5048/R Mancozeb Small Scale Trial-CCPIA

Small Scale Collaborative Study for the Determination of Mancozeb in TC and WP by HPLC

Limin Chemical Co., Ltd.
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Content page

1. Participants

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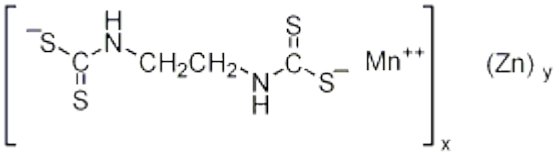
Chuanshan Yu	Beijing MNX Agro-chemical Testing Technology	Beijing, China
Lu Bin	Laprode (Zhejiang) Analysis Co., Ltd.	Zhejiang, China
Xu Mei	Limin Chemical Co., Ltd. QC lab	Xuzhou, China

2. Active Ingredient, General Information

IUPAC name: manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt
ISO common name: Mancozeb
CAS-Nr.:8018-01-7



Structure: x:y = 1:0.091
Molecular mass: 271.2(Based on composition)
Empirical formula:[C4H6MnN2S4]XZny

3. Samples

InMay 2016thefollowingsampleswere sent to theparticipants:

1. 201603084-03 Mancozeb Technical
2. 201603090-02 Mancozeb Technical
3. 201603080-02 Mancozeb Technical
4. 201603061-03 Mancozeb WP
5. 201603002-03 Mancozeb WP
6. 201603154-03 Mancozeb WP

In May 2016 results were obtained from 5 participants.

4. Method

4.1 Scope

Determination of the content of the active level in TC and WP

4.2 Principle

All teat sample listed will be analyzed using the HPLC assay method provided.

4.3 Procedureforthe collaborative trial

Each sample should be analyzed twice on two different days. The solutions should be injected twice and analyzed as follows:

Day 1:preparation (1 weighing) of the one standard solution.
Make 2 preparations (2 weighings) of each of the test samples.
Since there are six samples, this is a total of 12 weights, two each.
Analyze following the sequence provided below

Day 2:preparation (1 weighing) of the one standard solution.
Make 2 preparations (2 weighings) of each of the test samples.
Since there are six samples, this is a total of 12 weights, two each.
Analyze following the sequence provided below

4.4 Sample Analysis Sequence

The analysis sequence each day should be as follows:

- Duplicate injections of a calibration standard
- Duplicate injections of 1st sample type
- Duplicate injections of a calibration standard
- Duplicate injections of 2nd sample type
- Duplicate injections of a calibration standard
- Duplicate injections of 3rd sample type
- Duplicate injections of a calibration standard
- Duplicate injections of 4th sample type
- Duplicate injections of a calibration standard
- Duplicate injections of 5th sample type
- Duplicate injections of a calibration standard

- Duplicate injections of 6th sample type
- Duplicate injections of a calibration standard

