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Development and validation of HPLC method for estimation of lindane in its formulation

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ABSTRACT

A simple, economic, selective, precise and accurate High Performance liquid Chromatographic method used for the analysis of Lindane in its Formulations. Formulations was developed and validated in the present study. The mobile phase consists of mixed acetonitrile and water in the proportion 50:50 respectively. And this was found to give a sharp peak of Lindane at a retention time of 7.943 min. HPLC analysis of Lindane was carried out at a wavelength of 254 nm, With a flow rate of 2.08 ml min^{-1} linear regression analysis data for the calibration curve showed a good linear relationship with regression coefficient 0.999 in the concentration range of 50 ppm to 150 ppm. The linear regression equation was Y=1825×+484.8 the developed method was employed with a high degree of precision and accuracy for the analysis of Lindane. The method was validated for accuracy, precision, robustness, detection and quantification limits as for ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicate that this method is better for the quantification of Lindane

Key words: Lindane, HPLC, Validation.

INTRODUCTION

Lindane is the gamma isomer of hexachlorocyclohexane. Under the IUPAC system, lindane is named as Cyclohexane,1,2,3,4,5,6-hexachloro-,(1α,2α,3β,4α,5α,6β)-,γ-1,2,3,4,5,6Hexachlorocyclohexane[1]. This is a white, odorless, crystalline solid with molecular weight of 290.82 g/mole and molecular formula of $C_6H_6Cl_6$ and chemical structure as shown inFigure-1.Lindane, also known as gamma hexachlorocyclohexane (γ-HCH)[2] is an organ chlorine chemical that has been used both as an agricultural insecticide and as a pharmaceutical treatment for infestations of lice and scabies [3, 4] 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 [5]. Lindane is also used against ectoparasites in veterinary and pharmaceutical products [6].

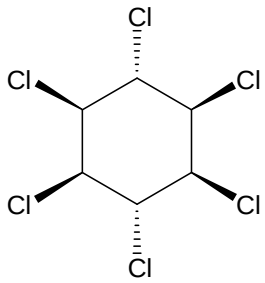


Figure -1.

Lindane (γ -hexachlorocyclohexane, γ-HCH) was used as a broad spectrum insecticide to control phytophagous and soil inhabiting insects since the 1940s[7]. Lindane is produced from technical grade HCH, which contains eight different isomers[8]. HCH is also marketed as an insecticide but since γ-HCH is the only Isomer that exhibits strong insecticidal properties, it has been common to refine and market it under the name "Lindane"[8]. Lindane, α-HCH and β-HCH were categorized as persistent organic pollutants (POPs) under the Stockholm convention in 2009[9]. The physico-chemical properties of lindane have been discussed by Xiao et al.[10]. HCH's are relatively volatile which has led to their global transport, even in locations such as the Arctic[8]. HCH's are one of the most widely detected organ chlorine compounds in environmental samples including air, surface water, soil, and living organisms. Bhatt et al[11]. Reported that HCHs can poten-tially impact on human health, due to impact on central nervous, endocrine, immune, and reproductive systems. It has also been reported that HCHs are probable carcinogens[9] . Heptachlor has been used since the 1950s as an insecticide to kill termites and other soil insects[12]. It is reported that heptachlor can persist in soil for as long as 14 years[13]. The main transforma-tion product is heptachlor epoxide, which exists in two isomeric forms: exo-heptachlor epoxide (isomer B) and endo-heptachlor epoxide (isomer A) with isomer B the more stable form in the environment[14]. As a result, heptachlor was listed on the original Stockholm Conventions "Dirty Dozen" POPs. The physico-chemical properties of heptachlor and heptachlor epoxide are reported by Shen and Wania[15]. The International Agency for Research and Cancer (IARC) has classified heptachlor as a possible human carcinogen[16] and residues are still encountered in the environment because of their high persistence and lipophilic properties[17], especially to sediments and terrestrial and aquatic organisms[14]. Heptachlor epoxide has been reported to be of greater toxicological significance because it is more stable and persists longer in the environment[18].

As a pharmaceutical lindane is an insecticide, larvicide and acaroids it is used topically in concentrations of 1 % for the treatment of scabies in some patients[19].It is administered differently to treat pediculosis. It is also used for the control of disease vectors, including control mosquitoes, lice and fleas[20].Previously published assays for lindane include spectrophotometry[21], high-pressure liquidchromatography[22], gas chromatography with electron capture (GC-EC) detection[23], and GC with nitrogen Selective detector[24&25].

