

FLORASULAM

Collaborative Study

Full Scale Collaborative Study

for the

Determination of Florasulam formulations by

High Performance Liquid Chromatography

Report to CIPAC

By

JIANGSU AGROCHEM LABORATORY CO., LTD

May 2020

1. Participants

In September 2019, Information Sheet No. 322 was sent out by the CIPAC Secretary, inviting members to participate in a collaborative study on the determination of florasulam by gradient reversed phase High Performance Liquid Chromatography.

By end of January 2020, 16 of the 16 respondents provided their results. Of the 16 laboratories, only 7 participants used the column material described in the CIPAC collaboration trial, 9 laboratories used a different column material.

The results of all 16 participants were presented and evaluated.

The participating 16 laboratories are listed in alphabetical order, whereas lab numbers in the result tables were assigned, chronologically, based upon receipt of results.

Agroscope Plant Protection Chemistry Mueller-Thurgau-Strasse 29, 8820 Waedenswil, Switzerland
ALCHIMEX SA ALCHIMEX SA, 63-ALEXANDRU CONSTANTINESCU-011472, BUCHAREST-1, ROMANIA
BASF BASF SE, RAA/AC - E210, Carl-Bosch-Strasse 38, 67056 Ludwigshafen, Germany
CORTEVA Corteva Agrisciences,9330 Zionsville Rd,Building 306, Lab B-1/202 Indianapolis, IN 46268,USA
Federal Office of Consumer Protection and Food Safety(BVL) Messeweg 11/12 D-38104 Braunschweig
HELM China Co., Ltd.(Germany) Sub-contract lab: Jiangsu Ever Test Co., Ltd. No. 31-1, Hengjing Road, Qixia District, Nanjing, Jiangsu, P. R. China.
Laboratory of Formulate Pesticides of the Ministerio de Ganadería Agricultura y Pesca Avenida Millán 4703 esq. Vedia C.P.:12900, Montevideo, Uruguay
LAPRODE(ZHEJIANG) ANALYSIS CO., LTD. 4/F, Building 6, No. 503 Xingguo Road, Yuhang District, Hangzhou, Zhejiang P.R.China.
National Center for Pesticide Quality Supervision and Inspection(ICAMA) Maizidian road No.22, Chaoyang district, Beijing, P. R. China.
National Institute of Health Department of Environment and Health ECASS Section, Via del Castro Laurenziano, 25 00161 Rome ,Italy
Pesticides Test Laboratory of Shenyang Research Institute of Chemical Industry No.8, Shen Liao Dong Road, Tie Xi district, Shen Yang City, P. R. China.
Rallis India Limited 2nd Floor Sharda Terraces, Plot No 65, Sector – 11, CBD Belapur, Navi Mumbai 400 614, Maharashtra, India
Rotam Research Laboratory No. 233, Songjiagang Road, Zhoushi Town, Kunshan, Jiangsu, P. R. China.

State Phytosanitary Laboratory
Ministry of Agriculture, Forestry and Water Economy
blvd. Aleksandar The Great bb pfah: 18 Post 1130, 1000 Skopje, Republic of Macedonia

The Pesticide Control Laboratory
The Pesticide Control Laboratory, Backweston Laboratory Complex, Backweston, Celbridge, Co.
Kildare, Ireland

Walloon Agricultural Research Centre
Carson Building Rue du Bordia, 11B-5030 GEMBLOUX, BELGIUM

2. Active substance: General Information

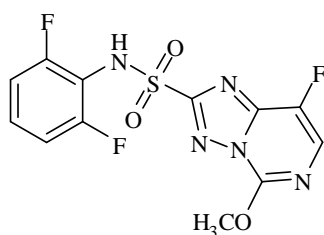
Chemical name: IUPAC 2',6',8-trifluoro-5-methoxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonanilide

CAS N-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4] triazolo [1,5-c] pyrimidine -2-sulfonamide

ISO common name: Florasulam

CAS-No.: 145701-23-1

Structure:



Molecular mass: 359.3

Empirical formula: C₁₂H₈F₃N₅O₃S

Activity: Herbicide

3. Samples

Five test samples, one analytical standard were sent to the participants:

1. Florasulam technical (TC-1)
2. Florasulam technical (TC-2)
3. Florasulam SC 50g/L (SC-1)
4. Florasulam SC 50g/L (SC-2)
5. Florasulam SC 50g/L (SC-3)

Florasulam, reference standard purity 99.84% (w/w)

4. Method

4.1 Scope

The determination of florasulam active substance content contained within two technical samples (TC) and three SC formulations.

4.2 Principle

The content of florasulam (g/kg) is determined by reversed phase high performance liquid chromatography using UV detection at 260nm and external standard calibration.

4.3 Procedure

Each sample was analyzed using four independent determinations. The samples were analyzed on two different days, each day involving duplicate injections of duplicate weights. Both test and reference solutions were freshly prepared on each day. Calibration curve was prepared by plotting the average peak area for each calibration solution versus the mass of florasulam in the calibration solutions. The method of least squares was used, to calculate the equation for the straight line that best fits the experimental calibration data. It was used to calculate the amount of florasulam in the test solution.

5. Remarks of the participants

Several participants provided comments about the method performance and also made a note of any deviations from the method:

Laboratory 1	Column:	ZORBAX Eclipse XDB-C18 (150×4.6 mm, 5 µm)
	Remarks:	Retention was approximately 4.9 minutes
Laboratory 2	Column:	Shim-Pack Gistwith C18 (150×4.6 mm, 5 µm)
	Remarks:	Retention was approximately 7.8 minutes
Laboratory 3	Column:	Zorbax SB C18 (250×4.6 mm, 5 µm)
	Remarks:	Reference wavelength was 200nm instead of 360nm Retention time was approximately 10.1 minutes
Laboratory 4	Column:	Eclipse XDB-C18 (150×4.6 mm, 5 µm)
	Remarks:	reference bandwidth was 100nm instead of 80nm Retention time was approximately 5.1 minutes
Laboratory 5	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	1.Column temperature was changed to 30°C, due to, room temperature was higher than column oven temperature. 2. Reference wavelength was not set.
Laboratory 6	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	1. Due to VWD detector of Agilent HPLC, the bandwidth of wavelength is not available. The signal peak width is " > 0.1min (2s resp. time)(5HZ) " 3. Due to VWD detector of Agilent HPLC, the reference wavelength is not available.
Laboratory 7	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	1. Retention time (day 1) was 5.9min; Retention time (day 2) was 6.1min; 2. The reference material was stored in the fridge starting Wednesday 27.11.2019. 3. By shaking the solutions (ACN & mobile Phase A) gas was generated. 4. The SC samples cannot be dissolved completely in pure ACN with ultra sonification. After adding mobile Phase A and approx. 60 minutes ultra sonification they are dissolved completely. I prepared every sample in the same way: 15

		Minutes in pure ACN, 45 minutes with added mobile phase A.
		<ol style="list-style-type: none"> 5. The peak shapes in calibration and sample runs are not ideal. The peak is integrated completely as one peak (no splitting). 6. In SC sample runs we find a second peak. This second peak was is not included in the evaluation.
Laboratory 8	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	<ol style="list-style-type: none"> 1. Type of HPLC: Waters Acquity UPLC H-Class Series. 2. Type of Integrator: Waters Empower 3. 3. Detection wavelength was 260nm (bandwidth 2.4nm <=> resolution =1.2nm) instead of 260nm (bandwidth 4nm) 4. Reference wavelength was not set. Not applicable with the detector used 5. We think that it would be preferable to divide the final concentration of solutions by a factor of two. 6. The cleaning gradient does not seem useful for the injection of calibration solutions. 7. The equilibration time of the column is very long. 8. We think that it would be preferable to put the same amount of acid in acetonitrile as in water in order to avoid pH variations due to the gradient. 9. Without having made the tests, the mobile phase A = water + 0.2% H₃PO₄ and the mobile phase B = acetonitrile + 0.2% H₃PO₄ could be a solution. 10. We find that the peak of Florasulam is pure and does not seem to co-elute with interfering compounds.
Laboratory 9	Column:	ZORBAX 300SB C18 (150×4.6 mm, 5 µm)
	Remarks:	Retention time difference (3.62mins Vs 6.4mins) possibly due to analytical column used.
Laboratory 10	Column:	BGB, LiChrospher, RP-18, C18, (125×4 mm, 5 µm)
	Remarks:	<ol style="list-style-type: none"> 1. Retention time was 4.5 minutes 2. Samples filtrated through a PET 0.20 µm filter
Laboratory 11	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	None
Laboratory 12	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	None
Laboratory 13	Column:	Phenomenex Luna C18 100A (150×4.6 mm, 5 µm)

	Remarks:	<ol style="list-style-type: none">1. No gradient in HPLC method, and run time was 10min.2. Column temperature was 40 °C instead of 25 °C3. Flow rate was 1.5mL/min instead of 1.0mL/min4. Inject volume was 20µL instead of 5µL5. Retention time was 3.6 minutes6. 50ml volumetric flask was used to prepare calibration solutions and sample solutions instead of 100ml. The concentration of these solutions were consistent with method
Laboratory 14	Column:	Merck LiChospher 100, RP-18 (150×4.6 mm, 5 µm)
	Remarks:	Retention time was 4.5 minutes
Laboratory 15	Column:	Thermo Hypersil Gold C18 (150×4.6 mm, 5 µm)
	Remarks:	Retention time was approximately 5.2 minute
Laboratory 16	Column:	ZORBAX SB C18 (150×4.6 mm, 5 µm)
	Remarks:	Inject volume was 1µL instead of 5 µL. (Our Ultimate 3000RS UHPLC system is equipped with a micro-cell of detection and from this reason our standard volume of injection is 1.0 microliter.)

