THIAMETHOXAM

Collaborative Study

Full Scale Collaborative Study
for the
Determination of
Thiamethoxam AI and formulations, by
High Performance Liquid Chromatography

Report to CIPAC
by
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1. Participants

In November 2011, Information Sheet No. 293 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Thiamethoxam, by high performance liquid chromatography.
By the middle of April 2012, 18 of the 20 respondents provided their results.

Participants are listed in alphabetical order whereas lab numbers in the result tables were assigned, chronologically, based upon receipt of results.

Cichy, Michael, Dr.	Bayer CropScience Aktiengesellschaft BCS AG-R&D-CPR-RT-AF-PC2 Industriepark Höchst, G836 D-65926 Frankfurt/Main Germany
de Aguila, Elizabeth C., Ing.	Coordinadora Laboratorios Control de Calidad de Plaguicidas MAG-OIRSA Ministerio de Agricultura y Ganadería Canton El Matazano, Soyapango San Salvador El Salvador
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Fries, Jürgen, Dr.	BASF SE, Agrarzentrum Limburgerhof 67117 Limburgerhof Germany
Grecu, Cornel	ALCHIMEX SA 58 - Stirbei Voda Street 010116 Bucharest Romania
Hansen, Steven W., PhD.	Du Pont Crop Protection Stine-Haskell Research Center, S315/2245 1090 Elkton Road Newark, DE 19711 U.S.A.
Haustein, Michael, Dr.	Currenta GmbH & Co. OHG CHEMPARK Dormagen, A559 41538 Dormagen Germany
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Manso, Luis	Laboratorio Arbitral Agroalimentario Ministerio de Agricultura, Alimentación y Medio Ambiente Aguarón, 13. Aravaca 28023 Madrid Spain
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Patrian, Bruno	Forschungsgruppe Pflanzenschutzchemie Eidgenössisches Volkswirtschaftsdepartement EVD Forschungsanstalt Agroscope Changins-Wädenswil ACW Forschungsbereich Produktequalität und -sicherheit, Ernährung und Gesundheit Schloss 1, Postfach, 8820 Wädenswil Schweiz
Schlosserová, Juliana, Dr. Dipl.-Ing.	Dept. of Environmental Protection and Organic Protection Hanulova 9A SK – 844 29 Bratislava Slovak Republic

Seidel, Erika, Dr. and Michel, Alexandra, Dipl.-Ing.	Bayer CropScience Aktiengesellschaft BCS AG-D-Formulation Technology Alfred-Nobel-Str.50 40789 Monheim, building 6820 Germany
Schuler, René	Syngenta Crop Protection Münchwilen AG Breiteloh 5 4333 Münchwilen Switzerland
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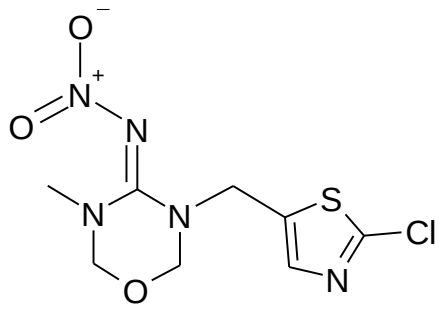
2. Active Ingredient: General Information

Chemical name: 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitro-amine

ISO common name: Thiamethoxam

CAS-Nr.: 153719-23-4

Structure:



Molecular mass: 291.7

Empirical formula: $C_8H_{10}ClN_5O_3S$

Activity: Insecticide

3. Samples

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

1. Thiamethoxam tech. 1
2. Thiamethoxam tech. 2
3. Thiamethoxam 25 WG
4. Thiamethoxam 240 SC
5. Thiamethoxam 350 FS

Thiamethoxam analytical standard, 98.9 % purity

4. Method

4.1 Scope

The determination of Thiamethoxam active ingredient content contained within Technical Grade Active Ingredient (TGAI) and WG, FS, and SC formulations.

4.2 Principle

Thiamethoxam content is determined using reversed phase high performance liquid chromatography incorporating UV detection at 230 nm with an external standard calibration.

4.3 Procedure

Each sample was analyzed using four independent determinations. The samples were analyzed on two different days, each day involving duplicate injections of duplicate weights. Both test and reference solutions were freshly prepared on each day. The four injections of each test solution were bracketed by duplicate injections of the calibration solution. The average response factor, used to calculate the amount of Thiamethoxam in the test solution, was calculated using the two injections before and after the test injections.

