# Analytical Method of Limit Test for Hexachlorobenzene in Picloram TC and Method Validation Data*

## Test Method (ABCTM-2010-01-07)

1. Apparatus

Electronic Balance
GC/MS System with NCI
Ultrasonic Water Bath
Constant Temperature Water Shaker

2. Reagents

Chloroform: AR grade
Water: Redistilled
Sodium Chloride: AR grade

3. Procedure

3.1 Conditions

| | | | |
|---|---|---|---|
| Column: | Agilent HP-5MS 5% phenyl methyl siloxane | | |
| Oven: | | 100℃ | 1min |
| | 25℃/min | 200℃ | 7min |
| Ion Source Temperature: | 150.0 ℃ | | |
| Quadrupole Temperature: | 150.0 ℃ | | |
| Methane Flow Rate: | 2.5 ml/min | | |
| Injection Volume: | 1.0 μl | | |
| Split rate: | 50:1 | | |
| Analysis Time: | 12 min | | |
| Thermal AUX: | 280℃ | | |
| MS Source: | NCI | | |

Acquistion mode: SIM

Plot Ion: 282, 284, 286

3.2.Standard Solution (1)

In a 10 ml volumetric flask transfer about 12.5 mg, accurately weighed, of Reference Item of Hexachlorobenzene. Dissolve and make to volume with Chloroform.

Pipette 400 µl of the solution into a 10 ml volumetric flask and make to volume with Chloroform.

Pipette 20 µl of the solution into a 100 ml volumetric flask again and make to volume with Chloroform.

3.3.Quality Control Solution (1)

In a 10 ml volumetric flask transfer about 12.5 mg, accurately weighed, of Reference Item of Hexachlorobenzene. Dissolve and make to volume with Chloroform.

Pipette 400 µl of the solution into a 10 ml volumetric flask and make to volume with Chloroform.

Pipette 20 µl of the solution into a 100 ml volumetric flask again and make to volume with Chloroform.

3.4.0.89 M NaCl Solution

An aqueous solution of sodium chloride (NaCl) will be prepared by dissolving 26g of NaCl in 500 ml of water.

3.5.Sample Solution (2 for each batch)

Each batch of Test Item was extracted by weighing 2.0 g, accurately weighed, of each batch of Test Item into a 40 ml vial and adding 10.0 ml of chloroform. The vials were then placed in an ultrasonic batch for 5 minutes. The vials were removed from the ultrasonic bath and 20 ml of 0.89 M NaCl solution will be added to each. The samples were shaken on a shaker for 2 hours and the phases allowed separating before a portion of the chloroform phase was transferred to an autosampler vial for GC/MS analysis.

3.6.Determination

After instrument equilibrium is reached, inject standard solution several times until the areas of the nearest two peaks agree within 1.5%. Inject standard solution (R), quality control solution (QC) and sample solutions in the following sequence: R, QC, 2×S-1-1, 2×S-1-2, R, QC, 2×S-2-1, 2×S-2-2...

3.7.Data and Result

The retention time of Hexachlorobenzene is about 8.1 min. Calculate the

content of Hexachlorobenzene by the following equation:

$$W'' = \frac{A}{A'} \times W' \times P \times 1.6 \times 10^{-4}$$

$$HCB(ppm) = \frac{W''}{W} \times 10^{6}$$

Where:

$A$ is the average peak area of Hexachlorobenzene in sample solution;

$A'$ is the average peak area of Hexachlorobenzene in standard solution;

$W$ is the mass of sample;

$W'$ is the mass of Reference Item of Hexachlorobenzene;

$P$ is the purity of Reference Item of Hexachlorobenzene.

## Method validation of the Limit Test for Hexachlorobenzene in Picloram Technical (ABCTM-2010-01-07)

**3.1.1.** Method Validation

***Linearity***

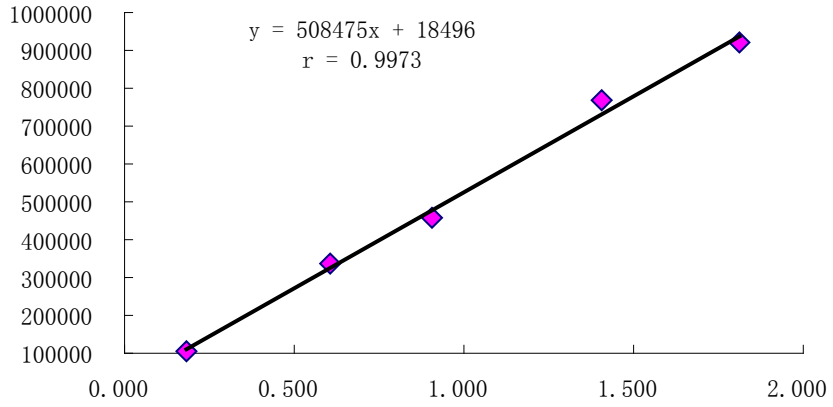
12.69 mg Reference Item of Hexachlorobenzene was accurately weighed and transferred in a 10 ml volumetric flask and was dissolved and made to volume with chloroform, the stock solution was labeled as 1001-R5-L-001. 1001-R5-L-001 was then diluted by pipetting 0.4 ml into a 10 ml volumetric flask, dissolving and making to volume with chloroform. The solution was labeled as 1001-R5-L-101. 1001-R5-L-101 was then diluted to 5 concentrations by pipetting 0.018 ml, 0.060 ml, 0.090 ml, 0.140 ml, 0.180 ml into five 5 ml volumetric flasks respectively, dissolving and making to volume with chloroform. The diluted solutions were labeled from 1001-R5-L-201 to 1001-R5-L-205.

