

# 4895/m. Relevant Impurity Toluene in Formulations

**Headspace GC-FID Method**

**DAPA small scale study**

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May 2013

## RELEVANT IMPURITY TOLUENE IN FORMULATIONS

### 1. Content

#### 1.1 Toluene by headspace gas chromatography

Determination of Toluene with internal standard, by standard addition mode.

##### 1.1.1 Apparatus

The following details are examples of suitable equipment and conditions for its use.

Headspace Sampler:	Agilent G1888
Oven temperature :	100°C
Loop temperature:	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/Agitation:	Low

Gas Chromatograph:	Agilent 7890A
Detector:	FID, output voltage: 1V
Column:	fused silica, 30 m length, 0.32 mm i.d. stationary phase: DB-624 film thickness: 1.8 µm available from: J & W Scientific
Injector liner:	Straight quartz liner
Column temperature:	50°C, 20 min isothermal, 50 - 75°C heating rate 1°C/min 75 – 280°C, heating rate 40°C/min, 4 min isothermal
Detector temperature:	300°C
Injector temperature:	220°C
Split ratio:	20:1
Carrier gas:	Helium, 2.0 ml / minute (constant flow)
Make-up gas	Nitrogen, 30 ml / minute
Size of sample:	1.0 ml of headspace gas from Section 2.1.2
Duration of chromatography:	approx. 65 minutes

### 1.1.2 Preparation of reference and test solutions

Reference substance: Toluene of known content

Internal standard: Ethylbenzene

Solvent: Dimethylsulfoxide (DMSO), GC Head Space Quality (eq. Sigma-Aldrich  
Cat.No. : 51779)

#### Internal standard stock solution of Ethylbenzene:

Weigh (to the nearest 0.1 mg) 45 – 55 mg of ethylbenzene into a 100 ml volumetric flask. Dissolve and make up to volume with solvent (DMSO) (1000 µg/ml).

#### Internal standard solution of Ethylbenzene:

Dilute Internal standard stock solution by a factor 10 with solvent (e.g. dilute 10.0 ml internal standard stock solution with solvent (DMSO) to 100.0 ml (50 µg/ml).

#### Reference stock solution of Toluene:

Weigh (to the nearest 0.1 mg) 90 – 110 mg of Toluene into a 100 ml volumetric flask. Dissolve and make up to volume with solvent (DMSO).

#### 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 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 active ingredient in the formulation (10 µg/ml). **Call this solution B.**

Dilute reference stock solution 1:40 with dimethylsulfoxide, corresponding to 0.25 % Toluene in 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 active ingredient in the formulation (50 µg/ml). **Call this solution D.**

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

#### Test solutions:

For preparation of the 2 test solutions per determination, weigh (to the nearest 0.1 mg) enough test substance to contain 20 mg active ingredient into headspace vials (20ml).

Prepare 2 solutions for each sample determination, herein after known as levels 0 – 5.

Pipette 2.0 ml each of pure DMSO, solution A, B, C, D and E into the headspace vials (20ml). Then add 2.0 ml Internal standard solution (IS solution) to each headspace vial. (See also following table)

Close the vial immediately.

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

The stability of the solutions was tested at room temperature. No degradation of the reference substances was observed after 1 week.

### 1.1.3 Procedure

Prepare 2 test solutions per determination Inject the highest level until the peak area ratios of Toluene to Internal Standard obtained for two consecutive chromatograms differs from one another by no more than 5%.

Then the following injection sequence is proposed:

.... L<sub>0\_1</sub> L<sub>0\_2</sub> L<sub>1\_1</sub> L<sub>1\_2</sub> L<sub>2\_1</sub> L<sub>2\_2</sub> L<sub>3\_1</sub> L<sub>3\_2</sub> L<sub>4\_1</sub> L<sub>4\_2</sub> L<sub>5\_1</sub> L<sub>5\_2</sub>

### 1.1.4 Retention times (guide values)

Component	Retention time [minutes]
Toluene	16.9
Internal Standard (Ethylbenzene)	31.8

**1.1.5 Calculations**

*These calculations reflect the specific requirements of the method. Other forms of this calculation may be used in different data systems and should give comparable results.*

**Calculate the mass of Toluene added ( $s_i$ ) for each of the levels.**

$$S_i = \frac{S_s \times P \times V_i}{100 \times V_s \times D}$$

Where:

- $S_i$  = mass of Toluene added per respective level (mg)
- $S_s$  = mass of Toluene in reference standard stock solution (mg)
- $P$  = purity of the Toluene (%)
- $V_i$  = volume of reference standard working solution used to spike each level (ml)
- $V_s$  = volume of the reference standard stock solution (= 100, if followed as described above)
- $D$  = dilution factor to obtain the reference standard working solution

These  $S_i$  values represent the x-values for the calculation of the slope.

**Calculate peak area ratios ( $R_w$ ) of the Toluene and the internal standard**

$$R_w = \frac{H_s}{I_q} - W_{ac} * f_{corr}$$

Where:

$R_w$  = peak area ratio of Toluene / ethylbenzene

$H_s$  = peak area of Toluene

$I_q$  = peak area of the internal standard

$W_{ac}$  = weight of actual sample which is evaluated

$f_{corr}$  = correction factor peak area ratio, calculated as:

$$f_{corr} = \frac{R_{o,av}}{W_{o,av}}$$

Where:

$R_{o,av}$  = average value of peak area ratios of Toluene / Ethylbenzene of level 0 determination

$W_{o,av}$  = average value of sample weight of level 0 determination

These  $R_w$  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")

$$a = \frac{\sum (x - \bar{x}) \times (y - \bar{y})}{\sum (x - \bar{x})^2}$$

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

$$b = \bar{y} - a \times \bar{x}$$

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

$$M_w = a * R_{0,av} + b$$

where:

$M_w$  = amount of Toluene present in the sample (mg)

$b$  = intercept of the linear regression line

$a$  = slope

$R_{0,av}$  = average value of Peak area ratios of Toluene of Level 0 measurement

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

$$Q_f = \frac{M_w \times 100}{W_{0,av}} \%$$

where:

$Q_f$  = content of Toluene relative to formulation (%)

$M_w$  = amount of Toluene present in the sample (mg)

$W_{0,av}$  = average value of sample weight of level 0 determination

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

$$Q_{AI} = \frac{Q_f \times 100}{C_{AI(w/w)}} \%$$

where:

$Q_{AI}$  = content of Toluene relative to active ingredient (%)

$Q_f$  = content of Toluene relative to formulation (%)

$C_{ai(w/w)}$  = content of AI in the formulation of interest (% w/w), calculated as:

$$C_{ai(w/w)} = \frac{C_{ai(w/v)}}{d}$$

Where:

$C_{ai(w/v)}$  = content of AI in the formulation of interest (% w/v)

$d$  = density of formulation