AMISULBROM (No.789)

CIPAC Collaborative Trial

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

by
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1. INTRODUCTION

1.1 Scope

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

1.2 Samples

1) Amisulbrom technical (TC-1)
2) Amisulbrom technical (TC-2)
3) Amisulbrom water dispersible granule (WG)
4) Amisulbrom suspension concentrate (SC-1)
5) Amisulbrom suspension concentrate (SC-2)

1.3 Participants

a)	Veronika Kmecl	Agricultural Institute of Slovenia (Slovenia)
b)	Bruno Patrian	Agroscope Changins-Wädenswil (Switzerland)
c)	Cornel Grecu	Alchimex S. A. (Romania)
d)	Robin Zou	Rotam Chemistry Co., Ltd. (China)
e)	Juliana Schlosserova	UKSUP / CCTIA (Slovakia)
f)	Florentina Vissarion	Central Phytosanitary Laboratory (Romania)
g)	Sune D. Nygaard	Technological Institute (Denmark)
h)	Takashi Watanabe	Food and Agricultural Materials Inspection Center (Japan)
i)	Sergio Grecco	Genbra Argentina S.A. (Argentina)
J)	A. Ramesh	International Institute of Biotechnology and Toxicology (India)
k)	Luis Manso	Laboratorio Arbitral Agroalimentario (Spain)
l)	Selma Belart	Laboratorio de plaguicidas y formulados (Argentina)
m)	George Balayiannis	Laboratory of Chemical Control of Pesticides (Greece)
n)	Lajos Benke	National Food Chain Safety Office (Hungary)
o)	Xiangdong Shao	Nutrichem Laboratory Co., Ltd. (China)
p)	Susan Marais	Pesticide Analytical Technology (South Africa)

q) Phil Cassidy — Ricerca Biosciences (USA)
r) Olga Novakova — State Phytosanitary Administration (Czech)
s) Jim Garvey — The Pesticides Control Laboratory (Ireland)
t) Vanessa Lecocq — Walloon Agricultural Research Centre (Belgium)

2. ANALYTICAL METHOD

2.1 Outline of Method

Amisulbrom in the test samples is determined by reversed phase high performance liquid chromatography using an ODS column, UV detection at 254 nm and external standardization as stated in CIPAC/4883/m.

2.2 Program of Work

The collaborators were requested to:

1) conduct duplicate determinations on two different days for each samples;
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) report the calibration curve and all chromatograms for each sample.

3. REMARKS OF PARTICIPANTS

3.1 Analytical Conditions

Lab	Liquid chromatograph	Column	Mobile phase	Flow rate (ml/min)	Column temp. (ºC)
Proposed Method		YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
1	Agilent 1260	Kinetex C18 100A (4.6 mm ID × 150 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40

3.1 Analytical Conditions (continued)

Lab	Liquid chromatograph	Column	Mobile phase	Flow rate (ml/min)	Column temp. (ºC)
2	Dionex	Zorbax SB C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	25
3	Agilent 1100	YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
4	Agilent 1100	YMC ODS-AQ (4.6 mm ID × 150 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 70 + 30 (v/v)	0.6	40
5	Waters Alliance 2695	Zorbax SB-C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
6	Agilent 1100	YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
7	Waters	YMC Pack Pro C18 (4.6 mm ID × 150 mm, 3 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
8	Perkin Elmer	YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
9	Shimadzu Prominence	YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
10	Waters	Microsorb 100-5C (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40

3.1 Analytical Conditions (continued)

Lab	Liquid chromatograph	Column	Mobile phase	Flow rate (ml/min)	Column temp. (ºC)
11	Shimadzu Prominence	Waters XBridge C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 65 + 35 (v/v)	1.0	40
12	Agilent 1100	Gemini C18 110A (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
13	Shimadzu LC 10	Supelcosil LC-18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
14	Shimadzu UFLC	Luna C-18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
15	Agilent 1200	Zorbax Eclipse XDB (4.6 mm ID × 150 mm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.2	40
16	Agilent 1100	Zorbax SB-C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
17	Agilent 1200	Prodigy ODS-3 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
18	Shimadzu LC-20A	Inertsil ODS-2 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
19	Dionex	LiChrospher 100-5 RP-18, C18 (4.0 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40
20	Varian Pro Star	Kinetex 2.6 µ XB-C18 100A (4.6 mm ID × 100 mm, 2.6 µm)	Acetonitrile - 0.01% v/v aq. phosphoric acid, 75 + 25 (v/v)	1.0	40

