AMISULBROM (No.789)

Small Scale Collaborative Trial

Small Scale Collaborative Study on the Determination of Amisulbrom in Amisulbrom 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 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) Amisulbrum suspension concentrate (SC-1)
5) Amisulbrum suspension concentrate (SC-2)

1.3 Participants

Takuto Minamisaki	Sumika Chemical Analysis Service, Ltd. Osaka Laboratory (JAPAN)
Makiko Mukumoto	Sumitomo Chemical Co., Ltd. Organic Synthesis Research Laboratory (JAPAN)
Toru Sugiyama	Nissan Chemical Industries, Ltd. Tsukuba Laboratory (JAPAN)

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/xxxx/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 Integrator	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 aqueous phosphoric acid, 75 + 25 (v/v)	1.0	40
1	Agilent 1200 Agilent ChemStation	YMC Pack Pro C18 (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aqueous phosphoric acid, 75 + 25 (v/v)	1.0	40
2	Shimadzu Prominence Shimadzu LCsolution	SUMIPAX ODS Z-CLUE (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aqueous phosphoric acid, 75 + 25 (v/v)	1.0	40
3	Shimadzu LC-10A Shimadzu LCsolution	L-column ODS (4.6 mm ID × 250 mm, 5 µm)	Acetonitrile - 0.01% v/v aqueous phosphoric acid, 75 + 25 (v/v)	Day 1: 1.0 Day 2: 0.9	40

3.2 Remarks

- Lab.3

  - On Day 2 the flow rate was changed to 0.9 ml/min to adjust to the retention time of 9 min.
  - The sonication time for the standard and technical was changed to 5 min.
  - On Day 2 further sonication for WG formulation was performed for 5 min.
  - Note that the standard and technical were easily affected by static electricity during sampling.

4. RESULTS AND DISCUSSION

Three data sets were obtained from three 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.
No stragglers and outliers were observed.

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 to be appropriate for the determination of amisulbrom in technical, water dispersible granule and suspension concentrate.
JAPAC proposes to proceed with a large scale collaborative trial.

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

	TC-1	TC-2	WG	SC-1	SC-2
Average (g/kg)	1001.95	1001.74	507.02	179.08	180.17
Number of laboratories	3	3	3	3	3
Repeatability standard deviation (S_r)	7.137	6.105	1.879	0.989	0.691
"Pure" between laboratory standard variation (S_L)	6.319	2.826	NC	0.691	NC
Reproducibility standard deviation (S_R)	9.532	6.727	1.778	1.206	0.616
Repeatability (r)	19.984	17.094	5.261	2.769	1.935
Reproducibility (R)	26.690	18.836	4.978	3.377	1.725
RSD_r	0.712	0.609	0.371	0.552	0.384
RSD_R	0.951	0.672	0.351	0.673	0.342
Horwitz's value	1.999	1.999	2.215	2.591	2.589

NC: Not calculable

Table 2-1 Amisulbrom Technical-1

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	998.7	1004.0				
	Day2	991.5	1000.2	998.60	997201.96	5.233	27.384
2	Day1	999.2	998.1				
	Day2	999.5	991.1	996.98	993969.12	3.963	15.705
3	Day1	999.6	1022.3				
	Day2	1015.5	1003.7	1010.28	1020665.68	10.474	109.705
S1 SUM	Yi =			3005.86			
S2 SUM	Yi^2 =				3011836.76		
S3 SUM	Si^2 =						152.794

1) Cochran's test (p=3, n=4)

$C = Si^2 max / S3$ = 0.718 < 0.798 (p=3, n=4, 5%)

2) Grubbs's test (P=3, n=4)

Yi min = 996.98 Yi max = 1010.28 Y = S1/p = 1001.95

S = 7.256

Y - Yi min = 4.97

Yi max - Y = 8.33

lower = (Y - Yi min)/S = 0.685 < 1.155 (p=3, 5%)

upper = (Yi max - Y)/S = 1.148 < 1.155 (p=3, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 1001.95

$S_r^2 = S3 / p$ = 50.931 S_r = 7.137

$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 39.924 S_L = 6.319

$S_R^2 = S_r^2 + S_L^2$ = 90.855 S_R = 9.532

$r = 2.8 \times S_r$ =	19.984
$R = 2.8 \times S_R$ =	26.690
$RSD_r = (S_r / mean) \times 100$ =	0.712
$RSD_R = (S_R / mean) \times 100$ =	0.951

Horwitz's value = 2 ^[1 - 0.5 x log (Y / 1000)] = 1.999

RSD_r and RSD_R < 1.999 (Horwitz's value)

