

# Analytical Method Validation for Quantitative Estimation of Chloropicrin by HPLC Method

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**ABSTRACT:** A simple, selective, precise and accurate High Performance Liquid Chromatographic method for the analysis of Chloropicrin in its formulations was developed and validated in the present study. The mobile phase consists of a mixture of acetonitrile and water in the proportion 50: 50 (v/v). This was found to give sharp peak of Chloropicrin at a run time of 10 min. HPLC analysis of Chloropicrin was carried out at a wave length of 220 nm with a flow rate of 1.0mL/ min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient 0.999 in the concentration range of 50% to 150%. The linear regression equation was  $y = 2055x - 109.6$ . The developed method was employed with a high degree of precision and accuracy for the analysis of Chloropicrin. The method was validated for accuracy, precision, robustness, ruggedness and specificity. The Precision, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is useful for the quantification of Chloropicrin.

**KEYWORDS:** Chloropicrin, HPLC Method Development and Validation.

## I. INTRODUCTION

Chloropicrin is also known as Trichloronitromethane, Nitrotrichloromethane, Chlorpikrin, Trichloronitromethan, Chloropicrine, and nitrochloroform. Chloropicrin is a widely used soil fumigant and an old chemical warfare agent and is a chemical compound commonly used as a broad-spectrum antimicrobial agent, fungicide, herbicide, insecticide, and nematicide. <sup>[1, 2]</sup>Chloropicrin was manufactured for use as poison gas in World War I<sup>[3]</sup> and a riot control agent. In agriculture, chloropicrin is injected into soil prior to planting a crop in order to fumigate soil. Chloropicrin affects a broad spectrum of fungi, microbes, and insects.<sup>[4,5]</sup> for the non-deciduous (falling off at maturity) fruits, and fumigant for crops like tomatoes, tobacco, potatoes, onions, beans, other field crops like cotton, peanuts, sugar beets, & deciduous fruits & nuts, other vegetables, floral crops, lawns, turf, & ornamentals, commodities, space, & structural pest control, miscellaneous soil fumigation. It is commonly used as a stand-alone treatment or in combination or co-formulation with methyl bromide and 1, 3-Dichloropropene<sup>[5, 6]</sup>. Chloropicrin is used as an indicator and repellent while fumigating residences for insects with sulfuryl fluoride which is an odorless gas.<sup>[7]</sup> <sup>[8]</sup>One of the oldest soil fumigants, chloropicrin's primary agricultural use in soils is as a fungicide, although it does have herbicidal and nematicidal activity. It is often added to 1, 3-D formulations in order to increase their fungicidal activity. Fumigation with Chloropicrin is used to control pests in buildings (structural fumigation), soil, grain, and also used during processing of goods to be imported or exported to prevent transfer of exotic organisms. This method also affects the structure itself, affecting pests that inhabit the physical structure, such as woodborers and dry wood termites.<sup>[9]</sup> The compound is acutely toxic and carcinogenic and is used in liquid formulations. In 1997, it was the 25th most abundantly used U.S. pesticide.<sup>[10]</sup> Chloropicrin (PS) is an irritant with characteristics of a tear gas, and it has an intensely irritating odor. Inhalation of 1 ppm causes eye irritation and can warn of exposure. Trade names of chloropicrin are Acquinite, Dojyopicrin, Dolochlor, Microlysin, Tri-Clor, Metapicrin, Picfume, Picride.<sup>[11]</sup>

Gas chromatograph with electron capture detector (GC-ECD) and Gas chromatograph-Mass spectrometer (GC-MS),<sup>[12]</sup> Gas chromatography-Mass spectrometry and Liquid chromatography-Tandem Mass spectrometry,<sup>[13]</sup> High-temperature automatic thermal desorption-Gas chromatography-Mass spectrometry,<sup>[14]</sup> Liquid-liquid extraction

