CLOTHIANIDIN (No.738)

CIPAC Collaborative Trial

CIPAC Collaborative Study on the Determination of Clothianidin in Clothianidin Technical and Formulations by High Performance Liquid Chromatography

by
Yasushi Asada
Sumitomo Chemical Co., Ltd.
Organic Synthesis Research Laboratory
3-1-98, Kasugade-naka, Konohanaku, Osaka
JAPAN

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1. INTRODUCTION

1.1 Scope

The results of the CIPAC collaborative trial for clothianidin technical product, clothianidin water dispersible granule, clothianidin suspension concentrate, clothianidin granule and clothianidin water soluble granule are reported.

1.2 Samples

1) Clothianidin technical (TC)
2) Clothianidin water dispersible granule (WG)
3) Clothianidin suspension concentrate (SC)
4) Clothianidin granule (GR)
5) Clothianidin water soluble granule (SG-1)
6) Clothianidin water soluble granule (SG-2)

1.3 Participants

Alain DuBois	Laboratoire fédéral pour la Sécurité alimentaire – AFSCA (Belgium)
Ana Gregorčič	Kmetijski inštitut Slovenije (Slovenia)
Bruno Patrian	Agroscope Changins-Wädenswil (Switzerland)
Erika Seidel	Bayer CropScience AG (Germany)
Heidrun Unterweger	Austrian Agency for Health and Food Safety (Austria)
Jim Garvey	Pesticide Control Laboratory (Ireland)
Juliana Schlosserova	Central Control and Testing Institute in Agriculture (Slovak)
Lajos Benke	Agricultural Office of County Fejér, Plant Protection and Soil Conservation Directorate (Hungary)

Luis Manso	Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Pescay Alimentacion (Spain)
Michael Haustein	CURRENTA GmbH & Co. OHG (Germany)
Nunchana Luetrakool	Pesticide Research Group, Agricultural production Science Research Development Office (Thailand)
Olga Novakova	State Phytosanitary Administration Pesticides Testing Laboratory (Czech)
Oliver O. Bennett, Jr.	Kansas Dept of Agriculture Laboratories (USA)
Ping Wan	Office of Indiana State Chemist (USA)
Ritva Mutanen	Finnish Food Safety Authority Evira, Chemistry and Toxicology Unit (Finland)
See Geok Heon	Sumitomo Chemical Enviro-Agro Asia Pacific SDN BHD (Malaysia)
Silke Peters	Bayer CropScience AG (Germany)
Teodora Iurascu	Central Laboratory for Phytosanitary Quarantine, Laboratory for Quality Control of Pesticides (Romania)
Vanessa Lecocq	Walloon Agricultural Research Centre (CRA-W), Pesticides Research Department (Belgium)

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/4658/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

The analytical conditions of the collaborators are summarized in Table 1.

3.2 Remarks

- Lab.1
  - Flow rate of 1.5mL/min used to help minimise peak fronting.
  - For preparation of calibration solutions and samples, 5ml of solution was taken and made up to 25mls instead of 10 mls to 50mls as stated in the method.

- Lab.2
  - For SC formulation (E08-12E801), the dilution was not complete. It remained a kind of cloudy suspension after the sonication.

- Lab.4
  - We adapted the mobile phase to acetonitrile - water - phosphoric acid, 200 + 800 + 1 (v/v/v) because we used a longer column (250 mm) instead of 150 mm. The retention time of clothianidin was about 9 min.
  - We observe that the solubility of the analytical standard after 10 min in the ultrasonic bath is not so good. So it is important that you make sure that all solid material will be solved.

- Lab.5
  - Concerning using a longer column and remaining to the given HPLC parameters, the retention time of Clothianidin was 13 min.

- Lab.6
  - Acetonitrile analytical reagent grade was used for preparation of solutions instead of HPLC grade.
  - The volumetric flasks were filled up to volume at 20°C ± 1°C and, for GR formulation, acetonitrile was added at 20°C ± 1°C instead of at room temperature.
  - The analytical standard did not appear homogeneous therefore the weighings had to be done twice each day.
  - A good repeatability of injections was difficult to obtain, and this on three different apparatus.
  - Peak symetry was not good with two new columns using the defined chromatographic parameters.
  - Peak purity changed depending on injections and an interference was present just before the active substance peak for SC formulation.
  - For SG and SC formulations, it would be preferable to add water to disperse the test item before adding acetonitrile.
  - Injection volume was 5 µL instead of 10 µL in order to prevent the overloading of the column.

- Lab.7
  - Samples were centrifuged prior to analysis using an Eppendorf Centrifuge 5424.
  - Content a.i.=98.5%. This is not appropriate for analysis of a TC with a nominal content a.i. of 98.7%.
  - Reference substance and a.i. in samples exhibited strong fronting when injected as described in the method. Reduction of the injection volume lead to improved peak shapes, indicating that the column was overloaded at the recommended amount.
  - We suggest to add some water during sample preparation for better solubilization of the samples in the case of WG, SC, GR and SG type formulations. In addition, we suggest to avoid dilution steps as described in the method.
  - The injection of 10 µL of reference compound and sample solutions, respectively, lead to integration problems because of pronounced peak asymmetry.
  - Preparation of eluent was performed using pump mixing (Agilent 1100 instrument, binary pump system, high pressure mixing).
  - Scaling of axes should be adjustable by the participants of the collaborative trial (peak areas are instrument-specific).

