**TOLUENE**

**Collaborative Study**

Full Scale Collaborative Study   
for the   
Determination of the  
Relevant Impurity Toluene in Formulations  
by Headspace Gas Chromatography and

Flame Ionisation or Mass Spectrometric Detection

Report to CIPAC

by

Syngenta Crop Protection AG  
in collaboration with DAPA

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June 2014

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# Participants

|  |  |
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Participants are listed in alphabetical sequence, lab numbers in the result tables were assigned in sequence of result receipt.

# Toluene, General Information

Chemical name: methylbenzene

ISO common name: toluene

CAS-Nr.: 108-88-3

Structure:



Molecular mass: 92.14

Empirical formula: C7H8

# Samples

In January 2014, Information Sheet No. 300 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of toluene as relevant impurity in formulations by headspace gas chromatography and flame Ionisation or mass spectrometric detection.

The participants who completed the study are listed in section 1.

Five test samples, the analytical standard and the internal standard were sent to the participants:

1. EC formulation (EC1)

1. EC formulation (EC2)
2. FS formulation (FS3)
3. SC formulation (SC4)
4. WG formulation (WG5)

Toluene analytical standard, 99.9 % purity and ethylbenzene internal standard

Until second half of May 2014 all 13 participants sent back their results.

# Method

## Scope

The content of toluene as relevant impurity of the active ingredient is determined at low levels in solid formulated products and in water and organic solvent based liquid formulated products. Please note that the scope of this method is different from other CIPAC Methods. The impurity toluene is measured in all different formulation types. The active ingredient does not interfere for this method.

## Principle

The Toluene content of the samples is determined by headspace capillary gas chromatography (column with low-medium polarity stationary phase: 6% cyanopropylphenyl/94% dimethylpolysiloxane, e.g. DB-624, or equivalent, 30 m x 0.32 mm (i.d.), 1.8 μm film thickness), helium carrier gas and flame ionisation or mass spectrometric detection. Quantification is done by internal standardization (standard addition method). When using MS-detection other types of stationary phases may be used, e.g. 5% Phenyl/95% dimethylpolysiloxane.

It is necessary to use headspace instrumentation with an appropriate injection system - either a fixed transfer line to the GC or a gastight syringe (PAL-autosampler or equivalent).

## Procedure

The samples were analyzed twice at two different days. All test solutions were prepared freshly on day 2. The Sample and five fortification levels were used to obtain a regression curve and to calculate the toluene content in the sample. Each level was prepared in duplicate.

# Remarks of the Participants

Several participants made comments about the performance of the method and noted deviations from the method:

Laboratory 1 FID detector, fixed transfer line

Laboratory 2 FID detector, fixed transfer line

Laboratory 3 FID detector, fixed transfer line  
Nitrogen instead of helium used as carrier gas.

Recommendation to extend the time of the temperature programme (formulation EC2).

Laboratory 4 FID detector, gastight syringe

Laboratory 5 MS detection, gastight syringe

Injection volume 500 µl instead of 1000 µl; chromatography shortened due to MS detection, toluene-d8 used as internal standard.

MS detection in SIM mode: ions for toluene m/z = 91 (quantifier), 92 (qualifier 1), 65 (qualifier 2), ions for toluene-d8 m/z = 98.

The data were also evaluated by external calibration (using the internal standard D8-toluene). The obtained results were comparable to those obtained by standard addition.   
Column: DB5, 30 m, 0.32 mm, film thickness 0.25 µm  
Temp. program: 40°C for 7 min, 55°C/min ramp to 240°C, hold for 2 min.  
Detector temp.: 220°C

Laboratory 6 MS detection, gastight syringe  
 Column: ZB-624, 60 m, 0.32 mm, film thickness 1.8 µm  
 Split ratio: 15:1

Laboratory 7 FID detector, gastight syringe

Laboratory 8 FID detector, gastight syringe

Carrier gas: hydrogen, shaking time: 12 s, split ratio 5:1

Laboratory 9 FID detector, gastight syringe  
 Analysis also performed with MS detection.

Laboratory 10 MS detection, gastight syringe  
Column: BGB 5, 30 m, 0.32 mm, film thickness 0.25 µm

Temp. program: 50°C for 2 min, 3°C/min to 62°C, 40°C/min to 240°C, hold for 2 min.  
Detector temp.: 230°C  
Split ratio: 50:1

Laboratory 11 FID detector, fixed transfer line

Laboratory 12 MS detector, gastight syringe  
Column: DB-624, 30 m, 0.25 mm, film thickness 1.4 µm

Laboratory 13 FID detector, fixed transfer line  
Hold time of final column temperature increased to 5 min.

# Evaluation and Discussion

## Evaluation of the Quality of Data and Chromatograms

The data obtained from each of the laboratories were reviewed to determine if there were any significant deviations regarding the chromatography which might affect the analysis results.

Visual examination of the chromatograms showed no evidence for invalid data.

All other changes and observations noted by the participants were not expected to affect the analysis results significantly.

