**AMETRYN**

**Collaborative Study**

Full Scale Collaborative Study
for the
Determination of Ametryn

In Technical Material and Formulations
by Gas Chromatography with

Flame Ionization Detection

Report to CIPAC

by

Syngenta Crop Protection
in collaboration with DAPA

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# Participants (Listed in Randomized Sequence)

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|  |  |

# Ametryn, General Information

Chemical name: 4-N-ethyl-6-methylsulfanyl-2-N-propan-2-yl-1,3,5-triazine-diamine

ISO common name: Ametryn

CAS Number: 834-12-8

Structure:

 

Molecular mass: 227.33

Empirical formula: C9H17N5S

# Samples

In November 2019, Information Sheet No. 325 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Ametryn as a technical material and in formulations by gas chromatography with flame ionization detection.

Five test samples (described below) including the Ametryn analytical reference standard (Batch 410430, 98.3% purity) and the internal standard (Dipropyl Phthalate Batch 1068214) were shipped to the participants:

A) Ametryn Technical Batch 729512

B) Ametryn Technical Batch 943744

C) Ametryn Water Dispersible Granule (WG) Batch 1092300

D) Ametryn Suspension Concentrate (SC) Batch 1120140

E) Ametryn Suspension Concentrate (SC) Batch 1120141

# Method

## Scope

The content of Ametryn in technical material and in formulated products (water dispersible granules, suspension concentrate)

## Principle

The Ametryn content of the samples is determined by capillary gas chromatography on a DB-WAX or equivalent fused silica column, 30 m x 0.25 mm (i.d.), 0.25 μm film thickness using helium carrier gas and flame ionization detection. Quantification is done by internal standard calibration.

## Procedure

Samples should be analyzed in duplicate at two different days resulting in a total of four individual test results for each sample. All test solutions should be prepared freshly on Day 2.

# Remarks of the Participants

Participants made comments about the performance of the method and noted deviations from the method. Below is a summary of specific method conditions provided by the participating laboratories.

Laboratory 1 Helium, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 2 Nitrogen, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 3 Helium, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 4 Nitrogen, DB-WAX (15 m x 0.25 mm id, 0.25 µm)

Laboratory 5 Nitrogen, VF-1ms (15 m x 0.25 mm id, 0.25 µm)

Laboratory 6 Helium, DB-WAX (30 m x 0.25 mm id, 0.25 µm), oven temp = 220°C

Laboratory 7 Helium, DB-WAX (30 m x 0.32 mm id, 0.32 µm), 4 mL/min flow rate

Laboratory 8 Helium, ZB-WAX (30 m x 0.25 mm id, 0.25 µm), 0.7 µL injection volume

Laboratory 9 Helium, DB-WAXETR (30 m x 0.25 mm id, 0.25 µm)

Laboratory 10 Nitrogen, RTX-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 11 Nitrogen, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 12 Helium, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 13 Helium, HP-INNOWax (30 m x 0.25 mm id, 0.25 µm)

Laboratory 14 Nitrogen, DB-WAX (30 m x 0.25 mm id, 0.25 µm)

Laboratory 15 Helium, HP-INNOWax (30 m x 0.25 mm id, 0.25 µm)

**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. Examination of provided chromatograms and raw data showed no evidence for invalid analysis results.

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

A different type of capillary GC column (VF-1ms; 100% dimethylpolysiloxane) was utilized by one of the participating laboratories (Laboratory 5) compared to the proposed Ametryn method high polarity crosslinked polyethylene glycol based stationary phase (DB-WAX column).

For the VF-1ms capillary GC column examination of provided chromatograms showed suitable resolution obtained between Ametryn/dipropyl phthalate internal standard peaks and potential sample matrix interferences such as Ametryn technical impurities. For analyses using the VF-1MS column, resolution between Ametryn/internal standard was deemed sufficient to enable accurate Ametryn quantitation in presence of potential sample matrix interference peaks.

However, based on overall evaluation of data obtained from this collaborative study it is recommended that only the specified DB-WAX column be considered for use in the proposed Ametryn provisional CIPAC method 5265/m.