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and Gas chromatography with electron capture detection,<sup>[15]</sup> chemical ionization (CI) method,<sup>[16]</sup> Gas-Liquid Chromatography<sup>[17]</sup> and Spectrophotometric determination.<sup>[18]</sup> Polarographic determination<sup>[19, 20]</sup> of chloropicrin and the quantitative determination of Chloropicrin has been performed by total chlorine method,<sup>[21]</sup> and Colorimetric method.<sup>[22]</sup> However, these methods require a great deal of trouble and most of these methods are complicated, tedious, used large amounts of solvent and time-consuming and expensive. Therefore, there is a need for better methods of analysis and this article describes the novel application of a modified QuEChERS method for Chloropicrin determination in its formulations.

The HPLC method described here is simple, sensitive, and reproducible for determination in formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters as mentioned in various gradients. For pesticide formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pesticide formulations.

### Structure:

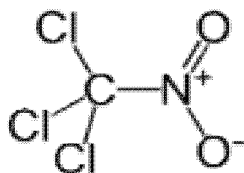


Figure-6.1: Chemical structure of Chloropicrin

**Chemical name:** Trichloro(nitro)methane [76-06-2]

**Molecular Formula:** CCl<sub>3</sub>NO<sub>2</sub>

**Molecular Weight:** 164.375 g/mol

## II. INSTRUMENTS REQUIRED

High performance liquid chromatography, with UV / PDA detector, HPLC Analytical column of RP - C18, 25mm x 4.6mm x 5μ, Analytical weighing balance - Mettler Toledo B204S, Millipore Nylon 0.2μm.

## III. CHEMICALS REQUIRED

Chloropicrin working Standard, Dow Agro sciences Chloropicrinare 96%, Acetonitrile – AR, Hydrochloric Acid, Sodium hydroxide and Millipore water.

## IV. CHROMATOGRAPHIC CONDITIONS

<b>Column</b>	: RP - C18, 25mm x 4.6mm x 5μ
<b>Mobile Phase</b>	: For isocratic system, prepared a mixture of Acetonitrile and water in the proportion 50: 50 respectively. Mixed well. Filtered through 0.2 μ Nylon membrane filter paper and degassed prior to use.
<b>Wavelength</b>	: 220 nm
<b>Flow Rate</b>	: 1.0 ml / minute
<b>Injection volume</b>	: 20 μl
<b>Run time</b>	: 10 minutes
<b>Blank solution</b>	: Acetonitrile
<b>Diluent</b>	: Acetonitrile

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## V. PREPARATION OF STANDARD SOLUTION

Accurately 50mg of Chloropicrin working standard is weighed and transferred to a 50ml volumetric flask. 10 ml of diluents was added and sonicated to dissolve. Diluted to volume with diluent and mixed. Transferred 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

## VI. PREPARATION OF TEST SOLUTION

Weighed accurately about 52 mg of sample and transferred to a 50 ml volumetric flask. Added 10 ml of diluent and sonicated to dissolve. Diluted to volume with diluent and mixed. Transferred 1.0 ml of solution into a 10 ml of volumetric flask and diluted to volume with the diluent and mixed.

## VII. SYSTEM SUITABILITY SOLUTION

**Procedure:** Chloropicrin standard working solution is used as system suitability solution. Equal volumes of blank, five replicate injections of system suitability solution were injected separately. Then injected two injections of test solution and recorded the chromatograms. Any peak due to blank in the test solution is disregarded. % RSD of five replicate injections of system suitability solution was calculated. Tailing factor and theoretical plates of the peak in the chromatogram obtained with 5<sup>th</sup> injection of system suitability solution is checked and results are recorded in table-1.1.

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%.

**Table -1.1: System suitability - Selectivity**

S. No.	Area of Chloropicrin
1	2088.67
2	2099.11
3	2095.82
4	2092.47
5	2049.53
<b>Mean</b>	<b>2085.12</b>
<b>Standard Deviation (±)</b>	<b>20.27</b>
<b>(%) Relative Standard Deviation</b>	<b>0.97</b>

## VIII. VALIDATION PARAMETERS

### (i). Specificity / Selectivity:

Selectivity was performed by injecting the diluent blank solution, excipient blend, system suitability solution, test solution and results are recorded in table-1.2.

