Metalaxyl and Metalaxyl-M

Collaborative study

Small scale collaborative study for the determination and differentiation of

METALAXYL and METALAXYL-M

in TC, SL, ES and WG formulation by LC

Report to CIPAC

By

Syngenta Crop Protection

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1. Participants

Participating Laboratories are listed in alphabetical order in the table below. Laboratory numbers in the result tables were assigned, chronologically, based upon receipt of results.

Company / Lab	Contact	Country
AGES*	Christoph Czerwenka	Austria
Agroscope#	Bruno Patrian, Ulrich Schaller	Switzerland
BASF Limburger Hof#	Simone Fuessl/Jürgen Fries	Germany
BASF Ludwigshafen*	Rolf Förster	Germany
Bayer Monheim*/#	Dirk Hoffmann	Germany
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit*	Claudia Vinke	Germany
Department of Agriculture, Food and the Marine (DAFM)*/#	Jim Garvey	Ireland
FMC*	Mary Ellen McNally	United States of America
Syngenta Crop Protection AG*/#	Christian Mink	Switzerland

*Chiral Analysis by LC
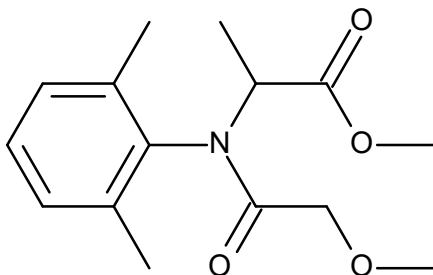# Assay by GC

2. General Information

Metalatyl

ISO common name: Metalaxyl

IUPAC name: methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate

Molecular mass: 279.3g mol^{-1}

Empirical formula: C15 H21 N O4
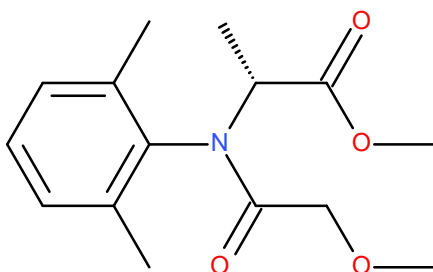
Structure:

Metalaxyl-M

ISO common name: Metalaxyl-M

IUPAC name: methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-D-alaninate

Molecular mass: 279.3g mol^{-1}

Empirical formula: C15 H21 N O4

Structure:

3. Samples

In total five samples, two TC samples and one SL, one ES and a WG formulated sample have been shipped together with reference standard and for laboratories participating in the GC collaborative trial internal standard.

- • Metalaxyl TC– sample A
- • Metalaxyl-M TC– sample B
- • Metalaxyl-M SL– sample C
- • Metalaxyl-M ES – sample D
- • Metalaxyl-M WG – sample E
- • Metalaxyl-M reference standard (purity 99.3 %w/w)
  CGA329351 (R-enantiomer) 96.1 %w/w
  CGA351920 (S-enantiomer) 3.22 %w/w
- • Benzyl benzoate (internal standard)

4. Method scope

The method is set up to determine the content of Metalaxyl and Metalaxyl-M by GC and to analyze for chiral purity by LC-UV (area%).

In a first step the overall assay (sum of S- and R-enantiomer) is determined by achiral GC with internal standard calibration. In a second step the chiral separation is carried out by chiral LC to discriminate between the racemic Metalaxyl and the enantiomerical enriched Metalaxyl-M. The sample is dissolved in acetonitrile and quantification is done against external standard, by liquid chromatography using UV detection.

This report will summarize both the achiral GC (Chapters 6 to 8) and the chiral LC (Chapters 9 to 11).

5. Procedure

For both techniques each sample was analyzed using four independent determinations: Two sample preparations double injected, analyzed on two different days.

In order to avoid that the chiral analysis is influenced by the assy deterimation a fixed assay was given for the chiral analysis. As a consequence, labs could also participate in the collaborative trial for the chiral analysis.

6. Remarks GC-FID

In table 1 the instruments, columns and chromatographic conditions noted by the participating laboratories are given for the overall assay (sum of S and R enantiomer) determination by GC-FID.

Table 1: Chromatographic conditions used by the participants.

Company or Institute	Instrument	Stationary phase	Length, diameter	Notable deviations or comments
Agroscope	Agilent 6890N	DB-5ms	30 m x 0.32 mm	centrifugation rather than filtration of solution; 5 minutes sonication
BASF Limburger Hof	Agilent 7890B	DB-5	30 m x 0.25 mm	Sample D was filtered through single-use-syrringe filters (0.22µm PTFE). Peakform requires manual integration
Bayer Monheim	Agilent 8860	DB-5ms	30 m x 0.25 mm	WG: the calibration solution was also filtered through a PTFE 0.45 µm filter because of turbidity
BVL	GC-FID	HP-5	30 m x 0.32 mm	Different analysts for Day 1 and Day 2, same calibration was used for both days
DAFM	Shimadzu	HP-5MSI	30m x 0.25 mm	We compared to our in-house method. (No internal standard, extract into Ethyl Acetate, analyse by GC-FID)
Syngenta	Agilent 7890B	DB-5 ms	30 m x 0.25 mm	

7. Evaluation and discussion GC-FID

Data review

In a first approach all deviations noted by the participating laboratories were deemed not to affect the analytical results. Therefore, all data sets were included within the statistical assessment. In a second attempt only the laboratories using the conditions outlined in the method were considered and in a third approach a statistical straggler has been excluded.