No one has developed RP-HPLC method for the determination of lindane in its formulations. So the author has developed RP-HPLC method for the determination of lindane in its formulations based on the use of symmetry column, without use of any internal standard. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines.

IUPAC NAME: gamma 1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexane

Physical Properties:

Table-1: Physical Properties of Lindane

S.No.	Property	Description
1	Molecular Weight	290.82
2	Appearance	Colorless to white crystalline powder/solid
3	Odor	Odorless to slight musty or aromatic odor
3	Density	1.85-1.90 g/cm 3
4	Melting Point	112.5 °C
5	Boiling Point	323.4 °C
6	Solubility in Water at 20 C	7.3 mg/l
7	Octanol/water partition coefficient as log Pow	3.61 - 3.72
8	Hazards LD_{50}	300 mg/kg
9	Henry's law constant at 20-25°C (Pa m^3mol^{-1})	0.140-0.52
10	CAS #Number	58-89-9
11	Vapor Pressure @25^{0}C	5.57 x 10-5 mm Hg
12	Specific gravity	1.9 (EPA, 1998)
13	Partition Coefficient	5,248
14	Adsorption Coefficient	1100
15	Maximum UV-vis absorption L mol^{-1} cm^{-1}	No absorption above 290nm in neutral medium

Solubility:

Table-1.1: Solubility Properties of Lindane

S.No.	Solvent	Solubility
1	Water at 20 ^{0}C g/l	0.007
2	Acetone at 20°C (g l^{-1})	435
3	Ethylacetate at 20°C (g l^{-1})	357
4	Benzene g/kg	289
5	Methanol at 20°C (g l^{-1})	29
6	Ether g/kg	208
7	Ethanol g/kg	64

CHECIMAL CLASS: *Organ chlorine insecticide*

LINDANE ASSAY BY HPLC-METHOD VALIDATION

Analysis of Lindane has mainly been accomplished by different chromatographic methods such as, gas chromatography (GC-ECD) and thin layer Chromatography TLC methods were more frequently employed for the analysis of Lindane in different Environmental samples.

However no reported RP-HPLC method for the analysis of Lindane in its technical grade and formulations. This chapter describes a validated RP-HPLC method for the quantitative determination of Lindane.

The author has developed RP-HPLC method based on the use of Waters symmetry C18 column, without use of any internal standard. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines.

INSTRUMENTS USED:

High performance liquid chromatography, with UV / PDA detector
HPLC Analytical column of Bondpark - C18, 300mm x 3.9mm x 5µm
Analytical weighing balance - Mettler Toledo B204S
Millipore Nylon 0.2µm
Laboratory accessory.

CHEMICALS USED

Lindane working standard
Gammexane powder
Acetonitrile -AR
Methanol-AR
Water-HPLC

REFERENCES

ICH Guideline number: Q2A & Q2B of CPMP / ICH / 281 / 95.

Table-1.2: ICH Guidelines

	Name	W.S No.	Purity on Dried Basis	LOD
1	Lindane	WS-005	99.81%	0.15%

ANALYTICAL METHOD:
The quantitative determination is carried out by HPLC system equipped with UV-detector.

Chromatographic conditions:

Column	:	Bondpark - C18, 300mm x 3.9mm x 5µm
Mobile Phase	:	Mixed Acetonitrile and water in the proportion 50:50 respectively. Mixed well. Filtered through 0.2 µm Nylon membrane filter paper and degas prior to use.
Wavelength	:	254 nm
Flow Rate	:	2.0 ml / minute
Injection volume	:	10 µl
Run time	:	15 minutes
Blank solution	:	Use Mobile phase as blank
Diluent	:	Use Mobile phase as diluent

Preparation of Lindane Standard Solution: Weighed accurately about 50 mg of Lindane working standard and transfered to a 50 ml volumetric flask. Added 10 ml of diluent and sonicated to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.(Dilution scheme: 50mg → 50.0 ml → 1 ml /10.0 ml)

Preparation of Test Solution: Weighed accurately about 50 mg of sample and transfered to a 50 ml volumetric flask. Added 10 ml of diluent and sonicated to dissolve. Diluted to volume with diluent and mixed. Transfered 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.(Dilution scheme: 50mg → 50.0 ml → 1 ml /10.0 ml)

System Suitability Solution:
Used Lindane standard working solution as system suitability solution.

