**Toluene**

**Determination of the Relevant Impurity Toluene  
in Formulations   
by Headspace Gas Chromatography and Flame Ionization or Mass Spectrometric Detection**

**CIPAC Collaborative Trial  
according to   
CIPAC Information Sheet No. 300**

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*ISO Common Name* Toluene

*Chemical Name* methylbenzene

*CAS Number* 108-88-3

*Empirical formula*  C7H8

*Molecular mass* 92.14

*Boiling point* 111°C

**TOLUENE**

**1 Sampling.** Take at least 100 g.

**2 Identity tests.**

**GLC**. Use the capillary GC method below and flame ionisation detection. The relative retention time of toluene with respect to the internal standard for the sample solution should not deviate by more than 2% from that of the calibration solution.

**GC-MS.** Use the capillary GC method below and mass spectrometric detection.

**3 Toluene**

OUTLINE OF METHOD

The content of toluene as relevant impurity of the active ingredient is determined at low levels by headspace capillary gas chromatography with flame ionisation or mass spectrometric detection. Quantification is done by internal standardization (standard addition method). The method can be used for solid and for liquid formulated products (water- and organic solvent-based). The active ingredient does not interfere with this analytical method. The maximum permitted concentration of toluene is dependent on the active ingredient in the formulation.

REAGENTS

*Toluene* reference standard, of known purity.

*Dimethylsulfoxide (DMSO)*, GC Head Space Quality.

*Ethylbenzene* internal standard. Must not contain impurities with the same retention time as toluene. When using MS-detection deuterated toluene (e.g. D8-toluene) may be used instead of ethylbenzene as internal standard.

*Internal Standard Solution*. Prepare a single stock of 0.5 mg/ml internal standard solution, of sufficient volume for all samples to be analysed. For example, to prepare 100 ml stock solution, dissolve 0.05 g of ethylbenzene in 100 ml dimethylsulfoxide. Dilute the stock solution by a factor of 10 with dimethylsulfoxide.

*Reference Stock Solution.* Weigh (to the nearest 0.1 mg) 90 - 110 mg of reference standard of toluene into a 100 ml volumetric flask. Dissolve and make up to volume with dimethylsulfoxide.

*Working standard solutions (corresponding to 0.05 %, 0.10 %, 0.25 %, 0.50 %, 1.00 % Toluene in active ingredient):*

Dilute reference stock solution 1:200 with dimethylsulfoxide, corresponding to 0.05 % Toluene in the active ingredient in the formulation (5 µg/ml). Call this solution A.

Dilute reference stock solution 1:100 with dimethylsulfoxide, corresponding to 0.1 % Toluene in the active ingredient in the formulation (10 µg/ml). Call this solution B. (This solution B for level 2 was used for validation purpose. In routine work it may be omitted).

Dilute reference stock solution 1:40 with dimethylsulfoxide, corresponding to 0.25 % Toluene in the active ingredient in the formulation (25 µg/ml). Call this solution C.

Dilute reference stock solution 1:20 with dimethylsulfoxide, corresponding to 0.5 % Toluene in the active ingredient in the formulation (50 µg/ml). Call this solution D. (This solution D for level 4 was used for validation purpose. In routine work it may be omitted).

Dilute reference stock solution 1:10 with dimethylsulfoxide, corresponding to 1 % Toluene in the active ingredient in the formulation (100 µg/ml). Call this solution E.

APPARATUS

*Headspace Gas chromatograph* equipped with the appropriate injection system – either a fixed transfer line or a gastight syringe (PAL-autosampler or equivalent) and a flame ionisation or mass spectrometric detector.

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| Examples of suitable equipment and conditions:  Headspace with fixed transfer line: |  |
| Headspace Sampler: | Agilent 7694E |
| Oven: | 100 °C |
| Loop | 180 °C |
| Transfer Line: | 200 °C |
| Equilibration time: | 30 min |
| Pressurize time: | 0.1 min |
| Loop fill time: | 0.1 min |
| Loop equilibration time: | 0.1 min |
| Injection time: | 1 min |
| Loop injection volume: | 1 ml |
| Shake: | on (low) |

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| --- | --- |
| Headspace with gastight syringe: |  |
| Headspace Sampler: | PAL or equivalent |
| Incubation temperature: | 100 °C |
| Incubation time: | 1800 s |
| Syringe temperature: | 110°C |
| Agitator speed: | 500 rpm |
| Fill speed: | 100 µl/s |
| Fill strokes: | 0 |
| Pull-up delay: | 6000 ms |
| Injection speed: | 500 µl/s |
| Pre inject delay: | 500 ms |
| Post inject delay: | 500 ms |
| Flush time: | 90 s |

*Capillary column* fused silica, length 30 m x 0.32 (i.d.) mm, film thickness: 1.8 μm, coated with crosslinked 6% cyanopropyl-phenyl/94% dimethyl polysiloxane (DB-624 or equivalent).