3.2 Remarks

- Lab.1
  - Kinetex C18 100A (4.6 mm ID × 150 mm, 5 µm) was used. The retention time of amisulbrom was 3.2 minutes.
- Lab.2
  - Zorbax SB C18 (4.6 mm ID × 250 mm, 5 µm) was used at 25ºC.
- Lab.4
  - About 1/5 weight of standard and samples has been prepared, except WG formulation, that more than 200 mg has been weighted and diluted afterwards in the same proportion.
  - YMC ODS-AQ (4.6 mm ID × 150 mm, 5 µm) was used. Rt: 6.2 min.
- Lab.5
  - We analysed Technical-1 and Technical-2 on Day 3 because of low repeatability on Day 2.
- Lab.7
  - YMC Pack Pro C18 (4.6 mm ID × 150 mm, 3 µm) was used.
- Lab.11
  - We used Waters XBridge C18 (4.6 mm ID × 250 mm, 5 µm).
  - As there was an impurity in front of the A.I. peak, we changed the composition of the mobile phase to acetonitrile - 0.01% v/v aqueous phosphoric acid, 65 + 35.
- Lab.14
  - There was a small time shift of the retention time (9.04 min on Day 1 → 8.67 min on Day 2).
- Lab.15
  - We use 50 mL volumetric flasks and therefore reduced the initial sample and standard messes by half throughout the study.
  - The flow rate was increased to shorten the retention time to approximately 5 minutes.
  - We used Zorbax Eclipse XDB-C18 (4.6 mm ID × 150 mm).
- Lab.16
  - Calibration and sample solutions were prepared by weighing half of the prescribed amounts into 50 mL volumetric flasks.
- Lab.18
  - We modified the reagents (MilliQ water instead of HPLC grade water)

and column (Inertsil ODS-2 instead of YMC Pack Pro C18).

- Lab.20
  - We used Kinetex 2.6 μ XB-C18 100A (4.6 mm ID × 100 mm, 2.6 μm).

4. RESULTS AND DISCUSSION

The samples were sent to twenty laboratories and all of them returned results. 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:

- TC-1

The variance of Lab 6, 7, 8, 10, 17 was identified as an outlier by Cochran's test. These data were retained because there were no reasons to remove them.

- TC-2

The variance of Lab 7, 8, 10 was identified as an outlier by Cochran's test. The mean of Lab 12 was identified as an outlier by Grubbs's test. These data were retained because there were no reasons to remove them.

- WG

The variance of Lab 12 was identified as an outlier by Cochran's test. The data was retained because there were no reasons to remove it.

- SC-1

The variance of Lab 13 was identified as a straggler by Cochran's test. The data was retained because there were no reasons to remove it.

- SC-2

The variance of Lab 10 was identified as a straggler by Cochran's test. The data was retained because there were no reasons to remove it.

5. CONCLUSION

For all samples, the values of RSD_R (reproducibility relative standard deviation) were smaller than those calculated by Horwitz's equation even if struggler and outlier were included. The proposed method is considered to be appropriate for the determination of amisulbrom in technical, water dispersible granule and suspension concentrate.
JAPAC proposes that the method is accepted as a provisional CIPAC method.

Table 1 Summary of statistical evaluation of amisulbrom large scale collaborative study

	TC-1	TC-2	WG	SC-1	SC-2
Average (g/kg)	994.99	993.00	501.11	177.45	179.17
Number of laboratories	20	20	20	20	20
Repeatability standard deviation (S_r)	6.571	5.569	4.046	1.152	1.216
"Pure" between laboratory Standard variation (S_L)	6.274	10.199	5.036	1.305	1.344
Reproducibility standard deviation (S_R)	9.085	11.620	6.460	1.740	1.812
Repeatability (r)	18.399	15.593	11.328	3.226	3.404
Reproducibility (R)	25.438	32.537	18.088	4.873	5.074
RSD_r	0.660	0.561	0.807	0.649	0.678
RSD_R	0.913	1.170	1.289	0.981	1.011
Horwitz's value	2.002	2.002	2.219	2.595	2.591