Procedure:
Five replicate injections of system suitability solution (Lindane standard working solution) and equal volumes of blank solution injected separately. Then injected two injections of test solution and recorded the chromatograms. Disregarded any peak due to blank in the test solution and calculated % RSD of five replicate injections of system suitability solution (Lindane standard working solution). Checked tailing factor and theoretical plates of the peak in the chromatogram obtained with 5^{th} injection of system suitability solution (Lindane standard working solution).

The limits are as below,
1) Theoretical plates should be not less than 3000.
2) Tailing factor should be less than 2.0.
3) % RSD should be not more than 2.0%.

Injection scheme:

Table-1.3: Injuction Scheme

Sr. No.	Solutions to be injected	No. of injections
01	Diluent Blank solution	1
02	System suitability solution (Lindane standard working solution)	5
03	Test Solution	2

Calculations:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{1}{10} \times \frac{50}{WT} \times \frac{10}{1} \times P$$

AT	Average Peak area of Lindane in test solution
AS	Mean peak area of Lindane in system suitability solution
WS	Weight of Lindane working standard taken in mg
WT	Weight of sample taken in mg
P	Assay of Lindane working standard in % on as is basis
	Express the results up to two decimals.

VALIDATION PARAMETERS

The HPLC method is evaluated for following validation parameters as per protocol followed by ICH guideline Quality topics Q2A & Q2B of CPMP / ICH / 281 / 95.

Table-1.4: Validation Parameters

S. No.	Validation Parameters	Assay
1	Specificity / Selectivity	+
2	Linearity & Range of Lindane Std from 50% to 150%	+
3	Precision i) System precision ii) Method precision iii) Intermediate precision (Ruggedness)	+
4	LOD & LOQ	+
5	Stability of analytical solutions	+

VALIDATION RESULTS:

The system suitability parameters were monitored throughout the validation study and are recorded in the validation report. The validation data is summarized below:

Specificity / Selectivity:

Selectivity was performed by injecting the diluent blank solution, system suitability solution, test solution.

Acceptance criteria:

The Lindane peak should be well resolved from any other peak and from each other.

The diluent blank solution should not show any peak at the retention time of the Lindane.

RESULTS

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution with Lindane peak.

LINEARITY :

Linearity and Range for standard:

For the linearity study five standard solutions of Lindane were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation. The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined.

Acceptance criteria:

Correlation coefficient should be greater than or equal to 0.999.

RESULTS

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table-1.6 for system suitability results).

Selectivity
The average peak area of Lindane peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of linearity study are as given in Table-1.7.

PRECISION:
System Precision:
Procedure:
The system precision was performed by injecting 10 replicate injections of system suitability solution and the chromatograms are reviewed for the system suitability criteria.

Acceptance criteria:
% RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per analytical method.

RESULTS

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The results are tabled in table-1.8.

Method Precision:
Procedure: Six test solutions of Lindane in Gammahexane powder were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated.

Acceptance criteria:
% RSD of the results of six test solutions should not be more than 2.0%.

RESULTS

The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. The results of assay obtained from six test solutions preparations are presented in Tables – 1.9&2.0.

Intermediate Precision:
Procedure:
Six test solutions of Gammahexane powder were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

Acceptance criteria:
% RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%.

RESULTS

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table -2.1 for system suitability results). The results of assay obtained from six test solutions are presented in Table – 2.2. % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table – 2.3.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

Observation: Limit of detection and Quantitation was established by injecting six times very low concentration of Lindane standard preparation i.e. 1ppm and 2ppm. The relative standard deviation for the peak response of Lindane obtained for six replicate injections is 0.33%. The results are tabled in tables-2.4 &2.5.

CONCLUSION

Hence the described method could be used to detect and quantify minimum of 5 ppm of Lindane for any given samples including in the samples collected for cleaning validation.

Stability of Analytical Solution:

Procedure:

