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## DEVELOPMENT AND VALIDATION OF GC METHOD FOR ESTIMATION OF LINDANE IN PHARMACEUTICAL FORMULATION

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### ABSTRACT

A simple, sensitive, and precise gas chromatographic method for the analysis of lindane has been developed, validated and used for the determination of compounds in commercial pharmaceutical products. Extraction of lindane from lindane lotion was done with chloroform. The extract was evaporated almost to dryness, the residue was dissolved in acetone and separation was carried on a glass capillary column (30m × 0.25mm, i.d packed with fused silica coated with Methyl 50% Phenyl Polysiloxane) and nitrogen as a carrier gas at a flow rate of 9 mL min<sup>-1</sup>. The oven temperature was programmed at 50°C for 3 min, with a rise of 20°C min<sup>-1</sup> up to 200°C (held for 2 min) and then increased to a final temperature of 270°C (held for 3min). The injector and detector port temperatures were maintained at 300°C. Detection was carried out using flame ionization detector. Methylene chloride was used as an internal standard. The calibration graphs were found linear in the concentration range of 2- 20 µg mL<sup>-1</sup>. The recovery amount was more than 99 %. The high recovery and low relative standard deviation confirms the suitability of the method for determination of lindane in pharmaceutical dosage forms.

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### Key Words

Lindane lotion, gas chromatography

## INTRODUCTION

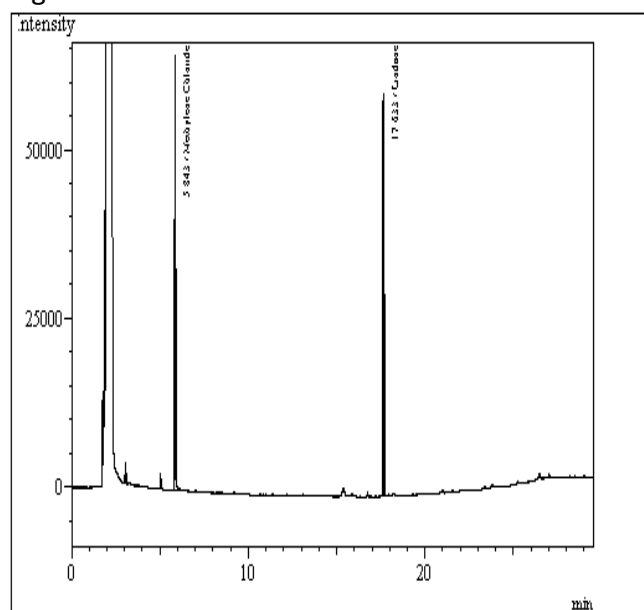
Lindane is the gamma isomer of hexachlorocyclohexane. Under the IUPAC system, lindane is named as Cyclohexane,1,2,3,4,5,6-hexachloro-, (1 $\alpha$ ,2 $\alpha$ ,3 $\beta$ ,4 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )-,  $\gamma$ -1,2,3,4,5,6-Hexachlorocyclohexane[58-89-9]<sup>1</sup>. Lindane, also known as gammahexachlorocyclohexane ( $\gamma$ -HCH)<sup>2</sup> is an organochlorine chemical that has been used both as an agricultural insecticide and as a pharmaceutical treatment for infestations of lice and scabies<sup>[3,4]</sup>. Lindane is a broad spectrum insecticide, which has been used since 1949 for agricultural and non agricultural purposes. Major agricultural use includes seed and soil treatment and wood and timber protection<sup>5</sup>. Lindane is also used against ectoparasites in veterinary and pharmaceutical products<sup>6</sup>. Lindane lotion is official in USP. As a pharmaceutical lindane is an insecticide, larvicide and acaricides. It is used topically in concentrations of 1 % for the treatment of scabies in some patients<sup>7</sup>. It is administered differently to treat pediculosis. It is also used for the control of disease vectors, including control mosquitoes, lice and fleas<sup>8</sup>. Previously published assays for lindane include spectrophotometry<sup>9</sup>, high-pressure liquid chromatography<sup>10</sup>, gas chromatography with electron capture (GC-EC) detection<sup>11</sup>, and GC with nitrogen selective detector<sup>[12,13]</sup>. GC-EC detection is a sensitive method; however, the published methods requires more than one extraction steps and inactivation of the GC column to obtain good chromatographic resolution and better peak shapes. The method given in USP is also time consuming and require prior separation of lindane by column chromatography<sup>1</sup>. We report here a GC-FID detection method that requires only a single extraction step and no preinactivation treatment of the column and that results in excellent chromatographic resolution with sharp and symmetrical peaks.