Table 2-2 Amisulbrom Technical-2

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	1007.3	997.2				
	Day2	1005.0	994.2	1000.93	1001860.86	6.228	38.788
2	Day1	999.0	996.9				
	Day2	996.9	999.4	998.05	996103.80	1.338	1.790
3	Day1	1012.4	1008.7				
	Day2	1010.1	993.8	1006.25	1012539.06	8.439	71.217
S1 SUM	Yi =			3005.23			
S2 SUM	Yi^2 =				3010503.72		
S3 SUM	Si^2 =						111.795

1) Cochran's test (p=3, n=4)

$C = Si^2 max / S3$ = 0.637 < 0.798 (p=3, n=4, 5%)

2) Grubbs's test (P=3, n=4)

Yi min = 998.05 Yi max = 1006.25 Y = S1/p = 1001.74

S = 4.160

Y - Yi min = 3.69

Yi max - Y = 4.51

lower = (Y - Yi min)/S = 0.887 < 1.155 (p=3, 5%)

upper = (Yi max - Y)/S = 1.084 < 1.155 (p=3, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 1001.74

$S_r^2 = S3 / p$ = 37.265 S_r = 6.105

$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 7.985 S_L = 2.826

$S_R^2 = S_r^2 + S_L^2$ = 45.250 S_R = 6.727

$r = 2.8 \times S_r$ =	17.094
$R = 2.8 \times S_R$ =	18.836
$RSD_r = (S_r / mean) \times 100$ =	0.609
$RSD_R = (S_R / mean) \times 100$ =	0.672

Horwitz's value = 2 ^[1 - 0.5 x log (Y / 1000)] = 1.999

RSD_r and RSD_R < 1.999 (Horwitz's value)

Table 2-3 Amisulbrom Water Dispersible Granule

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	506.2	511.4				
	Day2	505.7	506.9	507.55	257607.00	2.613	6.828
2	Day1	505.7	507.8				
	Day2	508.2	507.5	507.30	257353.29	1.105	1.221
3	Day1	504.3	506.2				
	Day2	506.1	508.2	506.20	256238.44	1.594	2.540
S1 SUM	Yi =			1521.05			
S2 SUM	Yi^2 =				771198.73		
S3 SUM	Si^2 =						10.589

1) Cochran's test (p=3, n=4)

$C = Si^2 max / S3$ = 0.645 < 0.798 (p=3, n=4, 5%)

2) Grubbs's test (P=3, n=4)

Yi min = 506.20 Yi max = 507.55 Y = S1/p = 507.02

S = 0.718

Y - Yi min = 0.82

Yi max - Y = 0.53

lower = (Y - Yi min)/S = 1.137 < 1.155 (p=3, 5%)

upper = (Yi max - Y)/S = 0.743 < 1.155 (p=3, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 507.02

$S_r^2 = S3 / p$ = 3.530 S_r = 1.879

$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = -0.368 S_L = NC

$S_R^2 = S_r^2 + S_L^2$ = 3.162 S_R = 1.778

$r = 2.8 \times S_r$ =	5.260
$R = 2.8 \times S_R$ =	4.979
$RSD_r = (S_r / mean) \times 100$ =	0.371
$RSD_R = (S_R / mean) \times 100$ =	0.351

Horwitz's value = 2 ^[1 - 0.5 x log (Y / 1000)] = 2.215

RSD_r and RSD_R < 2.215 (Horwitz's value)

Table 2-4 Amisulbrom Suspension Concentrate-1

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	179.2	180.1				
	Day2	177.6	178.3	178.80	31969.44	1.086	1.179
2	Day1	179.1	178.4				
	Day2	177.8	178.3	178.40	31826.56	0.535	0.286
3	Day1	179.8	178.5				
	Day2	181.4	180.4	180.03	32410.80	1.212	1.469
S1 SUM	Yi =			537.23			
S2 SUM	Yi^2 =				96206.80		
S3 SUM	Si^2 =						2.934

1) Cochran's test (p=3, n=4)

$C = Si^2 max / S3$ = 0.501 < 0.798 (p=3, n=4, 5%)

2) Grubbs's test (P=3, n=4)

Yi min = 178.40 Yi max = 180.03 Y = S1/p = 179.08

S = 0.849

Y - Yi min = 0.68

Yi max - Y = 0.95

lower = (Y - Yi min)/S = 0.801 < 1.155 (p=3, 5%)

upper = (Yi max - Y)/S = 1.119 < 1.155 (p=3, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 179.08