## Determination of Toluene

Results reported by the laboratories and the statistical evaluation are listed in tables 1-4 and displayed in figures 1-5.

The statistical evaluation of the data was done following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725. The data were examined for outliers and stragglers using Mandel’s k-statistics on the within-lab variance, followed by Mandel’s h-statistics on the lab means, and iterating where necessary. The tests were performed at an alpha level of 0.01 for outlier, and 0.05 for straggler.

Mandel’s k-statistics observed stragglers for the EC1, EC2, FS3 and WG5 formulations (marked with \* in Table 1). For the SC4 formulation an outlier according to Mandel’s k-statistics was observed (marked with \*\* in Table 1).

The Mandel’s h-statistic test identified outliers for the EC1, SC4 and WG5 formulation (marked with ++ in Table 2).

A comparison of the RSDR of this collaborative Study with the unmodified Horwitz equation showed that the relative reproducibility standard deviation (RSDR) is approximately at the Horwitz value for three formulations (FS3, SC4, WG5). For the other two formulations (EC1, EC2) the RSDR is slightly above the Horwitz value. Nevertheless this does not affect the validity of the results or the suitability of the analytical method. Due to the universal applicability of the method and the Headspace-technique a slightly higher coefficient of variation in this collaborative trial is acceptable.

**Table 1: Toluene concentration in the formulation (g/kg); results for each laboratory  
 on day 1 and day 2**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **EC1** | | **EC2** | | **FS3** | | **SC4** | | **WG5** | |
|  | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 |
| Lab 1 | 0.4798 | 0.4621 | 0.0792 | 0.0774 | 0.1960 | 0.1911 | 0.1012 | 0.0982 | 0.2146 | 0.2153 |
| Lab 2 | 0.5158 | 0.4791 | 0.0789 | 0.0792 | 0.1930 | 0.1873 | 0.0945 | 0.0942 | 0.2041 | 0.2064 |
| Lab 3 | 0.5050 | 0.4534 | 0.0908 | 0.0885 | 0.1880 | 0.1850 | 0.0987 | 0.0988 | 0.2302 | 0.2127 |
| Lab 4 | 0.4401 | 0.4149 | 0.0900 | 0.0943 | 0.1917 | 0.1937 | 0.1047 | 0.1001 | 0.1907 | 0.1746 |
| Lab 5 | 0.4554 | 0.4012 | 0.0820 | 0.0808 | 0.1826\* | 0.1492\* | 0.0844 | 0.0799 | 0.1919 | 0.2059 |
| Lab 6 | 0.5155 | 0.4880 | 0.0677\* | 0.0771\* | 0.1701 | 0.1568 | 0.0839 | 0.0909 | 0.1848\* | 0.2382\* |
| Lab 7 | 0.3201\* | 0.4007\* | 0.0860 | 0.0785 | 0.1688 | 0.1689 | 0.0806 | 0.0931 | 0.1984 | 0.1984 |
| Lab 8 | 0.4980 | 0.4981 | 0.0949 | 0.0990 | 0.1947 | 0.1929 | 0.0947 | 0.0919 | 0.2251 | 0.2163 |
| Lab 9 | 0.5060 | 0.5004 | 0.0887 | 0.0881 | 0.2243\* | 0.2013\* | 0.1212 | 0.1242 | 0.2217 | 0.2179 |
| Lab 10 | 0.3821 | 0.3782 | 0.0780 | 0.0778 | 0.1781 | 0.1766 | 0.0852 | 0.0835 | 0.2138 | 0.2234 |
| Lab 11 | 0.4768 | 0.4913 | 0.0744 | 0.0788 | 0.1739 | 0.1641 | 0.0909 | 0.0961 | 0.2000 | 0.2100 |
| Lab 12 | 0.3634 | 0.3119 | 0.0988\* | 0.0882\* | 0.2156\* | 0.1889\* | 0.0934\*\* | 0.1117\*\* | 0.2253 | 0.2370 |
| Lab 13 | 0.5165 | 0.5122 | 0.0715 | 0.0660 | 0.1765 | 0.1549 | 0.1106 | 0.1195 | 0.2498 | 0.2444 |