5. Remarks of the Participants

Several participants provided comments about the method performance and also made a note of any deviations from the method:

Laboratory 1	Column:	ZORBAX XDB-C18 (4.6×75 mm,3.5 µm)
	Remarks:	None
Laboratory 2	Column:	Nucleodur C18 (4.6×75 mm,3.5 µm)
	Remarks:	It should be discussed if the filtration step before dilution is necessary. To filter about 25 mL solution is not comfortable. Maybe the filtration step should be done after dilution. Could centrifugation be an alternative for filtration? Is the gradient necessary? We would like to recommend shortening the step from 4 to 10 min.
Laboratory 3	Column:	Nucleodur 100-3 (4.6×70 mm, 3 µm)
	Remarks:	We observed the small peak on the tail of thiamethoxam in samples of SC and FS formulations

Laboratory 4	Column:	Nucleodur 100-3 C18 ec (4.6×75 mm, 3 µm)
	Remarks:	None
Laboratory 5	Column:	Brownlee Analytical C 18 (4.6×150 mm, 5 µm)
	Remarks:	Start pressure 83atm, total run time 14 min, Thiamethoxam retention time about 3.1 min
Laboratory 6	Column:	Nucleodur C18 ec (4.6×75 mm, 3 µm)
	Remarks:	The retention time was 2.32 to 2.35 minutes
Laboratory 7	Column:	Phenomenex Gemini C18 110A (3.0×150 mm)
	Remarks:	Flow set to 1.2 ml/min It would not be necessary to inject the analytical standard so many times.
Laboratory 8	Column:	LiChrosphere 100 RP-18 endcapped (4.0×125 mm, 5 µm)
	Remarks:	Deviations in sample preparation: In the last diluting step we decided to take only 5.0 ml of the sample and calibration solution into a 20ml volumetric flask because of the very high consumption of acetonitrile. Whether this solvent is very expensive, we decided to decrease the volume. In our experience there is no influence in the accuracy.
Laboratory 9	Column:	Nucleodur C18 Gravity (4.6×70 mm, 3 µm)
	Remarks:	None
Laboratory 10	Column:	Nucleodur C18 ec (4.6×75 mm, 3 µm)
	Remarks:	None
Laboratory 11	Column:	Nucleodur C18 ec (4.6×75 mm, 3 µm)
	Remarks:	None
Laboratory 12	Column:	Supelcosil LC-18DB (4.6×100 mm, 5 µm)
	Remarks:	None
Laboratory 13	Column:	Zorbax SB-C8 (4.6×75 mm, 3.5 µm)
	Remarks:	None
Laboratory 14	Column:	Symmetry C18 (4.6×150 mm, 3.5 µm)
	Remarks:	None
Laboratory 15	Column:	Nucleodur C18 Gravity (4.0×150 mm, 3 µm)
	Remarks:	We found the method quite straightforward and easy to carry out.
Laboratory 16	Column:	Shodex C18-4C (4.6×100 mm, 3 µm)
	Remarks:	None

Laboratory 17

This lab initially used the slightly adapted methodology (set-up 1) before reverting to the different column and gradient conditions (set-up 2)

Set-up 1: Used only for one days analysis

Column: Nucleodur C18 ec, (4.6×70 mm, 3 µm)
Remarks: The volumetric flasks were filled up to volume at 20°C ± 1°C instead of at room temperature. Gradient was adapted because of problems of retention time (not repeatable) and of pressure stabilization after the run. The problems of repeatability of injections remains: it was very difficult to obtain 5 consecutive calibrations solutions repeatable. Selectivity problems for all formulations: Shoulder detected in thiamethoxam peak from SC solutions (probably an impurity not separated). Perturbation of thiamethoxam elution after injection of FS solutions during the following injection

:

Adapted gradient:

A: 0.1 % aqueous phosphoric acid
B: acetonitrile

Time (min.)	A [%]	B [%]
0	90	10
0.5	90	10
4	30	70
10	5	95
11	5	95
11.1	90	10
18	90	10

Set-up 2: Method used for two days and data submitted using these conditions

Column: Agilent Zorbax SB-C18, 5 µm, 250 x 4.6 mm i.d.