5. Analytical Methods

5.1 Analytical Conditions

Lab	Liquid Chromatograph Integrator	Column	Mobile Phase	Flow rate ml/min	Column temp (℃)
1	Agilent 1260 series HPLC UV	Agilent Extend C18, 150 mm×4.6 mm (i.d.), 5μm column	Methanol / buffer solution A (33:77 % v/v)Buffer solution: Water solution with 10mM EDTA,10mM Na2HPO4, 10 mMTetrabutylammonium Hydrogen Sulfate and 1g/L Na2SO3, adjust the pH of the solution to 9.5 ～ 10.0 with NaOH solution	0.9	29
2	Shimadzu LC-20AT with PDA detector	Agilent Extend-C18, 4.6×250mm,5 μm, SN :USHR01 6024 column	Methanol / buffer solution A (30:70 % v/v)Buffer solution: Water solution with 10mM EDTA,10mM Na2HPO4, 10 mMTetrabutylammonium Hydrogen Sulfate and 1g/L Na2SO3, adjust the pH of the solution to 9.5 ～ 10.0 with NaOH solution	1.0	30
3	Thermo Ultimate 3000 with DAD detector	Agilent Extend-C18, 150mm,4.6m m,5μm column	Methanol / buffer solution A (30:70 % v/v)Buffer solution: Water solution with 10mM EDTA,10mM Na2HPO4, 10 mMTetrabutylammonium Hydrogen Sulfate and 1g/L Na2SO3, adjust the pH of the solution to 9.5 ～ 10.0 with NaOH solution	1.0	25
4	Agilent 1260 HPLC with DAD detector	C18 pH 9.5-10.0 (250 mm x 4.6 mm) column	Methanol / buffer solution A (30:70 % v/v)Buffer solution: Water solution with 10mM EDTA,10mM Na2HPO4, 10 mMTetrabutylammonium Hydrogen Sulfate and 1g/L Na2SO3, adjust the pH of the solution to 9.5 ～ 10.0 with NaOH solution	1.0	29
5	Shimadzu 20A, UV detector	C18 pH 9.5-10.0 (250 mm x 4.6 mm) column	Methanol / buffer solution A (33:77 % v/v)Buffer solution: Water solution with 10mM EDTA,10mM Na2HPO4, 10 mMTetrabutylammonium Hydrogen Sulfate and 1g/L Na2SO3, adjust the pH of the solution to 9.5 ～ 10.0 with NaOH solution	0.9	29

5.2 Deviations from the analytical method

Lab1:
The 50 mL flasks were used when analysts were preparing sample solutions and standard solutions.

Lab2:
Injection volume: 20μL was changed to 10μL.
Mobile phase : Methanol + Buffer solution =33+77(v/v) was changed to Methanol + Buffer solution =30+70(v/v)
Column temperature: 29 °C was changed to 30 °C.
Flow rate: 0.9mL/min was changed to 1.0mL/min.

Lab3:
Injection volume: 20μL was changed to 5μL.
Mobile phase : Methanol + Buffer solution =33+77(v/v) was changed to Methanol + Buffer solution

=30+70(v/v) .
Column temperature: 29 °C was changed to 25°C.
Flow rate: 0.9mL/min was changed to 1.0mL/min.

Lab4:
Disodium hydrogen phosphate instead of Dipotassium phosphate.
Injection volume: 20μL was changed to 10μL.
Mobile phase: Methanol + Buffer solution =33+77(v/v) was changed to Methanol + Buffer solution=30+70(v/v) .
Flow rate: 0.9mL/min was changed to 1.0mL/min.

Lab5:No Deviations

5.3 Remarks about the analytical method
Lab1: No remarks
Lab2:1.It's better to find a solution in which the sample is more stable;
2.The sample is difficult to dissolve only by ultrasonic, should also be shaken by hand
Lab3:1.Sample solution should be prepared carefully, and make sure the sample and standard weighed dissolve thoroughly.
2.Buffer solution in water should be get filtered to protect HPLC system.
Lab4: No remarks
Lab5: No remarks

6. Evaluation and Discussion

Any deviations applied by the participants were not considered to have any adverse effect on the chromatography and consequently on the results.

The assay results obtained by the collaborators and the statistical evaluation are reported in Table1-4. The statistical evaluation was done in accordance with DIN ISO 5725.

Three results were identified as outlier(Grubbs test and Cochran variance homogeneity test). It is assumed that incomplete dissolution of the samplemay be responsible for these outlier and stragglers.

7. Conclusions

The RSD_R as determined from the collaborative study is not larger than RSDR(calc.), the method should be acceptable.Basedon the results of this pilot study, it is proposed to perform a CIPAC collaborative study to determine mancozeb in TC and WP by the HPLC method.

