

CLOTHIANIDIN (No.738)
Small Scale Collaborative Trial

Small Scale Collaborative Study on the Determination of
Clothianidin in Clothianidin Technical and Formulation
by High Performance Liquid Chromatography

by
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1. INTRODUCTION

1.1 Scope

The results of the small scale collaborative trial for clothianidin technical product and clothianidin water dispersible granule are reported.

1.2 Samples

- 1) Clothianidin technical (TC-1)
- 2) Clothianidin technical (TC-2)
- 3) Clothianidin water dispersible granule (WG-1)
- 4) Clothianidin water dispersible granule (WG-2)
- 5) Clothianidin water dispersible granule (WG-3)

1.3 Participants

Noriko Hamada	Hodogaya Contract Laboratory, Co., Ltd. (JAPAN)
Megumi Kawamura	Sumitomo Chemical Co., Ltd. Organic Synthesis Research Laboratory (JAPAN)
Yumiko Kozuki	Sumitomo Chemical Co., Ltd. Agricultural Chemicals Research Laboratory (JAPAN)
Yasuko Osaki	Taoka Chemical Analysis Center Co., Ltd. (JAPAN)
Takahide Ueno	Sumika Enviro-Science Co., Ltd. (JAPAN)
Hirofumi Ushino	Hayashi Agro Science, Ltd. (JAPAN)
Yuka Yoshida	Sumika Chemical Analysis Service, Ltd. Osaka Laboratory (JAPAN)

2. ANALYTICAL METHOD

2.1 Outline of Method

Clothianidin in the test samples is determined by reversed phase high performance liquid chromatography using an ODS column, UV detection at 269 nm and external standardization as stated in CIPAC/4604/m.

2.2 Program of Work

We requested the collaborators to:

- 1) conduct duplicate determinations on two different days for each sample;
- 2) inject each sample solution in duplicate and calculate the mean value;
- 3) check linearity before the determination;
- 4) describe operating conditions in detail; and
- 5) attach the calibration curve and all chromatograms for each sample.

3. REMARKS OF PARTICIPANTS

3.1 Analytical Conditions

Lab	Liquid chromatograph Integrator	Column	Mobile phase	Flow rate (ml/min)	Column temp. (°C)
	Proposed Method	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 µm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
1	Shimadzu LC-10Avp Shimadzu C-R7A plus	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 µm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
2	Shimadzu LC-10ADvp Shimadzu LC-solution	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 µm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40

3	Shimadzu Prominence Shimadzu LC-solution	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 μm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
4	Shimadzu LC-10ATvp Shimadzu CLASS-VP	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 μm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
5	Shimadzu LC-10Avp Shimadzu C-R8A	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 μm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
6	Agilent HP-1100 Agilent ChemStation	Develosil ODS-HG-5 C18 (4.6 mm ID × 15 cm, 5 μm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.0	40
7	Jasco 875-uv Shimadzu C-R7A plus	Zorbax Eclipse XDB-C18 (4.6 mm ID × 15 cm, 5 μm)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	1.1	40

4. RESULTS AND DISCUSSION

Seven data sets were obtained from seven participants. Summary and detailed statistical evaluations are shown in Tables 1 and 2-1 to 2-5. The statistical evaluations were carried out according to ISO 5725.

The discussion on stragglers and outliers is as follows:

- WG-1

The variance of Lab. 6 was identified as an outlier. The data were retained because there were no reasons to remove them.

- WG-3

The variance of Lab. 3 was identified as an outlier. The data were retained because there were no reasons to remove them.

5. CONCLUSION

For all samples, the values of RSD_R (reproducibility relative standard deviation) were smaller than those calculated by Horwitz's equation. The proposed method is considered appropriate for the determination of clothianidin in technical product and water dispersible granule. JAPAC proposes proceeding to a large scale collaborative trial.