## Determination of Ametryn in Technical and Formulations

Results reported by the laboratories and the statistical evaluation of the data are listed in Tables 1-5 and displayed in Figures 1-5.

The statistical evaluation of the data was completed following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725 [Dr. Pichen Hsu (Syngenta) is gratefully acknowledged for her work on statistical evaluation of the data]. The data was 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.

It is noted that all laboratories (with exception of Laboratory 5) used the specified method column

(DB-WAX, crosslinked polyethylene glycol based stationary phase), while Laboratory 5 used a non-equivalent VF-1ms column (non-polar). Therefore, all data obtained from Laboratory 5 are excluded from the statistical data analysis but still reported in the Tables (indicate as footnote in Table as a method deviation).

Mandel’s k-statistics stragglers (marked with \* in Table 1) and outliers (marked with \*\* in Table 1) were observed for the WG/SC formulations as well as for the technical material (TC). The Mandel’s h-statistic test identified outliers and no stragglers for the technical material and formulation samples (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) values in all five samples are above the Horwitz value without elimination of stragglers and outliers, while the corresponding Horwitz Ratios are below two (see Table 3). The RSDR further improved if stragglers and outliers are eliminated, and the Horwitz Ratios are below one in all five samples after elimination of Mandel’s h and k statistic outliers and stragglers (see Tables 4 and 5). No more than three values have been removed per sample (Table 5).

The validity of the results and the suitability of the Ametryn analytical method is shown. This Ametryn method collaborative trial is acceptable.

**Table 1: Ametryn Assay in TC and Formulations (g/kg); Results for each Laboratory on Day 1 and Day 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Ametryn SAMPLE A | Ametryn SAMPLE B | Ametryn SAMPLE C | Ametryn SAMPLE D | Ametryn SAMPLE E |
|   | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 | Day1 | Day2 |
| Laboratory 1 | 964.6 | 974.4 | 967.9 | 979.8 | 794.4 | 805.3 | 443.6 | 447.5 | 432.4 | 438.8 |
| Laboratory 2 | 970.8 | 970.5 |  969.6 | 972.3 | 796.9 | 799.3 | 445.9 | 446.2 | 435.7 | 439.8 |
| Laboratory 3 | 969.7 | 973.7 | 963.8 | 973.2 | 783.8 | 798.0 | 422.0\*\* | 443.5\*\* | 416.7\*\* | 436.3\*\* |
| Laboratory 4 | 971.3 | 971.1 | 971.1 | 970.0 | 800.2 | 799.1 | 446.1 | 445.7 | 436.0 | 432.8 |
| Laboratory 5 | 971.7\*\*\* | 972.0\*\*\* | 978.6\*\*\* | 980.8\*\*\* | 787.3\*\*\* | 790.0\*\*\* | 449.0\*\*\* | 451.5\*\*\* | 448.4\*\*\* | 445.5\*\*\* |
| Laboratory 6 | 955.8 | 972.5 | 966.2 | 968.8 | 801.8\* | 784.5\* | 449.2 | 440.1 | 436.7 | 423.9 |
| Laboratory 7 | 958.3 | 959.1 | 988.8 | 987.2 | 790.7 | 802.3 | 453.6 | 459.5 | 443.1 | 442.4 |
| Laboratory 8 | 969.7 | 973.2 | 971.8 | 967.2 | 804.6 | 804.2 | 450.9 | 450.3 | 434.4 | 437.9 |
| Laboratory 9 | 975.2 | 968.7 | 976.7 | 968.9 | 801.1 | 794.6 | 449.1 | 447.2 | 437.4 | 431.6 |
| Laboratory 10 | 971.8 | 967.7 | 971.3 | 972.1 | 798.0 | 793.3 | 450.5 | 447.7 | 438.5 | 435.7 |
| Laboratory 11 | 952.7 | 952.6 | 957.8 | 957.8 | 760.0 | 760.0 | 429.0 | 428.6 | 416.7 | 416.6 |
| Laboratory 12 | 972.1 | 972.0 | 971.0 | 971.1 | 793.3 | 791.9 | 450.8 | 453.5 | 440.7 | 441.3 |
| Laboratory 13 | 1009.0\*\* | 1061.3\*\* | 1001.2\*\* | 1067.6\*\* | 737.5 | 726.8 | 404.3 | 393.7 | 397.1 | 392.4 |
| Laboratory 14 | 969.7 | 970.4 | 979.0 | 978.1 | 797.5 | 796.9 | 446.3 | 446.3 | 435.6 | 435.0 |
| Laboratory 15 | 966.3 | 978.1 | 971.3 | 973.8 | 805.9 | 800.5 | 452.1 | 446.9 | 438.8 | 444.7 |

