**ISSN:** 2321-4902
Volume 1 Issue 3

Online Available at www.chemijournal.com

International Journal of Chemical Studies

# A Newer Validated and Stability Indicating GC Method for the Estimation of Lindane in Formulation

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A simple, selective, linear, precise and accurate GC Method was developed and validated for rapid assay of Lindane in Formulation. Isocratic elution at a flow rate of 1.0ml/min was employed on DB 1,30 m × 0.53 mm Capillary column at ambient temperature250 °C. Injection Volume was found to be 2 µl. The mobile phase consisted of Acetone: Chloroform 80:20 v/v. The UV detection wavelength was 240nm and 2µl sample was injected. The retention time for Lindane was 6.2 min. A linear regression curve was constructed, and the correlation coefficients ($R^2$) and assessment values calculated The percentage RSD was found to be 1.0%.The Accuracy of method ranges between 97.0 – 102%. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

*Keyword:* Lindane, GC, Recovery, Precise.

## 1. Introduction

Lidane is the gamma isomer of hexachlorocyclohexane. It contains not less than 99.0 percent and not more than 101.0 percent of ($\gamma$-$C_6H_6Cl_6$). $C_6H_6Cl_6$. A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and soluble in ethanol.

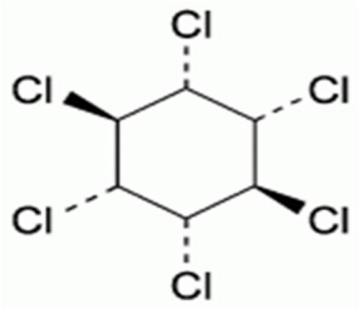


**Fig 1:** Structure of Lindane

Lindane IUPAC name is (1*r*,2*R*,3*S*,4*r*,5*R*,6*S*)-1,2,3,4,5,6-hexachlorocyclohexane. Lindane is a anorganochlorine chemical variant of hexachlorocyclohexane that has been used both as an agricultural insecticide and as a pharmaceutical treatment for lice and scabies [1,2]. A Simultaneous Determination by Gas Chromatography of Lindane and Carbaryl in Combined Formulations has been developed a glass capillary HP5 column (30 m x 0.32 mm; 0.25 µm), temperature programming with flame ionization detector and dibutylphthalate as an internal standard. The calibration graphs were found linear in the concentration range of 1 µg/mL to 1000 µg/mL for lindane with correlation coefficient of 0.999 and co-efficient of variation for intra-day and inter-day repeatability studies at different concentration levels was found to be less than 2%. The

accuracy of method ranges between 98.5% to 100.8% [3] . According to T. G. Kreindl, et.al., A method is described for the simultaneous quantitative determination of atrazine, pyrazon and lindane in potable water at the (sub-)ppb level. An adsorption column filled with Amberlite XAD-2 microporous resin advantageously replaces other preconcentration techniques. The concentrated eluate is analyzed by capillary gas chromatography without further purification. The recovery is 80% for atrazine and lindane at the 0.1 ppb level and 40% for pyrazon (1 ppb). The method was tested using tap water from the public water supply network [4] . Marili V.N. Rodrigues developed that Quantification was performed using GC-MS in the selected ion monitoring mode. Mean recovery rates of 70 to 124% were obtained. The inter-assay precision of a sample fortified with 200 ng $g^{-1}$ of each pesticide was in the range of 1.0 to 7.3%. The quantitation limits ranged from 3.0 to 30 ng $g^{-1}$ and were below the Maximum Residue Limit (MRL) for all the pesticides under study. The method was employed to analyze samples of *Mikania laevigata*, *Maytenus ilicifoli*a and *Cordia verbenacea* from an experimental field in Paulínia, SP, Brazil [5]. By the studies of Zorica Vujić *et al*., A method was developed to determine lindane (γ-HCH), the widely used organochlorine pesticide, and its impurity α-HCH in bulk and pharmaceutical products (shampoo, gel and emulsion). The system was able to successfully resolve the lindane peak from α-HCH and interfering components. The method displays an excellent linearity over the concentration range 0.5–15.0 μg $mL^{-1}$ for lindane and 0.005–0.15 μg $mL^{-1}$ for α-HCH and a precision better than 2.5% from intra- and inter-day analyses. LOQ and LOD were determined to be 0.45 and 0.15 μg $mL^{-1}$ for lindane and 0.005 and 0.002 μg $mL^{-1}$for α-HCH [6]. McManus SL described that The linearity of the method ranged from 0.015 to 5.0 μg L(-1), with correlation coefficients greater than 0.99. Recoveries ranged from 96 to 101% at several fortification levels with all coefficients of variation (CV%) less than 10.5%. The method was validated to the permitted limits laid down in the European Union drinking water directive (98/83/EC). The limit of quantitation (LOQ) was 0.015 μg L(-1) in groundwater samples. Samples had to be analysed within 24 h of collection otherwise degradation occurred and disposable SPME polyacrylate fibres lasted up to 51 injections [7].