**Table -1.2: Results of System - Selectivity**

S. No.	Area of Chloropicrin
1 (Blank)	Blank
2 (Standard 1)	2088.67
3 (Standard 2)	2099.11
4 (Standard 3)	2095.82
5 (Standard 4)	2092.47
6 (Standard 5)	2049.53
7 (Sample 1)	2093.81
8 (Sample 2)	2098.98

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<b>Mean</b>	<b>2088.34</b>
<b>Standard Deviation (±)</b>	<b>17.50</b>
<b>(%) Relative Standard Deviation</b>	<b>0.84</b>

**Acceptance criteria:** The Chloropicrin peak should be well resolved from any other peak and from each other. The diluent blank solution, excipient blend solution should not show any peak at the retention time of the Chloropicrin.

**(ii). Linearity**

For the linearity study five standard solutions of Chloropicrin 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. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined. The system suitability linearity standard solutions were injected and results are mentioned in table - 1.3.

**Table -1.3: System suitability - Linearity of standard**

S. No.	Area of Chloropicrin
1	2128.26
2	2132.99
3	2114.51
4	2119.75
5	2124.91
<b>Mean</b>	<b>2124.08</b>
<b>Standard Deviation (±)</b>	<b>7.21</b>
<b>(%) Relative Standard Deviation</b>	<b>0.34</b>

The average peak area of Chloropicrin 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 -1.4.

**Table -1.4: Results of linearity of standard**

Linearity Level	Sample Concentration (In %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1158.13	<b>0.999</b>
Level – 2	75	75	1644.43	
Level – 3	100	100	2143.31	
Level – 4	125	125	2658.05	
Level – 5	150	150	3220.30	

The linearity plot of peak area of Chloropicrin Vs. standard concentration in percentage is presented in figure-1.2 and the correlation coefficient was determined.

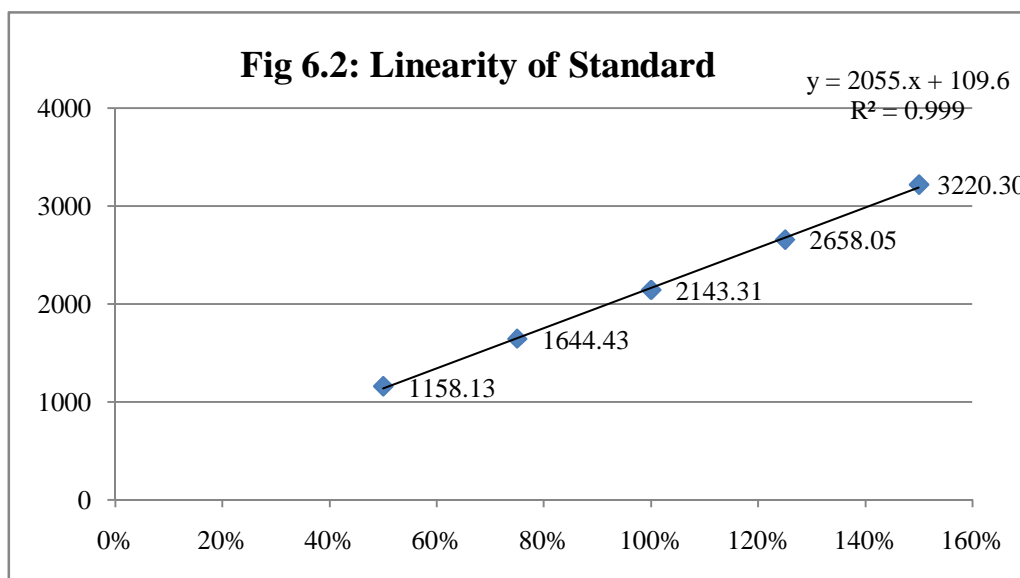
**Acceptance Criteria:** Correlation coefficient should be greater than or equal to 0.999.