A: 0.1 % aqueous phosphoric acid
B: 0.1 % phosphoric acid in acetonitrile

Time (min.)	A [%]	B [%]
0	85	15
8.5	85	15
8.6	5	95
14	5	95
14.1	85	15
18	85	15

Laboratory 18	Column:	Nucleodur C18 ec (4.6×75 mm, 3 µm)
	Remarks:	On Day 1 I missed the second injection and so the sequence was re-injected the next day to get the second injection.

6. Evaluation and Discussion

6.1 Data Review

The data obtained from each laboratory was visually reviewed to determine if there were any significant chromatography differences, from what was expected, which might affect the analytical results.
Visual examination of the chromatograms and data indicated no significant differences in all cases with the exceptions being Lab 3 and Lab 17.

The results of Lab 3 showed a significant variation of values generated on Day 1 compared to Day 2, with all values measured at Day 1 being lower than those of Day 2. This lab also highlighted the presence of a small peak on the tail of the Thiamethoxam peak for the SC and FS formulations but the area was a fraction of the main peak (< 1%) and not considered to be an issue.

Lab 17 reported issues with repeatability of retention time, post run pressure stabilization and injection repeatability when using the supplied method incorporating a modified gradient and a slightly shorter column (70mm). These issues had not been noted by other participants using the same column (Lab 3 and Lab 9). Lab 17 also remarked that a shoulder was visible when running the SC sample. Data was only generated for one day using this methodology. In order to overcome these issues Lab 17 applied some changes to the method which included the use of a longer column, a slight change to the eluent and a modified gradient before the samples were re-run.
The data generated using this longer column was the data incorporated into the statistical evaluation as this experiment was carried out over two days.

In summary it can be stated that the method deviations, noted by the participants, were deemed not to affect the analytical results significantly and therefore all data sets were included within the statistical assessment.

6.2 Determination of Thiamethoxam

The statistical evaluation of the data was accomplished following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725. Results reported by the laboratories and the statistical evaluation of these are listed in tables 1-3 and displayed in figures 1-5. These results are reported without any exclusion of outliers and/or stragglers.

The statistical evaluation in Table 3 shows that, without elimination of any outliers or stragglers, the between lab experimental Relative Reproducibility Standard Deviation, % RSD_R, is below the calculated acceptable Horwitz value, % $RSD_{R\ (Hor)}$, for all samples.

The data was examined for outliers and stragglers using Cochran’s test (within-lab variance), followed by Grubb’s test on the lab means (between lab variance) respectively. The tests were performed at an alpha level of 0.01 for outlier detection and at a 0.05 level for straggler detection. Based on this procedure, the Cochran variance homogeneity test identified Thiamethoxam tech.1 as an outlier in the data set from Laboratory 3. The Grubb’s test identified Thiamethoxam WG 25 as a straggler within the Laboratory 3 data set. No iterations were required during this outlier/straggler detection process as it was not necessary to eliminate any data after the first evaluation.

Determination of Thiamethoxam – no elimination of any outliers / stragglers

All results tabulated in table 1 and 2 are given in g/kg

Table 1 Results

	Thiamethoxam tech.1		Thiamethoxam tech.2		Thiamethoxam WG 25		Thiamethoxam SC 240		Thiamethoxam FS 350	
	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2
Laboratory 1	989	990	986	982	249	247	216	217	290	287
Laboratory 2	989	987	985	982	247	249	221	220	296	291
Laboratory 3	960*****	994	977	995	238	243	210	216	287	290
Laboratory 4	995	993	986	989	243	246	222	218	297	292
Laboratory 5	985	981	988	978	249	245	220	212	291	286
Laboratory 6	981	986	992	987	247	248	218	217	292	284
Laboratory 7	990	988	991	990	246	246	216	216	290	289
Laboratory 8	986	985	986	991	250	248	218	218	295	290
Laboratory 10	993	991	991	989	246	245	217	215	292	288
Laboratory 11	987	994	994	996	247	246	220	219	295	294
Laboratory 12	985	988	983	993	245	241	212	217	289	284
Laboratory 13	978	983	993	986	248	247	220	220	294	300
Laboratory 14	988	993	978	995	244	247	214	217	299	301
Laboratory 15	988	989	990	991	251	245	221	213	295	289
Laboratory 16	987	996	991	986	251	246	219	217	302	295
Laboratory 17	986	991	990	979	244	244	219	216	287	287
Laboratory 18	988	977	989	979	246	244	218	216	290	287
Laboratory 19	996	988	997	994	247	248	219	218	295	294

