoszyk GD, Hagan J, et al. Neurochemical evaluation of the novel 5-HT1A receptor partial agonist/serotonin reuptake inhibitor, vilazodone. Eur J Pharmacol. 2005; 510(1-2): 49-57.
3. Willard H, Merritt L, Dear J, Settle F, ed7, Instrumental method of analysis, CBS Publishers and Distributors, New Delhi, pp 118-158.
4. B. Parameswara Reddy, N. Pramod, P. Venkateswararao, A.M.S. Sudhakarbabu; Method developmentand validation for the assay of Vilazodone in bulk and formulation by using RPHPLC technique, International Journal of Biological & Pharmaceutical Research, 2012; 3(6): 789-795.
5. Yasser A. Z, Farag R. S, Elnawawy M, Ahmed M. A, Alsalam S. R, Spectrophotometric method for the determination of Trazodone hydrochloride in pharmaceutical formulations, International Journal of Pharmaceutical Research, 2011; 2(11): 2798-2800.
6. Reddy B. P, Pramod N, VenkateswararaoP, Sudhakarbabu A. M. S, Method development and validation for the assay of Vilazodone in bulk and formulation by using RP-HPLC technique, International Journal of Biological & Pharmaceutical Research, 2012; 3(6): 789-795.
7. Ravi Prakash P. V. D. L. S, Sumadhuri B, Srikanth M, Validated HPLC-MS/MS Method for Determination of Trazodone in Human Plasma, Open Access Scientific Reports, 2013; 2(2): 1-5.
8. Lovett L. J, Nygarda G. A, Khalil S. K, A Simple HPLC Method for the Determination of Trazodone Human Serum, Journal of Liquid Chromatography, 1987; 10(5): 909-919.
9. International conference on harmonization (ICH), Q2B: Validation of analytical procedure: Methodology, USFDA federal register. 1997.