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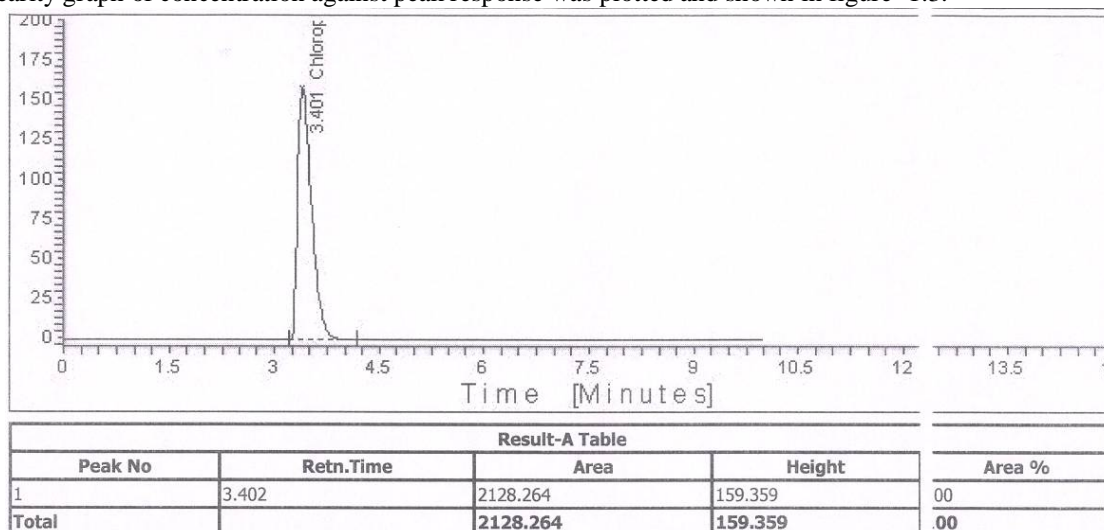
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**Figure 1.2: Linearity graph of Chloropicrin standard**

The linearity graph of concentration against peak response was plotted and shown in figure- 1.3.



**Figure-1.3: Chromatogram of Chloropicrin Sample**

**(iii). Precision:**

**a. 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 and results are recorded in table - 1.5.

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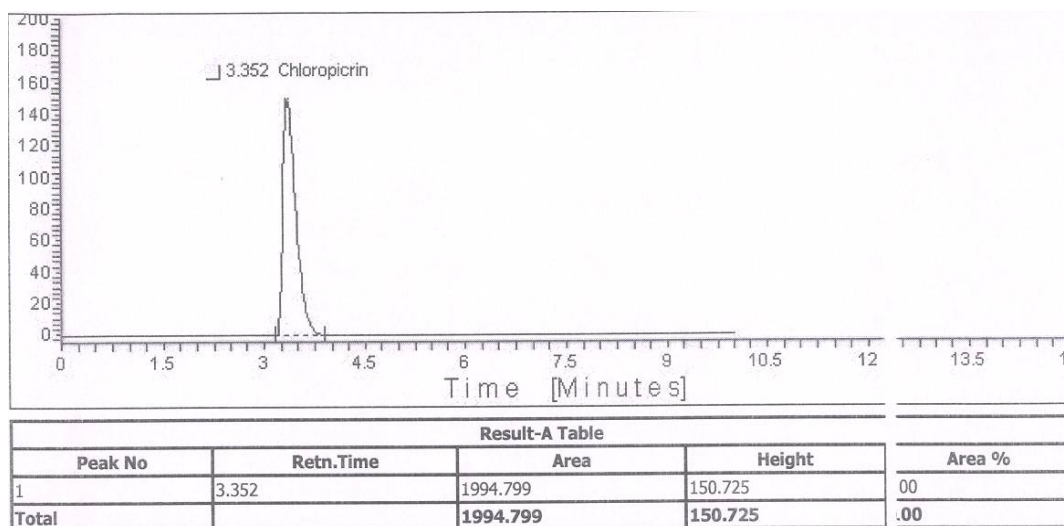
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**Table -1.5: System precision**