Table 2-1 Amisulbrom Technical-1

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	1002.7	995.6				
	Day2	1000.2	999.0	999.38	998750.39	2.951	8.709
2	Day1	998.3	998.3				
	Day2	997.3	998.9	998.20	996403.24	0.663	0.440
3	Day1	998.7	997.1				
	Day2	997.5	994.4	996.93	993859.46	1.815	3.296
4	Day1	997.6	997.8				
	Day2	996.8	996.5	997.18	994357.98	0.624	0.389
5	Day1	1005.4	1003.9				
	Day2	993.1	998.6	1000.25	1000500.06	5.588	31.230
6	Day1	962.6	984.7				**
	Day2	1000.0	1005.6	988.23	976588.65	19.232	369.869
7	Day1	997.6	985.9				**
	Day2	998.1	1006.0	996.90	993809.61	8.281	68.580
8	Day1	965.7	961.8				**
	Day2	964.8	978.7	967.75	936540.06	7.488	56.070
9	Day1	1001.9	1001.8				
	Day2	1001.8	1001.9	1001.85	1003703.42	0.058	0.003
10	Day1	978.2	986.5				**
	Day2	998.3	1011.5	993.63	987290.64	14.492	210.022
11	Day1	999.5	994.1				
	Day2	999.1	998.2	997.73	995455.18	2.477	6.136
12	Day1	1007.0	995.3				
	Day2	996.8	996.7	998.95	997901.10	5.410	29.270
13	Day1	998.0	997.2				
	Day2	995.6	997.4	997.05	994108.70	1.025	1.050
14	Day1	996.6	995.4				
	Day2	997.4	991.9	995.33	990671.86	2.427	5.889
15	Day1	992.2	992.9				
	Day2	991.4	989.8	991.58	983220.98	1.333	1.776
16	Day1	992.7	993.9				
	Day2	996.4	999.8	995.70	991418.49	3.138	9.847
17	Day1	1001.1	999.7				**
	Day2	988.2	990.4	994.85	989726.52	6.496	42.203
18	Day1	995.6	992.8				
	Day2	996.9	996.9	995.55	991119.80	1.933	3.737
19	Day1	999.3	992.6				
	Day2	999.8	998.7	997.60	995205.76	3.364	11.313
20	Day1	996.6	993.3				
	Day2	997	993.6	995.13	990273.77	1.945	3.783
S1 SUM	Yi =			19899.73			
S2 SUM	Yi^2 =				19800905.67		
S3 SUM	Si^2 =						863.612

** The variance of Lab 6, 7, 8, 10, 17 was identified as an outlier by Cochran's test but included in statistic analysis

1) Cochran's test (p=20, n=4)
   $C = Si^2 max / S3$ = 0.428 > 0.221 (p=20, n=4, 5%)

2) Grubbs's test (P=20, n=4)
   Yi min = 967.75 Yi max = 1001.85 Y = S1/p = 994.99
   S = 7.082
   Y − Yi min = 27.24
   Yi max − Y = 6.86
   lower = (Y − Yi min)/S = 3.846 > 2.709 (p=20, 5%)
   upper = (Yi max − Y)/S = 0.969 < 2.709 (p=20, 5%)

3) Calculation of r and R
   Mean; Y = S1 / p = 994.99
   $S_r^2 = S3 / p$ = 43.181 S_r = 6.571
   $S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 39.358 S_L = 6.274
   $S_R^2 = S_r^2 + S_L^2$ = 82.539 S_R = 9.085

$r = 2.8 \times S_r$ =	18.399
$R = 2.8 \times S_R$ =	25.438
$RSD_r = (S_r / mean) \times 100$ =	0.660
$RSD_R = (S_R / mean) \times 100$ =	0.913

Horwitz's value = 2 ^[1 − 0.5 x log (Y / 1000)] = 2.002

RSD_r and RSD_R < 2.002 (Horwitz's value)

Table 2-2 Amisulbrom Technical-2

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	996.7	988.5				
	Day2	999.0	997.6	995.45	990920.70	4.729	22.363
2	Day1	996.9	997.2				
	Day2	997.9	996.8	997.20	994407.84	0.497	0.247
3	Day1	1000.3	989.6				
	Day2	994.7	992.2	994.20	988433.64	4.569	20.873
4	Day1	991.1	994.6				
	Day2	996.8	997.8	995.08	990174.26	2.968	8.809
5	Day1	994.8	1002.8				
	Day2	1001.1	996.8	998.88	997751.27	3.709	13.756
6	Day1	995.1	991.2				
	Day2	1004.9	1000.2	997.85	995704.62	5.972	35.670
7	Day1	997.1	974.4			**	
	Day2	995.6	1005.5	993.15	986346.92	13.237	175.230
8	Day1	959.2	961.2			**	
	Day2	941.7	944.4	951.63	905590.14	9.996	99.922
9	Day1	1001.9	1001.6				
	Day2	1001.8	1001.8	1001.78	1003553.15	0.126	0.016
10	Day1	983.4	975.3			**	
	Day2	1001.6	1002.6	990.73	981536.03	13.551	183.623
11	Day1	991.3	997.8				
	Day2	996.3	998.1	995.88	991767.02	3.150	9.923
12	Day1	984.6	981.5	**			
	Day2	979.9	982.7	982.18	964667.73	1.982	3.929
13	Day1	997.5	996.1				
	Day2	987.1	994.4	993.78	987588.75	4.627	21.409
14	Day1	994.9	995.8				
	Day2	993.8	994.8	994.83	989676.78	0.818	0.669
15	Day1	990.0	989.7				
	Day2	990.5	992.7	990.73	981536.03	1.357	1.843
16	Day1	993.4	992.8				
	Day2	999.4	999.2	996.20	992414.44	3.589	12.880
17	Day1	998.8	1003.0				
	Day2	997.7	998.2	999.43	998850.33	2.425	5.883
18	Day1	995.5	993.4				
	Day2	996.2	994.8	994.98	989975.25	1.195	1.429
19	Day1	997.7	999.3				
	Day2	999.0	999.5	998.88	997751.27	0.810	0.656
20	Day1	998.8	996.7				
	Day2	996.8	996.5	997.20	994407.84	1.074	1.153
S1 SUM	Yi =			19859.98			
S2 SUM	Yi^2 =				19723054.00		
S3 SUM	Si^2 =						620.282