- Lab.9
  - Injection volume was reduced from 10 to 5 µL due to bad peak shape.

- Lab.10
  - For the response factor in day-1 analysis, we have taken as CA peak area the mean of the two injections made previously.
  - Peak area quantities have been adjusted to fit the worksheet scale.

- Lab.11
  - The changes from method are:
    (i) The chromatographic column: Luna 5µ C18, 4,6 mm i.d. x 150 mm.
    (ii) In preparation of sample solution the solvent (acetonitrile) was changed to mobile phase (150 acetonitrile + 850 water + 1 phosphoric acid).
    (iii) The weigh of clothianidin standard was approximately 50 mg into 100 ml volumetric flasks, and for preparation of samples solutions sufficient sample to contain about 50 mg of clothianidin.

- Lab.12
  - Reran SC day 1 the next day because relevant sample was not weighed correctly.

- Lab.16
  - At the beginning of the analyses we had really big problems with carryover, but afterwards we found that this carryover was in our case (by using Waters alliance system) supported by addiction of phosphoric acid to mobile phase. That´s why we didn´t use phosphoric acid during analyses. Then our situation was better. Consequently we had problems to get good reproducibility of standard injection. Areas of injected standards in sequence went down till time we shaked with vials than grown up. We realised that standards and sample had to by analysed as fast as possible after sonication, we suppose because of sorption of active ingredient to walls of glass flasks and also vials.
  - 0,45 µm PTFE filters were used for samples filtration.
  - Injection volume was reduced to 5 µL. At 10 µL injection peak was over-injected and wide.

- Lab.18
  - The retention time is very sensitive to only slight changes in the solvent mixture: Although we mixed the solvent every time very carefully the retention time of clothianidin in the 1st practice run was at 5.02 min, on the 1st day of the ring trial at 6.99 min and on the 2nd

day of the ring trial at 7.17 min. Therefore we recommend to state in the method that the retention time of clothianidin can lay between 5 and 8 minutes.
- In the method description point (b) Linearity check and (c) system equilibration should be changed as the system equilibration should take place before the linearity check.
- In the method description of Clothianidin Granule (d) "allow to cool to room temperature" After this sentence I would suggest to add: shake thoroughly and allow the particles to sink down before transferring 10.0 ml by pipette.
- The peak shape of clothianidin is not ideal. The peak is broad and shows leading. When reducing the amount injected from 10 µl to 5 µl the peak shape gets better.

- Lab.19
  - Because we don't have the Zorbax column we have used 250 mm long Spherisorb ODS 2 C18 column. Using this column, we had to change the flow rate to 1,7 ml/min.

4. RESULTS AND DISCUSSION

The samples were sent to nineteen laboratories and all of them returned results. Summary and detailed statistical evaluations are shown in Tables 2 and 3-1 to 3-6. The statistical evaluations were carried out according to ISO 5725. The discussion on stragglers and outliers is as follows:

- TC

  The variance of Lab 12 was identified as an outlier. The data were retained because there were no reasons to remove them.

- WG

  The variance of Lab 12 was identified as an outlier. The data were retained because there were no reasons to remove them.

- SC

  The variance of Lab 18 was identified as an outlier. The data were retained because there were no reasons to remove them.

- SG-1
  The variance of Lab 16 was identified as an outlier and the mean value of Lab 7 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, water dispersible granule, suspension concentrate, granule and water soluble granule.
JAPAC proposes that the method be accepted as a provisional CIPAC method.

Table 1 Summary of Analytical Conditions

Lab	Liquid chromatograph Integrator	Column	Mobile phase	Det. Wave-length (nm)	Flow rate (ml/ min)	Inj. Vol (µl)	Column temp. (°C)
Proposed Method		Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
1	Agilent 1100 Series Agilent Chemstation	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.5	10	40
2	VARIAN VarianStar, version 5.52	Phenomenex Luna C18 100A, 5 µm, 150 ×4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
3	Agilent 1200 Series Agilent ChemStation Rev. B.02.01	Lichrospher 100 RP-18, 5 µm, 250 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
4	Dionex Dionex Chromeleon Version 6.4	Zorbax Eclipse, XDB-C18, 5 µm, 250 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 800 + 200 +1	269	1.0	10	40

Lab	Liquid chromatograph Integrator	Column	Mobile phase	Det. Wave-length (nm)	Flow rate (ml/ min)	Inj. Vol (µl)	Column temp. (°C)
5	HP 1090 DAD II HP Chemstation Software Rev. B01.03	LiChrospher 100 RP-18e , 5 µm, 250 × 4 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
6	Waters Alliance 2695 Separation Module equipped with Waters 996 UV/visible Photodiode Array Detector Waters Empower	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	5	40
7	HP 1100 Agilent, Cerity	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
8	Agilent 1100 Series Chemstation	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
9	Agilent 1100 HPLC Dionex Chromeleon	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	5	40

Lab	Liquid chromatograph Integrator	Column	Mobile phase	Det. Wave-length (nm)	Flow rate (ml/ min)	Inj. Vol (µl)	Column temp. (°C)
10	Agilent 1100 HP Chemstation	Phenomenex Synergi, 4 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 800 + 200 +1	269	1.0	10	40
11	Varian Prostar Star software	Phenomenex Luna 5µ C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
12	Shimadzu LC-10ATvp EZstart data station	Alltech Prevail, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.87	10	40
13	HP 1100 Agilent Chemstation	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
14	Agilent 1100 Series Aglient Chemstation	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.1	10	40
15	Dionex HPLC system Chromeleon	Zorbax Eclipse XDB-C18, 5 µm, 250 ×3 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	0.7	5	25