6. Evaluation and Discussion

6.1 Data Review

The data obtained from each laboratory was visually reviewed to determine if there were any significant chromatography differences, from what was expected, which might affect the analytical results.

In summary it can be stated that the method deviations, noted by the participating 16 participants, were deemed not to affect the analytical results significantly and therefore all data sets were included within the statistical assessment.

6.2 Determination of florasulam

The statistical evaluation of the data was accomplished following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725. The testing for outliers/stragglers of the laboratory mean values were performed according to Grubbs test on a 1% significance level (see appendix B). All results reported by the 16 laboratories are reported and the statistical evaluation of these are listed in Tables 1-2 and displayed in Figures 1-5. These results are reported without any exclusion of outliers.

Determination of Florasulam-Full set of 16 participants

All results tabulated in table 1 are given in g/kg.

Table 1-Results*

Lab	Day	Florasulam Tech. TC-1		Florasulam Tech. TC-2		Florasulam SC 50g/L SC-1		Florasulam SC 50g/L SC-2		Florasulam SC 50g/L SC-3	
		value	Mean value	value	Mean value	value	Mean value	value	Mean value	value	Mean value
1	1	984.16	988.38	982.15	988.13	49.88	49.86	51.57	51.57	50.57	50.49
	2	992.60		994.10		49.84		51.56		50.40	
2	1	982.26	983.19	986.20	986.77	50.04	50.32	52.24	52.26	50.31	50.48
	2	984.12		987.34		50.59		52.27		50.65	
3	1	999.19	997.55	997.92	993.45	50.67	49.84	50.30	50.62	50.88	50.44
	2	995.90		988.98		49.01		50.94		49.99	
4	1	988.87	988.84	989.54	989.21	50.47	50.47	50.75	50.70	50.48	50.47
	2	988.80		988.87		50.46		50.64		50.46	
5	1	990.41	990.23	989.60	989.14	49.53	49.48	50.25	50.5	50.55	50.71

Lab	Day	Florasulam Tech. TC-1		Florasulam Tech. TC-2		Florasulam SC 50g/L SC-1		Florasulam SC 50g/L SC-2		Florasulam SC 50g/L SC-3	
		value	Mean value	value	Mean value	value	Mean value	value	Mean value	value	Mean value
	2	990.05		988.68		49.42		50.75		50.87	
6	1	989.73	988.65	990.61	990.05	50.37	50.15	51.44	51.60	51.45	51.06
	2	987.56		989.48		49.93		51.75		50.66	
7	1	993.27	990.60	995.02	992.72	50.63	50.43	52.62	52.41	51.10	51.32
	2	987.92		990.42		50.23		52.20		51.53	
8	1	987.18	987.56	999.54	997.34	49.92	50.02	51.76	51.94	50.83	50.90
	2	987.94		995.13		50.11		52.11		50.97	
9	1	987.09	990.05	991.37	988.99	50.19	50.45	52.24	52.28	51.31	51.38
	2	993.00		986.61		50.71		52.31		51.44	
10	1	973.26	979.02	971.29	973.87 ¹	49.74	49.92	51.48	51.68	50.37	50.61
	2	984.78		976.45		50.10		51.88		50.85	
11	1	988.46	993.46	989.43	990.03	50.06	50.21	51.68	51.92	50.27	50.59
	2	998.45		990.62		50.36		52.16		50.91	
12	1	988.43	987.30	995.65	994.33	49.56	49.72	51.54	51.56	50.43	50.48
	2	986.16		993.00		49.87		51.58		50.52	
13	1	987.14	988.13	987.94	987.83	49.82	49.79	50.99	50.92	50.23	50.29
	2	989.12		987.72		49.75		50.85		50.35	
14	1	982.13	983.25	985.93	985.22	46.73	49.33	48.53	50.56	48.01	49.49
	2	984.37		984.50		51.92		52.58		50.97	
15	1	989.63	988.89	991.16	989.99	50.05	50.02	51.54	51.68	50.48	50.63
	2	988.15		988.82		49.99		51.81		50.78	
16	1	991.41	992.62	991.89	990.16	50.49	50.72	52.00	52.20	50.86	50.89
	2	993.82		988.43		50.94		52.40		50.91	

¹ Result was detected as an outlier

***Note:** all raw data and calculation details were shown in Appendix C.

Table 2 – Summary of the statistical evaluation *

	Florasulam Tech. TC-1	Florasulam Tech. TC-2	Florasulam SC 50g/L SC-1	Florasulam SC 50g/L SC-1	Florasulam SC 50g/L SC-1
X _m [g/kg]	988.61	989.20	50.04	51.52	50.64
X _m [%, w/w]	98.861	98.92	5.004	5.152	5.064
L	16	16	16	16	16
r	10.01	9.19	2.76	2.11	1.67
R	14.04	15.61	2.23	2.38	1.71
S _r	3.58	3.28	0.99	0.75	0.60
S _R	5.01	5.58	0.79	0.85	0.61
RSD _r	0.51	0.56	1.58	1.65	1.2
RSD _R (Hor)	2.00	2.00	3.14	3.13	3.13
HorRat	0.26	0.28	0.50	0.53	0.38