***** Cochran outlier

Table 2 Mean values

	Thiamethoxam tech.1	Thiamethoxam tech.2	Thiamethoxam WG 25	Thiamethoxam SC 240	Thiamethoxam FS 350
Lab 1	989.5	984.0	248.0	216.5	288.5
Lab 2	988.0	983.5	248.0	220.5	293.5
Lab 3	977.0	986.0	240.5[+]	213.0	288.5
Lab 4	994.0	987.5	244.5	220.0	294.5
Lab 5	983.0	983.0	247.0	216.0	288.5
Lab 6	983.5	989.5	247.5	217.5	288.0
Lab 7	989.0	990.5	246.0	216.0	289.5
Lab 8	985.5	988.5	249.0	218.0	292.5
Lab 10	992.0	990.0	245.5	216.0	290.0
Lab 11	990.5	995.0	246.5	219.5	294.5
Lab 12	986.5	988.0	243.0	214.5	286.5
Lab 13	980.5	989.5	247.5	220.0	297.0
Lab 14	990.5	986.5	245.5	215.5	300.0
Lab 15	988.5	990.5	248.0	217.0	292.0
Lab 16	991.5	988.5	248.5	218.0	298.5
Lab 17	988.5	984.5	244.0	217.5	287.0
Lab 18	982.5	984.0	245.0	217.0	288.5
Lab 19	992.0	995.5	247.5	218.5	294.5

[+] Grubbs straggler

Table 3 Summary of the statistical evaluation (all data included)

	Thiamethoxam tech.1	Thiamethoxam tech.2	Thiamethoxam WG 25	Thiamethoxam SC 240	Thiamethoxam FS 350
x_m	987.4	987.9	246.1	217.3	291.9
L	19	19	19	19	19
S_r	6.59	5.73	2.06	2.53	3.09
S_L	2.57	2.39	1.43	1.59	1.76
S_R	6.43	5.41	2.59	2.64	4.55
r	18.46	16.04	5.76	7.10	8.67
R	18.00	15.15	7.26	7.41	12.75
RSD_r	0.67	0.58	0.84	1.17	1.06
RSD_R	0.65	0.55	1.05	1.22	1.56
$RSD_{R(Hor)}$	2.00	2.00	2.47	2.52	2.41

x_m = overall sample mean
L = number of laboratories
s_r = repeatability standard deviation
RSD_r = relative repeatability standard deviation
r = repeatability limit
s_R = reproducibility standard deviation
RSD_R = relative reproducibility standard deviation
R = reproducibility limit
s_L = “pure” between laboratory standard deviation