8. AppendixA
Tables and Figures for Mancozeb level

Table 1: Mancozebcontent of TC and WP [g/kg]

	TC-1		TC-2		TC-3		WP-1		WP-2		WP-3	
	-201603084		-201603090		-201603080		-20160308		-201603002		-201603154	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Lab 1	861.1	861.1	863.1	872.7	867.4	862.0	818.4	814.3	825.3	811.5	820.8	805.0
Lab 2	868.0	871.0	877.9	866.1	867.5	859.6	837.3	834.8	836.6	830.4	831.2	832.7
Lab 3	884.3	853.6	862.7	870.8	869.4	871.8	819.6	818.7	805.5	798.3	806.6	803.6
Lab 4	866.0	869.2	858.9	864.7	858.9	855.8	828.8	816.9	828.2	806.4	825.8	806.4
Lab 5	856.7	855.7	860.4	862.2	860.2	861.8	815.0	815.8	807.1	807.9	814.7	815.1

Table 2: Mean values of the Mancozeb concentration [g/kg]

	TC-1	TC-2	TC-3	WP-1	WP-2	WP-3
	-201603084	-201603090	-201603080	-20160308	201603002	-201603154
Lab 1	861.1	867.9	864.7	816.4	818.4	812.9
Lab 2	869.5	872.0	863.6	836.1++	833.5	832.0++
Lab 3	874.0++	866.8	870.6	819.2	801.9	805.1
Lab 4	867.6	861.8	857.4	822.9+	817.3	816.1
Lab 5	856.2	861.3	861.0	815.4	807.5	814.9

++Outlier according to the Grubbs test and Cochran variance homogeneity test.
+straggler according to the Cochran variance homogeneity test.

Table 3: Summary of the statistical evaluation no elimination of any outliers

	TC-1	TC-2	TC-3	WP-1	WP-2	WP-3
	-201603084	-201603090	-201603080	-20160308	-201603002	-201603154
Xm	865.7	866.0	863.4	822.0	815.7	816.2
L	5	5	5	5	5	5
Sr	6.699	5.778	3.309	4.076	8.698	7.984
S_L	5.191	1.811	4.301	7.882	10.405	8.006
S_R	8.474	6.055	5.427	8.873	13.562	11.306
r	18.757	16.178	9.265	11.413	24.354	22.355
R	23.727	16.954	15.196	24.844	37.974	31.657
RSDr	0.774	0.667	0.383	0.496	1.065	0.978
RSDR	0.979	0.699	0.628	1.079	1.663	1.385
$RSD_{R(Hor)}$	2.044	2.044	2.044	2.060	2.056	2.062

x_m = overallsample mean
L = numberoflaboratories
s_r = repeatabilitystandarddeviation
s_L = “pure” betweenlaboratorystandarddeviation
s_R = reproducibilitystandard deviation
r = repeatabilitylimit
R = reproducibilitylimit
RSD_r = relative repeatabilitystandard deviation
RSD_R = relative reproducibilitystandard deviation
$RSD_R(Hor)$ = relative reproducibilitystandard deviation (Horwitzequation)

Table 4: Summary of the statistical evaluation elimination of outliers

	TC-1	TC-2	TC-3	WP-1	WP-2	WP-3
	-201603084	-201603090	-201603080	-20160308	-201603002	-201603154
Xm	863.6	866.0	863.4	818.4	815.7	812.3
L	4	5	5	4	5	4
Sr	1.591	5.778	3.309	4.470	8.698	8.910
S_L	6.001	1.811	4.301	1.093	10.405	0
S_R	6.288	6.055	5.427	4.602	13.562	8.910
r	4.454	16.178	9.265	12.517	24.354	24.948
R	17.606	16.954	15.196	12.886	37.974	24.948
RSDr	0.184	0.667	0.383	0.546	1.065	1.097
RSDR	0.728	0.699	0.628	0.562	1.663	1.097
$RSD_{R(Hor)}$	2.044	2.044	2.044	2.061	2.056	2.064

Fig. 1: Results of the sampleTC-1(see table 3 for the evaluation)