Table 1 Summary of Statistical Evaluation of Clothianidin Small Scale Collaborative Study

	TC-1	TC-2	WG-1	WG-2	WG-3
Average (g/kg)	989.56	986.31	503.75	502.38	503.23
Number of labs.	7	7	7	7	7
Repeatability standard deviation (S_r)	5.967	6.069	7.494	4.314	4.011
"Pure" between laboratory standard variation (S_L)	-	0.580	4.246	2.163	1.160
Reproducibility standard deviation (S_R)	5.622	6.097	8.613	4.826	4.175
Repeatability (r)	16.708	16.993	20.983	12.079	11.231
Reproducibility (R)	15.742	17.072	24.116	13.513	11.690
RSD _r	0.603	0.615	1.488	0.859	0.797
RSD _R	0.568	0.618	1.710	0.961	0.830
Horwitz's value	2.003	2.004	2.217	2.218	2.218

Table 2-1 Clothianidin Technical -1

Lab	Analytical data (n=4)		Yi	(Yi) ²	Si	Si ²
1	Day1	979.5	982.7			
	Day2	997.0	992.9	988.03	976203.28	8.273
2	Day1	988.9	988.0			
	Day2	990.5	990.5	989.48	979070.67	1.239
3	Day1	991.6	1006.9			
	Day2	987.0	982.2	991.93	983925.12	10.696
4	Day1	987.3	992.6			
	Day2	991.1	992.6	990.90	981882.81	2.502
5	Day1	987.2	985.3			
	Day2	993.2	982.9	987.15	974465.12	4.400
6	Day1	983.6	988.2			
	Day2	984.1	992.5	987.10	974366.41	4.148
7	Day1	993.5	998.1			
	Day2	986.9	990.9	992.35	984758.52	4.697
S1 SUM	Yi =		6926.94			
S2 SUM	Yi ² =			6854671.93		
S3 SUM	Si ² =					249.270

p = 7

1) Cochran's test (p=7, n=4)

$$C = S_i^2 \max / S3 = 0.459 < 0.480 \text{ (p=7, n=4, 5\%)}$$

2) Grubbs' test (p=7, n=4)

Yi(min) =	987.10	Yi(max) =	992.35	Y = S1/p	989.56
				S =	2.214
Y - Yi(min) =	2.46				
Yi(max) - Y =	2.79				
lower = [Y - Yi(min)]/S =	1.113	<	2.020 (p=7, 5%)		
upper = [Yi(max) - Y]/S =	1.259	<	2.020 (p=7, 5%)		

3) Calculation of r and R

Mean; Y = S1 / p =	989.56	
Sr ² = S3 / p =	35.610	Sr = 5.967
SL ² = [(pS2 - S1 ²)/p(p-1)] - (Sr ² /n) =	-4.004	SL = -
SR ² = Sr ² + SL ² =	31.606	SR = 5.622

r = 2.8 x Sr =	16.708
R = 2.8 x SR =	15.742
RSDr = (Sr / mean) x 100 =	0.603
RSDR = (SR / mean) x 100 =	0.568

Horwitz's Value = $2 \sqrt{1 - 0.5 \times \log(Y / 1000)}$ = 2.003

RSDr and RSDR < 2.003 (Horwitz's Value)

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Table 2-2 Clothianidin Technical -2

Lab	Analytical data (n=4)	Yi	(Yi) ²	Si	Si ²
1	Day1 983.2 979.9				
	Day2 991.5 992.7	986.83	973833.45	6.257	39.150
2	Day1 978.0 987.2				
	Day2 986.7 992.8	986.18	972550.99	6.111	37.344
3	Day1 991.8 995.7				
	Day2 975.4 979.4	985.58	971367.94	9.711	94.304
4	Day1 988.8 989.3				
	Day2 988.4 987.3	988.45	977033.40	0.850	0.723
5	Day1 985.0 986.1				
	Day2 977.6 977.9	981.65	963636.72	4.527	20.494
6	Day1 982.0 985.2				
	Day2 989.6 979.7	984.13	968511.86	4.291	18.413
7	Day1 997.5 997.1				
	Day2 984.9 985.9	991.35	982774.82	6.885	47.403
S1 SUM	Yi =	6904.17			
S2 SUM	Yi ² =		6809709.18		
S3 SUM	Si ² =				257.831

p = 7

1) Cochran's test (p=7, n=4)

$$C = Si^2_{max} / S3 = 0.366 < 0.480 \text{ (p=7, n=4, 5\%)}$$

2) Grubbs' test (p=7, n=4)

