Metalaxyl and Metalaxyl-M

Collaborative study

Full scale collaborative study for the determination and differentiation of

METALAXYL and METALAXYL-M

in TC, SL, ES and WG formulation by GC and chiral LC

Report to CIPAC

By

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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**
Agentur für Gesundheit und Ernährungssicherheit GmbH AGES*	Christoph Czerwenka	Austria
Agroest*/#	Ioana Minea	Romania
Agroscope#	Bruno Patrian, Ulrich Schaller	Switzerland
CABB*/#	Christian Deist,Naomi Riviere	Switzerland
Chemark*/#	Dorottya Varju	Hungary
CRA-W*/#	Marie Baes	Belgium
Currenta#	Michael Haustein	Germany
Department of Agriculture, Food and the Marine (DAFM)*/#	Jim Garvey	Ireland
Fera*/#	Andrew Plumb	United Kingdom
FMC*/#	Mary Ellen McNally	United States of America
National phytosanitary authority#	Florentina Ciotea	Romania
Syngenta Crop Protection AG*/#	Christian Mink	Switzerland
Syngenta Crop Protection Goa*/#	Jayan Rappai	India
ÚSTŘEDNÍ KONTROLNÍ A ZKUŠEBNÍ ÚSTAV ZEMĚDĚLSKÝ (UKZUZ)#	Hana Šlampová	Czech Republic

*Chiral Analysis by LC
Assay by GC

2. General Information

Metalatyl

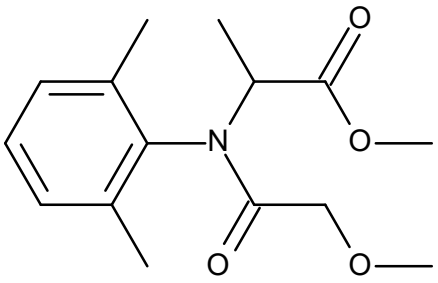
ISO common name: Metalaxyl

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

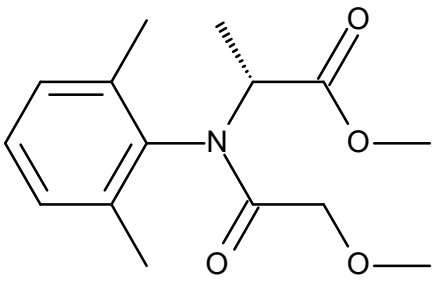
ISO common name: Metalaxyl-M

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 assay determination 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.

No.	Instrument	Stationary phase	Length, diameter	Notable deviations / Comments
1	Agilent 7890	DB5 MS	30 m x 0.25 mm x 0.25 µm	none
2	Agilent 8890	HP-5 MS UI	30 m x 0.25 mm x 0.25 µm	none
3		DB5 MS	30 m x 0.25 mm	Nitrogen as carrier gas; flow 2 mL/min
4	Agilent 7890B	HP5 MS	30 m x 0.25 mm x 0.25 µm	none
5	Agilent 6890	DB-5MS	30 m x 0.25 mm x 0.25 µm	Carrier gas Helium
6	Agilent 6890N	HP-5	30 m x 0.25 mm x 0.25 µm	Carrier gas Helium
7	Agilent 6850	DB-5MS	30 m x 0.25 mm x 0.25 µm	none
8	Agilent 6890N	HP-5MS UI	30 m x 0,25 mm x 0,25 µm	none
9	Agilent 6890N	DB5 MS	30 m x 0.32 mm x 0.25 µm	centrifugation insead of filtration
10	Thermo Trace 1310	DB5	30 m x 0.25 mm x 0.25 µm	none
11	SHIMADZU GC-2030	DB5 MS	30 m x 0.25 mm x 0.25 µm	none
12	Agilent 7890 A	HP-5MSI	30 m x 0.25 mm x 0.25 µm	Carrier gas Helium
13	Thermo Trace 1610	TG-5SILMS	30 m x 0.32 mm x 0.25 µm	Acetone as solvent

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 statistical outlier have been excluded (table 7) and in a third approach only the laboratories using the chromatographic conditions outlined in the method were considered (table 8).

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 [g/kg] of the different laboratories for Sample A (Metalaxyl TC) on day 1 and day 2.