$S_r^2 = S3 / p$ = 0.978 S_r = 0.989

$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = 0.477 S_L = 0.691

$S_R^2 = S_r^2 + S_L^2$ = 1.455 S_R = 1.206

$r = 2.8 \times S_r$ =	2.769
$R = 2.8 \times S_R$ =	3.377
$RSD_r = (S_r / mean) \times 100$ =	0.552
$RSD_R = (S_R / mean) \times 100$ =	0.673

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

RSD_r and RSD_R < 2.591 (Horwitz's value)

Table 2-5 Amisulbrom Suspension Concentrate-2

Lab		Analytical data (n=4)		Yi	Yi^2	Si	Si^2
1	Day1	181.0	181.2				
	Day2	179.5	179.6	180.33	32518.91	0.900	0.810
2	Day1	180.2	179.7				
	Day2	180.3	180.2	180.10	32436.01	0.271	0.073
3	Day1	180.9	180.2				
	Day2	179.1	180.1	180.08	32428.81	0.741	0.549
S1 SUM	Yi =			540.51			
S2 SUM	Yi^2 =				97383.73		
S3 SUM	Si^2 =						1.432

1) Cochran's test (p=3, n=4)

$C = Si^2 max / S3$ = 0.566 < 0.798 (p=3, n=4, 5%)

2) Grubbs's test (P=3, n=4)

Yi min = 180.08 Yi max = 180.33 Y = S1/p = 180.17

S = 0.139

Y - Yi min = 0.09

Yi max - Y = 0.16

lower = (Y - Yi min)/S = 0.647 < 1.155 (p=3, 5%)

upper = (Yi max - Y)/S = 1.151 < 1.155 (p=3, 5%)

3) Calculation of r and R

Mean; Y = S1 / p = 180.17

$S_r^2 = S3 / p$ = 0.477 S_r = 0.691

$S_L^2 = [(pS2-S1^2)/p(p-1)]-(S_r^2/n)$ = -0.098 S_L = NC

$S_R^2 = S_r^2 + S_L^2$ = 0.379 S_R = 0.616

$r = 2.8 \times S_r$ =	1.935
$R = 2.8 \times S_R$ =	1.725
$RSD_r = (S_r / mean) \times 100$ =	0.384
$RSD_R = (S_R / mean) \times 100$ =	0.342

Horwitz's value = 2 ^[1 - 0.5 x log (Y / 1000)] = 2.589

RSD_r and RSD_R < 2.589 (Horwitz's value)