$$Yi(\min) = 981.65 \quad Yi(\max) = 991.35 \quad Y = S1/p = 986.31$$

$$S = 3.089$$

$$Y - Yi(\min) = 4.66$$

$$Yi(\max) - Y = 5.04$$

$$\text{lower} = [Y - Yi(\min)]/S = 1.508 < 2.020 \text{ (p=7, 5\%)}$$

$$\text{upper} = [Yi(\max) - Y]/S = 1.631 < 2.020 \text{ (p=7, 5\%)}$$

3) Calculation of r and R

$$\text{Mean; } Y = S1 / p = 986.31$$

$$Sr^2 = S3 / p = 36.833 \quad Sr = 6.069$$

$$SL^2 = [(pS2 - S1^2)/p(p-1)] - (Sr^2/n) = 0.336 \quad SL = 0.580$$

$$SR^2 = Sr^2 + SL^2 = 37.169 \quad SR = 6.097$$

r = 2.8 x Sr =	16.993
R = 2.8 x SR =	17.072
RSDr = (Sr / mean) x 100 =	0.615
RSDR = (SR / mean) x 100 =	0.618

$$\text{Horwitz's Value} = 2 \sqrt{1 - 0.5 \times \log(Y / 1000)} = 2.004$$

RSDr and RSDR < 2.004 (Horwitz's Value)

Table 2-3 Clothianidin Water Dispersible Granule-1

Lab	Analytical data (n=4)		Yi	(Yi) ²	Si	Si ²
1	Day1	500.1	499.0			
	Day2	501.5	501.8	500.60	250600.36	1.299
2	Day1	504.6	505.7			
	Day2	506.3	518.5	508.78	258857.09	6.521
3	Day1	502.9	503.9			
	Day2	493.9	500.0	500.18	250180.03	4.498
4	Day1	504.0	503.6			
	Day2	500.6	501.2	502.35	252355.52	1.700
5	Day1	501.3	500.2			
	Day2	502.1	492.0	498.90	248901.21	4.665
6	Day1	506.7	537.2			
	Day2	505.9	507.5	514.33	264535.35	15.264
7	Day1	508.6	507.1			
	Day2	490.5	498.2	501.10	251101.21	8.427
S1 SUM	Yi =		3526.24			
S2 SUM	Yi ² =			1776530.77		
S3 SUM	Si ² =					393.098

p = 7

1) Cochran's test (p=7, n=4)

$$C = S_i^2 \max / S_3 = 0.593 > 0.480 \text{ (p=7, n=4, 5\%)} \\ > 0.568 \text{ (p=7, n=4, 1\%)}$$

Outlier Lab 6 was included in the following evaluation.

2) Grubbs' test (p=7, n=4)

$$Y_i(\min) = 498.90 \quad Y_i(\max) = 514.33 \quad Y = S_1/p = 503.75 \\ S = 5.663$$

$$Y - Y_i(\min) = 4.85 \\ Y_i(\max) - Y = 10.58 \\ \text{lower} = [Y - Y_i(\min)]/S = 0.856 < 2.020 \text{ (p=7, 5\%)} \\ \text{upper} = [Y_i(\max) - Y]/S = 1.869 < 2.020 \text{ (p=7, 5\%)}$$

3) Calculation of r and R

$$\text{Mean; } Y = S_1 / p = 503.75 \\ S_r^2 = S_3 / p = 56.157 \quad S_r = 7.494 \\ S_L^2 = [(pS_2 - S_1^2)/p(p-1)] - (S_r^2/n) = 18.029 \quad S_L = 4.246 \\ S_R^2 = S_r^2 + S_L^2 = 74.186 \quad S_R = 8.613$$

r = 2.8 x Sr =	20.983
R = 2.8 x SR =	24.116
RSDr = (Sr / mean) x 100 =	1.488
RSDR = (SR / mean) x 100 =	1.710

$$\text{Horwitz's Value} = 2 \sqrt{1 - 0.5 \times \log(Y / 1000)} = 2.217$$

RSDr and RSDR < 2.217 (Horwitz's Value)