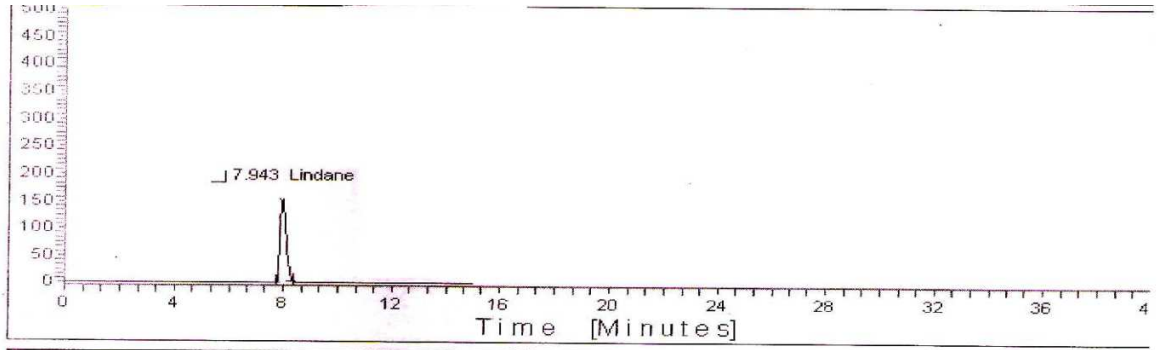
System suitability solution and test solution of Gammahexane powder were prepared on 0^{th},12^{th}, 24^{th}, 36^{th} and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of Gammahexane powder in the sample was calculated.

Acceptance criteria:

The analyte is considered stable if there is no significant change in % assay.

RESULTS

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method (Refer to Table2.6&2.7 –for system suitability results).



Result-A Table						
Peak No	**Retn.Time**	**Area**	**Height**	**Area %**	**Height %**	**Width@50%**
1	7.943	2447.955	153.285	100	100	0.266
Total		**2447.955**	**153.285**	**100**	**100**	