The above five diluted solutions were subjected to GC/MSD analysis with NCI mode in the condition described in *ABCTM-2010-01-07*. Each solution was injected twice. The results are listed below:

**Table 33**

The Linearity of the Test Method for Hexachlorobenzene in Picloram Technical

| Reference of Test Solutions | Amount Weighed (mg) | Conc. (mg/L) | Peak Area | Mean of Peak Area | Correlation Coefficient |
|---|---|---|---|---|---|
| 1001-R5-L-201 | 12.69 | 0.1810 | 106199 | 105211 | 0.9973 |
| | | | 104222 | | |
| 1001-R5-L-202 | 12.69 | 0.6033 | 338955 | 336835 | |
| | | | 334714 | | |
| 1001-R5-L-203 | 12.69 | 0.9050 | 452630 | 456882 | |
| | | | 461134 | | |
| 1001-R5-L-204 | 12.69 | 1.4078 | 770674 | 770154 | |
| | | | 769633 | | |
| 1001-R5-L-205 | 12.69 | 1.8100 | 919042 | 918535 | |
| | | | 918027 | | |




The correlation coefficient is greater than 0.99, indicating that the linearity is acceptable for Hexachlorobenzene. The range of linearity is 0.1810 ～ 1.8100 mg/L.

***Precision***

Pipette 0.020 ml of 1001-R5-L-101 into a 5 ml volumetric flask, dissolve and make to volume with chloroform. The diluted solution was labeled as 1001-R5-L-206.

Pipette 0.016 ml of 1001-R5-L-001 into a 100 ml volumetric flask and make to volume with chloroform and was labeled as 1001-R5-L-103

1.9918 g, 1.9972 g, 1.9876 g, 1.9958 g, 2.0030 g of Test Item (Batch Number: 20091046) were accurately weighed and transferred into a 50 ml volumetric flask respectively. Add 10 ml of the solution 1001-R5-L-103 into each flask and shake for 5min. Then add 25 ml of 0.89M NaCl solution, shake the flasks at 20 ℃ for 2 hours. The solutions were labeled from 1001-T3-L-003 to 1001-T3-L-007

The standard solution 1001-R5-L-206 and the chloroform phase in the above five solutions were subjected to GC/MSD analysis in the condition described in *ABCTM-2010-01-07*. Each solution was injected twice. The results are listed below:

**Table 34**

The Precision of the Test Method for Hexachlorobenzene in Picloram Technical

| Batch Number | Reference of Test Solutions | Amount Weighed (mg) | Peak Area | Mean of Peak Area | Amount Found (μg) | Impurity Content (ppm) | Mean of Impurity Content (ppm) | SD (%) | RSD (%) |
|---|---|---|---|---|---|---|---|---|---|
| 20090918 | 1001-R5-L-206 | 12.69 | 100161 | N/A | N/A | N/A | 1.23 | 4.92 | 4.00 |
| 20091046 | 1001-T3-L-003 | 1991.8 | 115429 | 111576 | 2.38 | 1.19 | | | |
| | | | 107722 | | | | | | |
| 20091046 | 1001-T3-L-004 | 1997.2 | 109258 | 109077 | 2.33 | 1.17 | | | |
| | | | 108896 | | | | | | |
| 20090918 | 1001-R5-L-206 | 12.69 | 88487 | N/A | N/A | N/A | | | |
| 20091046 | 1001-T3-L-005 | 1987.6 | 109403 | 108639 | 2.52 | 1.27 | | | |
| | | | 107874 | | | | | | |
| 20091046 | 1001-T3-L-006 | 1995.8 | 107239 | 107532 | 2.50 | 1.25 | | | |
| | | | 107825 | | | | | | |
| 20090918 | 1001-R5-L-206 | 12.69 | 84697 | N/A | N/A | N/A | | | |
| 20091046 | 1001-T3-L-007 | 2003.0 | 107987 | 108029 | 2.56 | 1.28 | | | |
| | | | 108070 | | | | | | |
| 20090918 | 1001-R5-L-206 | 12.69 | 84976 | N/A | N/A | N/A | | | |

*Amount of the Standard Solution of Hexachlorobenzene: 0.20304 mg/L

The relative standard deviation is less than 11%, indicating that the precision is acceptable for Hexachlorobenzene.