Lab. no.	1	2	3	4	5	6	7
day 1	991.3	1000.1	1002.5	1030.8	1045.2	998.3	991.1
day 2	995.6	1004.0	994.5	1019.5	1086.0	982.3	984.0
mean	993.5	1002.0	998.5	1025.2	1065.6	990.3	987.6

Lab. no.	8	9	10	11	12	13
day 1	980.3	1000.0	978.2	994.1	1002.8	999.8
day 2	979.0	997.4	985.0	998.1	994.8	968.3
mean	979.6	998.7	981.6	996.1	998.8	984.0

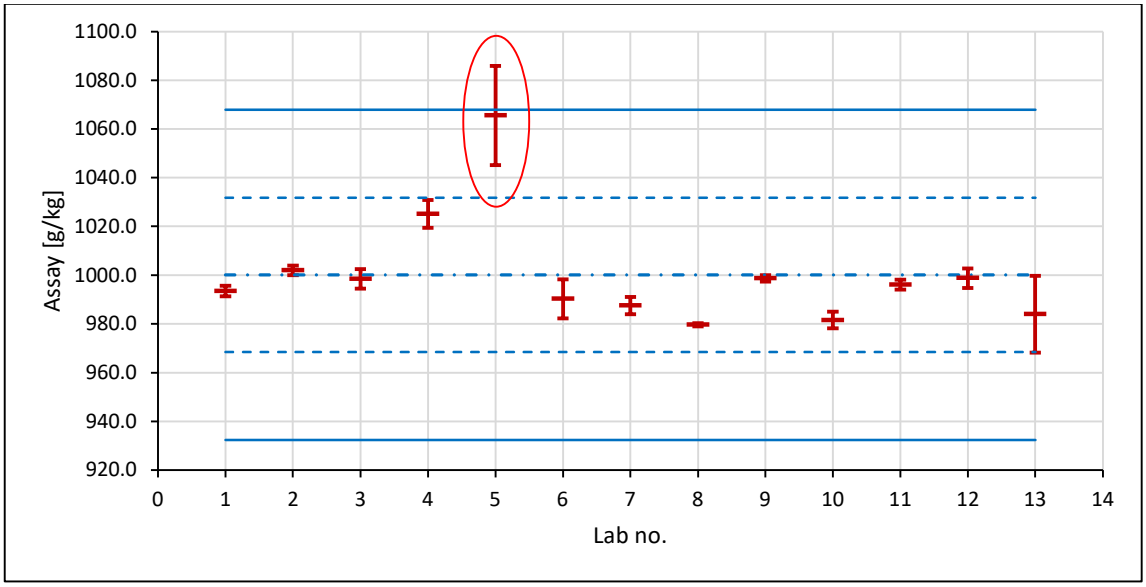


Figure 1: Graphical presentation of the results of the different laboratories for Sample A (Metalaxyl TC). For each laboratory (laboratories 1 to 13) the red bars represent day 1, day 2 and the average. Laboratory 5 is an outlier on both days.

Table 3: Results [g/kg] of the different laboratories for Sample B (Metalaxyl-M TC) on day 1 and day 2.

Lab. no.	1	2	3	4	5	6	7
day 1	970.5	965.3	971.4	991.1	1012.9	973.6	953.4
day 2	971.4	965.7	972.6	986.8	1058.3	964.7	939.5
mean	970.9	965.5	972.0	989.0	1035.6	969.1	946.5

Lab. no.	8	9	10	11	12	13
day 1	944.3	988.8	959.1	982.3	1008.9	977.8
day 2	946.0	983.6	965.8	988.5	964.9	979.3
mean	945.2	986.2	962.4	985.4	986.9	978.5

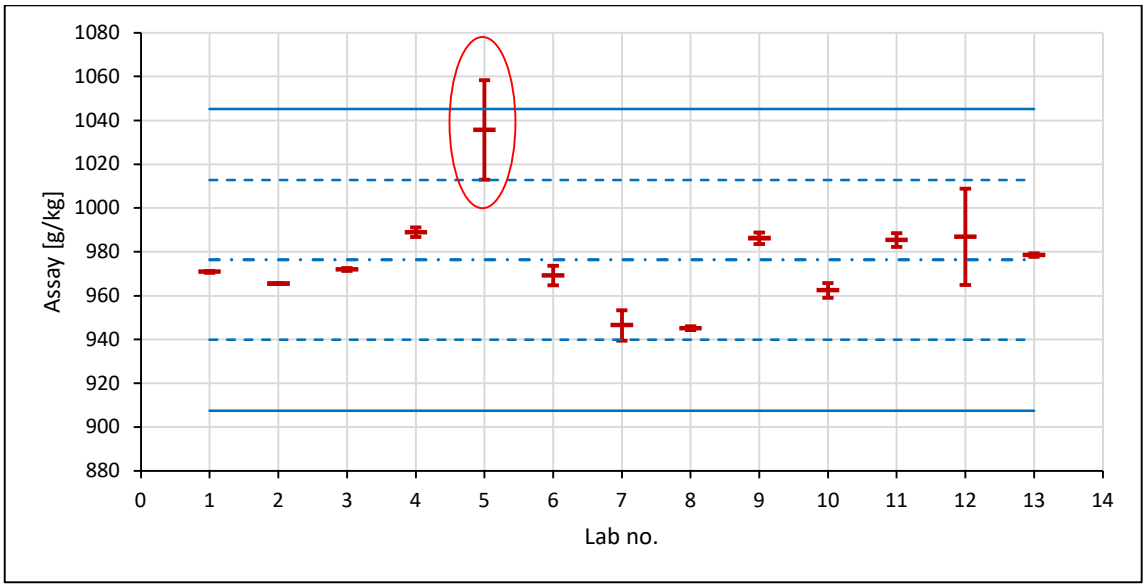


Figure 2: Graphical presentation of the results of the different laboratories for Sample B (TC). For each laboratory (laboratories 1 to 13) the red bars represent day 1, day 2 and the average. Laboratory 5 is an outlier on both days.