** The variance of Lab 7, 8, 10 was identified as an outlier by Cochran's test but included in statistic analysis
** The mean of Lab 12 was identified as an outlier by Grubbs's test but included in statistic analysis

1) Cochran's test (p=20, n=4)
C = Si^2 max / S3 = 0.296 > 0.221 (p=20, n=4, 5%)

2) Grubbs's test (P=20, n=4)
Yi min = 951.63 Yi max = 1001.78 Y = S1/p = 993.00
S = 10.572

Y − Yi min = 41.37
Yi max − Y = 8.78
lower = (Y − Yi min)/S = 3.913 > 2.709 (p=20, 5%)
upper = (Yi max − Y)/S = 0.830 < 2.709 (p=20, 5%)

3) Calculation of r and R
Mean; Y = S1 / p = 993.00
S_r^2 = S3 / p = 31.014 S_r = 5.569
$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 104.017 S_L = 10.199
$S_R^2 = S_r^2 + S_L^2$ = 135.031 S_R = 11.620

r = 2.8 x S_r =	15.593
R = 2.8 x S_R =	32.537
RSD_r = (S_r / mean) x 100 =	0.561
RSD_R = (S_R / mean) x 100 =	1.170

Horwitz's value = 2 ^[1 − 0.5 x log (Y / 1000)] = 2.002

RSD_r and RSD_R < 2.002 (Horwitz's value)

Table 2-3 Amisulbrom Water Dispersible Granule

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	500.7	504.8				
	Day2	496.6	495.5	499.40	249400.36	4.239	17.967
2	Day1	501.1	503.0				
	Day2	501.5	502.2	501.95	251953.80	0.835	0.697
3	Day1	507.4	511.0				
	Day2	511.7	503.4	508.38	258445.14	3.814	14.549
4	Day1	502.1	500.3				
	Day2	502.2	501.1	501.43	251427.03	0.900	0.809
5	Day1	507.9	506.7				
	Day2	501.9	504.4	505.23	255252.30	2.650	7.022
6	Day1	498.2	497.7				
	Day2	506.8	509.8	503.13	253134.77	6.103	37.249
7	Day1	496.6	496.4				
	Day2	486.1	485.6	491.18	241252.88	6.153	37.856
8	Day1	493.2	495.0				
	Day2	490.1	488.1	491.60	241670.56	3.089	9.540
9	Day1	506.8	506.9				
	Day2	507.1	506.9	506.93	256972.96	0.126	0.016
10	Day1	492.9	493.6				
	Day2	501.5	495.1	495.78	245792.85	3.925	15.409
11	Day1	507.2	507.8				
	Day2	507.8	506.5	507.33	257378.66	0.618	0.383
12	Day1	487.6	490.2				**
	Day2	507.9	502.0	496.93	246934.46	9.633	92.796
13	Day1	506.7	507.0				
	Day2	506.8	507.1	506.90	256947.61	0.183	0.033
14	Day1	499.0	500.2				
	Day2	499.0	496.3	498.63	248626.89	1.650	2.722
15	Day1	500.8	501.4				
	Day2	503.3	500.7	501.55	251552.40	1.207	1.457
16	Day1	503.7	502.2				
	Day2	510.1	506.1	505.53	255555.53	3.447	11.883
17	Day1	508.0	508.1				
	Day2	508.9	506.3	507.83	257886.23	1.094	1.196
18	Day1	502.8	502.4				
	Day2	498.2	496.8	500.05	250050.00	3.004	9.023
19	Day1	498.6	498.4				
	Day2	489.7	485.2	492.98	243024.35	6.639	44.082
20	Day1	504.4	502.6				
	Day2	495.4	495.3	499.43	249425.33	4.763	22.683
S1 SUM	Yi =			10022.10			
S2 SUM	Yi^2 =				5022684.10		
S3 SUM	Si^2 =						327.372