x_m = overall sample mean

L = number of laboratories

s_r = repeatability standard deviation

s_R = reproducibility standard deviation

r = repeatability limit

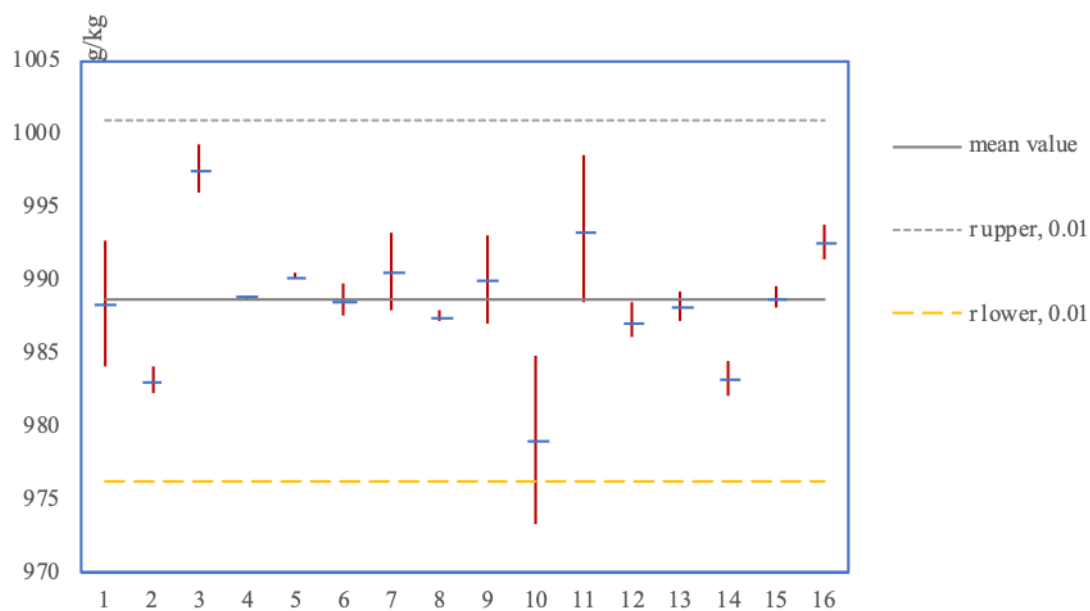
R = reproducibility limit

RSD_r = relative repeatability standard deviation

RSD_R = relative reproducibility standard deviation

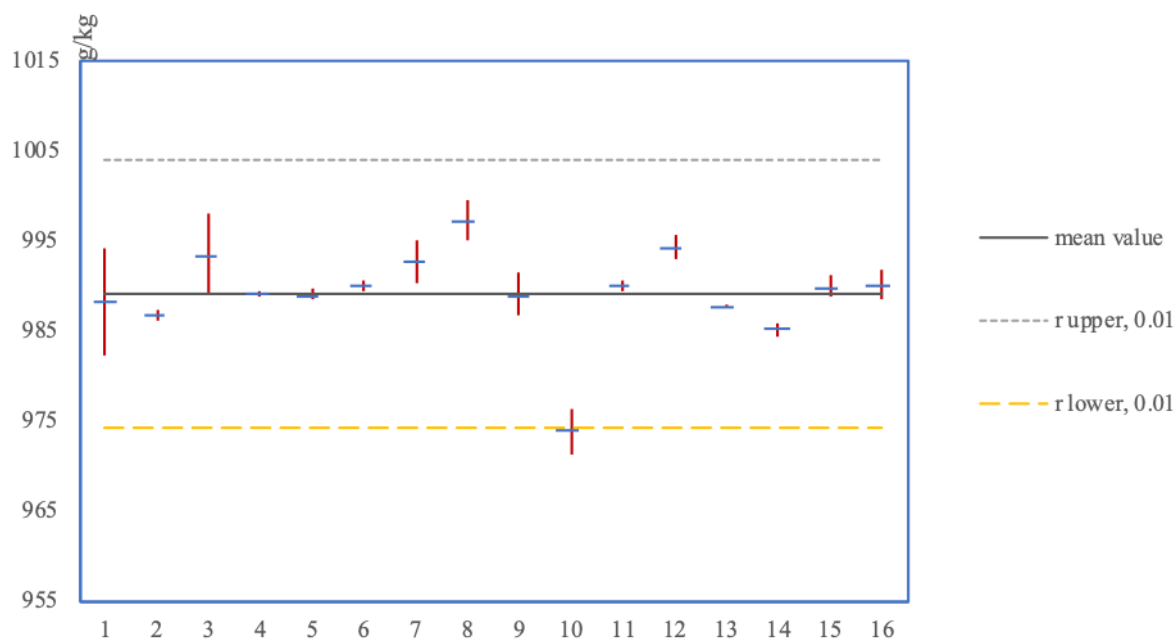
HorRat (Horwitz Ratio) = RSD_r/RSD_R (Hor)

**Note: calculation details were shown in Appendix A.*

Figure 1-Florasulam tech. (TC-1)

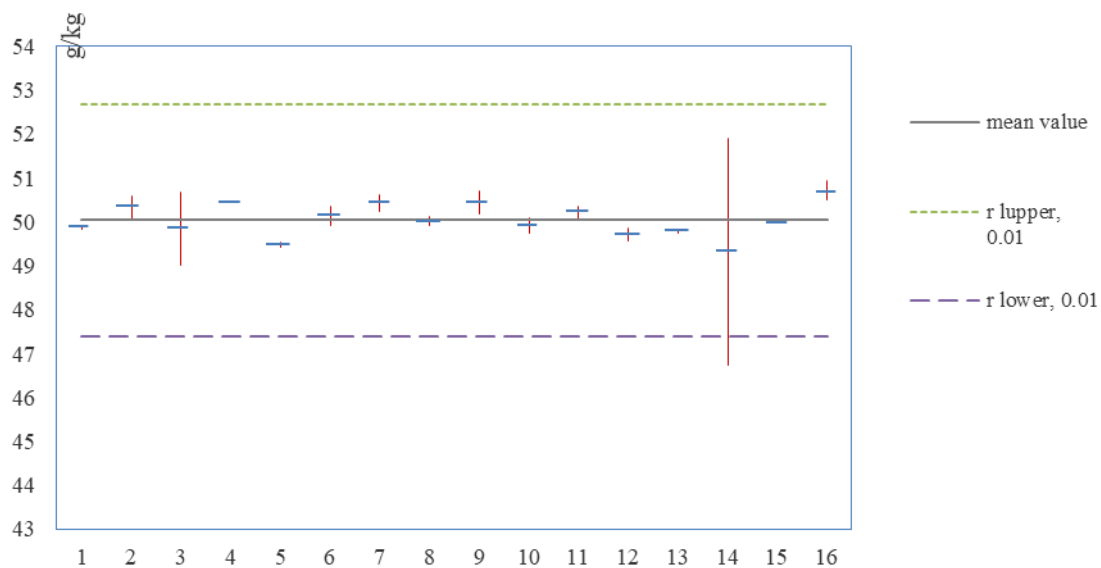
Mean value:	988.61g/kg
s_f :	3.58
s_R :	5.01
RSD_R :	0.51
$RSD_R(\text{Hor})$:	2.00
HorRat:	0.26
Upper of confidence interval (r upper, 0.01)*	1000.99g/kg
Lower of confidence interval (r lower, 0.01)*	976.22g/kg
Outlier (Grubbs) :	none

*Note: calculation details about confidence interval were shown in Appendix B.

Figure 2-Florasulam tech. (TC-2)

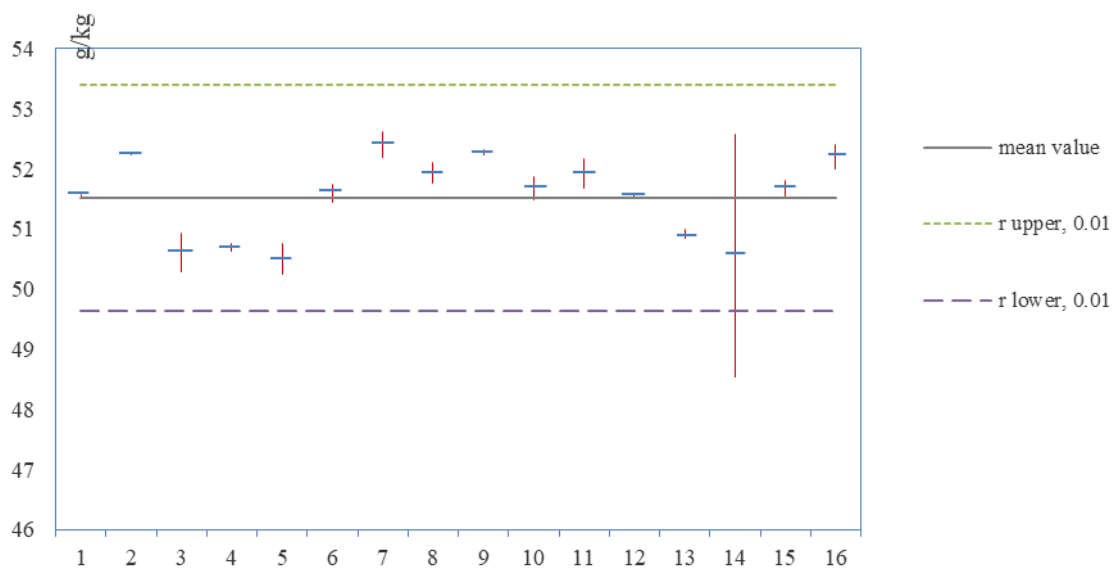
Mean value:	989.20g/kg
s_f :	3.28
s_R :	5.58
RSD_R :	0.56
$RSD_R(\text{Hor})$:	2.00
HorRat:	0.28
Upper of confidence interval (r upper, 0.01)*	1004.15g/kg
Lower of confidence interval (r lower, 0.01)*	974.25g/kg
Outlier (Grubbs) :	lower

*Note: calculation details about confidence interval were shown in Appendix B.

Figure 3-Florasulam SC 50g/L (SC-1)

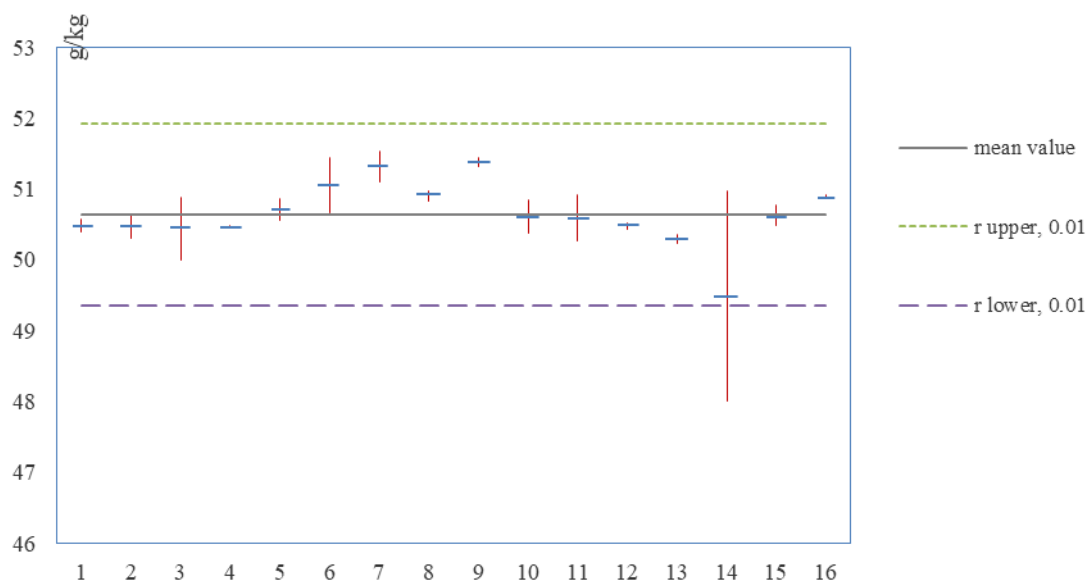
Mean value:	50.04g/kg
s_r :	0.99
s_R :	0.79
RSD_R :	1.58
$RSD_R(\text{Hor})$:	3.14
HorRat:	0.50
Upper of confidence interval (r upper, 0.01)*	52.69g/kg
Lower of confidence interval (r lower, 0.01)*	47.40g/kg
Outlier (Grubbs) :	none

*Note: calculation details about confidence interval were shown in Appendix B.