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

Lab. no.	1	2	3	4	5	6	7
day 1	460.5	456.6	459.6	481.7	481.2	458.8	457.1
day 2	457.4	458.5	462.6	466.1	498.9	457.4	444.3
mean	458.9	457.6	461.1	473.9	490.0	458.1	450.7

Lab. no.	8	9	10	11	12	13
day 1	447.9	459.0	453.4	461.2	469.8	456.9
day 2	437.9	467.5	453.3	457.1	453.5	459.5
mean	442.9	463.2	453.4	459.1	461.6	458.2

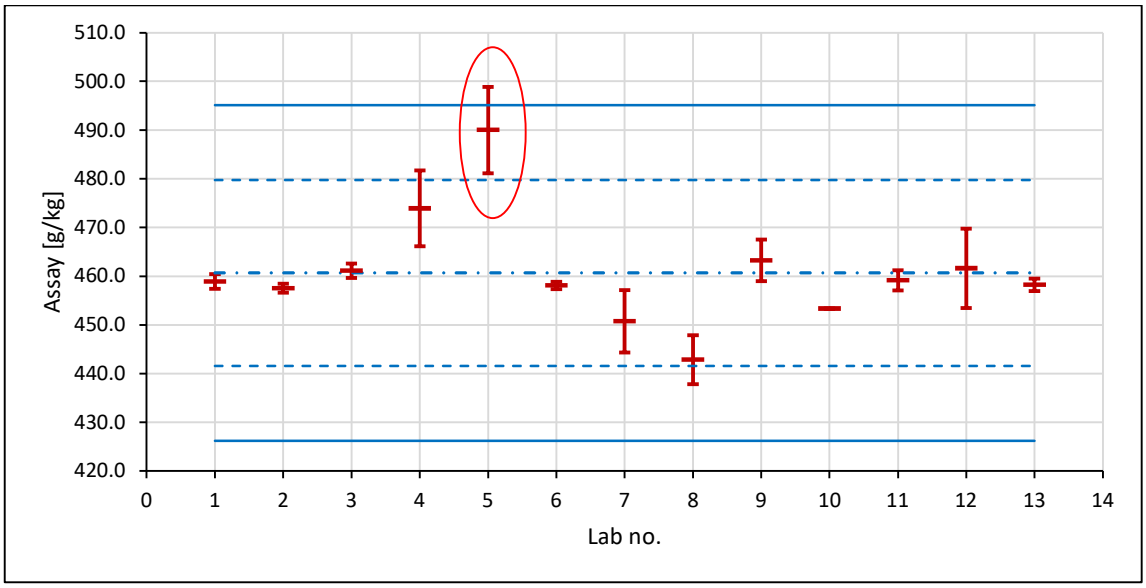


Figure 3: Graphical presentation of the results of the different laboratories for Sample C (SL). For each laboratory (laboratories 1 to 13) the red bars represent day 1, day 2 and the average. Laboratory 5 is an outlier on one day.

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

Lab. no.	1	2	3	4	5	6	7
day 1	318.3	319.8	320.5	324.4	335.0	319.1	322.5
day 2	314.3	320.0	317.5	327.2	351.2	319.1	311.1
mean	316.3	319.9	319.0	325.8	343.1	319.1	316.8

Lab. no.	8	9	10	11	12	13
day 1	305.5	320.7	314.5	318.2	320.7	317.6
day 2	301.3	328.8	315.4	316.9	314.0	325.3
mean	303.4	324.8	315.0	317.5	317.4	321.5