## EXPERIMENTAL

### Apparatus and conditions

A GC-2010 (Shimadzu) gas chromatograph (GC) equipped with a split/splitless injector and a flame ionization detector (FID) from Agilent Technologies Inc. was used in this study. The separation was carried

out on a OV-17 capillary column (30 m  $\times$  0.25 mm i.d., 0.25 $\mu$ m film thickness) from Agilent Technology. A volume of 3  $\mu$ L sample was injected, in the injector at 300 $^{\circ}$ C, in the mode at a split ratio of 10. GC oven temperature was initially maintained at 50 $^{\circ}$ C for 3min, then programmed to 200 $^{\circ}$ C at a rate 20 $^{\circ}$ C min<sup>-1</sup> and maintained for 2 min, finally it was raised with a rate of 5 $^{\circ}$ C min<sup>-1</sup> to a final temperature of 270 $^{\circ}$ C for 3 min. Nitrogen (ultrapure) was obtained from Pci nitrogen generator (Model no.NAG-02) and used as carrier gas at a constant flow rate of 9.0 mL min<sup>-1</sup>. The detector temperature was set at 300 $^{\circ}$ C. Control valve Prama Engineering Company (Mumbai) for providing the switch and adjustment of air to FID and 5 $\mu$ L gas-tight syringe (SGE Analytical science, Australia) were used. Under these conditions lindane was eluted at 17.633 minutes with a run time of 30 min. A typical chromatogram for estimation of lindane is illustrated in Fig. 1.



**Fig.1.** Gas chromatogram for standard solution containing lindane and Methylene Chloride (Internal standard). Peak at R.T.5.87 min is due to methylene chloride and at RT 17 .633 due to lindane.

## MATERIALS AND METHODS

Lindane lotion (1% w/v), was supplied by Gary Pharmaceutical Pvt. Limited (India) labeled to contain: 1% w/v of Gamabenzene Hexachloride I.P. HPLC grade Acetone was purchased from RANKEM (India) and used to prepare all solutions.

**Extraction of Lindane from lotion formulation**

The volume of lindane lotion equivalent to 100 mg of lindane taken in a separating funnel and 20 ml of water is added to dissolve the lotion and extracted with 2×25 ml of chloroform. The residue was dissolved in HPLC grade acetone and the solution was filtered through Whatman filter paper No.1. Before injection both standard and sample solution was filtered through 0.45µm syringe filter. Then 3 µl of standard and sample solutions were injected into injector and chromatogram was recorded.

**Standard Solution**

A standard stock solution of lindane (1 mg mL<sup>-1</sup>) were prepared in HPLC grade acetone and stored at 2-8°C. The solution was stable for 2 days at least. Working standard solutions were prepared daily by the appropriate dilution of the stock solution with the same solvent.

**Preparation of internal standard stock solutions:****Methylene chloride**

Approximately 1mL of methylene chloride was accurately taken and transferred into 100 mL volumetric flask, dissolved and diluted to volume using HPLC grade acetone.

**Preparation of reference standard solution**

100 mg of reference standard of lindane were weighed accurately and transferred into 100 mL volumetric flask. To this 10 mL of 1% v/v methylene chloride as an internal standard is added volume was made to 100 ml with HPLC grade acetone to make the concentration 1mg mL<sup>-1</sup>.

**RESULT AND DISCUSSION****Method Validation****Specificity (Selectivity)**

The selectivity of the GC method was checked by comparison of chromatograms obtained from samples and the corresponding placebo.