** The variance of Lab 12 was identified as an outlier by Cochran's test but included in statistic analysis

1) Cochran's test (p=20, n=4)
C = Si^2 max / S3 = 0.283 > 0.221 (p=20, n=4, 5%)

2) Grubbs's test (P=20, n=4)
Yi min = 491.18 Yi max = 508.38 Y = S1/p = 501.11
S = 5.427

Y − Yi min = 9.93
Yi max − Y = 7.27
lower = (Y − Yi min)/S = 1.830 < 2.709 (p=20, 5%)
upper = (Yi max − Y)/S = 1.339 < 2.709 (p=20, 5%)

3) Calculation of r and R
Mean; Y = S1 / p = 501.11
S_r^2 = S3 / p = 16.369 S_r = 4.046
$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 25.365 S_L = 5.036
$S_R^2 = S_r^2 + S_L^2$ = 41.733 S_R = 6.460

r = 2.8 x S_r =	11.328
R = 2.8 x S_R =	18.088
$RSD_r = (S_r / mean) \times 100$ =	0.807
$RSD_R = (S_R / mean) \times 100$ =	1.289

Horwitz's value = 2 ^[1 − 0.5 x log (Y / 1000)] = 2.219

RSD_r and RSD_R < 2.219 (Horwitz's value)

Table 2-4 Amisulbrom Suspension Concentrate-1

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2	
1	Day1	178.0	175.9					
	Day2	175.9	173.7	175.88	30932.02	1.756	3.082	
2	Day1	180.0	179.9					
	Day2	180.2	180.4	180.13	32445.02	0.222	0.049	
3	Day1	178.7	179.7					
	Day2	180.4	178.4	179.30	32148.49	0.920	0.847	
4	Day1	176.8	176.3					
	Day2	177.7	176.9	176.93	31302.46	0.580	0.336	
5	Day1	180.7	180.0					
	Day2	178.3	177.3	179.08	32067.86	1.554	2.416	
6	Day1	177.4	179.3					
	Day2	180.0	180.0	179.18	32103.68	1.228	1.509	
7	Day1	175.8	174.9					
	Day2	174.6	176.5	175.45	30782.70	0.866	0.750	
8	Day1	176.7	179.5					
	Day2	176.3	176.5	177.25	31417.56	1.509	2.277	
9	Day1	179.1	179.0					
	Day2	179.3	179.1	179.13	32085.77	0.126	0.016	
10	Day1	176.3	174.7					
	Day2	178.0	179.0	177.00	31329.00	1.896	3.593	
11	Day1	177.8	177.0					
	Day2	178.4	177.6	177.70	31577.29	0.577	0.333	
12	Day1	176.6	175.6					
	Day2	177.9	178.5	177.15	31382.12	1.303	1.697	
13	Day1	178.1	172.8					*
	Day2	173.2	175.5	174.90	30590.01	2.443	5.967	
14	Day1	176.1	176.7					
	Day2	176.1	177.2	176.53	31161.08	0.532	0.283	
15	Day1	176.7	175.3					
	Day2	176.3	175.6	175.98	30967.20	0.640	0.409	
16	Day1	177.5	176.7					
	Day2	177.4	177.4	177.25	31417.56	0.370	0.137	
17	Day1	179.0	179.0					
	Day2	178.8	178.5	178.83	31978.38	0.236	0.056	
18	Day1	176.9	177.6					
	Day2	176.9	176.7	177.03	31337.85	0.395	0.156	
19	Day1	176.8	176.7					
	Day2	176.5	176.4	176.60	31187.56	0.183	0.033	
20	Day1	178.5	179.7					
	Day2	176.9	176.1	177.80	31612.84	1.612	2.600	
S1 SUM	Yi =			3549.05				
S2 SUM	Yi^2 =				629826.44			
S3 SUM	Si^2 =						26.545	

* The variance of Lab 13 was identified as a straggler by Cochran's test but included in statistic analysis

1) Cochran's test (p=20, n=4)
C = Si^2 max / S3 = 0.225 > 0.221 (p=20, n=4, 5%)

2) Grubbs's test (P=20, n=4)
Yi min = 174.90 Yi max = 180.13 Y = S1/p = 177.45
S = 1.426

Y − Yi min = 2.55
Yi max − Y = 2.67
lower = (Y − Yi min)/S = 1.790 < 2.709 (p=20, 5%)
upper = (Yi max − Y)/S = 1.874 < 2.709 (p=20, 5%)

3) Calculation of r and R
Mean; Y = S1 / p = 177.45
S_r^2 = S3 / p = 1.327 S_r = 1.152
$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 1.702 S_L = 1.305
$S_R^2 = S_r^2 + S_L^2$ = 3.029 S_R = 1.740