*Electronic integrator* or *data system.*

PROCEDURE

*(a) Gas chromatographic conditions* (FID, typical):

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| *Column* | Fused silica, length 30 m x 0.32 mm internal diameter. DB-624 or equivalent phase, with film thickness of 1.8 μm (crosslinked 6% cyanopropyl-phenyl/94% dimethyl polysiloxane).  When using MS-detection other types of stationary phases may be used, e.g. 5% Phenyl/95% dimethylpolysiloxane (DB-5 or equivalent). |
| *Injection system*  Injector  Injection volume  Split ratio | split injection  1000 μl  20:1 |
| *Detector* | flame ionisation or mass spectrometer |
| *Temperatures*  Injection port  FID-Detector  Oven programme | 220 ºC  310 ºC  temp 1 50ºC, hold 2 min, ramp rate 3ºC/min  temp 2 110ºC, hold 0 min, ramp rate 40ºC/min  temp 3 240ºC, hold 2 min. |
| *Gas flow rates*  *Column:*  Helium (carrier)  *FID-Detector:*  Air  Hydrogen  Nitrogen (make up) | 2 ml/min (typically 86 kPa at 60°C), run at constant flow  400 ml/min  30 ml/min  to 30 ml/min |
| *Retention times*  Toluene  Internal standard | 11.1 min (approximate)  15.8 min (approximate) |

*(b) Gas chromatographic conditions* (GC-MS, typical):

See (a).

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| --- | --- |
| MS Detector: | SIM-mode at m/z 91 for toluene and ethylbenzene and at m/z 98 for D8-toluene  or  Full SCAN (e.g. m/z 30 - 260) with quantification at m/z 91 for toluene and ethylbenzene and at m/z 98 for D8-toluene  Standard instrument parameters that are appropriate for the mass spectrometer instrument used |

*(c) Sample preparation.* Homogenize the formulation prior to sample weighing. Weigh (to the nearest 0.1 mg) sufficient sample to contain 20 mg of active ingredient into a headspace vial (~20 ml).

Prepare 2 solutions for each sample determination. (levels 0 – 5: see table below).

Add by pipette 2.0 ml pure dimethylsulfoxide, solution A, B, C, D and E into the headspace vials (20 ml). Then add 2.0 ml internal standard (IS) solution to each headspace vial. Close the vial immediately.

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| Level | Test solutions (2 of each level) | 2.0 ml / 2.0 ml |
| 0 | Sample | DMSO/ IS solution |
| 1  2  3 | Sample fortified with solution A  Sample fortified with solution B  Sample fortified with solution C | Solution A/ IS solution  Solution B/ IS solution  Solution C/ IS solution |
| 4  5 | Sample fortified with solution D  Sample fortified with solution E | Solution D/ IS solution  Solution E/ IS solution |

*(d) Determination.* Prepare two test solutions per determination. Inject 1000 μl gas phase of each test solution as follows:

L0\_1 L0\_2 L1\_1 L1\_2 L2\_1 L2\_2 L3\_1 L3\_2 L4\_1 L4\_2 L5\_1 L5\_2

Determine the peak areas of toluene and the internal standard.

*(e) Remarks.* Adaptions to the method with regard to split ratio or injection volume may be necessary in order to remain within the linear range. Please do not use an injection volume of less than 10 to 20 % of the nominal volume of the gastight headspace syringe used.

*(f) Calculation.*

**Calculate the mass of toluene added (Si) for each of the levels.**

*Si*=

where:  
*Si* = mass of toluene added per respective level (mg)  
*Ss* = mass of toluene in reference standard stock solution (mg)  
*P* = purity of toluene (%)  
*Vi* = volume of reference standard working solution used to fortify each   
 level (ml)  
*Vs* = volume of the reference standard stock solution   
 (= 100, if followed as described above)  
*D* = dilution factor to obtain the reference standard working solution

These Si values represent the x-values for the calculation of the slope.

**Calculate peak area ratios (Rw) of the toluene and the internal standard**

*Rw*=

where:  
Rw = peak area ratio of toluene / ethylbenzene  
Hs = peak area of toluene   
Iq = peak area of the internal standard  
wac = weight of actual sample which is evaluated  
fcorr = correction factor peak area ratio, calculated as:

*fcorr*=

where:  
R0, av = average value of peak area ratios of toluene /  
 ethylbenzene of level 0 determination  
w0, av = average value of sample weight of level 0 determination

These Rw values represent the y-values for the calculation of the slope.

**Calculate slope a of the linear regression line** with the data points of all 6 levels (or using the Excel function “SLOPE”)

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*x*

*x*

*y*

*y*

*x*

*x*

*a*

**Calculate intercept b of the linear regression line** with the data points of all 6 levels (or using the Excel function “INTERCEPT”)



**Calculate the amount of the toluene present in the sample:**

*Mw* = 

where:  
*Mw* = amount of toluene present in the sample (mg)  
*b* = intercept of the linear regression line  
*a* = slope  
R0, av = average value of peak area ratios of toluene of level 0  
 measurement

**Calculate the content of toluene relative to formulation:**

*Qf* = %

where:  
*Qf* = content of toluene relative to formulation (%)  
*Mw* = amount of toluene present in the sample (mg)  
W0, av = average value of sample weight of level 0 determination

**Calculate the content of toluene relative to active ingredient:**

*QAI* = %

where:  
*QAI* = content of toluene relative to active ingredient (%)  
*Qf* = content of toluene relative to formulation (%)  
C ai(w/w) = content of AI in the formulation of interest (% w/w), calculated as:

*Cai(w/w)*=

where:  
C ai(w/v) = content of active ingredient in the formulation of interest (% w/v)  
d = density of formulation