**\*** Mandel’s k-statistic straggler

\*\* Mandel’s k-statistic outlier

\*\*\* Results from Laboratory 5 were not included in the data evaluation due to experimental method

 deviation.

**Table 2: Mean Values**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Ametryn SAMPLE A | Ametryn SAMPLE B | Ametryn SAMPLE C | Ametryn SAMPLE D | Ametryn SAMPLE E |
|   |   |   |   |   |   |
| Laboratory 1 | 969.5 | 973.9 | 799.9 | 445.6 | 435.6 |
| Laboratory 2 | 970.7 | 971.0 | 798.1 | 446.1 | 437.8 |
| Laboratory 3 | 971.7 | 968.5 | 790.9 | 432.8 | 426.5 |
| Laboratory 4 | 971.2 | 970.6 | 799.7 | 445.9 | 434.4 |
| Laboratory 5 | 971.9\*\*\* | 979.7\*\*\* | 788.7\*\*\* | 450.3\*\*\* | 447.0\*\*\* |
| Laboratory 6 | 964.2 | 967.5 | 793.2 | 444.7 | 430.3 |
| Laboratory 7 | 958.7 | 988.0 | 796.5 | 456.6 | 442.8 |
| Laboratory 8 | 971.5 | 969.5 | 804.4 | 450.6 | 436.2 |
| Laboratory 9 | 972.0 | 972.8 | 797.9 | 448.2 | 434.5 |
| Laboratory 10 | 969.8 | 971.7 | 795.7 | 449.1 | 437.1 |
| Laboratory 11 | 952.7 | 957.8 | 760.0 | 428.8 | 416.7 |
| Laboratory 12 | 972.1 | 971.1 | 792.6 | 452.2 | 441.0 |
| Laboratory 13 | 1035.2\*\* | 1034.4\*\* | 732.2\*\* | 399.0\*\* | 394.8\*\* |
| Laboratory 14 | 970.1 | 978.6 | 797.2 | 446.3 | 435.3 |
| Laboratory 15 | 972.2 | 972.6 | 803.2 | 449.5 | 441.8 |

\*\* Mandel’s h-statistic outlier (no Mandel’s h-statistic straggler observed)

\*\*\* Results from laboratory 5 were not included in the data evaluation due to experimental method

 deviation

**Table 3: Summary of the Statistical Evaluation - No Elimination of any
 Stragglers /Outliers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Sample A | Sample B | Sample C | Sample D | Sample E |
| Xm | 972.9 | 976.3 | 790.1 | 442.5 | 431.8 |
| L | 14 | 14 | 14 | 14 | 14 |
| Sr | 10.92 | 13.02 | 5.89 | 5.19 | 5.10 |
| SL | 17.17 | 15.41 | 19.34 | 13.96 | 12.06 |
| SR | 20.35 | 20.17 | 20.22 | 14.89 | 13.09 |
| r | 30.57 | 36.46 | 16.50 | 14.53 | 14.28 |
| R | 56.97 | 56.48 | 56.61 | 41.70 | 36.66 |
| RSDr | 1.12 | 1.33 | 0.75 | 1.17 | 1.18 |
| RSDR | 2.09 | 2.07 | 2.56 | 3.37 | 3.03 |
| RSDR(Hor) | 2.01 | 2.01 | 2.07 | 2.26 | 2.27 |
| HorRat | 1.04 | 1.03 | 1.24 | 1.49 | 1.33 |

Note: Results from Laboratory 5 are not included in the statistical evaluation for all samples due to experimental method deviation. It also applies to Table 4 and 5.