Statistical results

In the tables 2 to 6 and the figures 1 to 5 the full set of analytical results of all participating laboratories is shown.

Table 2: Results of the different laboratories for Sample A (Metalaxyl TC).

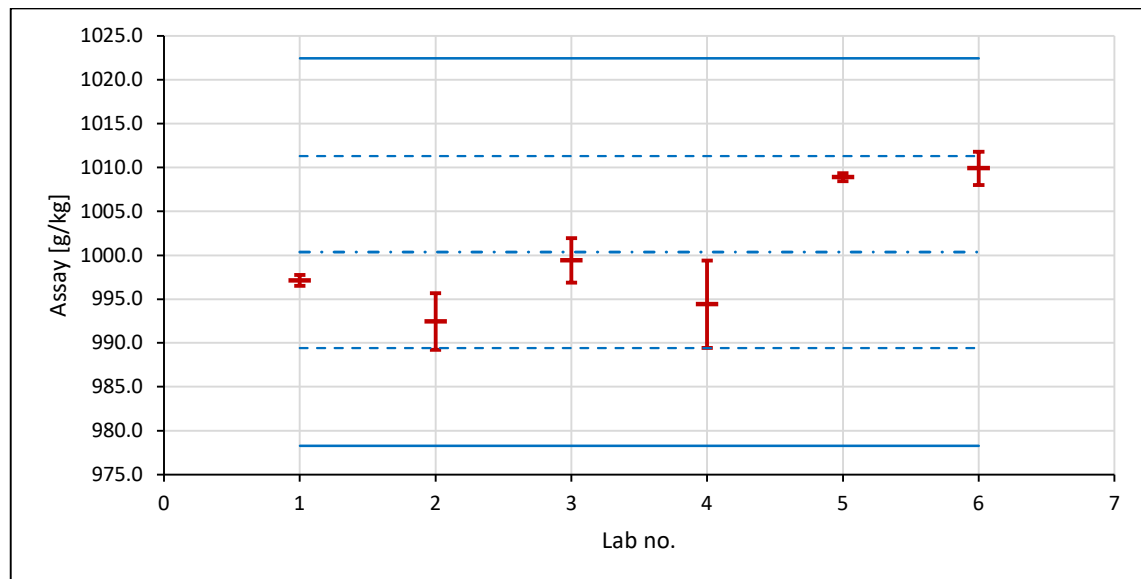| Laboratory no.	1	2	3	4	5	6
Assay [g/kg] day 1	996.51	995.66	996.86	989.44	1008.43	1011.80
Assay [g/kg] day 2	997.74	989.21	1001.95	999.38	1009.36	1007.99
mean	997.13	992.44	999.41	994.41	1008.90	1009.90

Figure 1: Graphical presentation of the results of the different laboratories for Sample A (TC). For each laboratory (laboratories 1 to 6) the red bars represent day 1, day 2 and the average.

Table 3: Results of the different laboratories for Sample B (Metalaxyl-M TC).

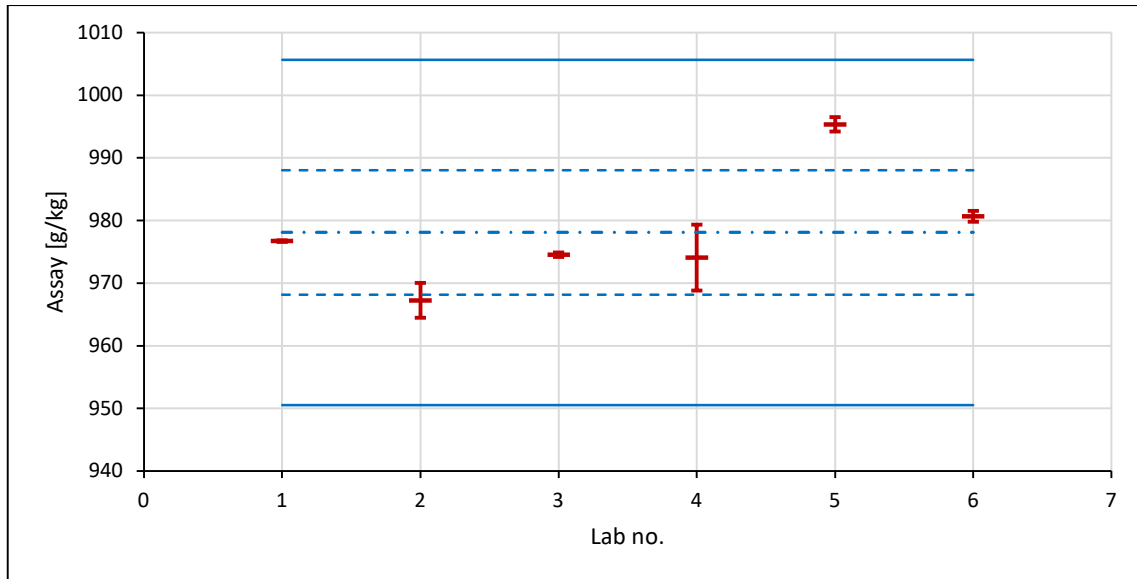| Laboratory no.	1	2	3	4	5	6
Assay [g/kg] day 1	976.85	970.02	974.89	979.34	996.51	981.53
Assay [g/kg] day 2	976.59	964.47	974.16	968.80	994.20	979.80
mean	976.72	967.25	974.53	974.07	995.36	980.67