Figure 4-Florasulam SC 50g/L (SC-2)

Mean value:	51.52g/kg
s_f :	0.75
s_R :	0.85
RSD_R :	1.65
$RSD_R(\text{Hor})$:	3.13
HorRat:	0.52
Upper of confidence interval (r upper, 0.01)*	53.40g/kg
Lower of confidence interval (r lower, 0.01)*	49.64g/kg
Outlier (Grubbs) :	none

*Note: calculation details about confidence interval were shown in Appendix B.

Figure 5-Florasulam SC 50g/L (SC-3)

Mean value:	50.64g/kg
s_r :	0.60
s_R :	0.61
RSD_R :	1.20
$RSD_R(\text{Hor})$:	3.13
HorRat:	0.38
Upper of confidence interval (r upper, 0.01)*:	51.92g/kg
Lower of confidence interval (r lower, 0.01)*:	49.35g/kg
Outlier (Grubbs) :	none

*Note: calculation details about confidence interval were shown in Appendix B.

7. Conclusions

The data of 16 different laboratories, who participated in this full scale CIPAC collaboration trial have been used for the statistical evaluation. The data presented in the statistical summary show that the method is suitable to gain acceptable and reproducible results for all samples tested and is therefore regarded to be robust.

JIANGSU AGROCHEM LABORATORY CO.,LTD consider this method to be suitable for the intended purpose, without further changes, and recommend accepting it as a provisional CIPAC method for the determination of Florasulam in technical samples and associated formulations: SC.

Appendix A:

Calculation for repeatability and reproducibility standard deviation and limit

$$T_1 = \sum n_i \bar{y}_i$$

$$T_2 = \sum n_i (\bar{y}_i)^2$$

$$T_3 = \sum n_i$$

$$T_4 = \sum n_i^2$$

$$T_5 = \sum (n-1) s_i^2$$

$$s_r^2 = \frac{T_5}{T_3 - p}$$

$$s_L^2 = \left[\frac{T_2 T_3 - T_1^2}{T_3(p-1)} - s_r^2 \right] \left[\frac{T_3(p-1)}{T_3^2 - T_4} \right]$$

$$s_R^2 = s_L^2 + s_r^2$$

$$r = 2.8 \times s_r$$

$$R = 2.8 \times s_R$$

In which:

- p : Number of laboratories
- n : Number of test results obtained in one laboratory at one level
- \bar{y}_i : The individual laboratory average
- s_r : repeatability standard deviation
- s_R : reproducibility standard deviation
- r : repeatability limit
- R : reproducibility limit

Results are calculated as follows, using florasulam Tech. TC-1 as example:

Florasulam Tech. TC-1	i	Level	Results	\bar{y}_i	s_i^2	n_i
	1	1	984.16	988.38	35.6168	2
		2	992.60			
	2	1	982.26	983.19	1.7298	2
		2	984.12			
	3	1	999.19	997.545	5.41205	2
		2	995.90			
	4	1	988.87	988.835	0.00245	2
		2	988.80			
	5	1	990.41	990.23	0.064800	2
		2	990.05			
	6	1	989.73	988.645	2.35445	2
		2	987.56			
	7	1	993.27	990.595	14.31125	2
		2	987.92			
	8	1	987.18	987.56	0.2888	2
2		987.94				
9	1	987.09	990.045	17.464050	2	
	2	993.00				
10	1	973.26	979.02	66.3552	2	
	2	984.78				
11	1	988.46	993.455	49.90005	2	
	2	998.45				
12	1	988.43	987.295	2.57645	2	
	2	986.16				
13	1	987.14	988.13	1.9602	2	
	2	989.12				
14	1	982.13	983.25	2.5088	2	
	2	984.37				
15	1	989.63	988.89	1.0952	2	
	2	988.15				
16	1	991.41	992.615	2.90405	2	
	2	993.82				

s : The individual laboratory standard deviation

$$T_1 = \sum n_i \bar{y}_i = 2 \times 998.38 + 2 \times 983.19 + 2 \times 997.545 + \dots + 2 \times 988.89 + 2 \times 992.615 = 36135.36$$

$$T_2 = \sum n_i (\bar{y}_i)^2 = 2 \times (988.38)^2 + 2 \times (983.19)^2 + 2 \times (997.545)^2 + \dots + 2 \times (988.89)^2 + 2 \times (992.615)^2 = 31275437.655$$

$$T_3 = \sum n_i = 2 \times 16 = 32$$

$$T_4 = \sum n_i^2 = 2^2 \times 16 = 64$$

$$T_5 = \sum (n-1)s_i^2 = (2-1) \times 35.6168 + (2-1) \times 1.7298 + (2-1) \times 5.41205 + \dots + (2-1) \times 1.0952 + (2-1) \times 2.90405 = 204.5444$$

$$s_r^2 = \frac{T_5}{T_3 - p} = \frac{204.5444}{32 - 16} = 12.784025$$

$$s_L^2 = \left[\frac{T_2 T_3 - T_1^2}{T_3(p-1)} - s_r^2 \right] \left[\frac{T_3(p-1)}{T_3^2 - T_4} \right]$$

$$= \left[\frac{31275437.655 \times 32 - 31635.36^2}{32 \times (16-1)} - 12.784025 \right] \left[\frac{31275437.655 \times (16-1)}{31275437.655^2 - 64} \right] = 12.3607275$$

$$s_R^2 = s_L^2 + s_r^2 = 12.3607275 + 12.784025 = 25.1447525$$

$$s_r = \sqrt{s_r^2} = \sqrt{12.784025} = 3.58$$

$$s_R = \sqrt{s_R^2} = \sqrt{25.1447525} = 5.01$$

$$r = 2.8 \times \sqrt{s_r^2} = 2.8 \times \sqrt{12.784025} = 10.01$$

$$R = 2.8 \times \sqrt{s_R^2} = 2.8 \times \sqrt{25.1447525} = 14.04$$

**Note: There was no rounding until the final reported results are achieved.*

Appendix B:**Calculation for Grubbs outlier test**

The testing for outliers of the laboratory mean values were performed according to Grubbs test on a 1% significance level.

Critical value on a 1% significance level is 2.852.

Upper of confidence interval ($r_{upper, 0.01}$) = total mean value + total standard deviation \times 2.852

Lower of confidence interval ($r_{lower, 0.01}$) = total mean value - total standard deviation \times 2.852

If test result $> r_{upper, 0.01}$, or test result $< r_{lower, 0.01}$, it will be considered as an outlier.

Using florasulam Tech. TC-1 as example:

	i	Level	Results	\bar{y}_i	Mean value	Standard deviation
Florasulam Tech. TC-1	1	1	984.16	988.38	988.605	4.343624.....
		2	992.60			
	2	1	982.26	983.19		
		2	984.12			
	3	1	999.19	997.545		
		2	995.90			
	4	1	988.87	988.835		
		2	988.80			
	5	1	990.41	990.23		
		2	990.05			
	6	1	989.73	988.645		
		2	987.56			
	7	1	993.27	990.595		
		2	987.92			
	8	1	987.18	987.56		
		2	987.94			
	9	1	987.09	990.045		
		2	993.00			
	10	1	973.26	979.02		
		2	984.78			
	11	1	988.46	993.455		
		2	998.45			
	12	1	988.43	987.295		
		2	986.16			
	13	1	987.14	988.13		
		2	989.12			
	14	1	982.13	983.25		
		2	984.37			
	15	1	989.63	988.89		
		2	988.15			
	16	1	991.41	992.615		
		2	993.82			

$$\text{Total mean value} = \frac{988.38 + 983.19 + 997.545 + \dots + 988.89 + 992.615}{16} = 988.605$$

Total standard deviation

$$= \frac{(988.38 - 988.605)^2 + (983.19 - 988.605)^2 + (997.545 - 988.605)^2 + \dots + (988.89 - 988.605)^2 + (992.615 - 988.605)^2}{16 - 1}$$

$$= 4.343624 \dots$$

$$\text{Upper of confidence interval (r upper, 0.01)} = 988.605 + \text{total standard deviation} \times 2.852 = 1000.99$$

$$\text{Upper of confidence interval (r upper, 0.01)} = 988.605 - \text{total standard deviation} \times 2.852 = 976.22$$