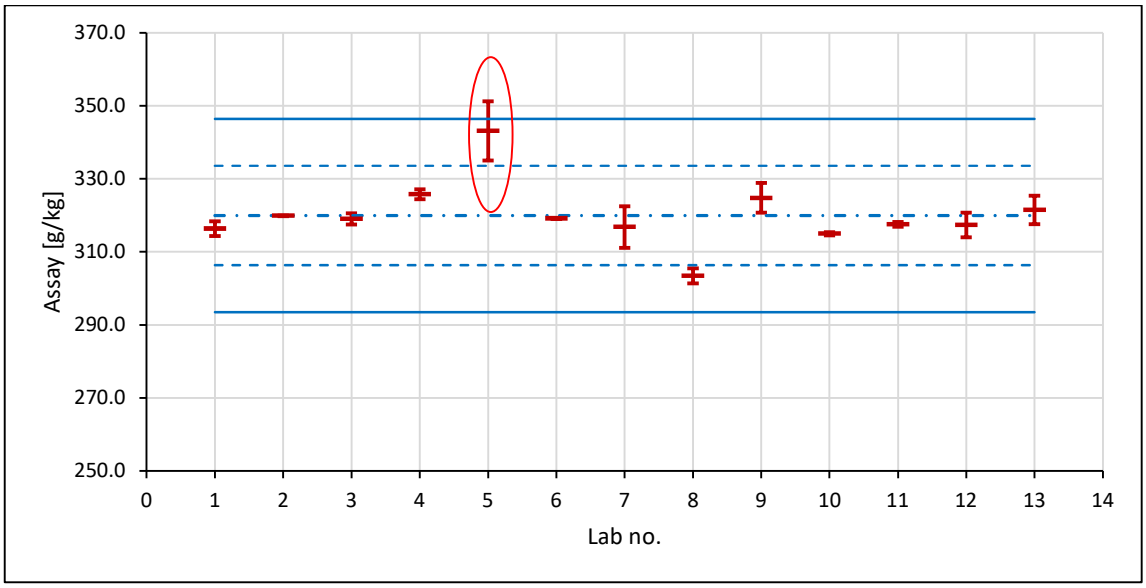


Figure 4: Graphical presentation of the results of the different laboratories for Sample D (ES 350). For each laboratory (laboratories 1 to 13) the red bars represent day 1 and day 2 and average. Lab 5 is an outlier

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

Lab. no.	1	2	3	4	5	6	7
day 1	38.6	38.8	37.6	39.7	37.1	38.7	40.6
day 2	38.7	40.9	37.1	39.5	38.9	39.1	41.1
mean	38.6	39.9	37.4	39.6	38.0	38.9	40.8

Lab. no.	8	9	10	11	12	13
day 1	39.2	38.7	39.3	39.2	40.4	38.2
day 2	38.9	39.9	41.0	39.7	39.4	37.8
mean	39.1	39.3	40.2	39.4	39.9	38.0

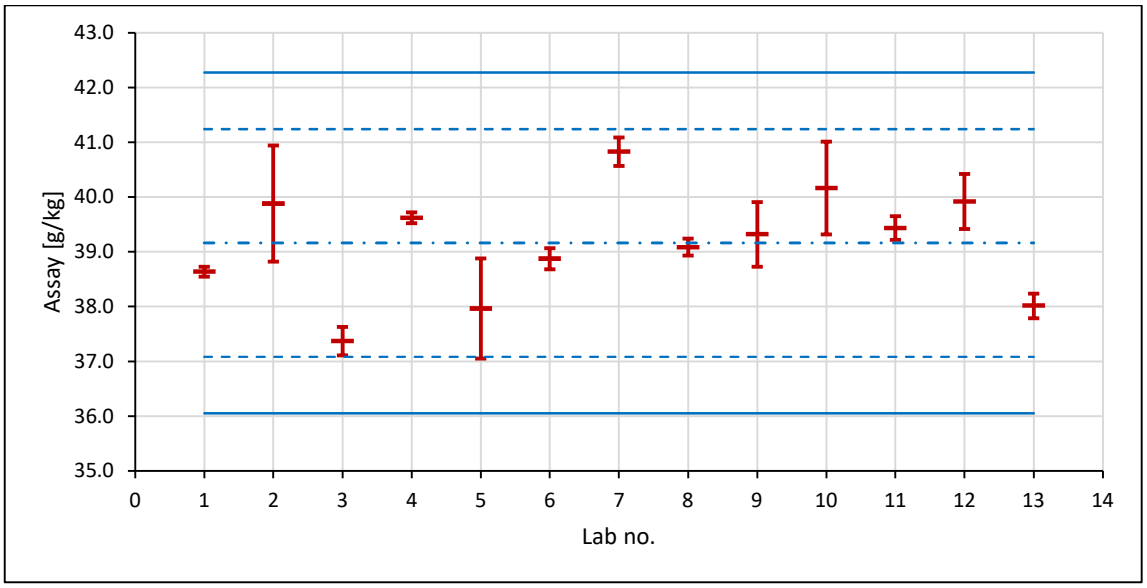


Figure 5: Graphical presentation of the results of the different laboratories for Sample E (WG 4). For each laboratory (laboratories 1 to 13 the red bars represent day 1 and day 2 and average.