Table 2-4 Clothianidin Water Dispersible Granule-2

Lab	Analytical data (n=4)		Yi	(Yi) ²	Si	Si ²
1	Day1	499.1	498.6			
	Day2	501.3	500.5	499.88	249880.01	1.245
2	Day1	506.7	503.4			
	Day2	507.0	513.5	507.65	257708.52	4.227
3	Day1	506.1	501.0			
	Day2	497.4	497.0	500.38	250380.14	4.219
4	Day1	502.1	501.2			
	Day2	501.3	500.0	501.15	251151.32	0.866
5	Day1	503.6	502.8			
	Day2	490.4	501.0	499.45	249450.30	6.131
6	Day1	502.0	508.6			
	Day2	505.4	504.4	505.10	255126.01	2.735
7	Day1	507.7	508.7			
	Day2	493.7	502.2	503.08	253089.49	6.872
S1 SUM	Yi =		3516.69			
S2 SUM	Yi ² =			1766785.79		
S3 SUM	Si ² =					130.261

p = 7

1) Cochran's test (p=7, n=4)

$$C = Si^2_{max} / S3 = 0.363 < 0.480 \text{ (p=7, n=4, 5\%)}$$

2) Grubbs' test (p=7, n=4)

$$Yi(\min) = 499.45 \quad Yi(\max) = 507.65 \quad Y = S1/p = 502.38$$

$$S = 3.055$$

$$Y - Yi(\min) = 2.93$$

$$Yi(\max) - Y = 5.27$$

$$\text{lower} = [Y - Yi(\min)]/S = 0.960 < 2.020 \text{ (p=7, 5\%)}$$

$$\text{upper} = [Yi(\max) - Y]/S = 1.723 < 2.020 \text{ (p=7, 5\%)}$$

3) Calculation of r and R

$$\text{Mean; } Y = S1 / p = 502.38$$

$$Sr^2 = S3 / p = 18.609 \quad Sr = 4.314$$

$$SL^2 = [(pS2 - S1^2)/p(p-1)] - (Sr^2/n) = 4.681 \quad SL = 2.163$$

$$SR^2 = Sr^2 + SL^2 = 23.289 \quad SR = 4.826$$

r = 2.8 x Sr =	12.079
R = 2.8 x SR =	13.513
RSDr = (Sr / mean) x 100 =	0.859
RSDR = (SR / mean) x 100 =	0.961

$$\text{Horwitz's Value} = 2 \sqrt{1 - 0.5 \times \log(Y / 1000)} = 2.218$$

RSDr and RSDR < 2.218 (Horwitz's Value)

Table 2-5 Clothianidin Water Dispersible Granule-3

Lab	Analytical data (n=4)		Yi	(Yi) ²	Si	Si ²
1	Day1	506.2	498.1			
	Day2	504.6	505.2	503.53	253542.46	3.676
2	Day1	503.7	503.8			
	Day2	510.9	506.7	506.28	256319.44	3.383
3	Day1	508.9	503.8			
	Day2	493.3	491.6	499.40	249400.36	8.320
4	Day1	502.7	504.1			
	Day2	502.7	503.3	503.20	253210.24	0.663
5	Day1	504.8	502.8			
	Day2	499.5	499.9	501.75	251753.06	2.509
6	Day1	508.2	503.1			
	Day2	507.0	504.2	505.63	255661.70	2.375
7	Day1	506.0	502.6			
	Day2	502.6	500.0	502.80	252807.84	2.460
S1 SUM	Yi =		3522.59			
S2 SUM	Yi ² =			1772695.10		
S3 SUM	Si ² =					112.608

p = 7

1) Cochran's test (p=7, n=4)

$$C = S_i^2 \max / S_3 = 0.615 > 0.480 \text{ (p=7, n=4, 5\%)} \\ > 0.568 \text{ (p=7, n=4, 1\%)}$$

Outlier Lab 3 was included in the following evaluation.

2) Grubbs' test (p=7, n=4)

$$Y_i(\min) = 499.40 \quad Y_i(\max) = 506.28 \quad Y = S_1/p = 503.23 \\ S = 2.317$$

$$Y - Y_i(\min) = 3.83 \\ Y_i(\max) - Y = 3.05 \\ \text{lower} = [Y - Y_i(\min)]/S = 1.652 < 2.020 \text{ (p=7, 5\%)} \\ \text{upper} = [Y_i(\max) - Y]/S = 1.318 < 2.020 \text{ (p=7, 5\%)}$$