## 2. Materials and Methods

### 2.1 Chemicals and reagents

The reference sample of Lindane was obtained from Ranbaxy, Mumbai. The Formulation Gambex Shampoo was purchased from the local market. Acetone and Chloroform used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

### 2.2 Instruments

A suitable Gas Chromatograph with an FID detector, Column - DB 1, 30 m × 0.53 mm Capillary column was employed for investigation. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

### 2.3 Preparation of Standard Solution

Accurately weigh 100 mg of Lindane into a 50 ml volumetric flask. Dissolve in 5 ml of Acetone and dilute to the mark with chloroform. Pipette 10.0 ml of this solution into a 100 ml volumetric flask. Dilute to volume with chloroform and mix well. For analysis 100 ppm standard solution was prepared, required concentrations were obtained from 100 ppm solution by appropriate dilution.

### 2.4 Preparation of Sample Solution

Weigh 2g of shampoo to 100 ml volumetric flask. Dissolve in 5 ml Acetone. Add 80 ml of chloroform mix well and dilute to volume with chloroform. Filter with No.40 filter paper into a clean 100 ml volumetric flask. Cover to minimise evaporation. For the determination of method accuracy, the same amount of placebos was spiked with standard lindane in a 100-mL volumetric flask to obtain spiked concentrations 0.8–1.2 lg mL-1.

### 2.5 Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant.

## 3. Test Validation Procedure, Requirements

The analytical performance of the method of analysis was checked for specificity, system suitability, accuracy and method precision

### 3.1 Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions must contain no components which co-elute with the Lindane. The peak purity results from the photo diode-array analysis must show that the Lindane peak is pure i.e. the purity angle (PA) must be less than the threshold angle (TH).The solutions were injected using the conditions specified in the method of analysis.

### 3.2 Chromatographic Results

For Chromatogram 1 the solvent used as chloroform. With this no Significant peak was detected. For Chromatogram 2.the Lindane was subjected at working concentrations. A peak was detected is due to lindane. For chromatogram 3 the product was stressed under UV light for 72 Hours, the peak is due to Lindane. It concluedes that Lindane is stable under UV light exposure. No components are seen to co-elute with the Lindane peak, and results indicate that the peak can therefore be considered spectrally pure.