Table 7: Overall statistics on all submitted results:

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	$RSD_{R\ (Hor)}$	HorRat
A	1000.12	13	26	11.31	21.39	24.19	31.66	67.74	2.42	2.00	1.21
A*	994.66	12	24	8.32	10.59	13.47	23.30	37.72	1.35	2.00	0.68
B	976.39	13	26	13.01	20.87	24.59	36.43	68.86	2.52	2.01	1.25
B*	971.46	12	24	9.88	13.17	16.47	27.67	46.10	1.69	2.01	0.84
C	460.67	13	26	6.81	10.26	12.32	19.08	34.49	2.67	2.25	1.19
C*	459.14	13	25	6.10	7.44	9.62	17.08	26.95	2.10	2.25	0.93
C**	458.22	12	24	6.10	6.07	8.60	17.08	24.09	1.88	2.25	0.83
D	319.96	13	26	4.86	8.11	9.46	13.61	26.48	2.96	2.37	1.25
D*	318.71	13	25	3.83	5.91	7.04	10.73	19.71	2.21	2.38	0.93
D**	318.03	12	24	3.83	4.96	6.27	10.73	17.55	1.97	2.38	0.83
E	39.16	13	26	0.74	0.83	1.11	2.08	3.11	2.84	3.26	0.87

SAMPLE A to E reflect the statistical evaluation of all laboratory results. Results marked with * reflect all results except the outlier. Data sets marked with ** reflect the results but lab 5.
After removal of the outlier results the HorRat is below 1 in all cases.

Table 8: Results of Labs 2, 5, 6 and 12 are removed as the carrier gas was changed

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	$RSD_{R\ (Hor)}$	HorRat
A	993.87	9	18	8.59	12.37	15.06	24.05	42.17	1.52	2.00	0.76
A*	989.96	8	16	8.66	4.71	9.86	24.25	27.59	1.00	2.00	0.50
B	970.66	9	18	4.28	16.15	16.71	11.98	46.78	1.72	2.01	0.86
C	457.94	9	18	5.88	7.57	9.58	16.46	26.84	2.09	2.25	0.93
D	317.78	9	18	4.13	5.89	7.19	11.57	20.15	2.26	2.38	0.95
E	39.16	9	18	0.54	0.98	1.12	1.53	3.14	2.87	3.26	0.88

SAMPLE A to E reflect the statistical evaluation of all laboratory results applying Hydrogen as carrier gas. A*: Results of Lab 4 have been identified as outlier and removed for the statistical evaluation. for Samples B to E no outliers have been identified in this subset. The Horrat ratio is not significantly improved compared to the results of Table 7 after removal of the outlier.

8. Summary and Conclusion GC-FID

A total of 13 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 8). For Lab 5 the results are much higher than the results from other labs. The summary in Table 7 the Horrat is below 1 after removal of the outlier. Sample code marked with *, for completeness for Samples C and D both results from Lab 5 have been removed even so 1 day was not an outlier. Table 8 summarized all data using Hydrogen as carrier gas.
In discussion with Lab 5 the reference standard was identified as the most likely root cause. Due to time constraints the analysis could not be repeated.

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 9: Chromatographic conditions used by the participants.

	Instrument	Stationary phase	Length, diameter	Notable deviations / Comments
1	Agilent 1260 DAD	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
2	Shimadzu N-Series XS	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
3	Agilent 1290 Infinity	CHIRAPACK IB 5µm	4.6 mm x 250 mm	0.800 mL/min flow, 10 µL injection volume
4	Waters Acquity UPLC H Class	Lux 3µ Cellulose-1	4.6 mm x 250 mm	0.800 mL/min flow, 10 µL injection volume
5	Thermo Vanquish	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
6	Agilent 1260 Infinity	CHIRAPACK IB 5µm	2.1 mm x 150 mm	0.800 mL/min flow, 2.5 µL injection volume
7	Agilent 1260 Infinity II	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
8	Thermo Vanquish flex	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
9	Thermo Vanquish duo	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume
10	Shimadzu	CHIRAPACK IB 5µm	4.6 mm x 150 mm	0.800 mL/min flow, 10 µL injection volume

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 10 to 14 and the figures 6 to 11 the full set of analytical results of all participating laboratories is shown. Table 11 summarizes the statistical data.

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

Lab. no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
day 1	498.40	496.10	498.50	497.50	498.20	498.30	497.00	497.00	497.20	500.90
day 2	498.60	495.60	497.70	497.30	498.20	498.40	498.00	496.10	499.10	498.60
mean	498.50	495.85	498.10	497.40	498.20	498.35	497.50	496.55	498.15	499.75