Fig. 1

Thiamethoxam tech. 1

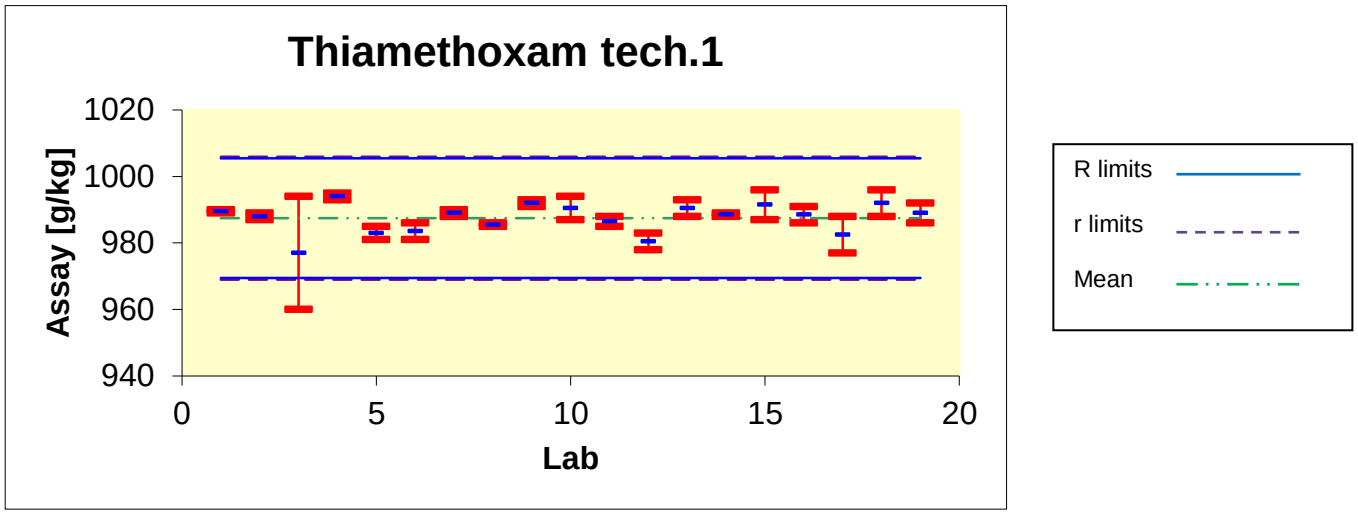


Fig. 2

Thiamethoxam tech. 2

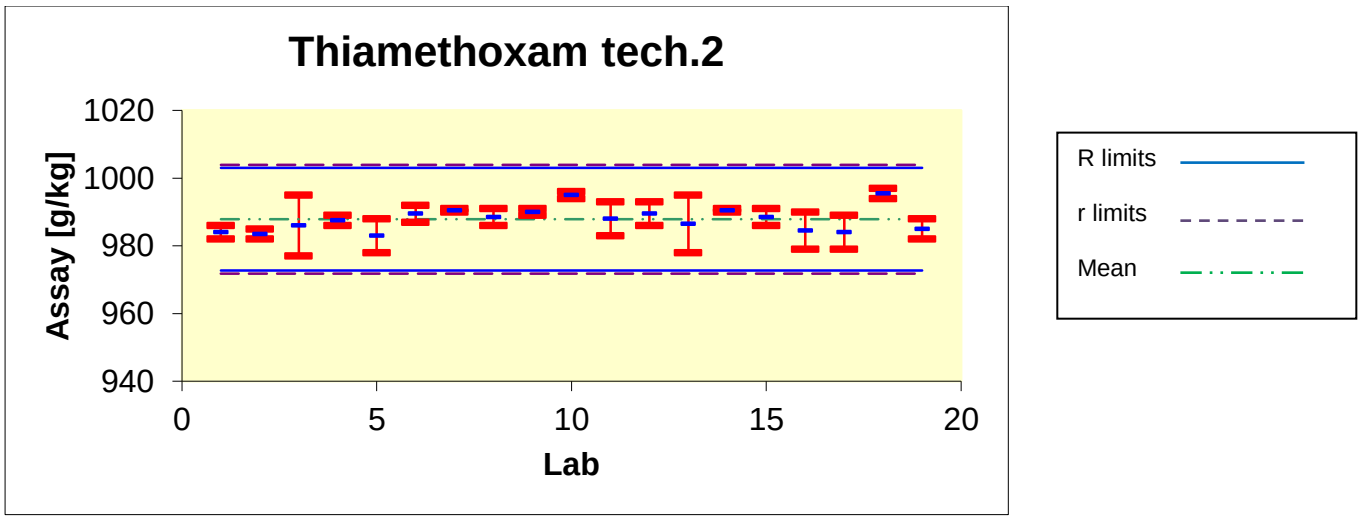


Fig. 3

Thiamethoxam WG 25

