METHOD DEVELOPMENT AND VALIDATION FOR IDENTIFICATION OF 2-PHENYL PHENOL IN SIAPTON 10L BY GAS CHROMATOGRAPHY MASS SPECTROMETRY

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ABSTRACT
Method was developed and validated for identification of 2-Phenyl Phenol by Gas Chromatography Mass Spectrometry in Siapton 10 L. The retention time of 2- Phenyl phenol was approximately 7.14 min with good peak shape. Instrument was linear for 2-phenyl phenol in concentration range from 0.05 – 1 mg/Kg, with correlation coefficient (r²) equals to 0.9993. The precision (%R.S.D.) at concentrations- 2 ppm, 1 ppm, 0.5 ppm, 0.2 ppm, 0.1 ppm, 0.05 ppm and 0.01 ppm was found to be 2.37, 1.12, 1.31, 3.19 and 2.99 respectively which shows good precision with S/N ratio 3.5:1 of the developed method. LOQ and LOD concentrations 0.01 mg/Kg, 0.05 mg/Kg respectively confirm good sensitivity of the method. The developed GC method with Mass detector was accurate, precise, reproducible and sensitive. All the validation parameters of the method show good result.

KEYWORDS: 2-Phenyl Phenol, DCM, LOQ & LOD, SIM & SCAN, GCMS.

INTRODUCTION
2-Phenylphenol, is an organic compound that consists of two linked benzene rings and a phenolic hydroxyl group.[1-2] Its IUPAC name: biphenyl-2-ol, CA: (1, 1’-biphenyl)-2-ol, other synonyms are ortho-biphenylolo, ortho-phenylphenol, OPP, ortho-hydroxybiphenyl, 2-hydroxybiphenyl with CAS No: 90-43-7, Molecular formula, C₁₂H₁₀O and molecular weight,
It is a fungicide generally applied after post-harvest. Waxing of citrus fruits and disinfection of seed boxes is done by this fungicide. European Union did not permit it as food additive but allowed its use after post harvest. In 1962, evaluation of 2-Phenylphenol (ortho-phenylphenol, OPP) was done for the post-harvest treatment of fruits and vegetables to protect against microbial damage during storage and distribution. As a general surface disinfectant, 2-Phenylphenol is used in households, hospitals, nursing homes, farms, laundries, barber shops and food processing plants. As lab reagent, it is used in rubber industry, polymer industry, in the manufacture of other fungicides, dye stuffs, resins and rubber chemicals. Eye and skin contact of this chemical can cause severe irritation and burns with possible eye damage. Hyperactive Children’s Support Group recommends that it should be eliminated from the diet of children. The sodium salt of orthophenyl phenol, is used as preservative to enhance shelf life. It is also used as a fungicide in food packaging which contaminate its contents.

Gas chromatography–mass spectrometry (GC-MS) is combination of gas-liquid chromatography and mass spectrometry to identify different constituents present within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. Also it helps to identify trace elements in materials that were previously thought to have disintegrated beyond identification. Many analytical methods for the detection and quantification of phenolic compounds are based on gas chromatography-mass spectrometry (GC-MS).

One of the objectives of the study was to develop a reliable method to identify presence of 2-Phenyl phenol in Siapton 10 L at the lowest possible detection limit in organic bio-product. The method for identification and quantification of 2-Phenylphenol was developed and validated using a simple, rapid, sensitive, precise, accurate and specific GC-MS method. It may be noted that several organically generated & certified Bio-products are commercially available to farmer to promote healthy & robust growth of the crop. The regulatory framework demands that the organically certified products should not have any synthetic product contamination even at ppm level. Incidentally 2-Phenyl phenol is regarded as one of the undesirable synthetic contaminant. A reliable analytical tool to identify contamination the
2- Phenyl phenol at ppm level is necessary to ensure the compliance of the Organic Product Certification. During this study first screening was done followed by optimization for method of analysis and sample preparation. The advantage is that the present method is simple, follows single step extraction, short run time and use of less toxic chemicals.

**MATERIALS AND METHODS**

**Material Required**
Hexane, Methanol, DCM (HPLC-grade), Micropipette (100-1000μl, 20-200μl, 10-20μl), Volumetric flask 10 ml, standard 2- Phenyl phenol (99.8%).