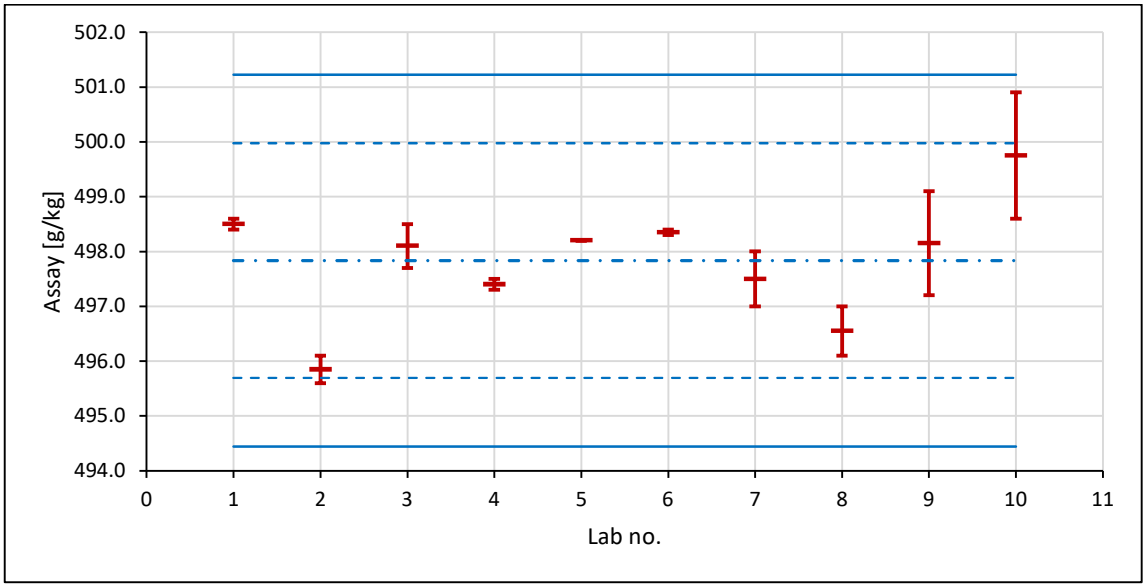


Figure 6: Graphical presentation of the results of the different laboratories for Sample A (TC). For each laboratory (laboratories 1 to 10) the red bars represent day 1, day 2 and the average.
No outlier or straggler has been identified.

Table 11: Results for R-enantiomer of the different laboratories for Sample B (Metalaxyl-M TC).

Lab. no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
day 1	948.80	951.50	949.10	944.40	949.10	948.00	947.50	950.00	949.70	947.60
day 2	948.90	951.90	949.10	944.50	949.10	948.80	948.80	951.60	957.80	947.30
mean	948.85	951.70	949.10	944.45	949.10	948.40	948.15	950.80	953.75	947.45

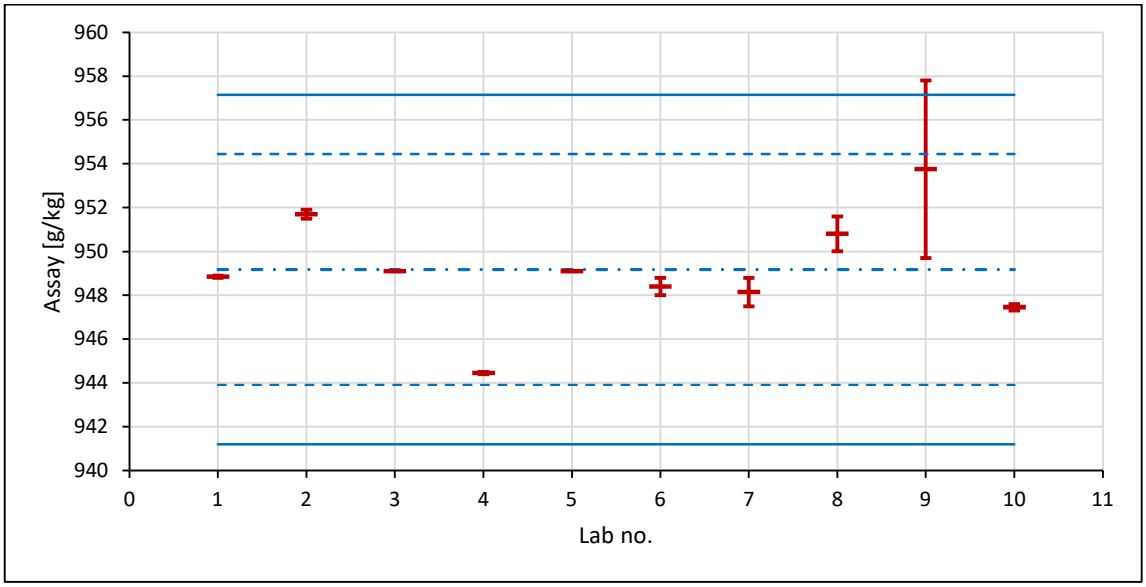


Figure 7: Graphical presentation of the results of the different laboratories for Sample B (TC). For each laboratory (laboratories 1 to 10) the red bars represent day 1, day 2 and the average.

Table 12: Results for R-enantiomer of the different laboratories for Sample C (SL 480).