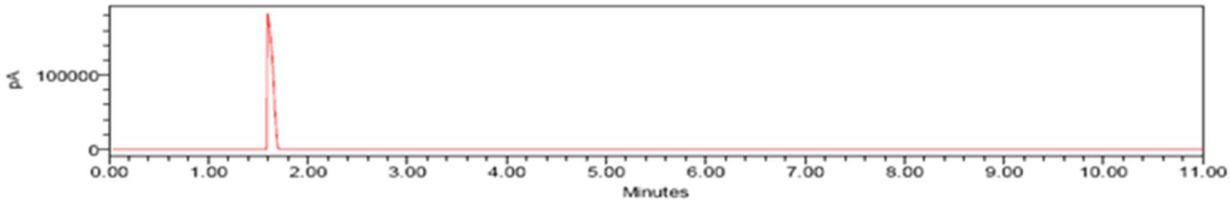


**Fig 2:** Chromatogram 1

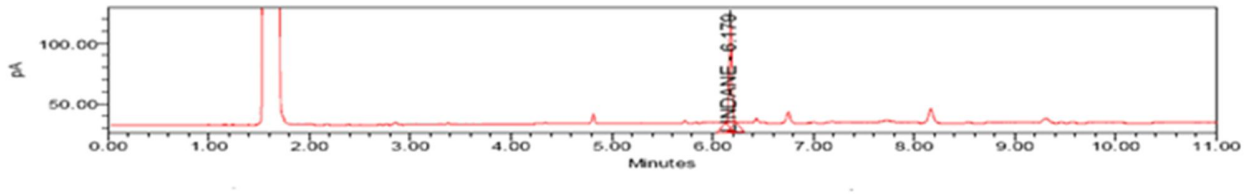


**Fig 3:** Chromatogram 2

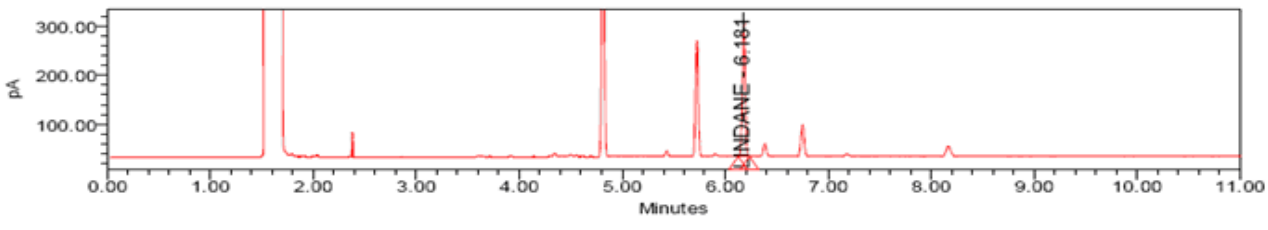


**Fig 4:** Chromatogram 3

### 3.3 System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to Lindane for the six replicate injections must be less than or equal to 2.0 %. Six replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. Therefore, the analytical system complies with the requirements specified by the system suitability The Results are tabulated in the Table:1

### 3.4 Linearity:

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug active in samples in a given range. Proof of linearity justifies the use of single-point calibrations. The correlation coefficient of the regression line for the Lindane should be greater than or equal to 0.999. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when +5 > z > -5.

**Table1:** System Suitability

| Sample | Lindane area |
|---|---|
| 1 | 287 |
| 2 | 282 |
| 3 | 282 |
| 4 | 283 |
| 5 | 285 |
| 6 | 290 |
| **Mean** | 285 |
| **% RSD** | 1.0 |

Five solutions containing 50, 75, 100, 125, and 150 % of the Lindane, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients ($R^2$) and assessment values calculated. From the linearity results,the correlation coefficients ($R^2$) and assessment value (Z) for the Lindane satisfy the acceptance criteria for linearity. Linearity Curve was shown in the Fig.2, and the results were tabulated in the Table:2.

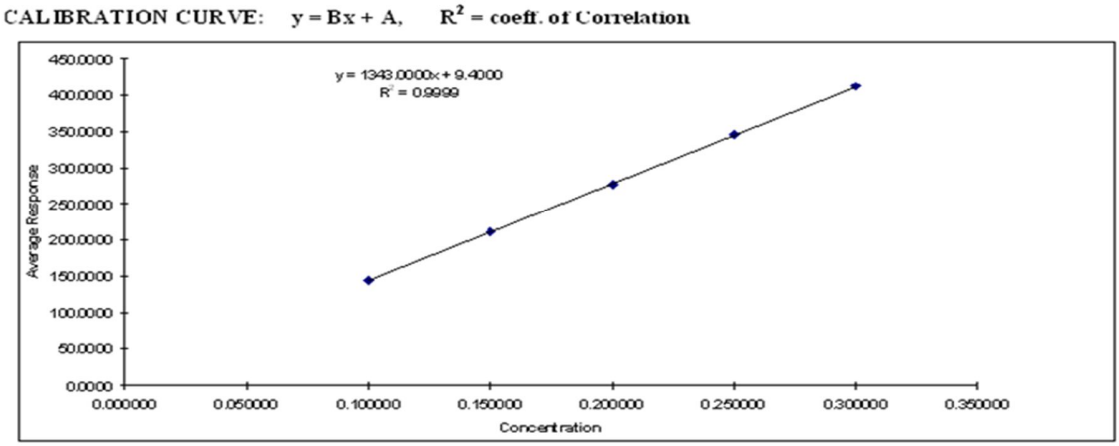


**Fig 5:** Linearity Curve

**Table 2:** Calibration Results

| Sample No. | Concentration | Response 1 | Response 2 | Average Response |
|---|---|---|---|---|
| 50% | 0.100000 | 143.0000 | 144.0000 | 143.5000 |
| 75% | 0.150000 | 210.0000 | 214.0000 | 212.0000 |
| 100% | 0.200000 | 273.0000 | 280.0000 | 276.5000 |
| 125% | 0.250000 | 347.0000 | 344.0000 | 345.5000 |
| 150% | 0.300000 | 438.0000 | 387.0000 | 412.6000 |