3) Calculation of r and R

$$\text{Mean; } Y = S_1 / p = 503.23 \\ S_r^2 = S_3 / p = 16.087 \quad S_r = 4.011 \\ S_L^2 = [(pS_2 - S_1^2)/p(p-1)] - (S_r^2/n) = 1.345 \quad S_L = 1.160 \\ S_R^2 = S_r^2 + S_L^2 = 17.432 \quad S_R = 4.175$$

r = 2.8 x Sr =	11.231
R = 2.8 x SR =	11.690
RSDr = (Sr / mean) x 100 =	0.797
RSDR = (SR / mean) x 100 =	0.830

$$\text{Horwitz's Value} = 2 \sqrt{1 - 0.5 \times \log(Y / 1000)} = 2.218$$

RSDr and RSDR < 2.218 (Horwitz's Value)

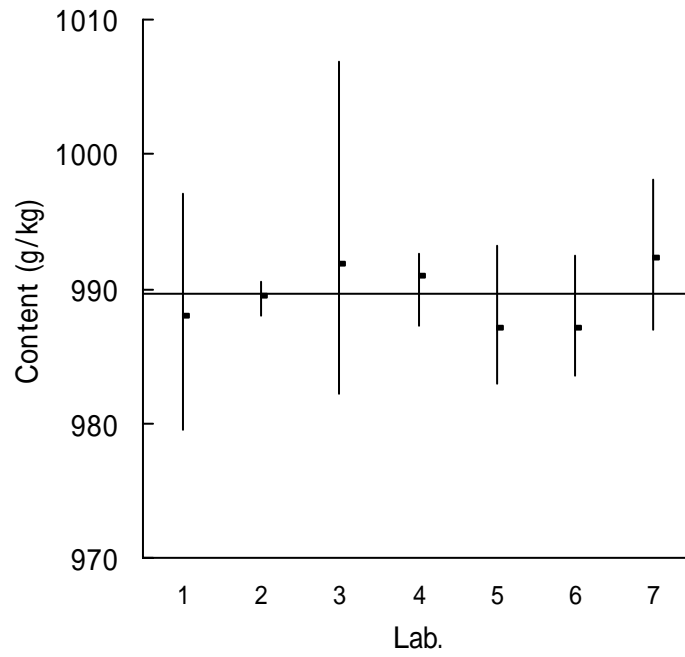


Fig. 1 Clothianidin Technical-1

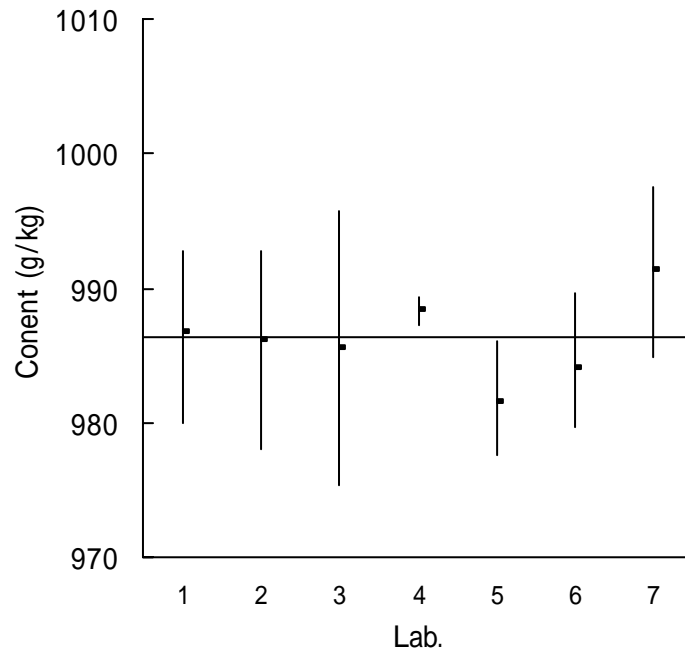


Fig. 2 Clothianidin Technical-2

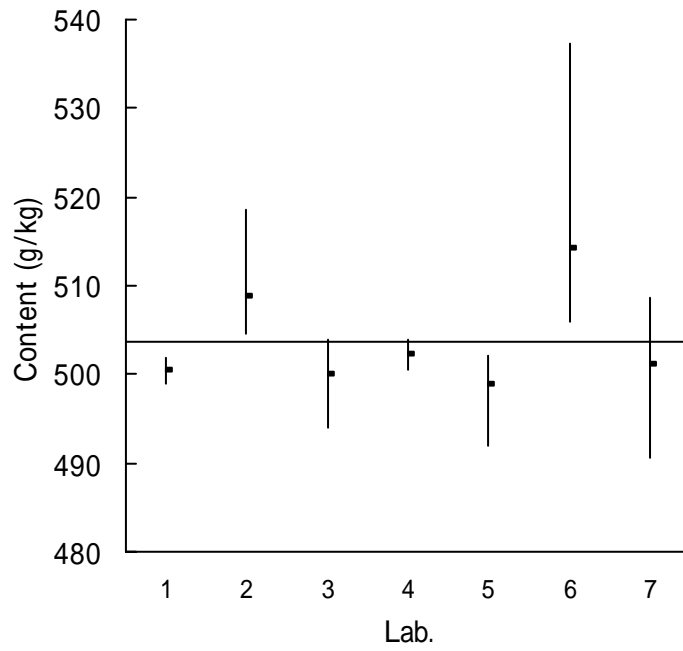


Fig. 3 Clothianidin Water Dispersible Granule-1

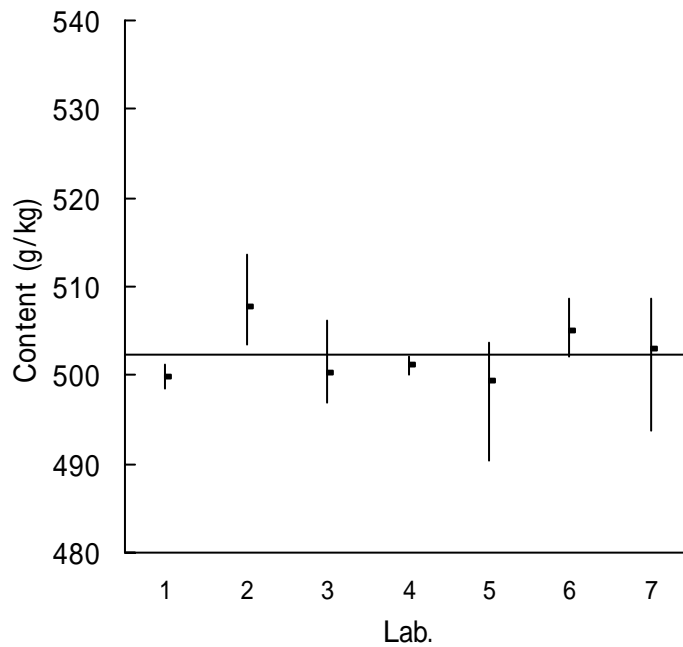


Fig. 4 Clothianidin Water Dispersible Granule-2

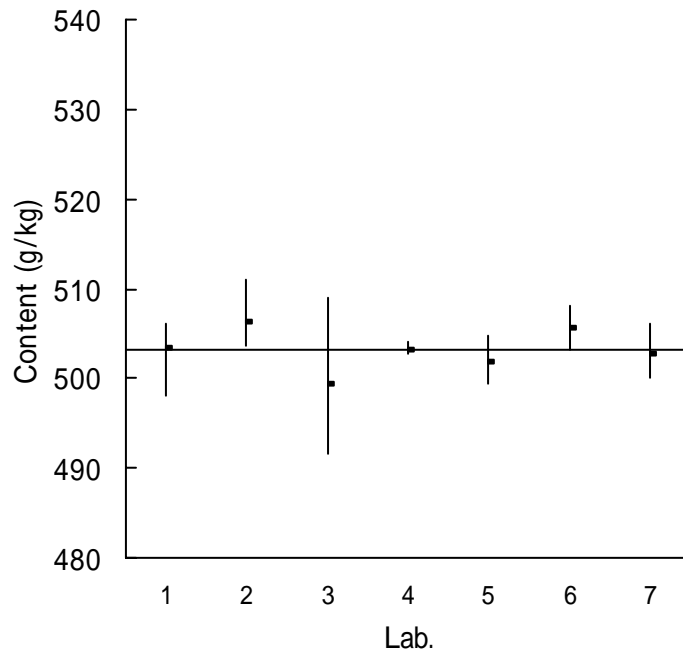


Fig. 5 Clothianidin Water Dispersible Granule-3