**Note: There was no rounding until the final reported results are achieved.*

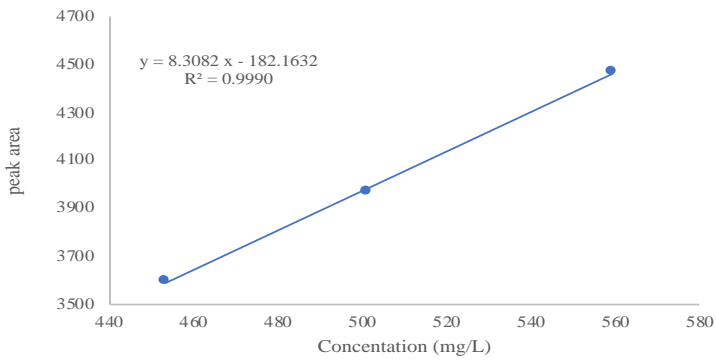
Appendix C:

Calculation of content determination for each laboratory

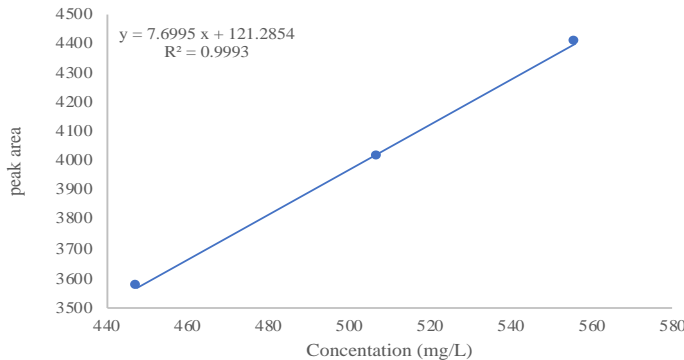
Laboratory 1 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1		
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$	
45.80	457.2672	3649.4319 3647.2004	3648.3162	0.99986	
50.20	501.1968	4007.4380 3973.4939	3990.4659		
55.10	550.1184	4416.3247 4372.6494	4394.4871		
Regression equation:	y=ax+b (a=8.0404; b=-32.1101)				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$		Average (g/kg)
TC-1	51.20	4006.0448	980.92	984.16	
	50.10	3945.3385	987.39		
TC-2	50.20	3910.2401	976.73	982.15	
	50.80	4001.6165	987.56		
SC-1	1010.90	4018.1835	49.83	49.88	
	1009.70	4020.8947	49.92		
SC-2	999.60	4114.5608	51.59	51.57	
	990.00	4071.6581	51.55		
SC-3	990.50	3991.4740	50.52	50.57	
	998.50	4031.8469	50.62		

Laboratory 1 (day2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.40	453.2736	3591.9978	3592.5334	0.99950
		3593.0691		
50.20	501.1968	3960.3398	3965.7257	
		3971.1116		
56.00	559.1040	4459.0552	4470.2671	
		4481.4790		
Regression equation:	$y = ax + b$ (a=8.3082; b=-182.1632)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	51.60	4082.6140	994.81	992.60
	51.80	4080.1084	990.39	
TC-2	51.70	4086.9050	993.88	994.10
	52.00	4113.5322	994.31	
SC-1	1007.80	3987.4168	49.80	49.84
	999.30	3957.8556	49.87	
SC-2	1009.40	4138.7058	51.52	51.56
	1002.00	4113.5518	51.60	
SC-3	1009.70	4045.1041	50.39	50.40
	1009.00	4043.6317	50.41	

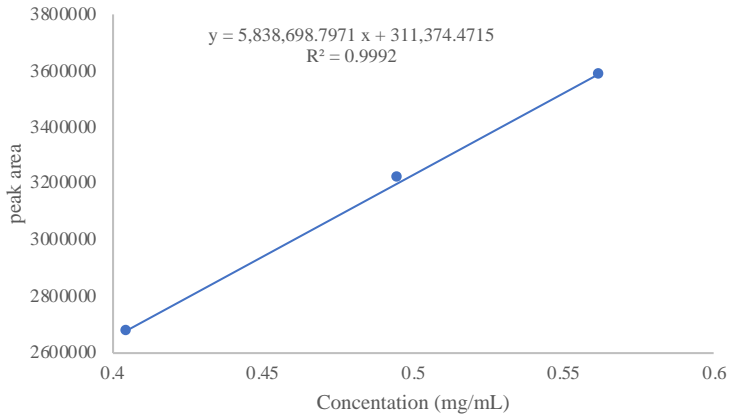
Laboratory 2 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient
				$r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.80	447.2832	3564.4000	3570.7000	0.99967
		3577.0000		
50.80	507.1872	4014.8000	4013.9500	
		4013.1000		
55.70	556.1088	4411.3000	4409.8500	
		4408.4000		
Regression equation:	y=ax+b (a=7.6995; b=121.2854)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	50.90	3975.2000	983.38	982.26
	51.10	3981.5000	981.13	
TC-2	51.30	4016.5000	986.17	986.20
	51.40	4024.3000	986.22	
SC-1	1005.00	3981.8000	49.89	50.04
	1025.60	4083.8000	50.18	
SC-2	1016.60	4179.2000	51.84	52.24
	1016.30	4240.3000	52.64	
SC-3	1029.30	4118.3000	50.43	50.31
	1014.80	4043.1000	50.19	

Laboratory 2 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.30	452.2752	3612.0000	3619.7000	0.99874
		3627.4000		
51.30	512.1792	4037.5000	4036.1000	
		4034.7000		
56.20	561.1008	4437.2000	4441.2500	
		4445.3000		
Regression equation:	$y = ax + b$ (a=7.5272; b=204.6510)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	51.20	4004.2000	985.14	984.12
	51.30	4003.7000	983.09	
TC-2	51.40	4025.3000	986.60	987.34
	51.80	4061.8000	988.08	
SC-1	1006.60	4023.8000	50.36	50.59
	1037.20	4180.5000	50.82	
SC-2	1018.30	4204.8000	52.08	52.27
	1017.80	4233.0000	52.46	
SC-3	1030.60	4148.1000	50.74	50.65
	1016.20	4076.0000	50.55	

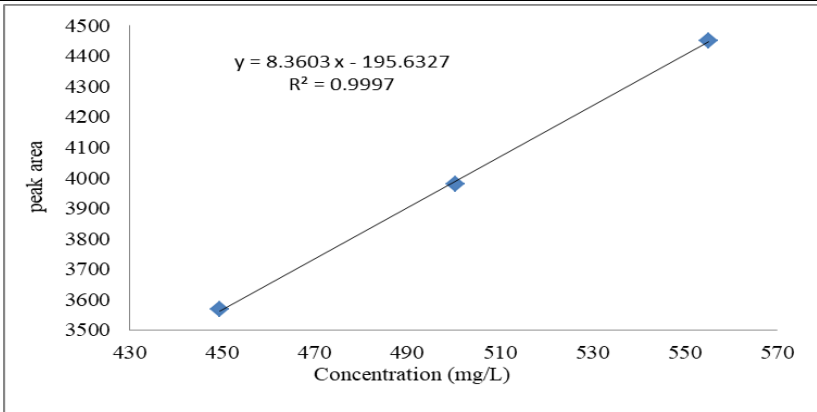
Laboratory 3 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
40.60	0.4054	2675544.7500	2671843.8350	0.99958
		2668142.9200		
49.60	0.4952	3210132.3600	3218014.7300	
		3225897.1000		
56.30	0.5621	3577798.9000	3584529.5800	
		3591260.2600		
Regression equation:	$y = ax + b$ (a=5838698.7971; b=311374.4715)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	49.40	3154924.2250	985.87	999.19
	49.50	3237694.0550	1012.51	
TC-2	50.50	3224604.2300	988.02	997.92
	50.60	3288818.5750	1007.81	
SC-1	1055.20	3462439.2200	51.15	50.67
	1048.20	3382556.4900	50.18	
SC-2	1021.50	3457013.1500	52.74	50.30
	1234.40	3760765.2450	47.86	
SC-3	1060.80	3443249.6700	50.57	50.88
	1049.50	3448390.0800	51.19	

Laboratory 3 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient
				$r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
47.50	0.4742	3144114.8400	3149143.0550	0.99561
		3154171.2700		
49.00	0.4892	3302856.0900	3297574.4950	
		3292292.9000		
57.00	0.5691	3744056.6900	3745785.0550	
		3747513.4200		
Regression equation:	$y = ax + b$ (a=6062100.5381; b=300777.8435)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	52.30	3500798.9700	1009.32	995.90
	48.50	3189351.3300	982.47	
TC-2	48.30	3185699.7700	985.29	988.98
	50.40	3333673.9200	992.67	
SC-1	989.40	3332009.1700	50.54	49.01
	879.80	2833340.2200	47.48	
SC-2	984.90	3374028.7700	51.47	50.94
	1080.30	3602271.0700	50.41	
SC-3	1022.00	3377353.2100	49.66	49.99
	1042.30	3479528.5600	50.31	

Laboratory 4 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient
				$r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.03	449.5795	3565.77	3567.2500	0.99987
		3568.73		
50.12	500.3981	3978.80	3979.6200	
		3980.44		
55.62	555.3101	4507.56	4450.8700	
		4394.18		
Regression equation:	$y = ax + b$ (a=8.3603; b=-195.6327)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	50.61	3991.9950	989.71	988.87
	50.64	3987.3450	988.03	
TC-2	50.80	4006.5950	989.45	989.54
	51.46	4061.9250	989.62	
SC-1	996.06	4002.3600	50.41	50.47
	998.42	4021.6400	50.52	
SC-2	1094.90	4467.8350	50.95	50.75
	1042.57	4210.0050	50.55	
SC-3	1029.19	4151.4000	50.52	50.48
	995.60	4001.5750	50.43	