Figure 2: Graphical presentation of the results of the different laboratories for Sample B (TC). For each laboratory (laboratories 1 to 6) the red bars represent day 1, day 2 and the average.

Table 4: Results of the different laboratories for Sample C (SL 480).

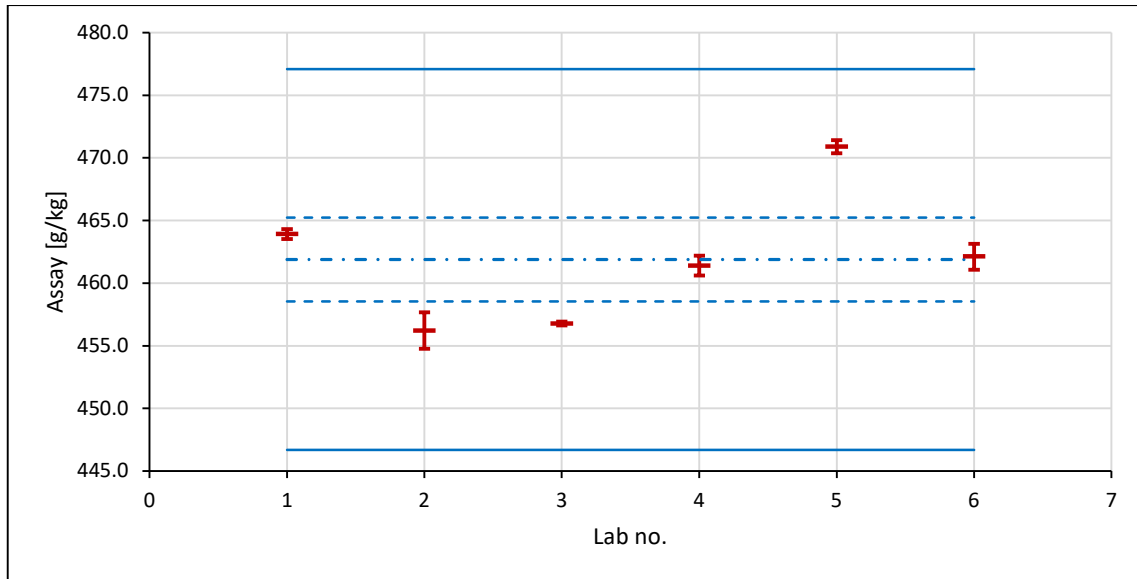| Laboratory no.	1	2	3	4	5	6
Assay [g/kg] day 1	463.53	457.66	456.93	460.61	470.37	463.15
Assay [g/kg] day 2	464.31	454.75	456.63	462.20	471.43	461.08
mean	463.92	456.21	456.78	461.41	470.90	462.12

Figure 3: Graphical presentation of the results of the different laboratories for Sample C (SL). For each laboratory (laboratories 1 to 6) the red bars represent day 1, day 2 and the average.

Table 5: Results of the different laboratories for Sample D (ES 35).