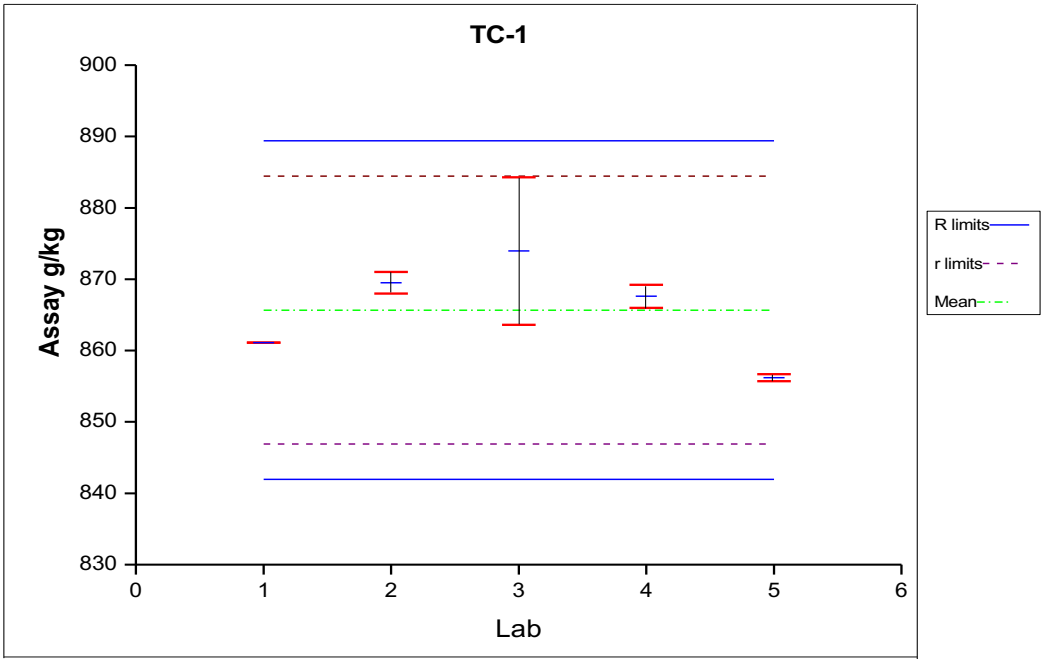


Fig. 2: Results of the sampleTC-2 (see table 3 for the evaluation)