S. No.	Area of Chloropicrin
1	1994.79
2	1986.48
3	1992.55
4	1979.77
5	1999.45
6	1993.76
7	1983.93
8	1978.35
9	1988.25
10	1985.69
<b>Mean</b>	<b>1988.30</b>
<b>Standard Deviation (±)</b>	<b>6.79</b>
<b>(%) Relative Standard Deviation</b>	<b>0.34</b>

**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.



**Figure-1.4: Chromatogram of Chloropicrin Sample (System Precision)**

**b. Method Precision:**

**Procedure:** The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. System suitability results are presented in table-1.6.

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**Table -1.6: System suitability - Method precision  
Analyst – 1, HPLC No.: EH/R&D/HPLC-024**

S. No.	Area of Chloropicrin
1	2104.25
2	2102.04
3	2103.03
4	2107.40
5	2118.60
<b>Mean</b>	<b>2107.06</b>
<b>Standard Deviation (±)</b>	<b>6.76</b>
<b>(%) Relative Standard Deviation</b>	<b>0.32</b>

**Method Precision of Test Solution:**

Six test solutions of Chloropicrin in Dow AgroSciences Chloropicrinare 96%and were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated. The results of assay obtained from six test solutions preparations are presented in table -1.7.

**Table -1.7: Results of Method precision**

Test Solution	% Assay of Chloropicrin
1	100.22
2	100.10
3	98.00
4	101.99
5	99.64
6	101.87
<b>Mean</b>	<b>100.30</b>
<b>Standard Deviation (±)</b>	<b>1.49</b>
<b>(%) Relative Standard Deviation</b>	<b>1.48</b>

**Acceptance Criteria:** % RSD of peak areas of six test solutions should not be more than 2.0%.

**c. Intermediate Precision:**

**Procedure:** The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Refer to table- 1.8 for system suitability results.

**Table -1.8: System suitability - Intermediate precision**

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

S. No.	Area of Chloropicrin
1	2306.35
2	2314.53
3	2311.61
4	2304.19
5	2320.76
<b>Mean</b>	<b>2311.49</b>
<b>Standard Deviation (±)</b>	<b>6.61</b>
<b>(%) Relative Standard Deviation</b>	<b>0.29</b>

Six test solutions of Dow AgroSciences Chloropicrinare 96% 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 results are presented in table- 1.9.



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**Table -1.9: Results of Intermediate precision**

Test Solution	% Assay of Chloropicrin
1	100.06
2	101.40
3	100.62
4	101.29
5	100.80
6	101.30
<b>Mean</b>	<b>100.91</b>
<b>Standard Deviation (±)</b>	<b>0.52</b>
<b>(%) Relative Standard Deviation</b>	<b>0.52</b>

**(iv). Ruggedness:**

The % RSD of assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated. Results are noted in table -1.10.

**Table -1.10: Results of twelve test solutions of Chloropicrin in Dow AgroSciences Chloropicrinare 96% (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 Chloropicrin
1	100.22
2	100.10
3	98.00
4	101.99
5	99.64
6	101.87
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column S. No.	015337030136 01
Test Solution	% Assay of Chloropicrin
7	100.06
8	101.40
9	100.62
10	101.29
11	100.80
12	101.30
<b>Mean of twelve samples</b>	<b>100.61</b>
<b>Standard Deviation (±)</b>	<b>1.11</b>
<b>(%) Relative Standard Deviation</b>	<b>1.10</b>

**Acceptance Criteria:** % RSD of peak areas of twelve test solutions (six of method precision & six of intermediate precision) should not be more than 2.0%.