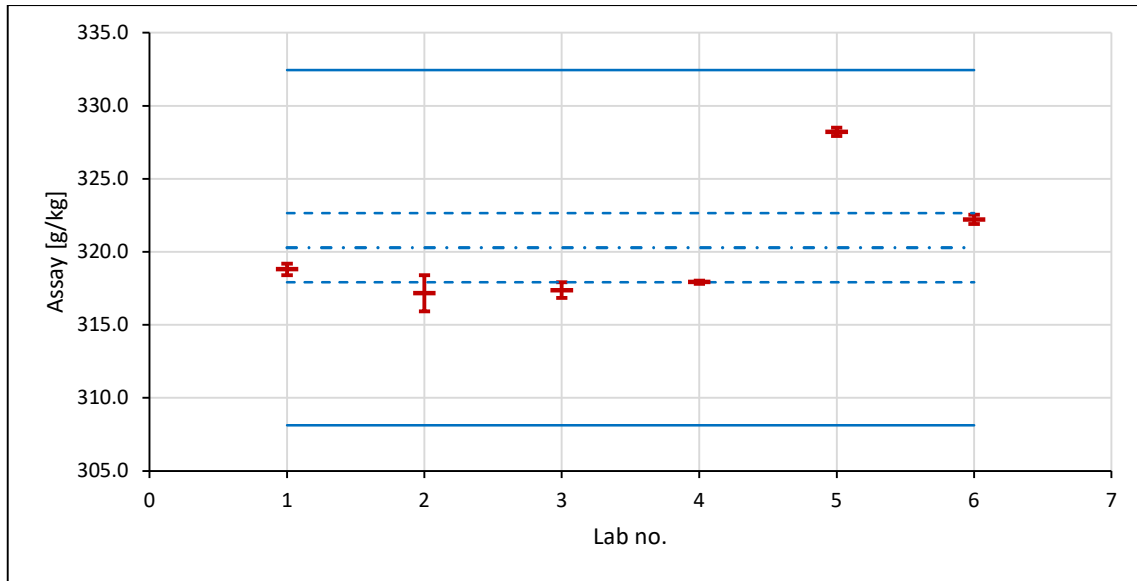| Laboratory no.	1	2	3	4	5	6
Assay [g/kg] day 1	319.20	318.40	316.85	317.81	328.49	322.53
Assay [g/kg] day 2	318.41	315.93	317.91	318.03	327.93	321.90
mean	318.81	317.17	317.38	317.92	328.21	322.22

Figure 4: Graphical presentation of the results of the different laboratories for Sample D (ES 350). For each laboratory (laboratories 1 to 6) the red bars represent day 1 and day 2 and average.

Table 6: Results of the different laboratories for Sample E (WG 4).

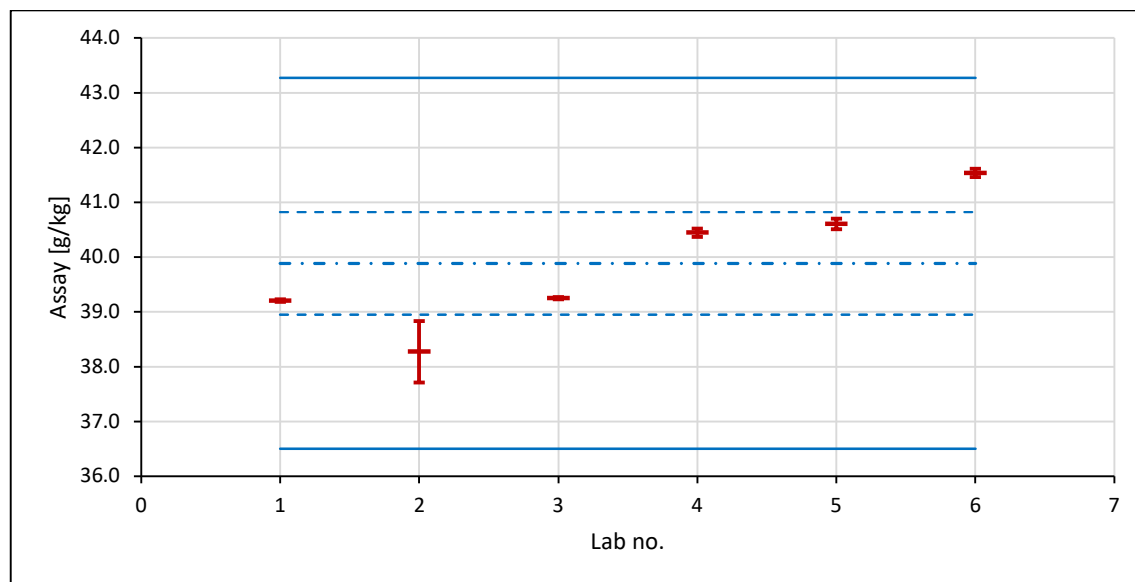| Laboratory no.	1	2	3	4	5	6
Assay [g/kg] day 1	39.18	38.83	39.23	40.52	40.51	41.61
Assay [g/kg] day 2	39.23	37.71	39.27	40.37	40.70	41.46
mean	39.21	38.27	39.25	40.45	40.61	41.54

Figure 5: Graphical presentation of the results of the different laboratories for Sample E (WG 4). For each laboratory (laboratories 1 to 6 the red bars represent day 1 and day 2 and average.