Laboratory 4 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.14	450.6778	3576.72	3577.5250	0.99955
		3578.33		
50.29	502.0954	3995.93	3991.6400	
		3987.35		
55.96	558.7046	4531.27	4497.9950	
		4464.72		
Regression equation:	$y = ax + b$ (a=8.5279; b=-274.1749)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	51.56	4075.0400	989.14	988.80
	50.55	3986.9150	988.46	
TC-2	50.67	4000.3800	989.23	988.87
	51.70	4084.1000	988.51	
SC-1	1019.92	4120.9400	50.53	50.46
	999.38	4020.5350	50.39	
SC-2	1181.96	4826.8900	50.61	50.64
	1038.57	4212.4500	50.66	
SC-3	1027.09	4159.9500	50.62	50.46
	1000.12	4015.8850	50.30	

Laboratory 5 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.13	0.4506	3514306	3515787.5	0.99986
		3517269		
50.41	0.5033	3927834	3927857.5	
		3927881		
55.63	0.5554	4307753	4312581.5	
		4317410		
Regression equation:	$y = ax + b$ (a=7603411.0766; b=93466.0540)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
TC-1	50.50	3896867	990.54	990.41
	49.52	3822029	990.27	
TC-2	50.11	3863439	989.48	989.60
	50.59	3900437	989.71	
SC-1	1014.61	3923138	49.64	49.53
	1024.70	3943147	49.41	
SC-2	1024.87	4016320	50.34	50.25
	993.55	3882327	50.15	
SC-3	1000.90	3915200	50.22	50.55
	1026.38	4063081	50.87	

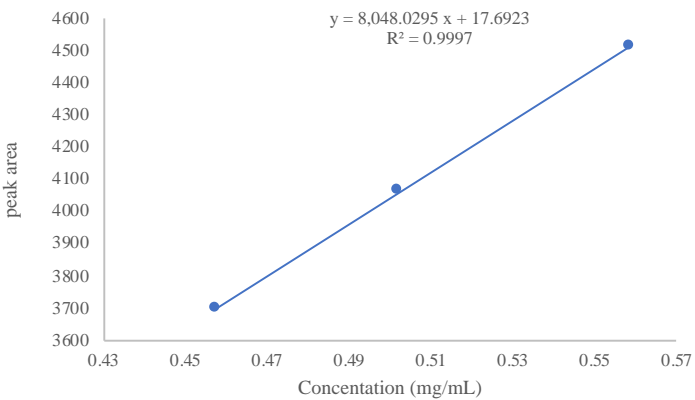
Laboratory 5 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.95	0.4488	3485821	3485907.5	1.00000
		3485994		
50.70	0.5062	3918413	3915269.5	
		3912126		
55.20	0.5511	4256539	4253968.0	
		4251397		
Regression equation:	$y = ax + b$ (a=7506660.7632; b=116454.4082)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	50.81	3891730	989.81	990.05
	51.72	3961202	990.29	
TC-2	50.86	3890840	988.61	988.68
	51.45	3935133	988.74	
SC-1	1005.95	3852554	49.48	49.42
	998.20	3815249	49.36	
SC-2	1001.67	3924791	50.65	50.75
	1034.55	4065294	50.85	
SC-3	1004.26	3947260	50.82	50.87
	1031.59	4059764	50.92	

Laboratory 6 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.03	0.4496	3630.66626	3632.2150	0.99983
		3633.76367		
50.34	0.5026	4073.03052	4071.8377	
		4070.64478		
54.99	0.5490	4432.03467	4432.5122	
		4432.98975		
Regression equation:	$y = ax + b$ (a=8057.0181; b=13.7898)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
TC-1	50.25	4017.70655	988.95	989.73
	50.82	4069.45606	990.50	
TC-2	51.08	4089.12769	990.24	990.61
	50.53	4048.25293	990.97	
SC-1	1000.16	4045.42701	50.03	50.37
	997.91	4089.77259	50.70	
SC-2	997.84	4112.68433	50.98	51.44
	1005.30	4216.72876	51.89	
SC-3	1003.47	4188.76489	51.64	51.45
	1000.73	4145.84888	51.25	

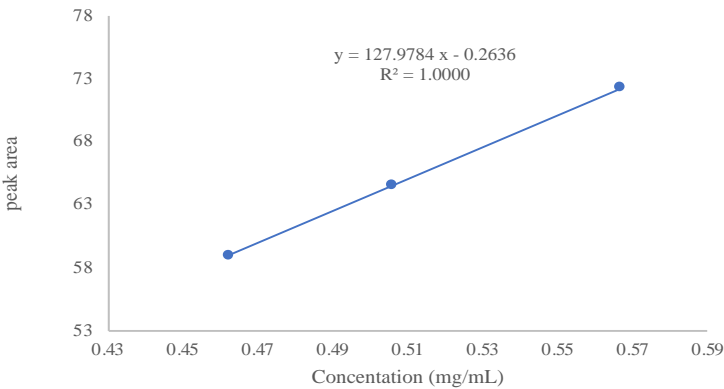
Laboratory 6 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.82	0.4575	3693.13403	3694.8208	0.99983
		3696.50757		
50.27	0.5019	4067.33105	4065.6306	
		4063.93018		
55.96	0.5587	4508.87500	4510.3391	
		4511.80322		
Regression equation:	$y = ax + b$ (a=8048.0295; b=17.6923)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	50.01	3994.27246	988.01	987.56
	50.00	3989.81641	987.10	
TC-2	49.91	3993.66944	989.84	989.48
	51.05	4081.52405	989.12	
SC-1	1002.39	4046.93506	49.95	49.93
	998.89	4029.32483	49.90	
SC-2	998.67	4176.10571	51.74	51.75
	1002.80	4194.68628	51.76	
SC-3	1001.88	4104.24683	50.68	50.66
	1004.12	4109.67822	50.64	

Laboratory 7 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.14	0.4407	56.2169	56.2040	0.99932
		56.1910		
49.00	0.4892	61.8545	61.9171	
		61.9797		
55.79	0.5570	71.0297	71.0576	
		71.0855		
Regression equation:	$y = ax + b$ (a=128.1716; b=-0.4665)			
<p>Scatter plot showing peak area (y-axis, 53 to 73) versus Concentration (mg/mL) (x-axis, 0.43 to 0.57). The regression equation is $y = 128.1716x - 0.4665$ and $R^2 = 0.9986$.</p>				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	52.35	66.1861	993.36	993.27
	55.44	70.1067	993.17	
TC-2	51.41	65.0860	994.83	995.02
	52.75	66.8196	995.20	
SC-1	1013.22	65.3070	50.65	50.63
	1013.90	65.2869	50.60	
SC-2	1012.90	67.9069	52.67	52.62
	1020.87	68.3066	52.56	
SC-3	1002.63	65.1768	51.08	51.10
	1003.43	65.2813	51.12	

Laboratory 7 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
46.33	0.4626	58.9399	58.9359	1.00000
		58.9318		
50.68	0.5060	64.5253	64.4992	
		64.4730		
56.78	0.5669	72.3066	72.2850	
		72.2633		
Regression equation:	$y = ax + b$ (a=127.9784; b=-0.2636)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	51.46	64.7974	987.90	987.92
	49.70	62.5738	987.93	
TC-2	52.36	66.0480	989.59	990.42
	50.44	63.7239	991.25	
SC-1	1020.75	65.3135	50.20	50.23
	1032.58	66.1538	50.26	
SC-2	1009.43	67.1521	52.19	52.20
	1010.62	67.2625	52.21	
SC-3	1003.93	65.8847	51.48	51.53
	1022.55	67.2206	51.57	

Laboratory 8 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
46.3	0.4623	3932829	3932729.50	0.99972
		3932630		
51.6	0.5152	4365741	4362966.50	
		4360192		
54.6	0.5451	4629582	4628947.00	
		4628312		
Regression equation:	$y = ax + b$ (a=8376657.6905; b=56781.3335)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
TC-1	49.7	4168588	987.66	987.18
	50.0	4189389	986.70	
TC-2	50.7	4307500	1000.88	999.54
	50.4	4270985	998.19	
SC-1	1006.8	4272070	49.98	49.92
	1010.1	4274395	49.85	
SC-2	996.8	4379877	51.77	51.76
	998.1	4382610	51.74	
SC-3	999.6	4310929	50.81	50.83
	1003.6	4331764	50.85	