Lab	Liquid chromatograph Integrator	Column	Mobile phase	Det. Wave-length (nm)	Flow rate (ml/ min)	Inj. Vol (µl)	Column temp. (°C)
16	Waters separation Alliance module 2695, PDA detector 2996 Empower (Build 1154)	Nova-Pack C18, 4 µm, 150 × 3.9 mm (i.d.)	Water - Acetonitrile, 850 + 150	269	1.0	5	40
17	Dionex Summit Chromeleon Software	Xbridge C18, 5 µm, 250 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.5	10	40
18	Agilent 1100 HPLC Dionex Chromeleon	Zorbax Eclipse XDB-C18, 5 µm, 150 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.0	10	40
19	Waters 2695 Separation Module equipped with Waters 2487 Dual λ Absorbance Detector Empower 2 software	Spherisorb ODS2 C18, 5 µm, 250 × 4.6 mm (i.d.)	Water - Acetonitrile - Phosphoric acid, 850 + 150 +1	269	1.7	10	40

Table 2 Summary of Statistical Evaluation of Clothianidin CIPAC Collaborative Study

	TC	WG	SC	GR	SG-1	SG-2
Average (g/kg)	989.07	502.57	187.09	9.86	169.76	470.65
Number of labs.	19	19	19	19	19	19
Repeatability standard deviation (S_r)	8.000	3.554	2.566	0.105	2.390	3.615
"Pure" between laboratory standard variation (S_L)	6.621	5.374	2.300	0.157	2.830	5.884
Reproducibility standard deviation (S_R)	10.385	6.443	3.446	0.189	3.704	6.906
Repeatability (r)	22.400	9.951	7.185	0.294	6.692	10.122
Reproducibility (R)	29.078	18.040	9.649	0.529	10.371	19.337
RSD_r	0.809	0.707	1.372	1.065	1.408	0.768
RSD_R	1.050	1.282	1.842	1.916	2.182	1.467
Horwitz's value	2.003	2.218	2.574	4.008	2.612	2.240

Table 3-1 Clothianidin Technical

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	1002.1	986.9				
	Day2	973.4	983.1	986.38	972945.50	11.925	142.206
2	Day1	981.8	989.0				
	Day2	982.6	984.8	984.55	969338.70	3.226	10.407
3	Day1	1000.1	986.2				
	Day2	994.8	987.4	992.13	984321.94	6.537	42.732
4	Day1	994.7	992.8				
	Day2	998.6	995.8	995.48	990980.43	2.424	5.876
5	Day1	971.1	975.2				
	Day2	975.4	971.2	973.23	947176.63	2.398	5.750
6	Day1	1000.2	1002.0				
	Day2	997.2	1002.2	1000.40	1000800.16	2.315	5.359
7	Day1	980.1	972.2				
	Day2	959.9	975.6	971.95	944686.80	8.660	74.996
8	Day1	990.8	982.2				
	Day2	1004.3	1005.0	995.58	991179.54	11.055	122.213
9	Day1	989.7	987.2				
	Day2	993.8	990.1	990.20	980496.04	2.722	7.409
10	Day1	987.1	985.3				
	Day2	985.7	988.0	986.53	973241.44	1.250	1.563
11	Day1	992.6	992.7				
	Day2	987.6	991.5	991.10	982279.21	2.396	5.741
12	Day1	997.2	1000.2				**
	Day2	959.5	1022.7	994.90	989826.01	26.200	686.440
13	Day1	983.8	983.7				
	Day2	987.9	987.2	985.65	971505.92	2.213	4.897
14	Day1	997.1	998.0				
	Day2	1001.4	1003.2	999.93	999860.00	2.863	8.197
15	Day1	987.3	986.3				
	Day2	986.7	987.1	986.85	973872.92	0.443	0.196
16	Day1	986.4	985.5				
	Day2	990.5	984.0	986.60	973379.56	2.782	7.740
17	Day1	987.4	986.6				
	Day2	993.3	998.0	991.33	982735.17	5.360	28.730
18	Day1	988.5	976.9				
	Day2	982.5	983.9	982.95	966190.70	4.779	22.839
19	Day1	1000.9	998.3				
	Day2	998.7	988.1	996.50	993012.25	5.715	32.661
S1 SUM		Yi =		18792.24			
S2 SUM		Yi^2 =			18587828.92		
S3 SUM		Si^2 =					1215.952
							p = 19

1) Cochran's test (p=19, n=4)

C = Si^2max / S3 = 0.565 > 0.230 (p=19, n=4, 5%)
> 0.276 (p=19, n=4, 1%)

Outlier Lab 12 was included in the following evaluation.