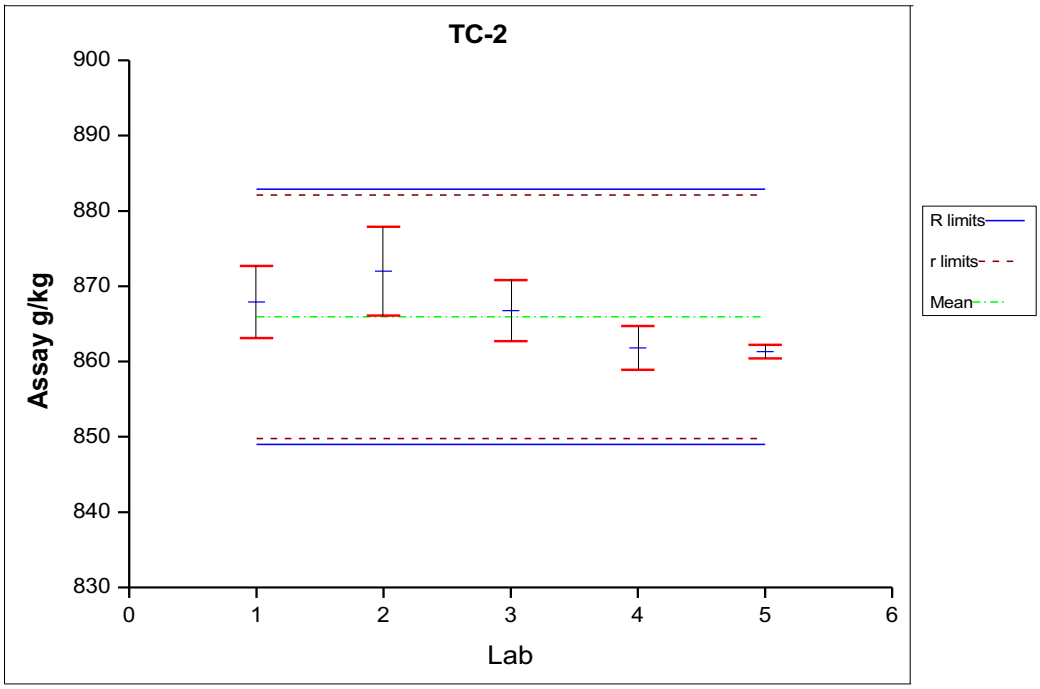


Fig. 3: Results of the sampleTC-3(see table 3 for the evaluation)

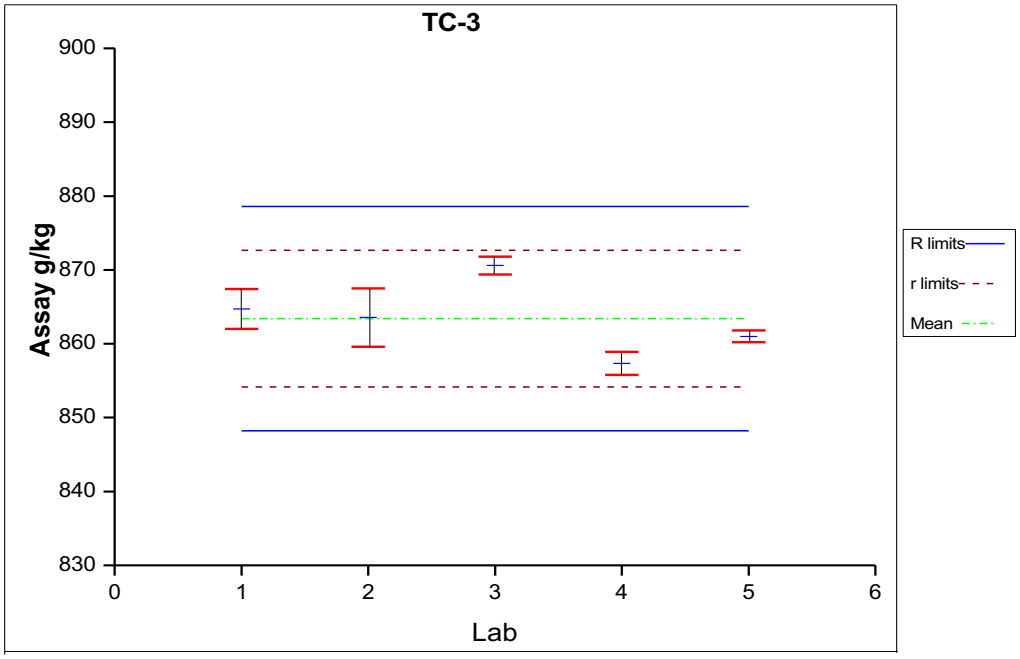


Fig. 4: Results of the sample WP-1(see table 3 for the evaluation)