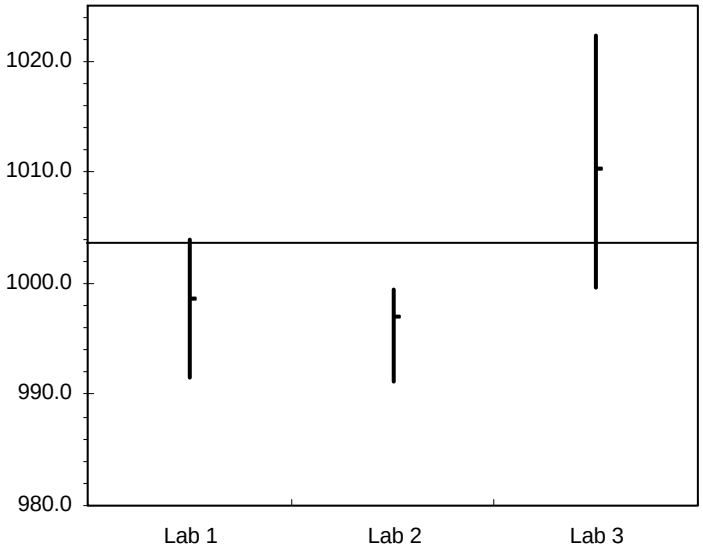

Figure 1 Amisulbrom Technical-1

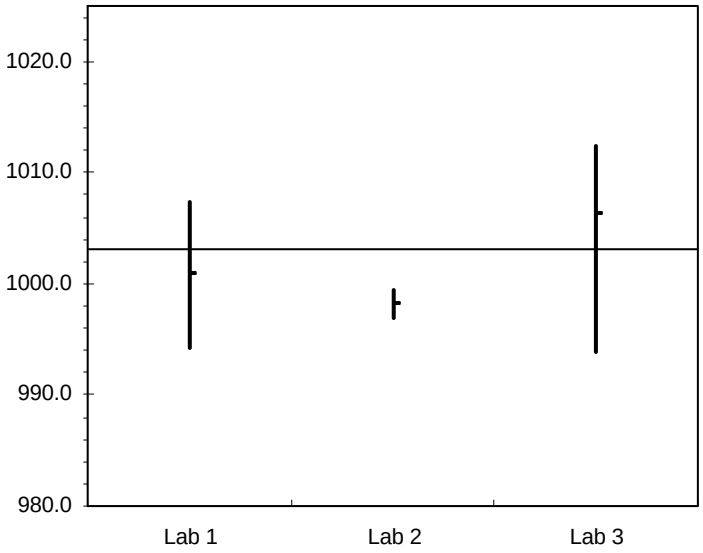

Figure 2 Amisulbrom Technical-2

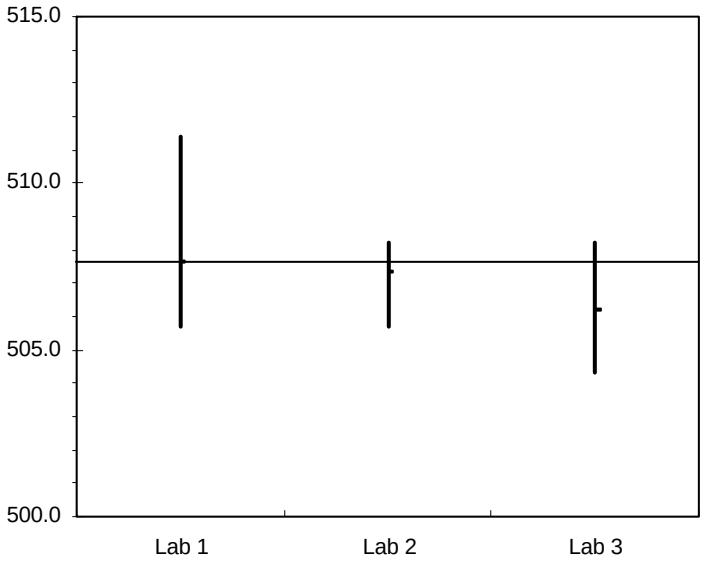

Figure 3 Amisulbrom Water Dispersible Granule

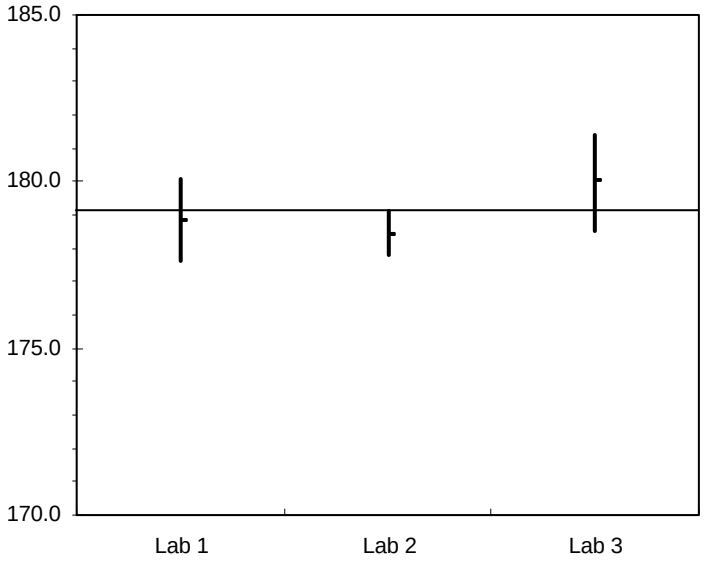

Figure 4 Amisulbrom Suspension Concentrate-1

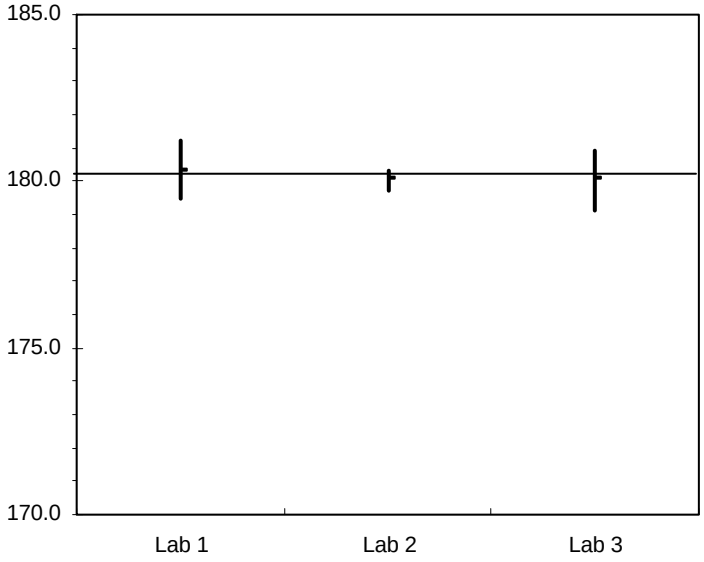

Figure 5 Amisulbrom Suspension Concentrate-2