**\*** Mandel’s k-statistic straggler

\*\* Mandel’s k-statistic outlier

**Table 2: Mean values of the toluene concentration in the formulation (g/kg)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **EC1** | **EC2** | **FS3** | **SC4** | **WG5** |
| Lab 1 | 0.4710 | 0.0783 | 0.1936 | 0.0997 | 0.2150 |
| Lab 2 | 0.4975 | 0.0791 | 0.1902 | 0.0944 | 0.2053 |
| Lab 3 | 0.4792 | 0.0897 | 0.1865 | 0.0988 | 0.2215 |
| Lab 4 | 0.4275 | 0.0922 | 0.1927 | 0.1024 | 0.1827 |
| Lab 5 | 0.4283 | 0.0814 | 0.1659 | 0.0822 | 0.1989 |
| Lab 6 | 0.5018 | 0.0724 | 0.1635 | 0.0874 | 0.2115 |
| Lab 7 | 0.3604 | 0.0823 | 0.1689 | 0.0869 | 0.1984 |
| Lab 8 | 0.4981 | 0.0970 | 0.1938 | 0.0933 | 0.2207 |
| Lab 9 | 0.5032 | 0.0884 | 0.2128 | 0.1227++ | 0.2198 |
| Lab 10 | 0.3802++ | 0.0779 | 0.1774 | 0.0844 | 0.2186 |
| Lab 11 | 0.4841 | 0.0766 | 0.1690 | 0.0935 | 0.2050 |
| Lab 12 | 0.3377++ | 0.0935 | 0.2023 | 0.1026 | 0.2312 |
| Lab 13 | 0.5144 | 0.0688 | 0.2023 | 0.1151++ | 0.2471++ |
| **Overall mean** | **0.453** | **0.083** | **0.183** | **0.097** | **0.214** |

++Mandel’s h-statistic straggler

**Table 3: Summary of the statistical evaluation - no elimination of any outliers /  
 stragglers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **EC1** | **EC2** | **FS3** | **SC4** | **WG5** |
|  |  |  |  |  |  |
| **Xm [g/kg]** | 0.453 | 0.083 | 0.183 | 0.097 | 0.214 |
| **L** | 13 | 13 | 13 | 13 | 13 |
| **Sr** | 0.0264 | 0.0037 | 0.0110 | 0.0053 | 0.0027 |
| **SL** | 0.0569 | 0.0082 | 0.0139 | 0.0112 | 0.0161 |
| **SR** | 0.0628 | 0.0090 | 0.0177 | 0.0124 | 0.0163 |
| **r** | 0.0738 | 0.0103 | 0.0309 | 0.0147 | 0.0076 |
| **R** | 0.1752 | 0.0251 | 0.0497 | 0.0346 | 0.0458 |
| **RSDr** | 5.83 | 4.44 | 6.03 | 5.40 | 1.26 |
| **RSDR** | 13.87 | 10.83 | 9.68 | 12.71 | 7.65 |
| **RSDR(Hor)** | 6.38 | 8.23 | 7.30 | 8.03 | 7.14 |
|  |  |  |  |  |  |

**Table 4: Summary of the statistical evaluation with elimination of outliers /  
 stragglers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **EC1** | **EC2** | **FS3** | **SC4** | **WG5** |
|  |  |  |  |  |  |
| **Xm [g/kg]** | 0.480 | 0.083 | 0.180 | 0.092 | 0.211 |
| **L** | 10 | 11 | 10 | 10 | 11 |
| **Sr** | 0.0211 | 0.0026 | 0.0063 | 0.0038 | 0.0074 |
| **SL** | 0.0265 | 0.0079 | 0.0117 | 0.0063 | 0.0129 |
| **SR** | 0.0339 | 0.0083 | 0.0133 | 0.0074 | 0.0149 |
| **r** | 0.0591 | 0.0074 | 0.0176 | 0.0107 | 0.0206 |
| **R** | 0.0950 | 0.0234 | 0.0372 | 0.0207 | 0.0416 |
| **RSDr** | 4.39 | 3.17 | 3.49 | 4.15 | 3.49 |
| **RSDR** | 7.06 | 10.07 | 7.38 | 8.00 | 7.05 |
| **RSDR(Hor)** | 6.32 | 8.23 | 7.32 | 8.10 | 7.15 |

xm = overall sample mean

L = number of laboratories

sr  = repeatability standard deviation

RSDr = relative repeatability standard deviation

r = repeatability limit

sR = reproducibility standard deviation

RSDR = relative reproducibility standard deviation

R = reproducibility limit

sL = “pure” between laboratory standard deviation

RSDR(Hor) = relative reproducibility standard deviation (Horwitz equation)

**Figures 1 – 5 (all results)**

**Fig. 1:**

R limits

r limits 

Mean

**Fig. 2:**

R limits

r limits

Mean

**Fig. 3:**

R limits

r limits

Mean

**Fig. 4:**

R limits

r limits

Mean

**Fig. 5:**

R limits

r limits

Mean

## 

# Conclusions

13 different laboratories participated in this collaborative study.

As shown in the statistical summary given in Table 4, for the WG, SC and FS formulation samples the between lab experimental Relative Reproducibility Standard Deviation (% RSDR) meets the calculated acceptable value (% RSDR (Hor)) based on the Horwitz equation.

For both EC-formulations tested, the RSDR is slightly above the Horwitz value. Nevertheless this does not affect the validity of the results or the suitability of the analytical method. Due to the universal applicability of the method and the Headspace-technique a slightly higher coefficient of variation in this collaborative trial is acceptable.

Therefore, we consider this method to be suitable without further changes and recommend accepting it as a provisional CIPAC MT-method for the determination of toluene as relevant impurity of the active ingredient at low levels in solid formulated products and in water and organic solvent based liquid formulated products.