Lab. no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
day 1	444.20	443.40	445.50	445.20	444.00	443.50	443.73	444.30	444.60	441.90
day 2	444.10	443.40	445.50	445.30	444.00	443.60	444.14	445.40	440.80	442.40
mean	444.15	443.40	445.50	445.25	444.00	443.55	443.94	444.85	442.70	442.15

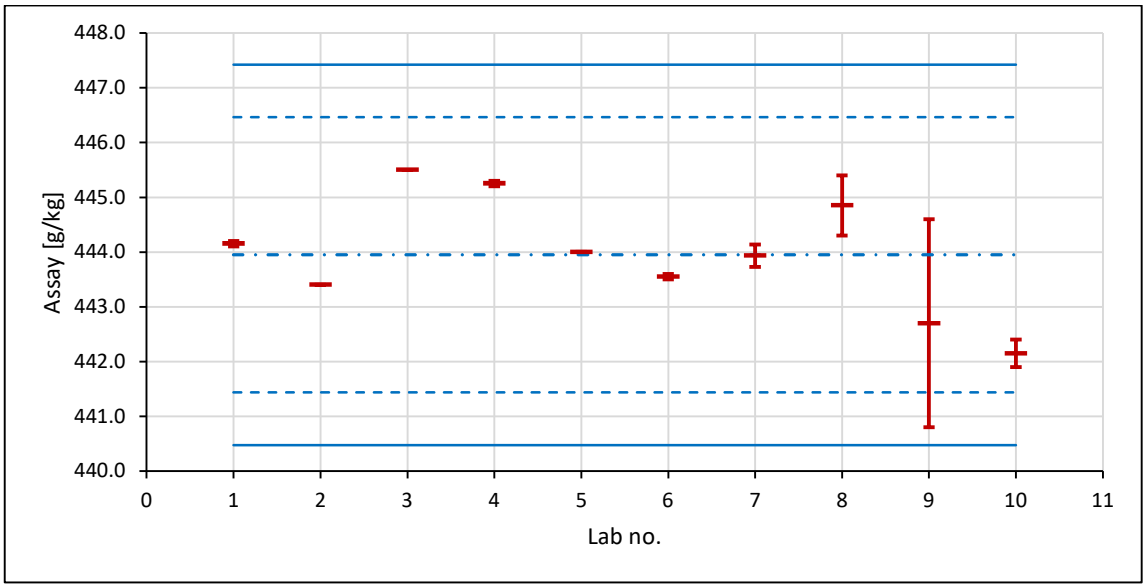


Figure 8: Graphical presentation of the results of the different laboratories for Sample C (SL). For each laboratory (laboratories 1 to 10) the red bars represent day 1, day 2 and the average.

Table 13: Results for R-enantiomer of the different laboratories for Sample D (ES 35).

Lab no.	1	2	3	4	5	6	7	8	9	10
day 1	308.40	308.70	308.60	308.20	308.70	308.10	308.42	309.20	306.10	308.10
day 2	308.40	308.20	308.80	308.30	308.50	308.40	308.47	309.90	305.10	307.90
mean	308.40	308.45	308.70	308.25	308.60	308.25	308.45	309.55	305.60	308.00

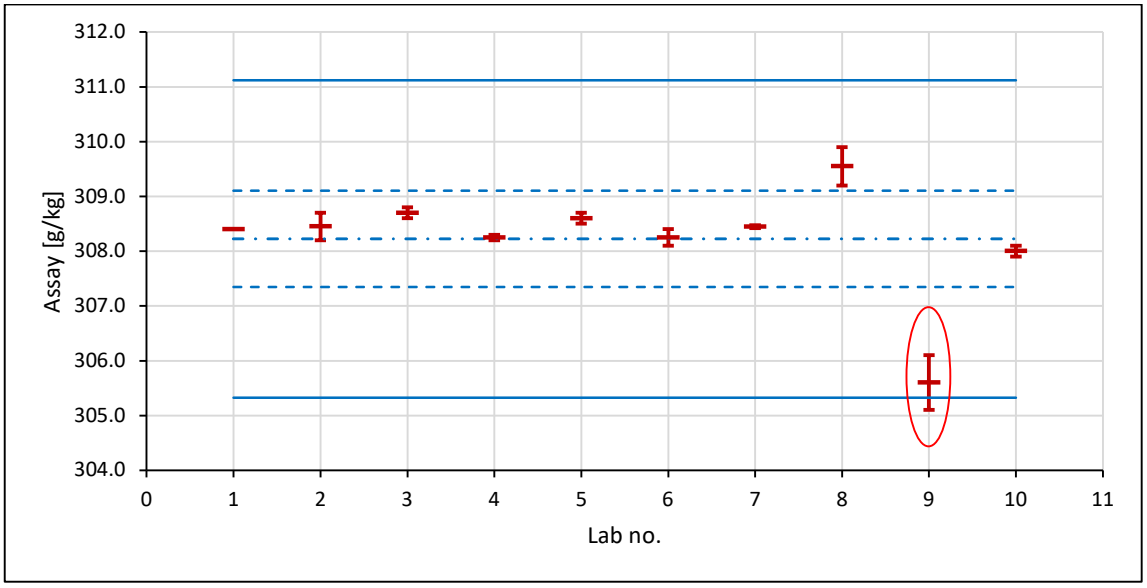


Figure 9: Graphical presentation of the results of the different laboratories for Sample D (ES 350). For each laboratory (laboratories 1 to 10) the red bars represent day 1 and day 2 and average. Lab No. 9 is an outlier

Table 14: Results for R-enantiomer of the different laboratories for Sample E (WG 4).