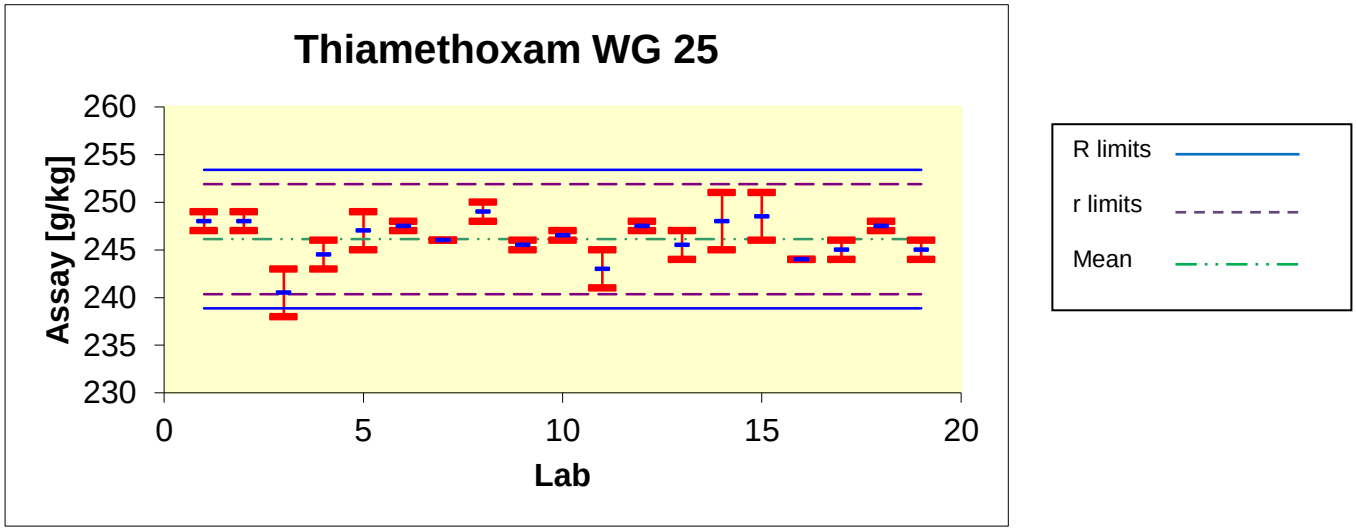


Fig. 4

Thiamethoxam SC 240

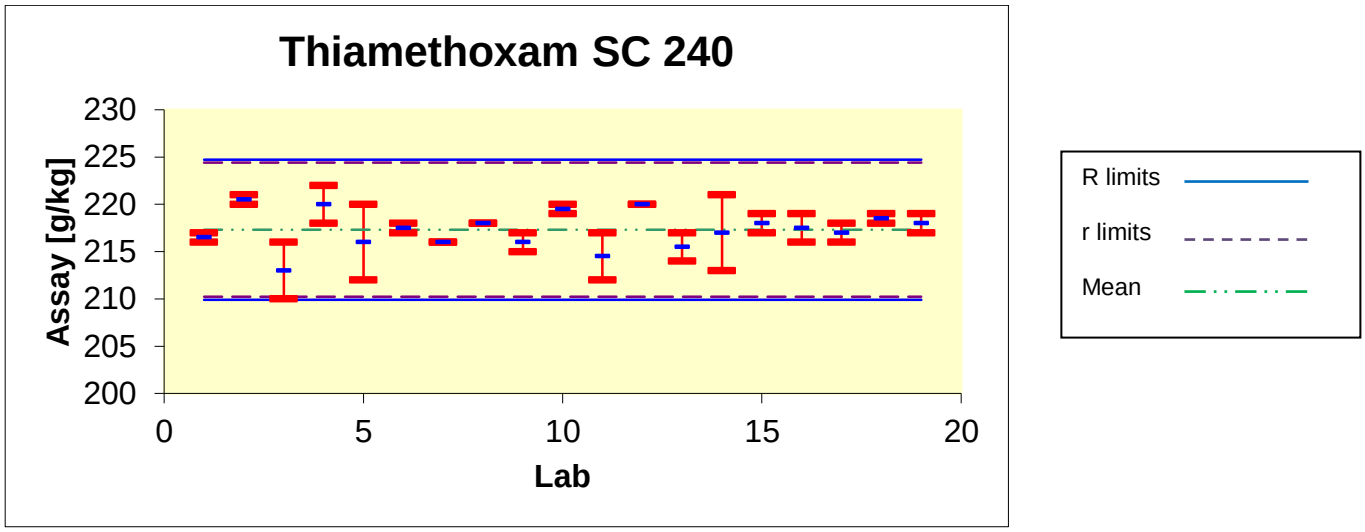
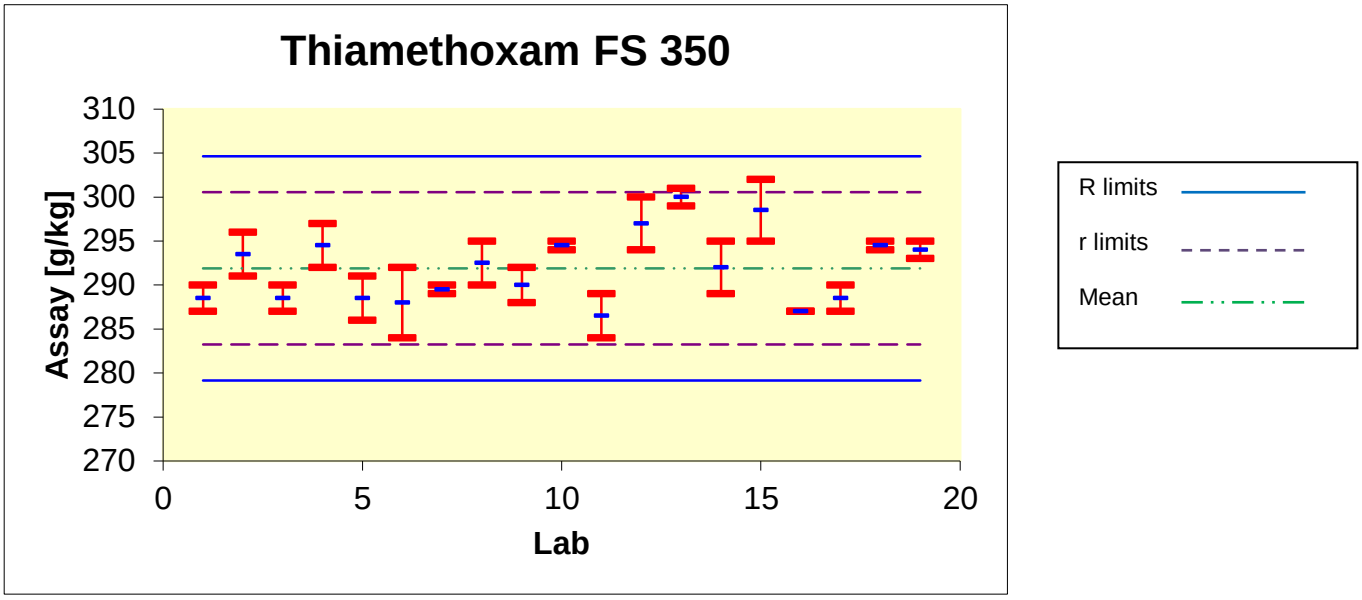