2) Grubbs' test (p=19, n=4)

Yi(min) = 971.95 Yi(max) = 1000.40 Y = S1/p 989.07
S = 8.630

Y - Yi(min) = 17.12
Yi(max) - Y = 11.33
lower = [Y-Yi(min)]/S = 1.983 < 2.681 (p=19, 5%)
upper = [Yi(max)-Y]/S = 1.313 < 2.681 (p=19, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 989.07
Sr^2 = S3 / p = 63.997 Sr = 8.000
SL^2 = [(pS2-$S1^2$)/p(p-1)]-(Sr^2/n) = 43.841 SL = 6.621
SR^2 = Sr^2 + SL^2 = 107.838 SR = 10.385

r = 2.8 x Sr =	22.400
R = 2.8 x SR =	29.078
RSDr = (Sr / mean) x 100 =	0.809
RSDR = (SR / mean) x 100 =	1.050

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 2.003

RSDr and RSDR < 2.003 (Horwitz's Value)

Table 3-2 Clothianidin Water Dispersible Granule

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	490.4	502.4				
	Day2	498.5	498.1	497.35	247357.02	5.023	25.231
2	Day1	501.7	502.2				
	Day2	501.2	501.6	501.68	251682.82	0.411	0.169
3	Day1	506.3	504.0				
	Day2	503.3	502.9	504.13	254147.06	1.520	2.310
4	Day1	503.0	504.4				
	Day2	509.5	508.8	506.43	256471.34	3.211	10.311
5	Day1	505.0	501.2				
	Day2	508.2	502.9	504.33	254348.75	3.015	9.090
6	Day1	510.7	509.5				
	Day2	509.9	512.1	510.55	260661.30	1.147	1.316
7	Day1	495.8	496.4				
	Day2	502.8	495.6	497.65	247655.52	3.450	11.903
8	Day1	498.6	505.6				
	Day2	506.2	510.5	505.23	255257.35	4.926	24.265
9	Day1	501.1	501.6				
	Day2	504.5	505.4	503.15	253159.92	2.121	4.499
10	Day1	503.2	501.1				
	Day2	500.3	500.3	501.23	251231.51	1.370	1.877
11	Day1	487.3	490.6				
	Day2	489.2	489.8	489.23	239345.99	1.406	1.977
12	Day1	515.3	524.6				**
	Day2	523.7	503.9	516.88	267164.93	9.611	92.371
13	Day1	495.9	494.9				
	Day2	500.7	500.7	498.05	248053.80	3.087	9.530
14	Day1	503.1	507.3				
	Day2	500.7	506.5	504.40	254419.36	3.066	9.400
15	Day1	501.8	502.1				
	Day2	501.9	501.8	501.90	251903.61	0.141	0.020
16	Day1	498.7	501.3				
	Day2	498.8	499.8	499.65	249650.12	1.207	1.457
17	Day1	501.8	505.4				
	Day2	503.3	496.0	501.63	251632.66	4.030	16.241
18	Day1	499.2	501.5				
	Day2	498.1	500.9	499.93	249930.00	1.559	2.430
19	Day1	509.8	503.5				
	Day2	507.3	500.9	505.38	255408.94	3.951	15.610
S1 SUM		Yi =		9548.78			
S2 SUM		Yi^2 =			4799482.00		
S3 SUM		Si^2 =					240.007
						p =	19

1) Cochran's test (p=19, n=4)

$C = Si^2max / S3$ = 0.385 > 0.230 (p=19, n=4, 5%)
> 0.276 (p=19, n=4, 1%)

Outlier Lab 12 was included in the following evaluation.

2) Grubbs' test (p=19, n=4)

Yi(min) = 489.23 Yi(max) = 516.88 Y = S1/p 502.57
S = 6.509

Y - Yi(min) = 13.34
Yi(max) - Y = 14.31
lower = [Y-Yi(min)]/S = 2.049 < 2.681 (p=19, 5%)
upper = [Yi(max)-Y]/S = 2.199 < 2.681 (p=19, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 502.57
$Sr^2 = S3 / p$ = 12.632 Sr = 3.554
$SL^2 = [(pS2-S1^2)/p(p-1)]-(Sr^2/n)$ = 28.884 SL = 5.374
$SR^2 = Sr^2 + SL^2$ = 41.516 SR = 6.443

r = 2.8 x Sr =	9.951
R = 2.8 x SR =	18.040
RSDr = (Sr / mean) x 100 =	0.707
RSDR = (SR / mean) x 100 =	1.282

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 2.218

RSDr and RSDR < 2.218 (Horwitz's Value)

Table 3-3 Clothianidin Suspension Concentrate

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	186.1	180.6				
	Day2	184.7	186.1	184.38	33995.98	2.602	6.770
2	Day1	171.0	180.5				
	Day2	184.5	183.0	179.75	32310.06	6.062	36.748
3	Day1	186.9	187.4				
	Day2	187.1	186.9	187.08	34998.93	0.236	0.056
4	Day1	188.3	188.9				
	Day2	189.6	189.8	189.15	35777.72	0.686	0.471
5	Day1	189.0	188.6				
	Day2	186.7	189.4	188.43	35505.86	1.195	1.428
6	Day1	189.8	189.9				
	Day2	191.5	188.2	189.85	36043.02	1.348	1.817
7	Day1	183.3	179.1				
	Day2	183.7	187.3	183.35	33617.22	3.356	11.263
8	Day1	191.1	187.7				
	Day2	189.0	188.5	189.08	35751.25	1.452	2.108
9	Day1	186.9	188.0				
	Day2	187.9	189.3	188.03	35355.28	0.984	0.968
10	Day1	185.9	185.9				
	Day2	186.3	186.3	186.10	34633.21	0.231	0.053
11	Day1	185.3	185.1				
	Day2	186.5	185.1	185.50	34410.25	0.673	0.453
12	Day1	191.2	187.7				
	Day2	188.5	190.7	189.53	35921.62	1.690	2.856
13	Day1	183.5	185.9				
	Day2	183.3	184.2	184.23	33940.69	1.181	1.395
14	Day1	188.9	186.7				
	Day2	188.1	190.1	188.45	35513.40	1.427	2.036
15	Day1	188.2	188.0				
	Day2	188.4	187.9	188.13	35392.90	0.222	0.049
16	Day1	191.9	189.1				
	Day2	192.5	185.7	189.80	36024.04	3.109	9.666
17	Day1	186.8	186.5				
	Day2	187.6	187.7	187.15	35025.12	0.592	0.350
18	Day1	184.6	180.3				**
	Day2	194.6	192.5	188.00	35344.00	6.700	44.890
19	Day1	188.6	190.5				
	Day2	188.1	187.4	188.65	35588.82	1.328	1.764
S1 SUM		Yi =		3554.64			
S2 SUM		Yi^2 =			665149.37		
S3 SUM		Si^2 =					125.141