Laboratory 8 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.5	0.4443	3776424	3773190.50	0.99973
		3769957		
49.5	0.4942	4179290	4180006.50	
		4180723		
55.1	0.5501	4673323	4674163.00	
		4675003		
Regression equation:	$y = ax + b$ (a=8522277.3086; b=-19634.0005)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
TC-1	50.4	4239960	991.70	987.94
	50.9	4249584	984.18	
TC-2	50.9	4305664	997.11	995.13
	52.2	4398506	993.15	
SC-1	999.5	4244621	50.06	50.11
	997.3	4242741	50.15	
SC-2	1001.2	4421898	52.05	52.11
	1003.2	4439676	52.16	
SC-3	1000.3	4323588	50.95	50.97
	1000.6	4327804	50.98	

Laboratory 9 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.55	444.7872	3637807.0000	3640263.0000	0.99999
		3642719.0000		
49.94	498.6010	4074646.0000	4075231.0000	
		4075816.0000		
54.46	543.7286	4431781.0000	4435780.0000	
		4439779.0000		
Regression equation:	$y = ax + b$ (a=8041.6354; b=64140.9418)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	49.92	4007399.0000	982.28	987.09
	49.33	3998926.0000	991.89	
TC-2	50.32	4048375.0000	984.60	991.37
	49.51	4038099.0000	998.13	
SC-1	1006.70	4129623.0000	50.22	50.19
	1004.42	4115826.0000	50.16	
SC-2	1002.05	4277463.0000	52.29	52.24
	1003.74	4275738.0000	52.18	
SC-3	1001.76	4202321.0000	51.37	51.31
	1000.6	4327804	50.98	

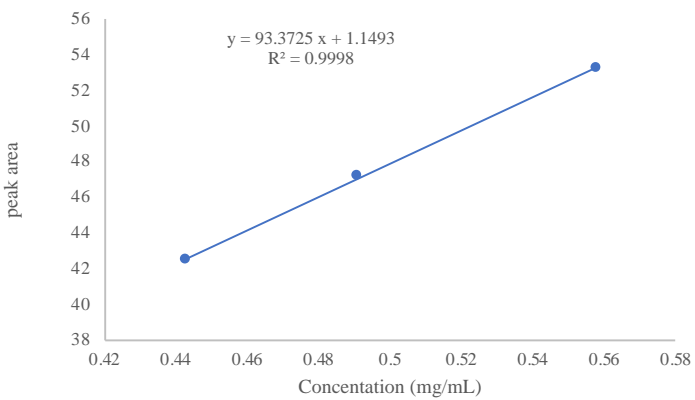
Laboratory 9 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
46.53	464.5555	3768817.0000	3769037.5000	0.99920
		3769258.0000		
49.79	497.1034	4018840.0000	4017693.5000	
		4016547.0000		
55.24	551.5162	4506104.0000	4503302.0000	
		4500500.0000		
Regression equation:	$y = ax + b$ (a=8493.0331; b=-187137.7282)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	51.43	4132174.0000	988.86	993.00
	51.62	4184403.0000	997.13	
TC-2	51.01	4065780.0000	981.68	986.61
	49.90	4015020.0000	991.54	
SC-1	1003.61	4136303.0000	50.72	50.71
	1007.19	4148699.0000	50.69	
SC-2	1000.42	4258336.0000	52.32	52.31
	1005.41	4279121.0000	52.30	
SC-3	1009.09	4221468.0000	51.44	51.44
	1002.88	4193619.0000	51.43	

Laboratory 10 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
43.10	0.4303	40.9970	40.9330	1.00000
		40.8690		
51.34	0.5126	48.8140	48.8035	
		48.7930		
56.22	0.5613	53.5300	53.5030	
		53.4760		
Regression equation:	$y = ax + b$ (a=95.9203; b=-0.3479)			
<p>Scatter plot showing peak area (y-axis, 38 to 56) versus Concentration (mg/mL) (x-axis, 0.42 to 0.58). The regression equation is $y = 95.9203x - 0.3479$ and $R^2 = 1.0000$.</p>				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
	TC-1	49.12	46.0175	
	50.74	47.9085	977.21	973.26
TC-2	51.69	48.5090	971.36	971.29
	50.21	47.1235	971.22	
SC-1	992.58	47.7425	49.78	49.74
	1026.44	49.2735	49.69	
SC-2	996.67	49.6005	51.52	51.48
	985.94	48.9860	51.43	
SC-3	983.19	47.8750	50.40	50.37
	994.04	48.3465	50.34	

Laboratory 10 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.37	0.4430	42.4930	42.4600	0.99989
		42.4270		
49.19	0.4911	47.0930	47.0960	
		47.0990		
55.92	0.5583	53.2440	53.2410	
		53.2380		
Regression equation:	$y = ax + b$ (a=93.3725; b=1.1493)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{sp})$	Average (g/kg)
TC-1	49.57	46.7620	985.48	984.78
	50.07	47.1560	984.07	
TC-2	49.91	46.5920	975.12	976.45
	52.80	49.3540	977.77	
SC-1	1015.09	48.6590	50.13	50.10
	1006.06	48.1720	50.06	
SC-2	998.92	49.5250	51.87	51.88
	1000.61	49.6325	51.89	
SC-3	992.89	48.2825	50.84	50.85
	991.58	48.2385	50.86	

Laboratory 11 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
46.20	461.2608	61.4412	61.5451	0.99999
		61.6489		
51.60	515.1744	68.2199	68.2558	
		68.2917		
56.30	562.0992	74.2155	74.1688	
		74.1220		
Regression equation:	$y = ax + b$ (a=0.1252; b=3.7972)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
	$(y-b)*V*1000/(a*W_{spl})$			
TC-1	51.70	68.4903	999.45	998.45
	50.40	66.7365	997.44	
TC-2	50.60	66.8980	996.05	990.62
	51.10	66.8264	985.18	
SC-1	1010.70	67.4903	50.33	50.36
	1010.40	67.5317	50.38	
SC-2	1019.30	70.3476	52.15	52.16
	1001.10	69.1830	52.17	
SC-3	1005.00	67.7838	50.85	50.91
	1019.90	68.8733	50.96	

Laboratory 12 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (g)	Concentration of calibration solution C (g/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
0.04505	0.4498	3551.6182	3562.3524	0.99997
		3573.0867		
0.05011	0.5003	3967.1799	3962.6467	
		3958.1135		
0.05545	0.5536	4395.9619	4397.2629	
		4398.5640		
Regression equation:	$y = ax + b$ (a=8044.4757; b=-58.0720)			
Sample code	Mass of sample W_{spl} (g)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	0.05104	3990.8640	986.13	988.43
	0.05220	4102.2144	990.73	
TC-2	0.05201	4098.6992	993.51	995.65
	0.05100	4035.5513	997.79	
SC-1	1.05776	4162.0669	49.60	49.56
	1.02430	4021.1863	49.51	
SC-2	1.01448	4159.3618	51.68	51.54
	1.01782	4149.4868	51.39	
SC-3	1.05338	4217.3384	50.45	50.43
	1.01140	4043.5273	50.41	

Laboratory 12 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (g)	Concentration of calibration solution C (g/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
0.04545	0.4538	3607.0359	3608.8925	0.99996
		3610.7490		
0.05028	0.5020	3967.1194	3967.7837	
		3968.4480		
0.05479	0.5470	4302.3091	4313.8835	
		4325.4580		
Regression equation:	$y = ax + b$ (a=7562.8800; b=175.0212)			
Sample code	Mass of sample W_{spl} (g)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	0.05249	4096.6074	987.87	986.16
	0.05092	3966.1389	984.45	
TC-2	0.05137	4034.4102	993.39	993.00
	0.05051	3966.7734	992.60	
SC-1	1.00569	3968.1157	49.87	49.87
	1.00895	3980.0349	49.87	
SC-2	1.03343	4205.9580	51.57	51.58
	1.02968	4191.9268	51.58	
SC-3	1.04644	4185.0190	50.67	50.52
	1.04298	4147.1792	50.36	

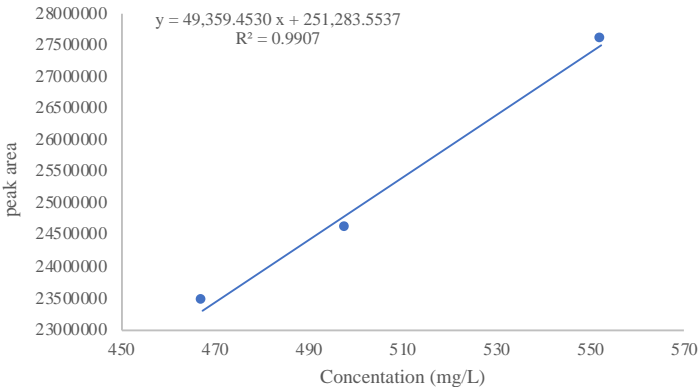
Laboratory 13 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	50	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
22.90	0.4573	8360	8359.50	0.99961
		8359		
24.90	0.4972	9171	9167.00	
		9163		
27.70	0.5531	10196	10191.00	
		10186		
Regression equation:	$y = ax + b$ (a=19066.4877; b=-342.3789)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg) $(y-b)*V*1000/(a*W_{spl})$	Average (g/kg)
TC-1	25.30	9179	986.91	987.14
	27.70	10087	987.37	
TC-2	24.50	8868	985.80	987.94
	26.50	9663	990.07	
SC-1	502.00	9231	50.01	49.82
	642.00	11805	49.62	
SC-2	535.00	10084	51.10	50.99
	630.00	11881	50.88	
SC-3	630.00	11687	50.07	50.23
	639.00	11936	50.39	