Lab. no.	1	2	3	4	5	6	7	8	9	10
day 1	39.00	38.70	38.50	38.60	38.80	38.70	38.34	36.10	37.80	38.50
day 2	39.00	39.00	38.50	38.80	38.50	38.70	38.75	38.00	38.90	38.40
mean	39.00	38.85	38.50	38.70	38.65	38.70	38.55	37.05	38.35	38.45

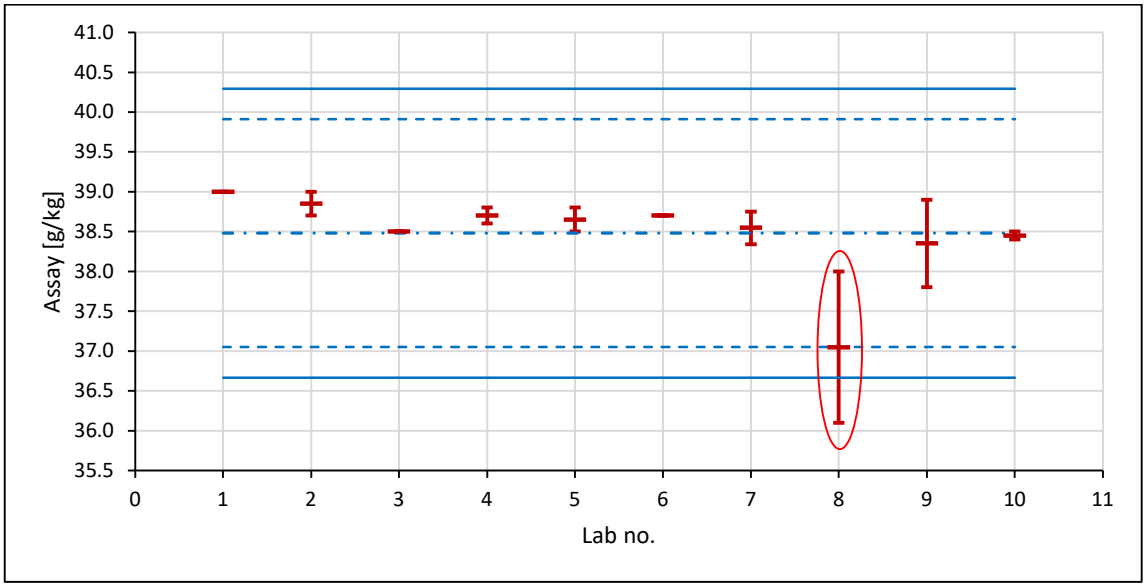


Figure 10: Graphical presentation of the results of the different laboratories for Sample E (WG 4). For each laboratory (laboratories 1 to 10 the red bars represent day 1 and day 2 and average.

Table 15: Overall statistics for R-enantiomer on all submitted results:

Sample	x_m [g/kg]	L	N	s_r	s_L	s_R	r	R	RSD_R	$RSD_{R\ (Hor)}$	HorRat
A	497.84	10	20	0.76	0.94	1.21	2.14	3.39	0.24	2.22	0.11
B	949.18	10	20	1.88	2.14	2.85	5.27	7.97	0.30	2.02	0.15
C	443.95	10	20	0.90	0.86	1.24	2.51	3.47	0.28	2.26	0.12
D	308.22	10	20	0.31	0.99	1.03	0.88	2.90	0.34	2.39	0.14
D*	308.52	9	18	0.23	0.41	0.47	0.65	1.31	0.15	2.39	0.06
E	38.48	10	20	0.51	0.40	0.65	1.43	1.81	1.68	3.27	0.52
E*	38.60	10	19	0.30	0.12	0.32	0.84	0.90	0.83	3.26	0.26

For sample D Lab No 9 is an outlier on both days. Still the Horrat is well below 1. For sample E Lab 8 is an outlier on Day 1. Still the Horrat is below 1 already without elimination of the outlier result.
D* and E* show the results after elimination of the outlier result.

11. Summary and Conclusion chiral LC

A total of 10 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 10 to 14). In all cases shown in table 15 the Horrat is well below 1. Grubbs straggler has been identified for Sample D and E. 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.