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)

HorRat = Horwitz Ratio (HorRat)

**Table 4: Summary of the Statistical Evaluation - with elimination of Mandel’s k
 Statistic Stragglers /Outliers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Sample A | Sample B | Sample C | Sample D | Sample E |
| Xm | 968.2 | 971.8 | 789.9 | 443.3 | 432.2 |
| L | 13 | 13 | 13 | 13 | 13 |
| Sr | 4.81 | 3.60 | 5.09 | 3.35 | 3.64 |
| SL | 5.00 | 6.25 | 20.26 | 14.54 | 12.75 |
| SR | 6.94 | 7.22 | 20.88 | 14.92 | 13.26 |
| r | 13.48 | 10.09 | 14.24 | 9.38 | 10.18 |
| R | 19.43 | 20.21 | 58.48 | 41.79 | 37.12 |
| RSDr | 0.50 | 0.37 | 0.64 | 0.76 | 0.84 |
| RSDR | 0.72 | 0.74 | 2.64 | 3.37 | 3.07 |
| RSDR(Hor) | 2.01 | 2.01 | 2.07 | 2.26 | 2.27 |
| HorRat | 0.36 | 0.37 | 1.28 | 1.49 | 1.35 |

Sample A: Results of Lab 13 eliminated; Sample B: Results of Lab 13 eliminated; Sample C: Results of Lab 6 eliminated; Sample D: Results of Lab 3 eliminated; Sample E: Results of Lab 3 eliminated.

**Table 5: Summary of the Statistical Evaluation - with elimination of Mandel’s h and k
 Statistic Stragglers /Outliers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | Sample A | Sample B | Sample C | Sample D | Sample E |
| Xm | 968.2 | 971.8 | 794.7 | 446.9 | 435.3 |
| L | 13 | 13 | 12 | 12 | 12 |
| Sr | 4.81 | 3.60 | 4.82 | 2.73 | 3.66 |
| SL | 5.00 | 6.25 | 11.05 | 6.35 | 6.33 |
| SR | 6.94 | 7.22 | 12.06 | 6.91 | 7.31 |
| r | 13.48 | 10.09 | 13.50 | 7.65 | 10.25 |
| R | 19.43 | 20.21 | 33.77 | 19.35 | 20.46 |
| RSDr | 0.50 | 0.37 | 0.61 | 0.61 | 0.84 |
| RSDR | 0.72 | 0.74 | 1.52 | 1.55 | 1.68 |
| RSDR(Hor) | 2.01 | 2.01 | 2.07 | 2.26 | 2.27 |
| HorRat | 0.36 | 0.37 | 0.73 | 0.69 | 0.74 |

Sample A: Results of Lab 13 eliminated; Sample B: Results of Lab 13 eliminated; Sample C: Results of Lab 6 and Lab 13 eliminated; Sample D: Results of Lab 3 and Lab 13 eliminated; Sample E: Results of Lab 3 and Lab 13 eliminated.

**Figures 1 – 5 (All Results)**

**Figure 1:**

**Figure 2:**

**Figure 3:**

**Figure 4:**

**Figure 5:**

# Conclusions

Fifteen different laboratories participated in this collaborative study. The results from the laboratories are provided in Tables 1-2, the statistical summary is included in Tables 3-5. The results for the samples evaluated are illustrated in Figures 1-5.

Without elimination of any outliers or stragglers, the between lab experimental Relative Reproducibility Standard Deviation (% RSDR) values of all five samples are above the calculated acceptable values based on the Horwitz curve calculation (% RSDR (Hor)). With elimination of the outliers and stragglers, the % RSDR is below % RSDR (Hor) in all samples. The minimum number of considered results after elimination of stragglers and outliers was twelve. Horwitz ratio values obtained for this Ametryn method collaborative trial are considered acceptable.

Taking into account the relatively high number of participating laboratories a broad basis was given even after elimination of the outliers. Therefore, we consider this Ametryn method as presented to be suitable. We recommend accepting this method as a provisional CIPAC MT-method for the determination of Ametryn in technical material and its associated formulated products (WG, SC).