p = 19

1) Cochran's test (p=19, n=4)

$C = Si^2max / S3$ = 0.359 > 0.230 (p=19, n=4, 5%)
> 0.276 (p=19, n=4, 1%)

Outlier Lab 18 was included in the following evaluation.

2) Grubbs' test (p=19, n=4)

Yi(min) = 179.75 Yi(max) = 189.85 Y = S1/p 187.09
S = 2.913

Y - Yi(min) = 7.34
Yi(max) - Y = 2.76
lower = [Y-Yi(min)]/S = 2.518 < 2.681 (p=19, 5%)
upper = [Yi(max)-Y]/S = 0.949 < 2.681 (p=19, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 187.09
$Sr^2 = S3 / p$ = 6.586 Sr = 2.566
$SL^2 = [(pS2-S1^2)/p(p-1)]-(Sr^2/n)$ = 5.291 SL = 2.300
$SR^2 = Sr^2 + SL^2$ = 11.877 SR = 3.446

r = 2.8 x Sr =	7.185
R = 2.8 x SR =	9.649
RSDr = (Sr / mean) x 100 =	1.372
RSDR = (SR / mean) x 100 =	1.842

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 2.574

RSDr and RSDR < 2.574 (Horwitz's Value)

Table 3-4 Clothianidin Granule

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	9.69	9.54				
	Day2	9.72	9.57	9.63	92.74	0.088	0.008
2	Day1	10.12	10.08				
	Day2	10.01	9.94	10.04	100.80	0.079	0.006
3	Day1	9.98	9.73				
	Day2	9.63	9.63	9.74	94.87	0.165	0.027
4	Day1	10.14	9.92				
	Day2	9.86	10.01	9.98	99.60	0.122	0.015
5	Day1	9.55	9.69				
	Day2	9.47	9.55	9.57	91.58	0.091	0.008
6	Day1	10.13	10.34				
	Day2	9.95	10.06	10.12	102.41	0.164	0.027
7	Day1	9.64	9.81				
	Day2	9.96	9.89	9.83	96.63	0.138	0.019
8	Day1	9.60	9.75				
	Day2	9.55	9.90	9.70	94.09	0.158	0.025
9	Day1	9.94	9.84				
	Day2	9.85	9.90	9.88	97.61	0.046	0.002
10	Day1	9.66	9.70				
	Day2	9.76	9.61	9.68	93.70	0.063	0.004
11	Day1	10.08	10.03				
	Day2	9.98	9.93	10.01	100.20	0.065	0.004
12	Day1	9.95	10.24				
	Day2	10.07	10.20	10.12	102.41	0.132	0.017
13	Day1	9.68	9.85				
	Day2	9.76	9.78	9.77	95.45	0.070	0.005
14	Day1	9.98	10.07				
	Day2	9.93	9.91	9.97	99.40	0.071	0.005
15	Day1	9.87	9.89				
	Day2	9.87	9.90	9.88	97.61	0.015	0.000
16	Day1	10.13	9.98				
	Day2	10.14	9.97	10.06	101.20	0.093	0.009
17	Day1	9.98	10.01				
	Day2	9.72	9.89	9.90	98.01	0.130	0.017
18	Day1	9.60	9.70				
	Day2	9.70	9.59	9.65	93.12	0.061	0.004
19	Day1	9.92	9.97				
	Day2	9.77	9.83	9.87	97.42	0.090	0.008
S1 SUM		Yi =		187.40			
S2 SUM		Yi^2 =			1848.85		
S3 SUM		Si^2 =					0.210
						p =	19

1) Cochran's test (p=19, n=4)

$C = Si^2max / S3$ = 0.129 < 0.230 (p=19, n=4, 5%)

2) Grubbs' test (p=19, n=4)

Yi(min) = 9.57 Yi(max) = 10.12 Y = S1/p 9.86
S = 0.183

Y - Yi(min) = 0.29
Yi(max) - Y = 0.26
lower = [Y-Yi(min)]/S = 1.602 < 2.681 (p=19, 5%)
upper = [Yi(max)-Y]/S = 1.403 < 2.681 (p=19, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 9.86

$Sr^2 = S3 / p$ = 0.011 Sr = 0.105

$SL^2 = [(pS2-S1^2)/p(p-1)]-(Sr^2/n)$ = 0.025 SL = 0.157

$SR^2 = Sr^2 + SL^2$ = 0.036 SR = 0.189

r = 2.8 x Sr =	0.294
R = 2.8 x SR =	0.529
RSDr = (Sr / mean) x 100 =	1.065
RSDR = (SR / mean) x 100 =	1.916

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 4.008

RSDr and RSDR < 4.008 (Horwitz's Value)