**Linearity**

Linearity of the method was determined by plotting a calibration curve of lindane for concentration vs.

detector response (area counts in mV). From the reference standard stock solution of lindane containing lindane at concentration level 100mg/ml, aliquots of 0.2, 0.4, 0.6, 0.8, 1 and 2 mL were taken into five individual 100mL volumetric flask. The total volume was made up to the mark with HPLC grade acetone. This gave a series of calibration standard solutions of lindane having concentrations of 2 µg, 4µg, 6 µg, 8 µg, 10 µg and 20 µg. From each of these calibration standards 3 µl was injected into the GC. The calibration curve obtained was subjected to regression analysis by the least square method to calculate the calibration equation and the correlation coefficient (r). The best fit for the calibration curve could be achieved by a linear regression equation of lindane found to be  $y = 17830x + 763.5$  and the regression coefficient values (R<sup>2</sup>) were found to be 0.998. The average retention time for lindane was found to be 17.633 ± 0.03 (Table 1).

Standard Plot for lindane  $y = 17830x - 763.5$   
R<sup>2</sup> = 0.998

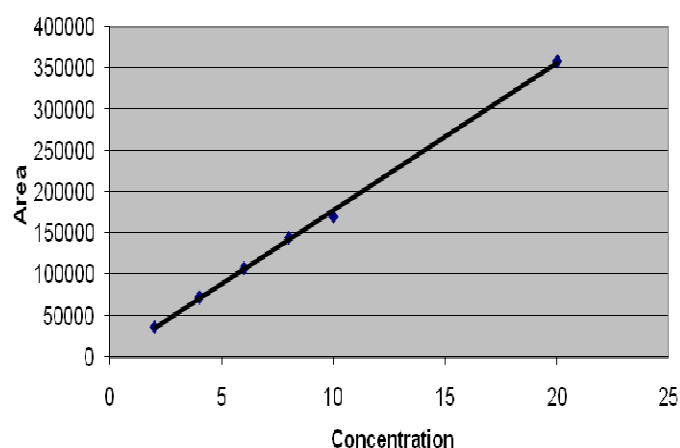


Fig.2. Calibration Curve

**Precision and accuracy**

The precision of the method was determined in terms of repeatability or reproducibility and intermediate precision studies. Repeatability was determined by evaluating five replicates of the three different concentrations *i.e.* 5µg, 10µg and 20µg of the calibration standard solution of lindane on the same day (intra-day under the mentioned chromatographic conditions. The

intermediate precision of the method was assured by performing the analysis on three different days (inter-day) and also by different analysts in the same laboratory (between analysts). Co-efficient of variation or the percent RSD was calculated in each case. (Table1) The accuracy of the method was evaluated by spiking different known concentrations of lindane into the pre-analyzed sample. One of the five sample solutions containing approx.10 µg each of lindane was spiked with varying standard concentrations of lindane *i.e.* 5µg of each, 10µg of each and 15µg of each so as to give a total concentration of 15 µg, 20 µg and 25 µg of lindane. 3µL of each of these solutions was injected onto gas chromatograph and the closeness of the results to the true value was determined. (Table1)

**Table 1:** Summary of validation parameters for the proposed method

Validation Parameters	Lindane
Linearity µg mL <sup>-1</sup>	2-20
LOD (µg mL <sup>-1</sup> )	0.0547
LOQ (µg mL <sup>-1</sup> )	0.164
Repeatability (% RSD)	0.278
Accuracy (%)	98.96–99.64
Precision (% RSD)	
Intraday (n=5)	0.56 - 0.72
Interday (n=7)	0.39 - 0.68

RSD = relative standard deviation

n = number of determination

### Limits of Detection and Quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted. The LOD for Lindane was 0.0547 µg mL<sup>-1</sup> and LOQ was 0.164 µg mL<sup>-1</sup>. (Refer Table 1).

### Analysis of Pharmaceutical Dosage Form (Lotion)

The values of analysis of Lotion obtained by the proposed method were between 99.15 % and 99.66 %, which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%. (Refer Table 2).

**Table 2:** Results of analysis of formulation and recovery studies

Drug	Quantity Claimed	Quantity Found	% Recovery ±SD
Lindane (% w/v)	1	0.9934	99.34 (±1.64)

\*Recovery amount was the average of six determinants

### CONCLUSION

Newly developed GC-FID method was developed for determining Lindane in pharmaceutical dosage forms. This GC-FID detection procedure is an excellent method for determining levels of lindane in lotions and creams. Furthermore, the single-step extraction procedure and the use of a fused silica capillary column that precludes the need for column inactivation make the assay easy to perform as well as very efficient in terms of sample processing and analysis time.

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