## 3.5 Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compound, for each solution prepared, must be within 95.0 – 105.0 % of the actual amount. Sample solutions were weighed with known concentrations of the Lindane to result in concentrations representing respectively 50, 75, 100, 125, and 150 % relative to the working concentrations. The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for the Lindane satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. The Results are tabulated in Table:3.

**Table 3:** Accuracy Results

| Sample | Theoretical | Actual | % Recovery | Average % Recovery |
|---|---|---|---|---|
| 50% | 0.48 | 0.48 | 100 | 101 |
| 50% | 0.48 | 0.49 | 102 | |
| 75% | 0.71 | 0.72 | 101 | 102 |
| 75% | 0.71 | 0.73 | 103 | |
| 100% | 0.95 | 0.97 | 102 | 102 |
| 100% | 0.95 | 0.96 | 101 | |
| 125% | 1.19 | 1.18 | 99 | 100 |
| 125% | 1.19 | 1.19 | 100 | |
| 150% | 1.43 | 1.38 | 97 | 97 |
| 150% | 1.43 | 1.39 | 97 | |

## 3.6 Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

## 3.7 Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to the Lindane for the six samples must be less than or equal to 2.0 %. Six separate sample preparations of batch 252826 were analysed according to the method of analysis. The % RSD

due to the Lindane concentration for the assay meets the requirements for repeatability at 1.6 %. The Results are tabulated in the Table: 4.

**Table 4:** Repeatability Values

| Sample number | Lindane Results (% m / v) |
|---|---|
| 1<br>2<br>3<br>4<br>5<br>6 | 0.94<br>0.95<br>0.94<br>0.96<br>0.98<br>0.95 |
| Mean | 0.95 |
| % RSD | 1.6 |

## 3.8 Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed by a different analyst on a different day, and using different reagents, mobile phases and solvents. The % RSD due to the Lindane concentration for the six samples must be less than or equal to 2.0 %. The mean results obtained in the repeatability, and the intermediate precision must not differ by more than 3.0 %. Six separate sample preparations of batch 252826 were assayed according to the method of analysis. The % RSD for intermediate precision is 0.8 % for the Lindane. The intermediate precision and repeatability comply as they differ by 0.7 % for the Lindane. The Results are indicated in the Table 5 and Table 6.

**Table 5:** Intermediate Precision

| Sample | Results (% m / v) |
|---|---|
| | Lindane |
| 1<br>2<br>3<br>4<br>5<br>6 | 0.94<br>0.94<br>0.93<br>0.94<br>0.95<br>0.95 |
| Mean | 0.94 |
| % RSD | 0.8 |

**Table 6:** Repeatability Intermediate Precision

| Sample | Mean Results (% m / v) |
|---|---|
| | Lindane |
| Repeatability<br>Intermediate Precision | 0.95<br>0.94 |
| Mean | 0.95 |
| % RSD | 0.7 |

## 3.9 Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the assay of the Lindane 0.5 –1.5 % m / v which represents 50 % to 150 % of the working concentration for the active.

## 3.10 LOD and LOQ

The LOD and LOQ data calculated on the basis of extraction of 10 serum blanks at a signal - to-noise ratio of 3 and 10 respectively. The results were tabulated in the Table: 7.

**Table 7:** LOD and LOQ

| **Sample** | **LOD** | **LOQ** |
|---|---|---|
| **Lindane** | 0.09 | 0.29 |

## 3.11 Ruggedness

The degradation of the compounds when taking the extract into dryness. We measured that the degradation of the compounds varying the time we left the extract once it was dry (5, 15 and 30 minutes). Degradation of Lindane: 23, 21 and 40 % as time increase.

## 4. Declaration on the validity of the method

The method for the assay of Lindane complies with the requirements for specificity, system suitability, linearity, accuracy and method precision across the range of 50 % to 150 %. The method is therefore acceptable as valid and stability indicating.

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