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**(v). Robustness:**

Prepare two test solutions of the same lot of Chloropicrin in Dow AgroSciences Chloropicrinare 96% as per analytical method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

- Change in column lot (same make, different serial no.)
- Change in flow rate ( $\pm 0.2$  ml/minute)
- Change in wavelength ( $\pm 2$  nm)
- Change in composition of mobile phase ( $\pm 20$ ml)

**a. Change in Column Lot:**

Normal Experimental Condition: RP - C18, 25mm x 4.6mm x 5 $\mu$ . The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Results recorded in table -1.11 for system suitability.

**Table -1.11: System suitability - Robustness with Change in Column Lot**

S. No.	Area of Chloropicrin	
	Same column	Diff column
1	2114.36	2028.26
2	2107.06	2052.37
<b>Mean</b>	<b>2110.71</b>	<b>2040.32</b>
<b>Standard Deviation (<math>\pm</math>)</b>	<b>5.16</b>	<b>17.05</b>
<b>(%) Relative Standard Deviation</b>	<b>0.24</b>	<b>0.84</b>

The assay results obtained with different Column lot conditions are as given in table -1.12.

**Table -1.12: Results for Change in Column Lot**

Flow rate $\rightarrow$	Same column	Diff column
<b>Sample</b>	<b>% Assay</b>	
Test solution	100.17	99.54
Average assay result from Method precision	100.30	100.30
<b>Mean</b>	<b>100.24</b>	<b>99.92</b>
<b>Standard Deviation (<math>\pm</math>)</b>	<b>0.09</b>	<b>0.54</b>
<b>(%) Relative Standard Deviation</b>	<b>0.09</b>	<b>0.54</b>

**b. Change in Flow Rate ( $\pm 0.2$  mL/minute):**

Normal Experimental Condition: 1.0ml/minute. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Results are recorded in table -1.13 for system suitability.

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**Table -1.13: System suitability - Robustness with change in flow rate**

S. No.	Area of Chloropicrin	
	0.8mL/minute	1.2 mL/minute
1	2122.45	2132.99
2	2134.16	2116.64
<b>Mean</b>	<b>2128.31</b>	<b>2124.82</b>
<b>Standard Deviation (±)</b>	<b>8.28</b>	<b>11.56</b>
<b>(%) Relative Standard Deviation</b>	<b>0.39</b>	<b>0.54</b>

The assay results obtained with different flow rate conditions are as given in table- 1.14.

**Table -1.14: Results for change in flow rate**

Flow rate →	0.8mL/minute	1.2 mL/minute
<b>Sample</b>	<b>% Assay</b>	
Test solution	99.50	100.04
Average assay result from Method precision	100.30	100.30
<b>Mean</b>	<b>99.90</b>	<b>100.17</b>
<b>Standard Deviation (±)</b>	<b>0.57</b>	<b>0.18</b>
<b>(%) Relative Standard Deviation</b>	<b>0.57</b>	<b>0.18</b>

**c. Change in Wavelength (± 2 nm):**

Normal Experimental Condition: 220nm. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Results are recorded in table-1.15 for system suitability.

**Table -1.15: System suitability - Robustness with change in wavelength**

S. No.	Area of Chloropicrin	
	218 nm	222 nm
1	1974.74	2068.74
2	2052.81	2071.82
<b>Mean</b>	<b>2013.78</b>	<b>2070.28</b>
<b>Standard Deviation (±)</b>	<b>55.20</b>	<b>2.18</b>
<b>(%) Relative Standard Deviation</b>	<b>2.74</b>	<b>0.11</b>

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The assay results obtained with different wavelength conditions are as given in table -1.16.

**Table -1.16: Results for change in wavelength**

Wavelength →	218 nm	222 nm
Sample	% Assay	
Test solution	100.05	98.20
Average assay result from Method precision	100.30	100.30
<b>Mean</b>	<b>100.18</b>	<b>99.25</b>
<b>Standard Deviation (±)</b>	<b>0.18</b>	<b>1.48</b>
<b>(%) Relative Standard Deviation</b>	<b>0.18</b>	<b>1.50</b>

**d. Change in composition of Mobile Phase (± 20ml):**

Normal Experimental Condition: Water: Acetonitrile = 500ml: 500ml. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Results are recorded in table-1.17 for system suitability.