Table 3-5 Clothianidin Water Soluble Granule -1

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	171.2	172.9				
	Day2	170.1	169.9	171.03	29251.26	1.374	1.888
2	Day1	169.1	169.7				
	Day2	170.5	171.1	170.10	28934.01	0.879	0.773
3	Day1	168.3	167.4				
	Day2	170.7	170.0	169.10	28594.81	1.517	2.301
4	Day1	169.9	170.1				
	Day2	170.4	170.4	170.20	28968.04	0.245	0.060
5	Day1	171.3	170.4				
	Day2	169.7	170.1	170.38	29029.34	0.680	0.462
6	Day1	173.6	172.6				
	Day2	174.0	167.9	172.03	29594.32	2.812	7.907
7	Day1	158.0	153.3	**			
	Day2	159.6	165.7	159.15	25328.72	5.120	26.214
8	Day1	167.3	167.3				
	Day2	172.4	172.3	169.83	28842.23	2.916	8.503
9	Day1	170.7	168.8				
	Day2	172.9	172.7	171.28	29336.84	1.926	3.709
10	Day1	171.6	172.0				
	Day2	170.8	172.2	171.65	29463.72	0.619	0.383
11	Day1	168.9	170.9				
	Day2	170.4	170.1	170.08	28927.21	0.850	0.723
12	Day1	174.5	169.3				
	Day2	172.9	175.8	173.13	29974.00	2.812	7.907
13	Day1	169.4	168.0				
	Day2	169.5	170.5	169.35	28679.42	1.028	1.057
14	Day1	166.5	168.1				
	Day2	166.3	166.5	166.85	27838.92	0.839	0.704
15	Day1	172.1	171.8				
	Day2	171.9	171.7	171.88	29542.73	0.171	0.029
16	Day1	167.6	175.6				**
	Day2	162.8	163.6	167.40	28022.76	5.856	34.293
17	Day1	171.6	166.9				
	Day2	168.3	165.1	167.98	28217.28	2.749	7.557
18	Day1	172.2	173.6				
	Day2	169.1	171.7	171.65	29463.72	1.881	3.538
19	Day1	172.1	172.8				
	Day2	173.0	171.4	172.33	29697.63	0.727	0.529
S1 SUM		Yi =		3225.40			
S2 SUM		Yi^2 =			547706.96		
S3 SUM		Si^2 =					108.537
							p = 19

1) Cochran's test (p=19, n=4)

$C = Si^2max / S3$ = 0.316 > 0.230 (p=19, n=4, 5%)
> 0.276 (p=19, n=4, 1%)

Outlier Lab 16 was included in the following evaluation.

2) Grubbs' test (p=19, n=4)

Yi(min) = 159.15 Yi(max) = 173.13 Y = S1/p 169.76
S = 3.351

Y - Yi(min) = 10.61
Yi(max) - Y = 3.37
lower = [Y-Yi(min)]/S = 3.165 > 2.681 (p=19, 5%)
> 2.968 (p=19, 1%)
upper = [Yi(max)-Y]/S = 1.006 < 2.681 (p=19, 5%)

Outlier Lab 7 was included in the following evaluation.

3) Calculation of r and R

Mean; Y = S1 / p = 169.76
$Sr^2 = S3 / p$ = 5.712 Sr = 2.390
$SL^2 = [(pS2-S1^2)/p(p-1)]-(Sr^2/n)$ = 8.008 SL = 2.830
$SR^2 = Sr^2 + SL^2$ = 13.720 SR = 3.704

r = 2.8 x Sr =	6.692
R = 2.8 x SR =	10.371
RSDr = (Sr / mean) x 100 =	1.408
RSDR = (SR / mean) x 100 =	2.182

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 2.612

RSDr and RSDR < 2.612 (Horwitz's Value)

Table 3-6 Clothianidin Water Soluble Granule -2

Lab		Analytical data (n=4)		Yi	$(Yi)^2$	Si	Si^2
1	Day1	468.2	472.5				
	Day2	464.8	460.3	466.45	217575.60	5.171	26.739
2	Day1	464.1	466.2				
	Day2	465.8	470.3	466.60	217715.56	2.629	6.912
3	Day1	473.1	478.4				
	Day2	478.2	474.6	476.08	226652.17	2.642	6.980
4	Day1	464.5	464.4				
	Day2	464.3	464.6	464.45	215713.80	0.129	0.017
5	Day1	469.1	469.2				
	Day2	472.4	471.3	470.50	221370.25	1.623	2.634
6	Day1	480.0	483.3				
	Day2	476.6	482.4	480.58	230957.14	2.994	8.964
7	Day1	464.1	449.4				
	Day2	457.7	466.1	459.33	210984.05	7.524	56.611
8	Day1	474.5	474.2				
	Day2	471.0	473.9	473.40	224107.56	1.619	2.621
9	Day1	469.4	473.7				
	Day2	473.0	474.0	472.53	223284.60	2.125	4.516
10	Day1	463.7	461.6				
	Day2	459.9	461.8	461.75	213213.06	1.555	2.418
11	Day1	483.8	482.5				
	Day2	479.7	480.4	481.60	231938.56	1.889	3.568
12	Day1	474.5	474.7				
	Day2	480.0	480.7	477.48	227987.15	3.333	11.109
13	Day1	463.9	468.1				
	Day2	468.6	467.4	467.00	218089.00	2.124	4.511
14	Day1	474.2	474.3				
	Day2	472.9	476.0	474.35	225007.92	1.271	1.615
15	Day1	466.8	466.3				
	Day2	467.0	466.8	466.73	217836.89	0.299	0.089
16	Day1	473.4	470.3				
	Day2	466.4	469.9	470.00	220900.00	2.865	8.208
17	Day1	478.5	479.5				
	Day2	471.3	465.1	473.60	224296.96	6.742	45.455
18	Day1	477.5	482.0				
	Day2	469.4	469.5	474.60	225245.16	6.224	38.738
19	Day1	461.7	468.9				
	Day2	461.7	468.6	465.23	216438.95	4.072	16.581
S1 SUM		Yi =		8942.26			
S2 SUM		Yi^2 =			4209314.38		
S3 SUM		Si^2 =					248.286