Siapton 10 L was supplied by Isagro Asia Agrochemical Pvt. Ltd.

**INSTRUMENTATION**

**Chromatographic Conditions**

GC Model: **GC-2010(Shimadzu)**

Column: DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness)

Carrier: Helium
Flow Control Mode: Linear
Flow rate: 1 mL/min
Pressure: 61.8
Total Flow: 12.6 ml/min
Linear velocity: 34.8 cm/sec
Purge flow: 3.0 ml/min
Injector temperature: 280°C
Injection volume: 2 micro litre
Injection mode: splitless
Solvent cut time: 5 min

**Oven temperature program.**

<table>
<thead>
<tr>
<th>Rate °C /min.</th>
<th>Final Temp (°C)</th>
<th>Hold time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>2min</td>
</tr>
<tr>
<td>20°C /min</td>
<td>280</td>
<td>0 min</td>
</tr>
</tbody>
</table>

Total program time: 11 min.
MS Conditions
Detector: GC-MSD (Mass Selective Detector, GC-QP 2010 plus MSD Shimadzu Make)
Mode: Electron Impact Mode (EI) with electron energy 70eV.
Ionsource temperature: 250°C
Interface temperature: 280°C
Acq. Mode: Selective Ion Mode.
Event time: 7.15 RT
Selective Ions: 170, 141, 115

PREPARATION OF SOLUTIONS

Stock Standard preparation
5.78 mg of 2-Phenyl phenol was weighed in Analytical Precision Balance (Saritorius make, range 0.01mg-220g) in standard 10 ml volumetric flask and the volume was maintained in hexane (HPLC grade) to prepare 578 ppm stock standard solution.

Working Standard preparation
Working standard solutions of concentrations- 2 ppm, 1 ppm, 0.5 ppm, 0.2ppm, 0.1 ppm, 0.05 ppm and 0.01 ppm were prepared in 10 ml volumetric flask using hexane (HPLC grade).

Sample preparation
Standard was checked for solubility and it was found soluble in methanol. The standard solution was made up to the mark using Dichloro methane (DCM) which was filtered and used for GCMS injection in SIM and SCAN mode. Through SCAN mode, Retention Time (7.14) and major fragmentation ions of Standard were selected. By SIM mode, method was developed to search fragment ions 170, 141, 115.

RESULTS AND DISCUSSION
In order to provide a simple, accurate and economical analytical method, the chromatographic conditions were optimized which can be employed for routine quality control of 2-Phenyl phenol in Siapton 10L. The injection port, detector temperature and oven temperature program were set accordingly as mentioned in instrumentation section. Proper solvent, column and acquisition parameters were selected for method development which can result in excellent separation. The retention time of 2-Phenyl phenol was approximately 7.14 min with good peak shape as shown in Figure 1.
Figure 1: Chromatogram showing the elution of 2-phenyl phenol at their respective retention times.

A five point calibration curve was constructed with working standards and was found linear ($R^2 = 0.999$). The developed GC method with Mass detector was accurate, precise, reproducible and sensitive. All the validation parameters of the method are showing good results.

Accuracy and precision were determined by elaboration of standard calibration curve. The precision (％R.S.D.) at concentrations- 2 ppm, 1 ppm, 0.5 ppm, 0.2 ppm, 0.1 ppm, 0.05 ppm and 0.01 ppm was found to be 2.37, 1.12, 1.31, 3.19 and 2.99 respectively which shows good precision of the developed method. LOQ and LOD concentrations confirm sensitivity of the methods. The method was specific as none of the excipients interfered with the analytes of interest. Hence, the method was suitably employed for assaying of 2-Phenyl phenol in a commercial formulation like Siapton 10L.

Validation of the Proposed Method

Four parameters were chosen for the method development work are given under

1. Calibration Curve (Linearity of Instrument)
2. Precision (Instrument)
3. Limit of Detection (LOD) & Limit of Quantification (LOQ)
4. Specificity and Selectivity
Calibration Curve (Linearity of Instrument)

2 phenyl phenol standard received from ISAGRO (ASIA) Agrochemical Pvt. Ltd. was used for the standard solution preparation. HPLC grade n-Hexane from Merck was used as solvent for the preparation of stock solution of 100 mg/Kg concentration. Stock solution was used for preparation of various concentration levels following serial dilution for calibration. A calibration curve of five points (viz. 1, 0.5, 0.2, 0.1 & 0.05 mg/Kg) was plotted and presented in Figure 2.