Fig. 5

Thiamethoxam FS 350



7. Conclusions

A total of 19 different laboratories have participated in this full scale collaborative study.
Upon visual review of the chromatographic and calculated data all the results appeared to be valid and were used within the statistical assessment.

Without elimination of any outliers or stragglers the between lab experimental Relative Reproducibility Standard Deviation, % RSD_R, for all Thiamethoxam samples (Table 3), is below the acceptable Horwitz value, % $RSD_{R\ (Hor)}$.

The statistical evaluation confirmed the visual inspection, which indicated that the biggest day to day variation of all the labs was within the Lab 3 data. A Grubbs “straggler” was evident in the Lab 3 data for Thiamethoxam WG and a Cochran outlier for Thiamethoxam tech. 1. These data points were not discarded as the Horwitz criteria was fulfilled with them included within the statistical evaluation. The fact that Lab 3 observed a small peak on the tail of the Thiamethoxam peak, for SC and FS formulations, did not raise any issues as this peak was less than 1% of the main peak and no other lab reported this issue. This could be explained either as a system artifact or due to peak splitting.

Lab 17 reported difficulties when using a column of 70 mm length and these included:- retention time repeatability, post run pressure stabilization and the appearance of a shoulder on the Thiamethoxam peak for the SC formulation. These issues were not reported by any other lab, even though a wide variety of C18-columns had been used. Data was only generated for one day and therefore could be included within the statistical analysis. Lab 17 consequently developed a separate methodology and supplied the results from this method as the experiment was conducted over two days.

The fact that Lab 17 provided data using modified conditions confirms that the method is very robust to changes in column and chromatographic conditions.

In general it was noted that the liquid formulations, Thiamethoxam SC 240 and especially Thiamethoxam FS 350, showed slightly higher variability than either the Thiamethoxam TGAI or the Thiamethoxam WG 25. The implication of this is that careful sample homogenization, before weighing, is crucial in order to achieve more reliable results. A remark to reflect this has been added to the method.

Syngenta consider this method to be suitable for the intended purpose, without further changes, and recommend accepting it as a provisional CIPAC method for the determination of Thiamethoxam in TGAI and associated formulations:- WG, SC and FS.