Figure.1: Chromatogram of Lindane

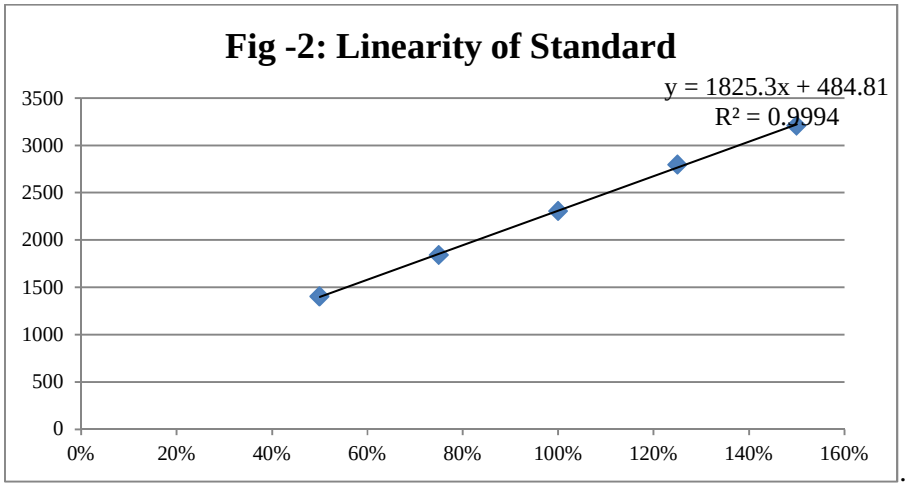


Figure-2: Linearity graph of Lindane standard

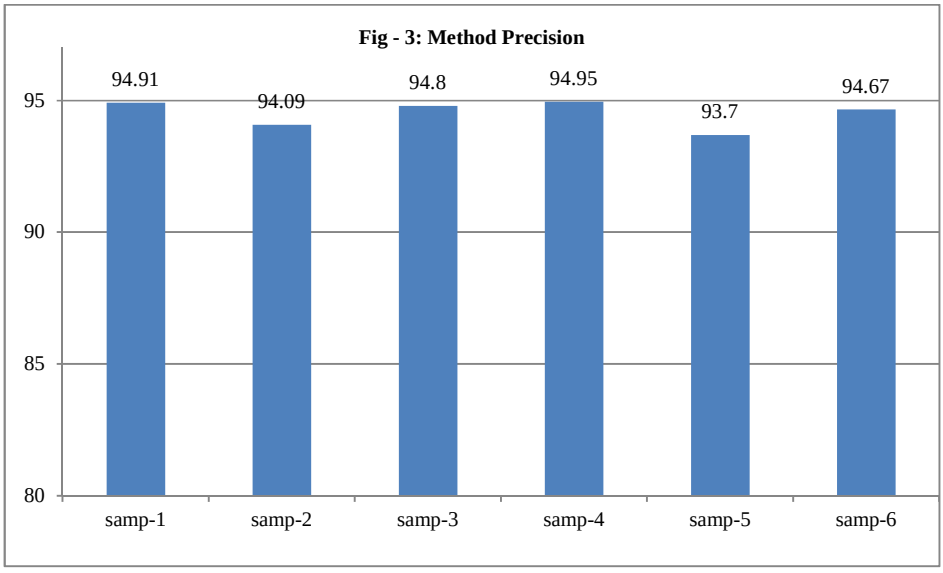


Figure-3: Method Precision

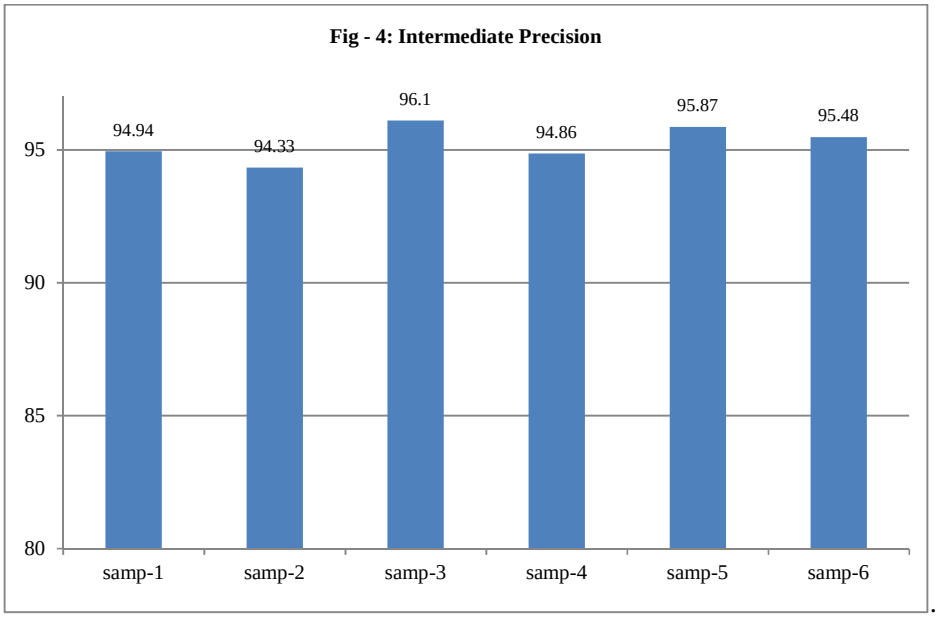


Figure-4: Intermediate Precision

Table-1.5: System suitability - Selectivity

Sr. No.	Area of Lindane
1	2820.47
2	2845.89
3	2835.88
4	2805.11
5	2841.97
Mean	**2829.87**
Standard Deviation (±)	**16.89**
(%) Relative Standard Deviation	**0.60**

Table-1.6: System suitability - Linearity of standard

Sr. No.	Area of Lindane
1	2447.96
2	2440.35
3	2415.59
4	2434.72
5	2462.18
Mean	**2440.16**
Standard Deviation (±)	**17.17**
(%) Relative Standard Deviation	**0.70**

Table-1.7: Results of linearity of standard

Linearity Level	SampleConcentration (in%)	Sample Concentration (inppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1401.83	
Level – 2	75	75	1841.12	
Level – 3	100	100	2305.93	**0.999**
Level – 4	125	125	2795.29	
Level – 5	150	150	3206.38	

The linearity plot of peak area of Lindane Vs. standard concentration in percentage is presented in figure-1.

Table-1.8: System precision

Sr. No.	Area of Lindane
1	2483.26
2	2486.44
3	2484.17
4	2432.67
5	2407
6	2458.07
7	2464.49
8	2508.58
9	2447.96
10	2446.39
Mean	**2461.9**
Standard Deviation (±)	**29.92**
(%) Relative Standard Deviation	**1.22**

Analyst – 1

HPLC No.: EH/R&D/HPLC-024

Table-1.9: System suitability - Method precision

Sr. No.	Area of Lindane
1	2722.70
2	2793.40
3	2790.05
4	2748.06
5	2762.14
Mean	**2763.27**
Standard Deviation (±)	**29.59**
(%) Relative Standard Deviation	**1.07**

Table-2.0: Results of method precision

Test Solution	% Assay of Lindane
1	94.91
2	94.09
3	94.80
4	94.95
5	93.70
6	94.67
Mean	**94.52**
Standard Deviation (±)	**0.51**
(%) Relative Standard Deviation	**0.54**

Analyst – 2 **HPLC No.: EH/R&D/HPLC-023**

Table-2.1: System suitability - Intermediate precision

Sr. No.	Area of Lindane
1	5235.94
2	5221.11
3	5240.62
4	5270.70
5	5250.90
Mean	**5243.85**
Standard Deviation (±)	**18.44**
(%) Relative Standard Deviation	**0.35**