![Linearity Curve](image)

Figure 2: Linearity curve of 2- Phenyl phenol at 5 concentration levels.

Precision

Precision of the instrument was measured at the 5-concentration levels considering average/mean peak area of standard (M), standard deviation (SD) and relative standard deviation (RSD) are presented in Table 1.

Table 1: Presenting average/mean peak area of standard (M), standard deviation (SD) and relative standard deviation (RSD) at 5-concentration levels of 2- Phenyl phenol (mg/kg).

<table>
<thead>
<tr>
<th>Concentration levels (2-Phenyl phenol), (mg/kg)</th>
<th>Area1</th>
<th>Area2</th>
<th>Area3</th>
<th>Mean</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>930</td>
<td>980</td>
<td>980</td>
<td>963.3</td>
<td>28.87</td>
<td>2.99</td>
</tr>
<tr>
<td>0.2</td>
<td>2066</td>
<td>2195</td>
<td>2095</td>
<td>2118.6</td>
<td>67.68</td>
<td>3.19</td>
</tr>
<tr>
<td>0.5</td>
<td>5277</td>
<td>5405</td>
<td>5391</td>
<td>5357.6</td>
<td>70.21</td>
<td>1.31</td>
</tr>
<tr>
<td>1</td>
<td>11612</td>
<td>11390</td>
<td>11390</td>
<td>11464</td>
<td>128.17</td>
<td>1.12</td>
</tr>
<tr>
<td>2</td>
<td>29680</td>
<td>29712</td>
<td>28489</td>
<td>29293.67</td>
<td>697.05</td>
<td>2.37</td>
</tr>
</tbody>
</table>
Limit of Detection (LOD) & Limit of Quantification (LOQ)

LOD of the instrument for 2-Phenyl phenol was calculated as concentration of analyte which gives signal to noise (S/N) ratio of 3.5:1 are shown in Table 2.

Table-2: Shows the chromatogram of the analyte at 0.05 and 0.01 mg/kg conc. with calculation of signal to noise (S/N) ratio.

LOQ was taken as the concentration of analyte equals to the 5 times of LOD value. Figure 3 & 4 shows the chromatogram of the analyte at 0.05 and 0.1 mg/kg conc. with calculation of signal to noise (S/N) ratio.

Figure 3: TIC of 2-Phenyl phenol (0.05 mg/Kg).
Figure 4: Limit of Detection (LOD): TIC of 2-phenylphenol at 0.01 mg/Kg concentration.

Specificity & Selectivity
NIST library of Gas chromatography mass spectrometry was used to confirm the presence of analyte 2- Phenyl phenol. Spectra match with reference spectra of NIST library shows 84 % matching with retention time 7.14, molecular weight 98, target ion 170 with qualifier ions 115, 141. Figure 5. shows the total ion chromatogram of the analyte with its mass fragmentation pattern.

Figure 5 Total ion chromatogram of 2- Phenyl phenol and its EI mass spectra.
CONCLUSION

The developed GC method with Mass detector was accurate, precise, reproducible and sensitive. All the validation parameters of the method are showing good result. The retention time of 2-Phenyl phenol was approximately 7.14 min with good peak shape. Instrument was linear for 2-Phenyl phenol in concentration range from 0.05 – 1 mg/Kg, with correlation coefficient ($r^2$) equals to 0.9993. The precision as percent relative standard deviation (% R.S.D.) at concentrations- 2 ppm, 1 ppm, 0.5 ppm, 0.2 ppm, 0.1 ppm, 0.05 ppm and 0.01 ppm was found to be 2.37, 1.12, 1.31, 3.19 and 2.99 respectively which shows good precision with S/N ratio 3.5:1 of the developed method. LOD and LOQ concentrations 0.01 mg/Kg, 0.05 mg/Kg respectively confirm good sensitivity of the method.

The optimised GC Mass method was suitably employed for assaying 2-Phenyl phenol in the commercial Bio-product formulation namely Siapton 10L. It was conclusively and unambiguously established by analyzing samples of the Siapton 10 L.

ACKNOWLEDGEMENT

The authors are highly grateful to the Director, Institute of Pesticide Formulation Technology for providing the Research Facilities for work.

REFERENCES