$r = 2.8 \times S_r$ =	3.226
$R = 2.8 \times S_R$ =	4.873
$RSD_r = (S_r / mean) \times 100$ =	0.649
$RSD_R = (S_R / mean) \times 100$ =	0.981

Horwitz's value = 2 ^[1 − 0.5 x log (Y / 1000)] = 2.595

RSD_r and RSD_R < 2.595 (Horwitz's value)

Table 2-5 Amisulbrom Suspension Concentrate-2

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2	
1	Day1	177.9	178.4					
	Day2	177.6	176.7	177.65	31559.52	0.714	0.510	
2	Day1	181.5	182.3					
	Day2	183.3	182.5	182.40	33269.76	0.739	0.547	
3	Day1	181.3	180.2					
	Day2	179.6	181.7	180.70	32652.49	0.970	0.940	
4	Day1	177.9	178.4					
	Day2	178.9	178.9	178.53	31871.18	0.479	0.229	
5	Day1	180.5	180.3					
	Day2	179.7	178.9	179.85	32346.02	0.719	0.517	
6	Day1	180.4	180.2					
	Day2	181.2	181.4	180.80	32688.64	0.589	0.347	
7	Day1	181.6	176.3					
	Day2	179.4	179.4	179.18	32103.68	2.179	4.749	
8	Day1	174.8	177.5					
	Day2	175.8	177.8	176.48	31143.43	1.422	2.022	
9	Day1	179.8	179.7					
	Day2	179.9	179.8	179.80	32328.04	0.082	0.007	
10	Day1	173.1	177.8					*
	Day2	179.7	177.5	177.03	31337.85	2.792	7.796	
11	Day1	178.7	176.6					
	Day2	177.0	178.2	177.63	31550.64	0.988	0.976	
12	Day1	180.8	181.8					
	Day2	181.3	181.1	181.25	32851.56	0.420	0.177	
13	Day1	181.1	181.3					
	Day2	177.4	177.9	179.43	32193.33	2.061	4.249	
14	Day1	178.0	178.4					
	Day2	177.8	178.6	178.20	31755.24	0.365	0.133	
15	Day1	179.1	178.7					
	Day2	177.7	177.6	178.28	31781.98	0.741	0.549	
16	Day1	179.7	178.3					
	Day2	178.6	179.2	178.95	32023.10	0.624	0.390	
17	Day1	180.6	180.3					
	Day2	179.8	180.0	180.18	32463.03	0.350	0.122	
18	Day1	178.7	179.2					
	Day2	178.4	179.1	178.85	31987.32	0.370	0.137	
19	Day1	178.7	179.3					
	Day2	178.2	177.8	178.50	31862.25	0.648	0.420	
20	Day1	181.7	181.5					
	Day2	178.4	177.4	179.75	32310.06	2.176	4.737	
S1 SUM	Yi =			3583.40				
S2 SUM	Yi^2 =				642079.13			
S3 SUM	Si^2 =						29.553	

* The variance of Lab 10 was identified as a straggler by Cochran's test but included in statistic analysis

1) Cochran's test (p=20, n=4)
C = Si^2 max / S3 = 0.264 > 0.221 (p=20, n=4, 5%)

2) Grubbs's test (P=20, n=4)
Yi min = 176.48 Yi max = 182.40 Y = S1/p = 179.17
S = 1.475

Y − Yi min = 2.69
Yi max − Y = 3.23
lower = (Y − Yi min)/S = 1.827 < 2.709 (p=20, 5%)
upper = (Yi max − Y)/S = 2.190 < 2.709 (p=20, 5%)

3) Calculation of r and R
Mean; Y = S1 / p = 179.17
S_r^2 = S3 / p = 1.478 S_r = 1.216
$S_l^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 1.807 S_L = 1.344
$S_R^2 = S_r^2 + S_L^2$ = 3.284 S_R = 1.812

$r = 2.8 \times S_r$ =	3.404
$R = 2.8 \times S_R$ =	5.074
$RSD_r = (S_r / mean) \times 100$ =	0.678
$RSD_R = (S_R / mean) \times 100$ =	1.011

Horwitz's value = 2 ^[1 − 0.5 x log (Y / 1000)] = 2.591

RSD_r and RSD_R < 2.591 (Horwitz's value)