Laboratory 13 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	50	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
21.10	0.4213	7415	7410.50	0.99995
		7406		
25.30	0.5052	8884	8886.00	
		8888		
27.40	0.5471	9661	9652.00	
		9643		
Regression equation:	$y = ax + b$ (a=17784.8241; b=-86.4056)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	22.40	7787	988.11	989.12
	25.60	8930	990.12	
TC-2	26.70	9309	989.29	987.72
	32.30	11244	986.15	
SC-1	323.00	5648	49.91	49.75
	610.00	10674	49.59	
SC-2	477.00	8544	50.86	50.85
	609.00	10924	50.83	
SC-3	615.00	10916	50.30	50.35
	623.00	11080	50.39	

Laboratory 14 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
46.79	467.1514	23132328.0000	23459059.0000	0.99535
		23785790.0000		
49.88	498.0019	24554876.0000	24597908.0000	
		24640940.0000		
55.31	552.2150	27303184.0000	27593353.0000	
		27883522.0000		
Regression equation:	$y = ax + b$ (a=49359.4530; b=251283.5537)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	52.33	25648942.0000	983.86	982.13
	48.78	23842660.0000	980.40	
TC-2	49.98	24254332.0000	973.56	985.93
	52.94	26321850.0000	998.29	
SC-1	101.22	2536642.0000	45.77	46.73
	113.00	2909282.0000	47.68	
SC-2	108.00	2817609.0000	48.17	48.53
	109.24	2885445.0000	48.88	
SC-3	115.48	3039804.0000	48.95	48.01
	98.42	2536600.0000	47.07	

Laboratory 14 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
44.85	447.7824	17551030.0000	17574175.0000	0.99035
		17597320.0000		
50.23	501.4963	20401060.0000	20368960.0000	
		20336860.0000		
59.31	592.1510	23023734.0000	23238303.0000	
		23452872.0000		
Regression equation:	$y=ax+b$ (a=38438.9300; b=643509.9049)			
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	49.02	19219834.0000	985.86	984.37
	49.04	19171112.0000	982.87	
TC-2	48.70	19095894.0000	985.72	984.50
	48.83	19099164.0000	983.27	
SC-1	100.80	2580075.0000	49.98	51.92
	104.93	2815865.0000	53.86	
SC-2	94.00	2506253.0000	51.55	52.58
	97.64	2655240.0000	53.60	
SC-3	90.22	2409593.0000	50.93	50.97
	90.10	2409682.0000	51.00	

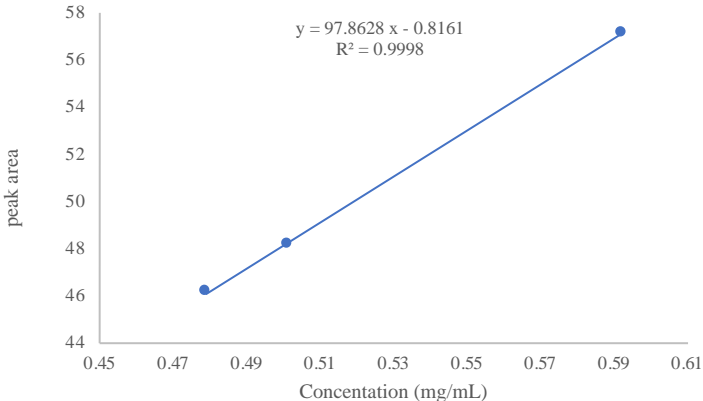
Laboratory 15 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.57	454.9709	3709.7	3709.05	0.99994
		3708.4		
49.97	498.9005	4070.9	4068.95	
		4067.0		
55.60	555.1104	4511.9	4511.35	
		4510.8		
Regression equation:	$y = ax + b$ (a=8.0054; b=69.7972)			
<p>Scatter plot showing peak area (y-axis, 3600 to 4600) versus Concentration (mg/L) (x-axis, 430 to 570). The regression equation is $y = 8.0054x + 69.7972$ and $R^2 = 0.9999$.</p>				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	52.11	4201.8000	990.50	989.63
	53.48	4302.9000	988.75	
TC-2	51.79	4185.1000	992.60	991.16
	51.64	4161.3000	989.72	
SC-1	1008.15	4122.7000	50.22	50.05
	1081.56	4388.1000	49.87	
SC-2	1019.60	4277.7000	51.55	51.54
	1051.01	4405.4000	51.53	
SC-3	1037.59	4269.9000	50.57	50.48
	1109.30	4544.2000	50.39	

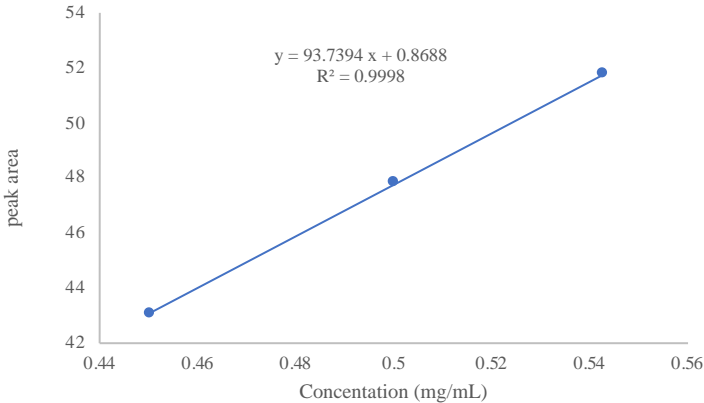
Laboratory 15 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (L):	0.1	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/L)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.71	456.3686	3704.5	3705.50	1.00000
		3706.5		
50.64	505.5898	4098.6	4097.90	
		4097.2		
54.80	547.1232	4429.8	4429.80	
		4429.8		
Regression equation:	$y = ax + b$ (a=7.9806; b=63.2759)			
<p>Scatter plot showing peak area (y-axis, 3600 to 4500) versus Concentration (mg/L) (x-axis, 430 to 570). The regression equation is $y = 7.9806x + 63.2759$ and $R^2 = 1.0000$.</p>				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	50.67	4069.0	990.59	988.15
	50.57	4041.4	985.71	
TC-2	51.18	4102.6	988.95	988.82
	52.05	4170.2	988.69	
SC-1	970.33	3936.3	50.01	49.99
	991.38	4015.7	49.96	
SC-2	1050.72	4405.1	51.78	51.81
	1041.41	4370.8	51.83	
SC-3	1023.45	4208.1	50.75	50.78
	1019.36	4197.0	50.81	

Laboratory 16 (day 1)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
48.00	0.4792	46.1011	46.1442	0.99992
		46.1873		
50.20	0.5012	48.2632	48.1528	
		48.0424		
59.30	0.5921	57.0203	57.1441	
		57.2678		
Regression equation:	$y = ax + b$ (a=97.8628; b=-0.8161)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y - b) * V * 1000 / (a * W_{spl})$	
TC-1	52.10	49.6628	990.04	991.41
	50.20	47.9562	992.78	
TC-2	50.60	48.2857	991.58	991.89
	49.90	47.6359	992.19	
SC-1	1004.90	48.7937	50.45	50.49
	994.00	48.3249	50.52	
SC-2	1008.70	50.3365	51.82	52.00
	1014.10	50.9555	52.17	
SC-3	1003.20	49.0722	50.82	50.86
	991.30	48.5565	50.89	

Laboratory 16 (day 2)

Purity of reference substance (%):	99.84	Volume of Solution, V (mL):	100	
Mass of reference substance (mg)	Concentration of calibration solution C (mg/mL)	Peak area	Average peak area A	Correlation Coefficient $r = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum(C_i - \bar{C})^2 \sum(A_i - \bar{A})^2}}$
45.10	0.4503	43.0518	43.0465	0.99990
		43.0411		
50.10	0.5002	47.7797	47.8291	
		47.8784		
54.40	0.5431	51.6602	51.7401	
		51.8199		
Regression equation:	$y=ax+b$ (a=93.7394; b=0.8688)			
				
Sample code	Mass of sample W_{spl} (mg)	Average of Peak area y	Content (g/kg)	Average (g/kg)
			$(y-b)*V*1000/(a*W_{spl})$	
TC-1	49.10	46.5475	992.45	993.82
	53.60	50.8715	995.19	
TC-2	50.30	47.4384	987.67	988.43
	50.60	47.7880	989.19	
SC-1	1011.10	49.0315	50.82	50.94
	1019.30	49.6487	51.05	
SC-2	1001.00	50.0242	52.39	52.40
	1007.50	50.3637	52.41	
SC-3	1012.60	49.2374	50.96	50.91
	1001.50	48.6199	50.86	