Table 7: Overall statistics on all submitted results:

	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	SAMPLE E
x_m [g/kg]	1000.4	978.1	461.9	320.3	39.9
L	6.0	6.0	6.0	6.0	6.0
N	12.0	12.0	12.0	12.0	12.0
s_r	3.9	3.5	1.2	0.8	0.3
s_L	6.9	9.2	5.3	4.3	1.2
s_R	7.9	9.8	5.4	4.3	1.2
r	10.9	9.9	3.3	2.4	0.9
R	22.1	27.6	15.2	12.2	3.4
RSD_R	0.8	1.0	1.2	1.4	3.0
$RSD_{R (Hor)}$	2.0	2.0	2.2	2.4	3.2
HorRat	0.4	0.5	0.5	0.6	0.9

No Grubbs straggler or outlier have been identified. Even without elimination of any result the between laboratory experimental Relative Reproducibility Standard Deviation (RSDR) is below the acceptance limit based on the Horwitz curve calculation (RSDR(Hor)) for all samples.

8. Summary and Conclusion GC-FID

A total of 6 laboratories participated in the trial, came back in time and provided results. The data sets from all these laboratories have been considered for the statistical evaluation (Figure 1 to 5 and Tables 2 to 7). In all cases shown in Tables 7 the Horrat is well below 1. No Grubbs straggler or outlier has been identified.

Syngenta considers this method to be suitable for the intended purpose and recommends going for a full collaborative trial for the determination of Metalaxyl-M in TC as well as SL, ES and WG formulated material.

9. Remarks chiral LC

In table 8 the instruments, columns and chromatographic conditions noted by the participating laboratories are given for the chiral LC separating the R enantiomer and the S-enantiomer from each other.

Table 8: Chromatographic conditions used by the participants.

Company or Institute	Instrument	Stationary phase	Length, diameter	Notable deviations or comments
Ages	Agilent 1260 Infinity II	Chiralpak IB	2.1 mm (i.d.) 150 mm x 3 µm	0.17 mL/min, 2.5 µL injection volume
BASF Ludwigshafen	Agilent 1200	Chiralpak IB	3 mm (i.d.) 150 mm x 3 µm	0.6 mL/min, 10 µL injection volume
Bayer Monheim	Agilent 1260 Infinity II	Chiralpak IB	4.6 mm (i.d.) 150 mm x 5 µm	For TC, SL and ES 80 mL acetonitrile was added and after sonication the sample and allow the mixture to reach ambient temperature, the 100 mL volumetric flask was filled to the mark with acetonitrile. The WG formulation was also filled to the mark with acetonitrile
DAFM, Ireland	Shimadzu	Chiralpak IB	4.6mm (i.d.) 150 mm x 5 µm	40°C is at the upper limit of the column operating conditions. No problems on Day 1 but issues were encountered on Day 2. Days of flushing the column regenerated the column. The experiment was tried at lower temperatures (25°C, 30°C and 35°C), which showed improved separation. The best separation was achieved at the lowest temperature. Separation at 40°C between R and S was ca 1.0 mins. Separation at 35°C between R and S was ca 1.9 mins

FMC	Aglient 1290 Infinity II	Chiralpak IB N-5	250 mm, 4.6mm (i.d.)	Retention time shifted to [CGA351920- approx.5.17 min] [CGA329351-approx. 5.99 min] For Sample B Day 2 one injection is significantly lower 926.9 g/kg than the second (949.5 g/kg), which fits nicely to the average of Day 1 (947.4 g/kg).
Syngenta	Agilent 1260	Chrialpak IB	150 mm, 5 µm 4.6mm (i.d.)	none
Syngenta	Thermo 3000	Chiralpak IB	150 mm, 5 µm 4.6mm (i.d.)	Different Days, Different weighings but same column than for the Agilent 1260

10. Evaluation and discussion chiral LC

Data review

In a first approach all deviations noted by the participating laboratories were deemed not to affect the analytical results. Therefore, all data sets were included within the statistical assessment. In a second attempt only the laboratories using the conditions outlined in the method were considered and in a third approach a statistical straggler has been excluded.

Statistical results

In the tables 9 to 13 and the figures 6 to 11 the full set of analytical results of all participating laboratories is shown.

Table 9: Results for R-enantiomer of the different laboratories for Sample A (Metalaxyl TC).