p = 19

1) Cochran's test (p=19, n=4)

$C = Si^2max / S3$ = 0.228 < 0.230 (p=19, n=4, 5%)

2) Grubbs' test (p=19, n=4)

Yi(min) = 459.33 Yi(max) = 481.60 Y = S1/p 470.65
S = 6.861

Y - Yi(min) = 11.32
Yi(max) - Y = 10.95
lower = [Y-Yi(min)]/S = 1.649 < 2.681 (p=19, 5%)
upper = [Yi(max)-Y]/S = 1.597 < 2.681 (p=19, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 470.65

$Sr^2 = S3 / p$ = 13.068 Sr = 3.615

$SL^2 = [(pS2-S1^2)/p(p-1)]-(Sr^2/n)$ = 34.626 SL = 5.884

$SR^2 = Sr^2 + SL^2$ = 47.693 SR = 6.906

r = 2.8 x Sr =	10.122
R = 2.8 x SR =	19.337
RSDr = (Sr / mean) x 100 =	0.768
RSDR = (SR / mean) x 100 =	1.467

Horwitz's Value = 2 ^[1 - 0.5 x log(Y / 1000)] = 2.240

RSDr and RSDR < 2.240 (Horwitz's Value)

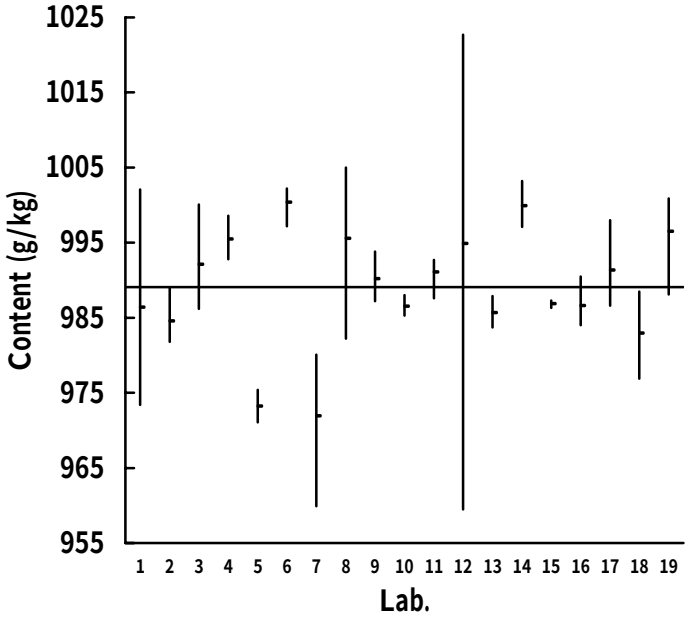

Figure 1 Clothianidin Technical

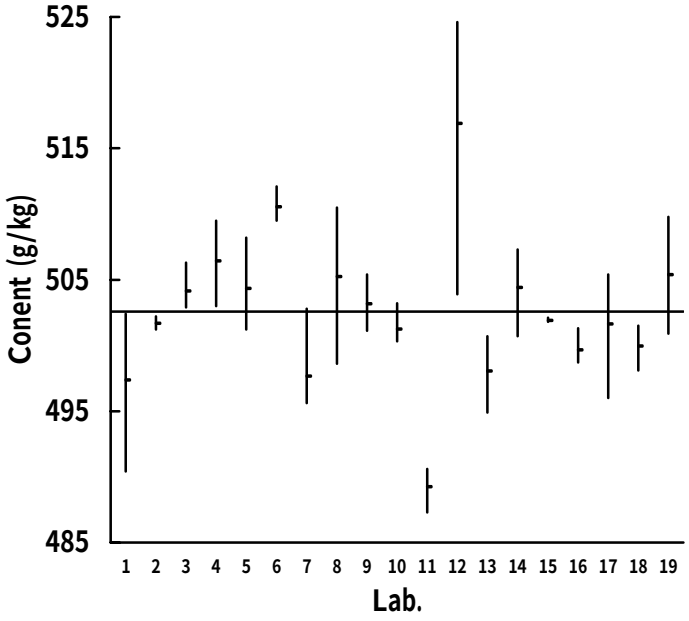

Figure 2 Clothianidin Water Dispersible Granule

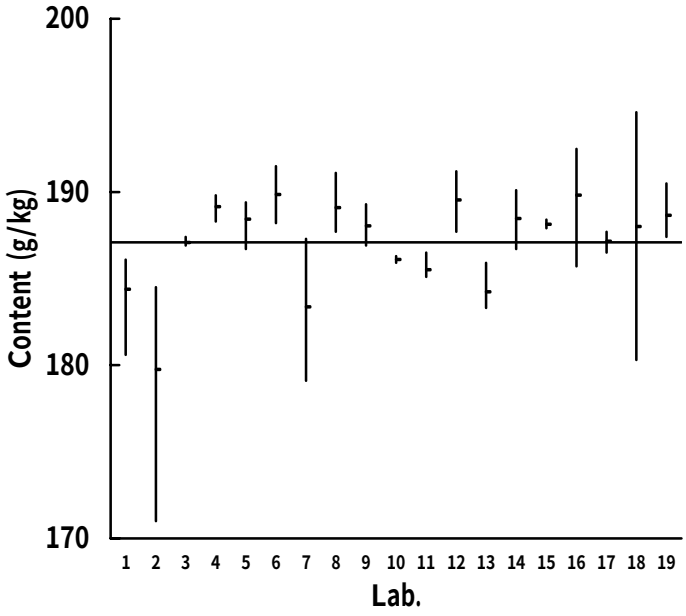

Figure 3 Clothianidin Suspension Concentrate

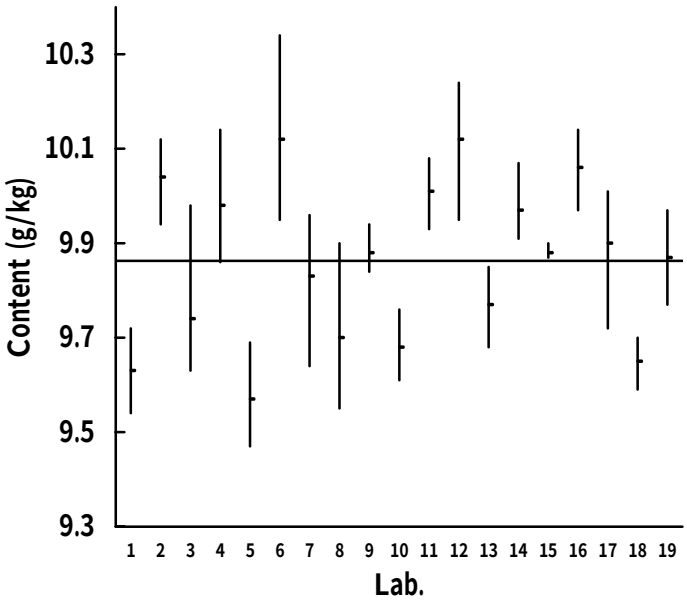

Figure 4 Clothianidin Granule

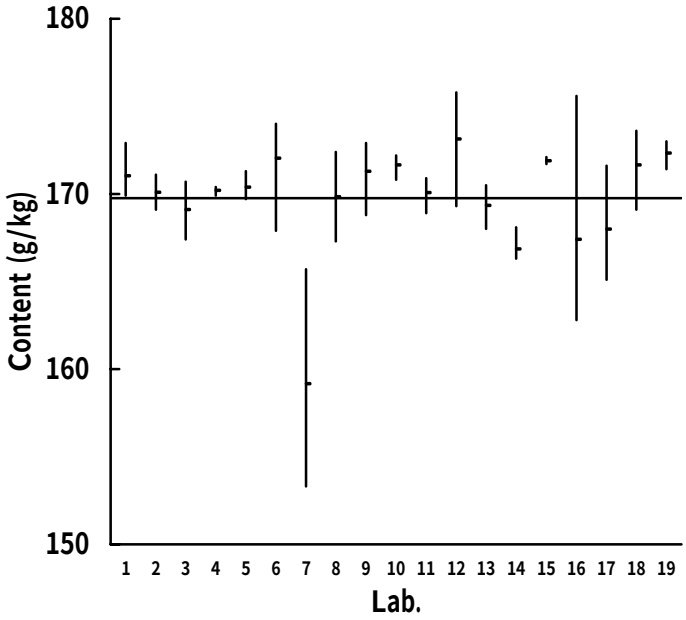

Figure 5 Clothianidin Water Soluble Granule-1

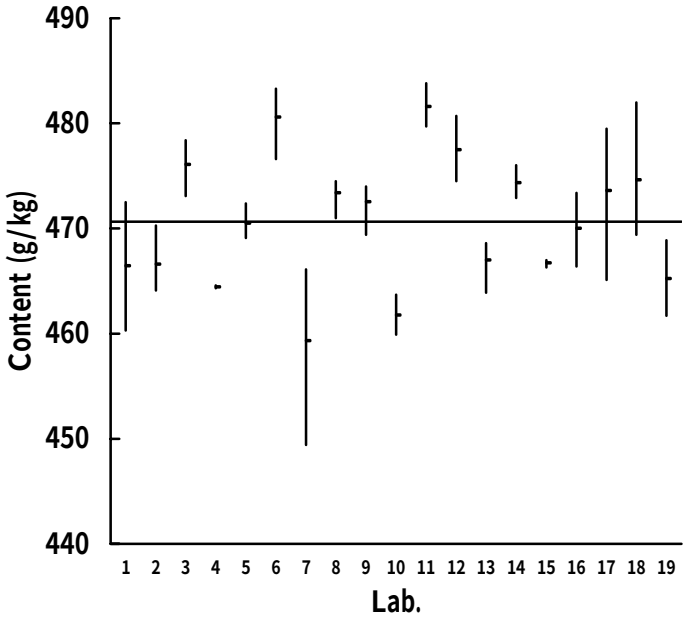

Figure 6 Clothianidin Water Soluble Granule-2