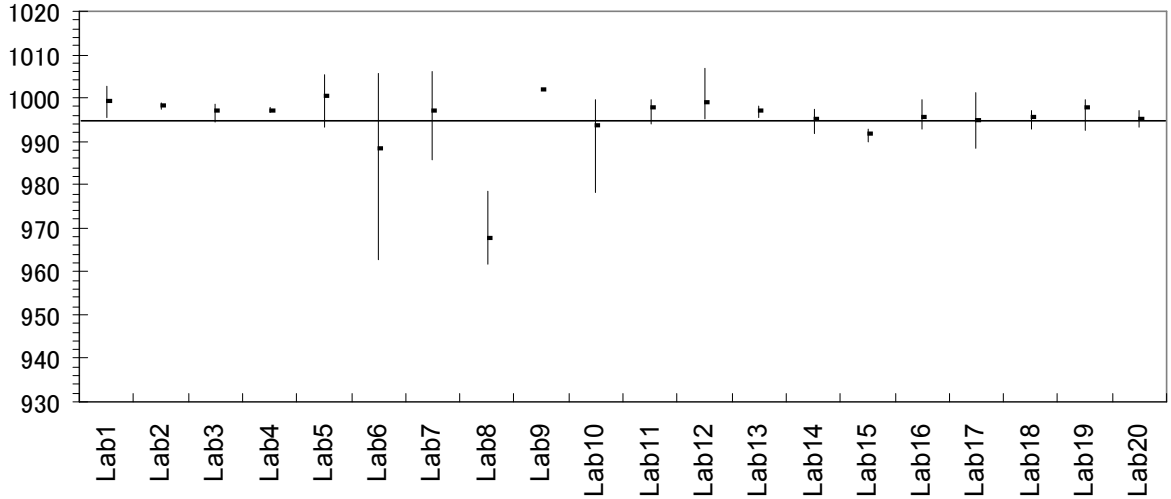

Figure 1 Amisulbrom Technical-1

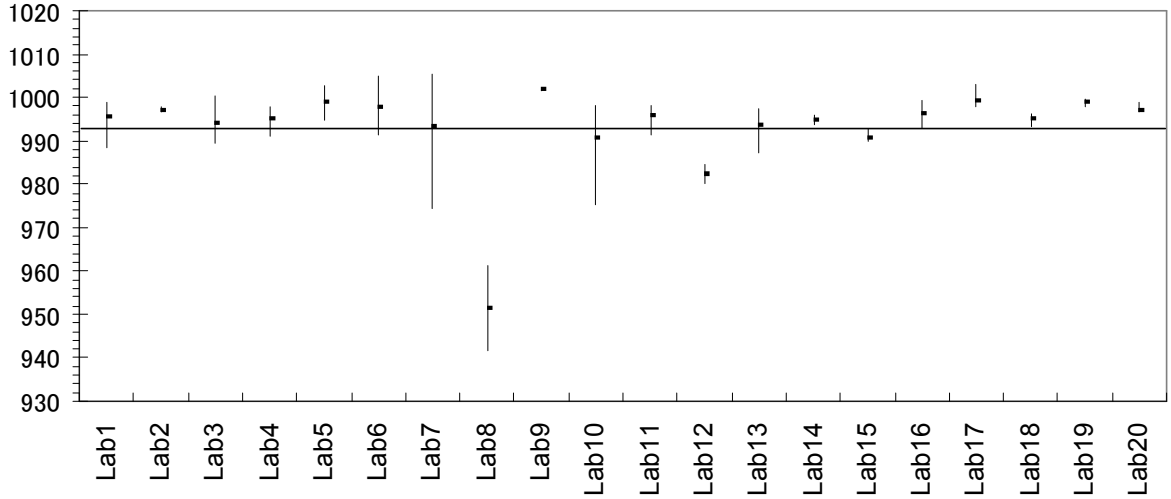

Figure 2 Amisulbrom Technical-2

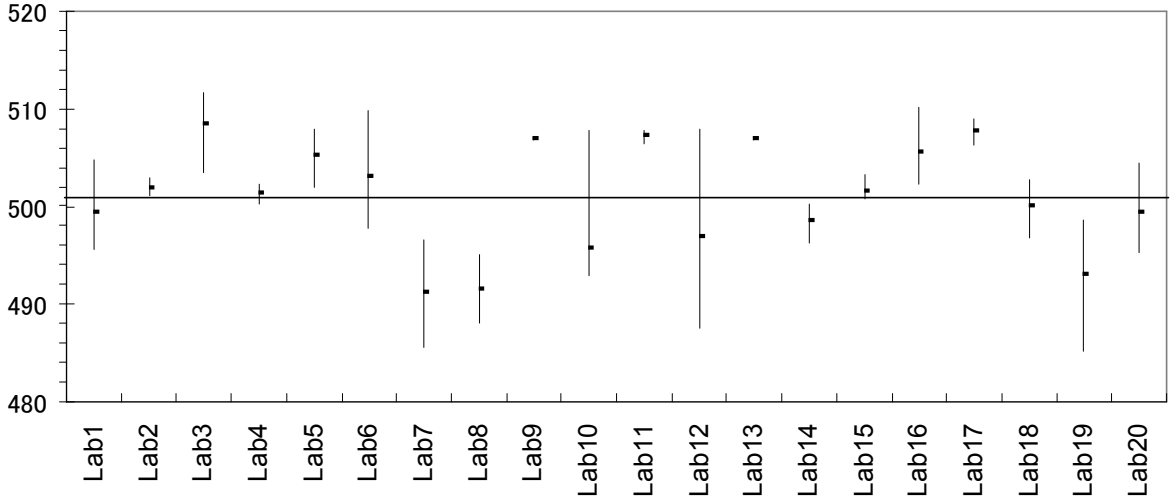