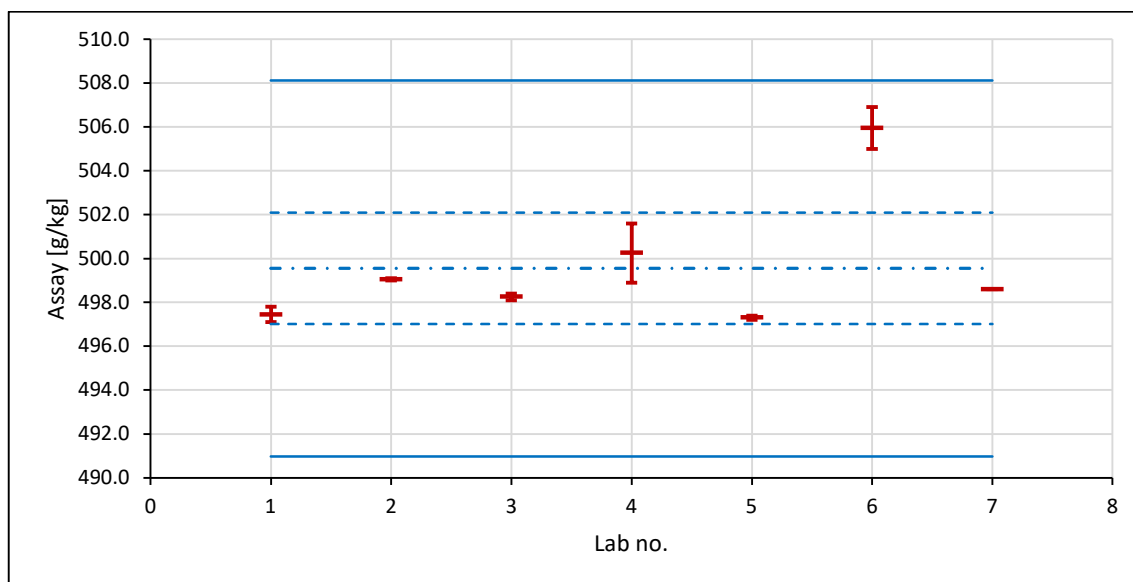| Laboratory no.	1	2	3	4	5	6	7
Assay [g/kg] day 1	497.10	499.00	498.40	498.90	497.40	506.90	498.60
Assay [g/kg] day 2	497.80	499.10	498.10	501.60	497.20	505.00	498.60
mean	497.45	499.05	498.25	500.25	497.30	505.95	498.60

Figure 6: Graphical presentation of the results of the different laboratories for Sample A (TC). For each laboratory (laboratories 1 to 7) the red bars represent day 1, day 2 and the average.
Lab 6 is a Grubbs straggler.

Table 10: Results for R-enantiomer of the different laboratories for Sample B (Metalaxyl-M TC).

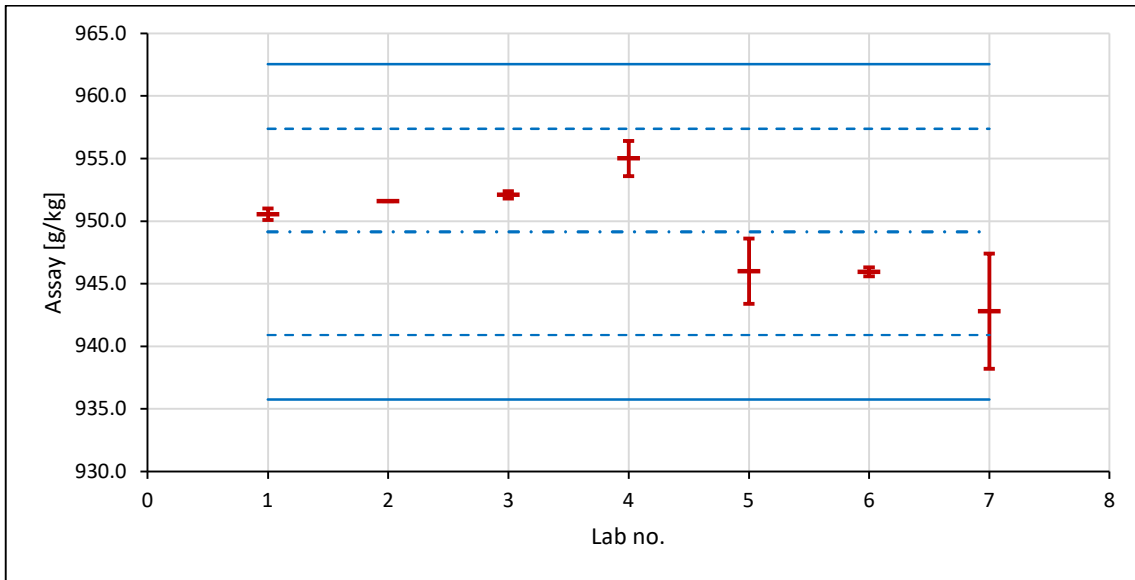| Laboratory no.	1	2	3	4	5	6	7
Assay [g/kg] day 1	951.00	951.60	952.40	953.60	948.60	946.30	947.40
Assay [g/kg] day 2	950.10	951.60	951.80	956.40	943.40	945.60	938.20
mean	950.55	951.60	952.10	955.00	946.00	945.95	942.80

Figure 7: Graphical presentation of the results of the different laboratories for Sample B (TC). For each laboratory (laboratories 1 to 6) the red bars represent day 1, day 2 and the average.

Table 11: Results for R-enantiomer of the different laboratories for Sample C (SL 480).