**Table -1.17: System suitability - Robustness with change in composition of mobile phase**

S. No.	Area of Chloropicrin	
	480ml W:520ml A	520mlW:480mlA
1	1996.15	1988.25
2	1986.48	1982.69
<b>Mean</b>	<b>1991.32</b>	<b>1985.47</b>
<b>Standard Deviation (±)</b>	<b>6.84</b>	<b>3.93</b>
<b>(%) Relative Standard Deviation</b>	<b>0.34</b>	<b>0.20</b>

The assay results obtained with change in composition of mobile phase are as given in table-1.18.

**Table -1.18: Results for change in composition of mobile phase**

Composition of Acetonitrile & water	480Wml:520Aml	520Wml:480Aml
Sample	% Assay	
Test solution	99.31	99.45
Average assay result from Method precision	100.30	100.30
<b>Mean</b>	<b>99.81</b>	<b>99.88</b>
<b>Standard Deviation (±)</b>	<b>0.70</b>	<b>0.60</b>
<b>(%) Relative Standard Deviation</b>	<b>0.70</b>	<b>0.60</b>

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### IX. FORCED DEGRADATION

The forced degradation studies are performed to establish the stability indicating nature of the assay Method and to observe any degraded compounds. Chloropicrin working standard and Samples are subjected to stress with 5N HCl, 5N NaOH, Thermal degradation and UV degradation. The stress conditions are followed for degradation mentioned in table- 1.19.

**Table - 1.19: Conditions – Forced Degradation**

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105°C for 12 hours in Hot-Air oven
UV degradation	expose to UV-radiation for 7 days

All the above solutions are analyzed and recorded the chromatograms and results recorded in table-1.20.

**Table -1.20: System suitability – Forced Degradation**

S. No.	Area of Chloropicrin
1	2063.30
2	2072.04
3	2058.64
4	2053.67
5	2066.99
<b>Mean</b>	<b>2062.93</b>
<b>Standard Deviation (±)</b>	<b>7.14</b>
<b>(%) Relative Standard Deviation</b>	<b>0.35</b>

**Acceptance criteria:** The degradation peaks should be well separated from each other. The peak purity should pass.

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. The results are presented in table-1.21.

**Table -1.21: Results - Forced Degradation**

Acid Stress	% Degradation
Standard	0.666
Sample	0.017
Alkali Stress	% Degradation
Standard	0.029
Sample	0.025
Thermal Stress	% Degradation
Standard	0.011
Sample	0.009
UV Stress	% Degradation
Standard	0.011
Sample	0.007

### X. STABILITY OF ANALYTICAL SOLUTION

System suitability solution and test solution of Dow AgroSciences Chloropicrinare 96% were prepared on 0<sup>th</sup>, 12<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 48<sup>th</sup> hour of experiment and stored these solutions at room temperature for every time interval up to

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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 results are presented in table-1.22.

**Table -1.22: Results of Analytical solution Stability**

TIME	Std Area	Avg std area	Spl area	Avg Spl area
0 <sup>th</sup> hr	2017.27	2015.105	2001.20	2008.04
	2012.94		2014.88	
12 <sup>th</sup> hr	2017.37	2022.74	2025.10	2023.4
	2028.11		2021.70	
24 hr	2033.13	2035.98	2029.78	2031.07
	2038.83		2032.36	
36 hr	2048.41	2041.465	2059.62	2057.245
	2034.52		2054.87	
48 hr	2050.65	2042.18	2055.32	2059.755
	2033.71		2064.19	
<b>Mean</b>	<b>2031.49</b>	<b>2031.49</b>	<b>2035.90</b>	<b>2035.90</b>
<b>Standard Deviation (±)</b>	<b>12.83</b>	<b>12.03</b>	<b>21.38</b>	<b>22.25</b>
<b>(%) Relative Standard Deviation</b>	<b>0.63</b>	<b>0.59</b>	<b>1.05</b>	<b>1.09</b>

The assay of Dow AgroSciences Chloropicrinare 96% in the sample was calculated. The assay results obtained during solution stability experiment are as given in table-1.23.