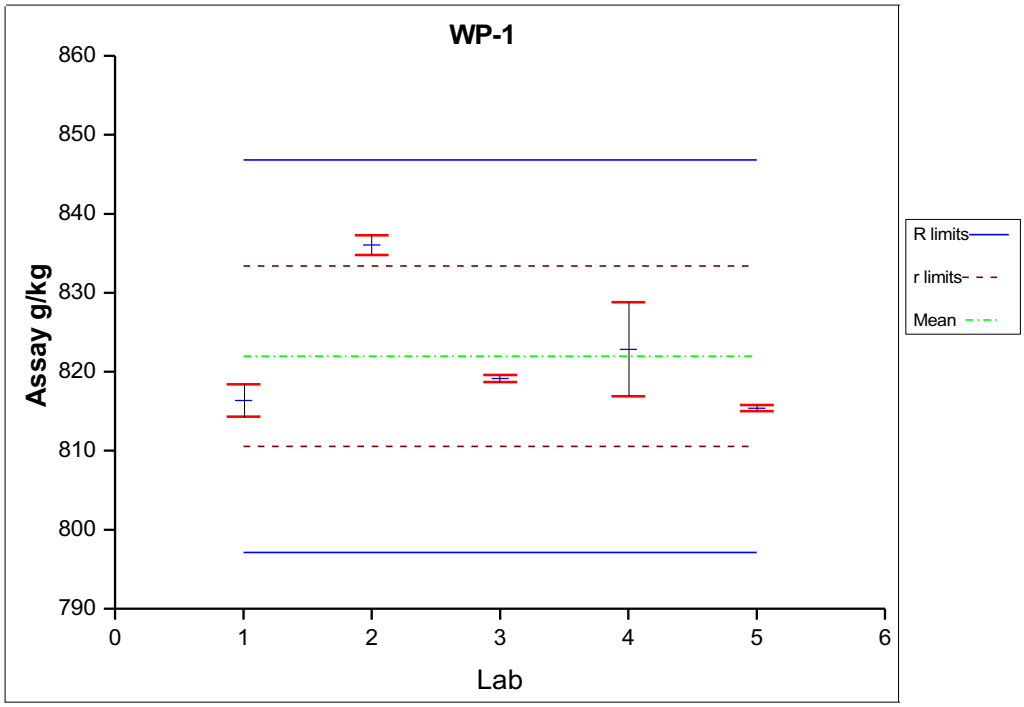


Fig. 5: Results of the sample WP-2(see table 3 for the evaluation)

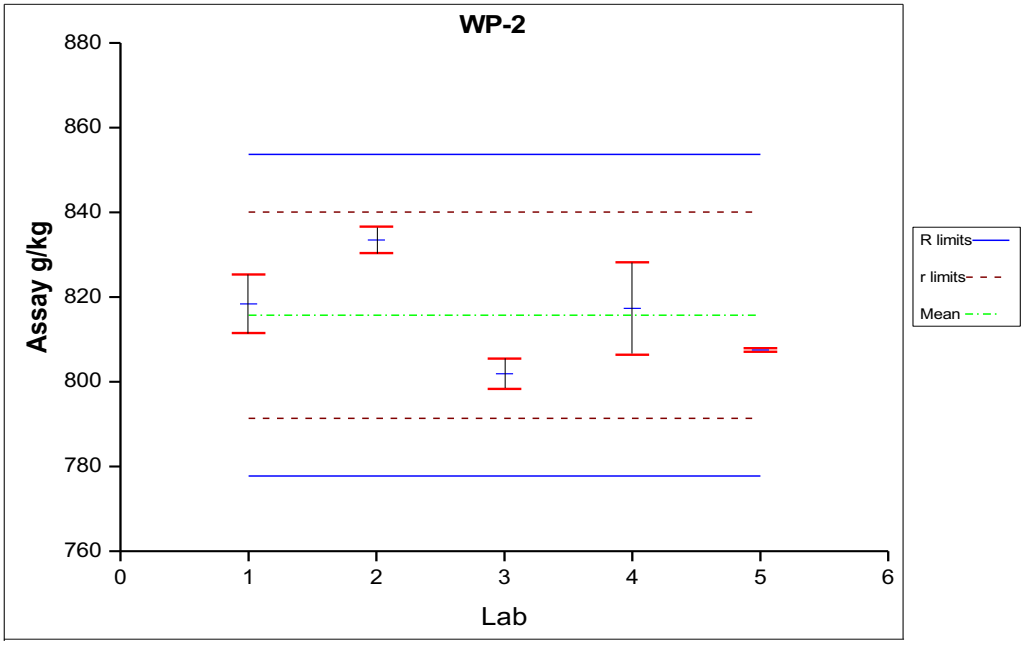


Fig. 6: Results of the sample WP-3(see table 3for the evaluation)