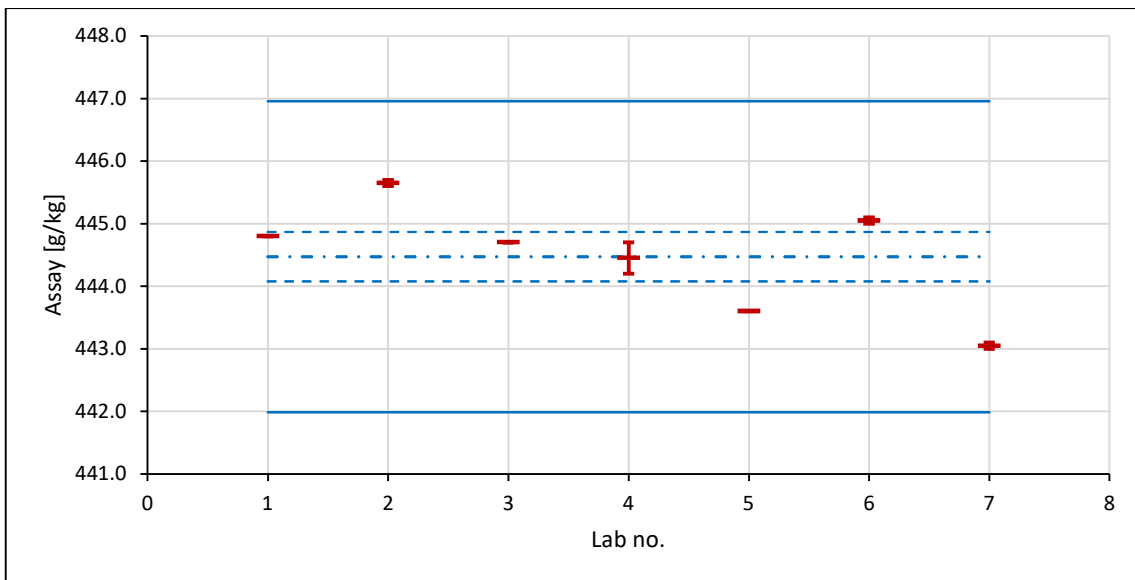| Laboratory no.	1	2	3	4	5	6	7
Assay [g/kg] day 1	444.80	445.70	444.70	444.70	443.60	445.10	443.10
Assay [g/kg] day 2	444.80	445.60	444.70	444.20	443.60	445.00	443.00
mean	444.80	445.65	444.70	444.45	443.60	445.05	443.05

Figure 8: Graphical presentation of the results of the different laboratories for Sample C (SL). For each laboratory (laboratories 1 to 6) the red bars represent day 1, day 2 and the average.

Table 12: Results for R-enantiomer of the different laboratories for Sample D (ES 35).

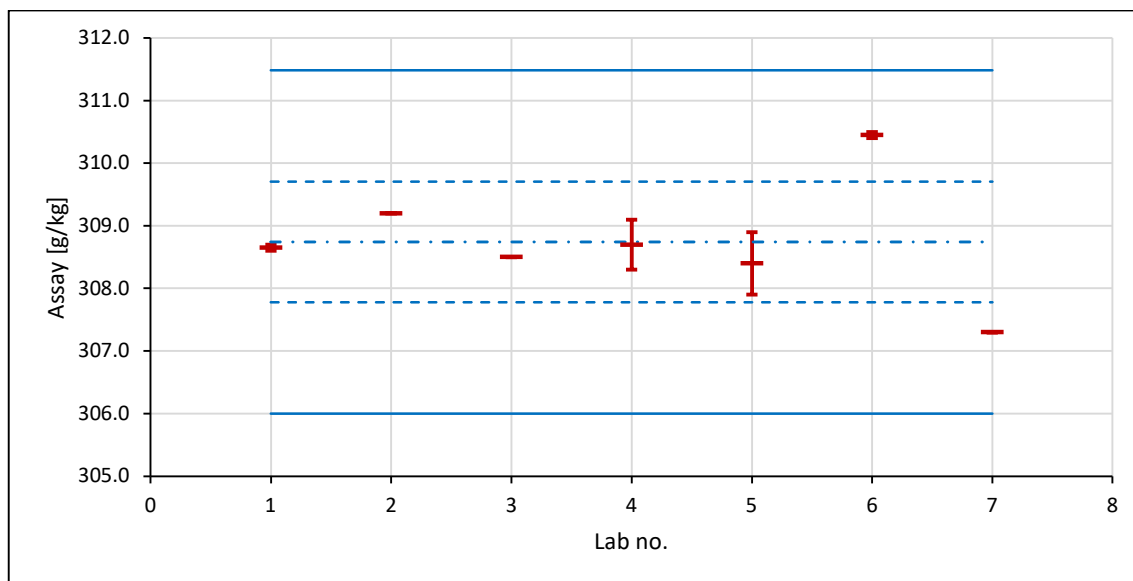| Laboratory no.	1	2	3	4	5	6	7
Assay [g/kg] day 1	308.60	309.20	308.50	308.30	307.90	310.50	307.30
Assay [g/kg] day 2	308.70	309.20	308.50	309.10	308.90	310.40	307.30
mean	308.65	309.20	308.50	308.70	308.40	310.45	307.30

Figure 9: Graphical presentation of the results of the different laboratories for Sample D (ES 350). For each laboratory (laboratories 1 to 6) the red bars represent day 1 and day 2 and average.