Figure 3 Amisulbrom Water Dispersible Granule

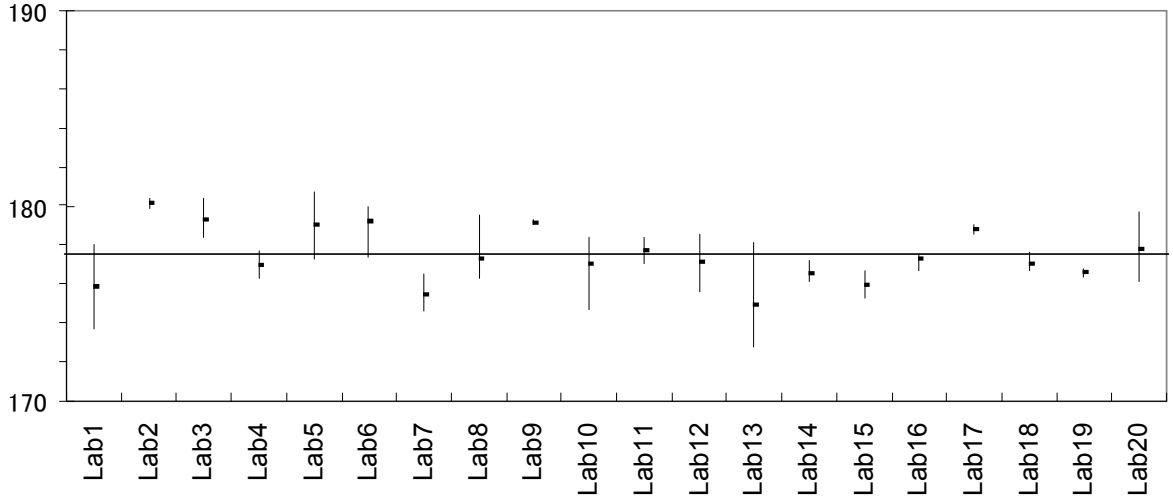

Figure 4 Amisulbrom Suspension Concentrate-1

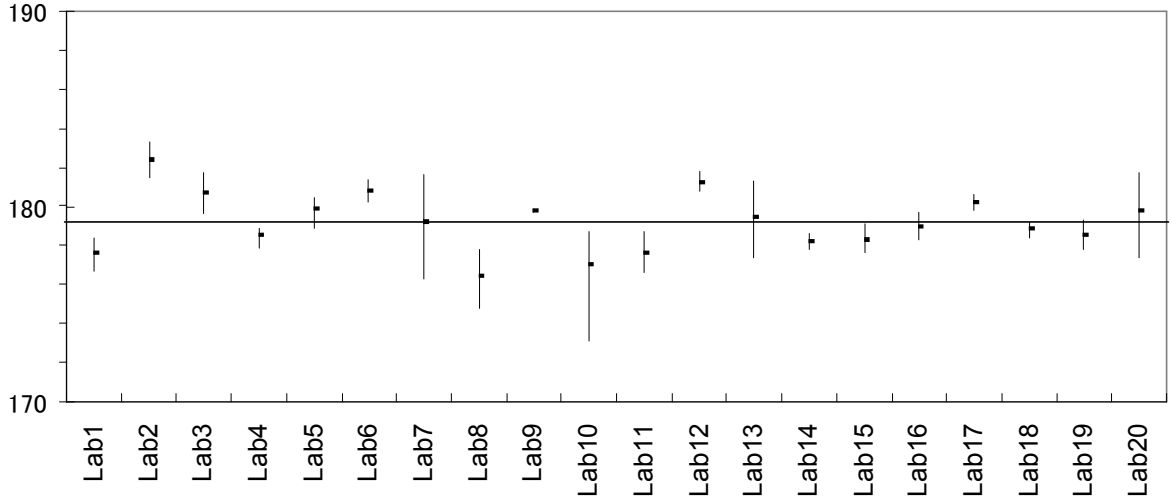

Figure 5 Amisulbrom Suspension Concentrate-2