**Table -1.23: Results for solution stability**

% Assay results calculated against the freshly prepared system suitability standard	
Sample	% Assay of Chloropicrin
0 <sup>th</sup> hr	99.48
12 <sup>th</sup> hr	99.84
24 hr	99.59
36 hr	100.60
48 hr	100.69
<b>Mean</b>	<b>100.04</b>
<b>Standard Deviation (±)</b>	<b>0.57</b>
<b>(%) Relative Standard Deviation</b>	<b>0.57</b>

**Acceptance Criteria:** The analyte is considered stable if there is no significant change in the assay.

**Table-6.24: Performance calculations, detection characteristics, system precision and method precision of the proposed method for Chloropicrin.**

Parameter	HPLC method
Wavelength(nm)	220
Retention time (t)min	3.401
Linearity range (in %)	50-150
LOD (µg/mL)	1.20
LOQ (µg/mL)	3.75
Regression equation	$y = 2055x - 109.6$
Slope (a)	2055x
Intercept (b)	- 109.6
Correlation coefficient (r <sup>2</sup> )	0.999
Standard deviation	7.21
Relative Standard deviation(%RSD)	0.34

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## XI. RESULTS AND DISCUSSION

### i). System selectivity:

All the injections were processed at the wavelength provided in the method. There was no interference observed from diluents blank solution, excipients blend solution with Chloropicrin peak. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. Hence this method is selective.

### ii). Linearity and Range of Standard:

Linearity graph of the average area at each level against the concentration in v/v% is plotted and is found to be a straight line graph. The correlation coefficient is found to be more than 0.999. Hence it is concluded that the method is found to be linear in the range of 50% to 150% of the working concentration.

Correlation coefficient= 0.999

Range= 50% to 150%

### iii). Precision:

The analysis was carried out on six test solutions of the same lot of the pesticide by two different analysts using two different equipments within the same laboratory using two different columns of the same make but having different serial numbers on two different days. The % RSD of the twelve assay results which six of method precision and six from intermediate precision is found to be less than 2.0%. Thus, the method is found to be rugged and precise.

**System precision= % RSD= 0.34**

**Method precision= % RSD= 1.48**

**Intermediate precision= % RSD= 0.52**

### iv). Robustness:

The analysis of the same lot of Dow AgroSciences Chloropicrinare 96% was carried out at different conditions of column lot, flow rate, wavelength, and change in composition of mobile phase. The % RSD between results obtained with changed condition and average result of method precision is not more than 2.0%. The analytical method meets the pre-established acceptance criteria for robustness study. Thus, the method is robust.

### v). Forced degradation:

There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well separated from each other. The peak purity criteria were found to pass at each condition of degradation for both standard and sample. Hence, the method is very precise, selective and specific to the estimation of assay of Chloropicrin in Dow AgroSciences Chloropicrinare 96% by HPLC, as the degraded products are well separated from Chloropicrin and as well from each adjacent peak.

### vi). Stability of Analytical solution:

The % RSD between assay results obtained for freshly prepared test solution and the stored test solutions is less than 2.0%. There is no significant change in assay level observed up to 48 hours for test solution at room temperature. The system suitability was found to meet the pre-established criteria and it can be concluded that the solution is stable up to 48 hours at room temperature.

## XII. SUMMARY AND CONCLUSION

The above summary and the validation data summarized in this paper shows that the analytical method of assay of Chloropicrin in Dow AgroSciences Chloropicrinare 96% by HPLC is found to be suitable, selective, specific, precise, linear, accurate and robust. The analytical solution is found to be stable up to 48 hours 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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