Table 13: Results for R-enantiomer of the different laboratories for Sample E (WG 4).

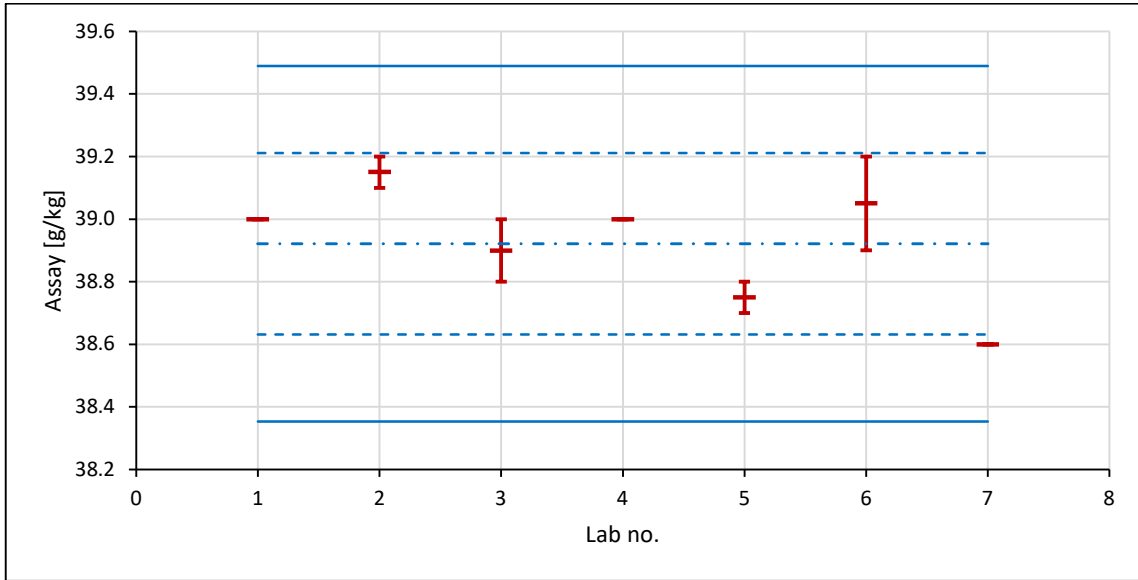| Laboratory no.	1	2	3	4	5	6	7
Assay [g/kg] day 1	39.00	39.10	38.80	39.00	38.80	39.20	38.60
Assay [g/kg] day 2	39.00	39.20	39.00	39.00	38.70	38.90	38.60
mean	39.00	39.15	38.90	39.00	38.75	39.05	38.60

Figure 10: Graphical presentation of the results of the different laboratories for Sample E (WG 4). For each laboratory (laboratories 1 to 6 the red bars represent day 1 and day 2 and average.

Table 14: Overall statistics for R-enantiomer on all submitted results:

	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	SAMPLE E
xm [g/kg]	499.6	949.1	444.5	308.7	38.9
L	7	7	7	7	7
N	14	14	14	14	14
sr	0.91	2.94	0.14	0.34	0.10
sL	2.92	3.77	0.88	0.92	0.17
sR	3.06	4.78	0.89	0.98	0.20
r	2.54	8.24	0.40	0.96	0.29
R	8.57	13.40	2.48	2.74	0.57
RSDR	0.61	0.50	0.20	0.32	0.52
RSDR (Hor)	2.22	2.02	2.26	2.39	3.26
HorRat	0.28	0.25	0.09	0.13	0.16

Lab 6 has been identified as a Grubbs outlier/straggler for SAMPLE A on both days. Even without elimination of any result the between laboratory experimental Relative Reproducibility Standard Deviation (RSDR) is below the acceptance limit based on the Horwitz curve calculation (RSDR(Hor)) for all samples.

11. Summary and Conclusion chiral LC

A total of 7 laboratories participated in the trial, came back in time and provided results. The data sets from all these laboratories have been considered for the statistical evaluation (figure 6 to 10 and tables 9 to 15). In all cases shown in tables 14 and 15 the Horrat is well below 1. Only one Grubbs straggler has been identified for SAMPLE A. Even without removing it the HorRat is well below 1.

Syngenta considers this method to be suitable for the intended purpose and recommends going for a full collaborative trial for the determination of Metalaxyl-M in TC as well as SL, ES and WG formulated material.