Table-2.2: Results of intermediate precision

Test Solution	% Assay of Lindane
1	94.94
2	94.33
3	96.1
4	94.86
5	95.87
6	95.48
Mean	**95.26**
Standard Deviation (±)	**0.67**
(%) Relative Standard Deviation	**0.70**

Table-2.3: Results of twelve test solutions of Lindane in Gammexane powder (six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Lindane**
1	94.91
2	94.09
3	94.80
4	94.95
5	93.70
6	94.67
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Lindane**
7	94.94
8	94.33
9	96.10
10	94.86
11	95.87
12	95.48
Mean of twelve samples	**94.91**
Standard Deviation (±)	**0.68**
(%) Relative Standard Deviation	**0.72**

Table-2.4: System suitability – LOD & LOQ

Sr. No.	Area of Lindane
1	2827.42
2	2823.07
3	2814.41
4	2837.04
5	2814.95
Mean	**2823.38**
Standard Deviation (±)	**9.41**
(%) Relative Standard Deviation	**0.33**

Table-2.5: Results for LOD & LOQ

Injection No.	Peak Response of LOD 1ppm	Peak Response of LOQ 2ppm
1	112.68	241.18
2	103.95	241.84
3	103.58	252.27
4	103.82	257.24
5	105.30	243.33
6	111.22	243.85
Average	**106.76**	**246.62**
Standard Deviation	**4.09**	**6.57**
%RSD	**3.83**	**2.66**

Table-2.6: System suitability - Solution stability

Time	Std Area	Avg std area	Spl area	Avg Spl area
0^{th} hr	2496.29		2491.79	
	2488.6	2492.45	2497.08	2491.79
12^{th} hr	2448.31		2440.6	
	2436.96	2442.64	2437.14	2440.6
24 hr	2425.59		2422.26	
	2428.76	2427.17	2432.32	2422.26
36 hr	2414.45		2407	
	2411.02	2412.74	2406.18	2407
48 hr	2456.19		2463.1	
	2458.42	2457.3	2464.49	2463.1
Mean	**2445.13**	**2446.46**	**2444.16**	**2444.95**
Standard Deviation (□)	**29.09**	**30.64**	**32.15**	**33.53**
(%) Relative Standard Deviation	**1.18**	**1.25**	**1.31**	**1.37**

Table-2.7: Results for solution stabiliy

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Lindane**
0^{th} hr	95.99
12^{th} hr	95.76
24 hr	95.92
36 hr	95.67
48 hr	96.17
Mean	**95.9**
Standard Deviation (□)	**0.19**
(%) Relative Standard Deviation	**0.19**

CONCLUSION

Table-2.8: Summary and conclusion

S. No.	Parameter	Result	Acceptance Criteria
1	**Specificity: Selectivity**	The Lindane peak in test solution was found to be well resolved from peaks due to diluent blank solution. The diluent blank do not show any peak at the retention time of the Lindane.	The Lindane peak all should be well resolved from any other peak and from each other. The diluent blank solution should not show any peak at the retention time of the Lindane.
2	**Linearity and Range of Standard**	Correlation coefficient = 0.999 Range = 50 ppm to 150 ppm	Correlation coefficient should be greater than or equal to 0.999.
3	**System precision**	% RSD = 1.22	% RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per analytical method.
4	**Method precision**	% RSD = 0.54	% RSD of the results of six test solutions should not be more than 2.0%.
5	**Intermediate precision**	% RSD = 0.70	% RSD of the results of twelve test solutions (six of Method Precision and six of Intermediate Precision) should not be more than 2.0%.
6	**LOD**	% RSD = 3.83	% RSD of the results of six test solutions should not be more than 10.0%.
7	**LOQ**	% RSD =2.66	% RSD of the results of twelve test solutions (six of Method Precision and six of Intermediate Precision) should not be more than 5.0%.
8	**Stability of analytical solution**	No significant change was observed in the % assay upto 48 Hrs. Hence the solution is found to be stable up to 48 Hours at room temperature.	The analyte is considered stable if there is no significant change in % assay.

CONCLUSION

The above summary and the validation data summarized in this document shows that the analytical method of assay of Lindane in Gammahexane powder by HPLC is found to be suitable, selective, specific, precise, linear and robust. The analytical solution is found to be stable up to 48 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

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