***Accuracy***

Pipette 0.048 ml of 1001-R5-L-001 into a 100 ml volumetric flask and make to volume with chloroform. The solution was labeled as 1001-R5-L-104.

1.9957 g, 1.9951 g, 1.9977 g, 1.9976 g, 1.9974 g of Test Item (Batch Number: 20091046) were accurately weighed and transferred into five 50 ml volumetric flasks respectively. Add 10 ml of 1001-R5-L-104 into each flask and shake for 5min. Then add 25 ml 0.89M NaCl solution and shake the flasks at 20 ℃ for 2 hours. The solutions were labeled from 1001-T3-L-008 to 1001-T3-L-012.

Pipette 0.080 ml of 1001-R5-L-001 into a 100 ml volumetric flask and make to volume with chloroform. The solution was labeled as 1001-R5-L-105.

2.0058 g, 1.9993 g, 1.9978 g, 1.9980 g, 2.0045 g of Test Item (Batch Number: 20091046) were accurately weighed and transferred in five 50 ml volumetric flasks respectively. Add 10 ml of 1001-R5-L-105 into each flask and shake for 5min. Then add 25 ml 0.89M NaCl solution and shake the samples at 20 ℃ for

2 hours. The solutions were labeled from 1001-T3-L-013 to 1001-T3-L-017

The chloroform phase in the above ten solutions were subjected to GC/MSD NCI analysis in the condition described in *ABCTM-2010-01-07*. Each solution was injected twice. The results are listed less than:

**Table 35**

The Accuracy of the Test Method for Hexachlorobenzene in Picloram Technical

| Reference of Test Solutions | Amount Weighed (mg) | Spike Level (mg) | Peak Area | Mean of Peak Area | Amount Found (mg) | Recovery | Mean of Recoveries | SD (%) | RSD (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1001-T3-L-008 | 1995.7 | 0.004061 | 363777<br>364795 | 364286 | 0.006801 | 107.03% | 104.23% | 2.05 | 1.97 |
| 1001-T3-L-009 | 1995.1 | 0.004061 | 355442<br>349684 | 352563 | 0.006570 | 101.36% | | | |
| 1001-T3-L-010 | 1997.7 | 0.004061 | 359917<br>359701 | 359809 | 0.006712 | 104.77% | | | |
| 1001-T3-L-011 | 1997.6 | 0.004061 | 353659<br>361274 | 357467 | 0.006666 | 103.64% | | | |
| 1001-T3-L-012 | 1997.4 | 0.004061 | 359132<br>358567 | 358850 | 0.006694 | 104.34% | | | |
| 1001-T3-L-013 | 2005.8 | 0.008122 | 474805<br>485039 | 479922 | 0.009075 | 81.36% | 80.20% | 1.52 | 1.90 |
| 1001-T3-L-014 | 1999.3 | 0.008122 | 463377<br>465080 | 464229 | 0.0088 | 78.07% | | | |
| 1001-T3-L-015 | 1997.8 | 0.008122 | 470721<br>470546 | 470634 | 0.0089 | 79.32% | | | |
| 1001-T3-L-016 | 1998.0 | 0.008122 | 474018<br>486520 | 480269 | 0.0091 | 81.78% | | | |
| 1001-T3-L-017 | 2004.5 | 0.008122 | 489830<br>466208 | 478019 | 0.0090 | 80.45% | | | |

The mean recoveries are between 80~110% and their relative standard deviations are less than 11%, indicating that the accuracy is acceptable for Hexachlorobenzene.

***Limit of Quantitation***

Pipette 0.016 ml, 0.024 ml of 1001-R5-L-101 into two 5 ml volumetric flasks respectively, dissolve and make to volume with chloroform. The diluted solutions were labeled as 1001-R5-L-208 and 1001-R5-L-209.

1001-R5-L-206 and the above two solutions were subjected to GC/MSD analysis in the condition described in *ABCTM-2010-01-07*. Each solution was injected twice. The results are listed below:

**Table 36**

The LOQ of the Test Method for Hexachlorobenzene in Picloram Technical

| Reference of Test Solutions | Cencentration (mg/L) | Peak Area | Mean of Peak Area | Amount Found (mg) | Recovery | Mean of Recovery | SD (%) | RSD (%) |
|---|---|---|---|---|---|---|---|---|
| 1001-R5-L-206 | 0.20 | 87294 | N/A | N/A | N/A | 108.75% | 5.09 | 4.68 |
| 1001-R5-L-208 | 0.16 | 74005 | 73703 | 13.344 | 105.15% | | | |
| | | 73400 | | | | | | |
| 1001-R5-L-209 | 0.24 | 118044 | 118119 | 14.257 | 112.35% | | | |
| | | 118194 | | | | | | |
| 1001-R5-L-206 | 0.20 | 87933 | N/A | N/A | N/A | | | |

The mean recovery is between 80~110% and the relative standard deviation is less than 11%, indicating that the assumed LOQ, 0.20 mg/L, corresponding to 1 ppm in wt. % Hexachlorobenzene in the Test Item.