GIBBERELLIC ACID

Collaborative Study

Full Scale Collaborative Study for the Determination of
Gibberellic acid in TC by HPLC

Report to CIPAC by
Chinese Pesticide Analytical Committee（CHIPAC）

Method Developed by
Zhejiang Qianjiang Biochemical Co., Ltd.

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Content

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1. Participants

20 laboratories enrolled in the collaborative trial on the determination of Gibberellic acid according to CIPAC Information Sheet No. 339. But due to the shipment blocking, 1 laboratory could not receive the sample. By mid of March 2024, 19 laboratories provided their results. The results for the 19 participants are presented in the following section.

Participating laboratories are listed in the table below.

Contact person	Participating Laboratory	Country
Moustafa A. Khalifa	AL-Sharahan Laboratory for Chemical Testing, Al Sharhan Industries, Kuwait	Kuwait
Liang Huang	Anhui Fengle Agrochemical Co., Ltd. Product Testing Center	China
Cornel Grecu	Biochem - ROMANIA Quality Control Laboratory	Romania
Dagmar Julínková	Central institute for supervising and testing in agriculture National Reference Laboratory, Department of testing plant protection products	Czech Republic
Chunqing Hou, Hongfeng Sun	China National Pesticide Quality Inspection and Testing Center (Shenyang)	China
Lisha Zhang	Chongqing Chemical Pesticide Quality Supervision and Inspection Station	China
Frank Fan, Rongmei Chen	GreenTech Laboratory Co., Ltd.	China
Lu Huang	Hunan Research Institute of Chemical Industry Testing Technology Co., Ltd.	China
Peize Li	Institute for the Control of Agrochemicals, Ministry of Agricultrue and Rural Affairs	China
Jianzhong Yu	Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences	China
Angela Santilio	National Institute of Health (Istituto Superiore di Sanità)	Italy
Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.	China
Yily Yan	Jiangsu Rotam Chemistry Co., Ltd.	China
Hongxia Li, Mingwei Zhu	Nutrichem (Shandong) Laboratory Co., Ltd	China
Yuying Wang, Daifeng Wang	Pesticide Quality Supervision, Inspection and Testing Center in Shenyang, MOA	China

| Agus Salim	PT Agriculture Construction (AGRICON)	Indonesia
Christian Mink	Syngenta Crop Protection AG	Switzerland
Yongfei Guo	Testing Technology Center of Sino-Agri Leading Bioscience Co., Ltd.	China
Vanessa Lecocq	Walloon Agricultural Research Centre (CRA-W) Protection, control products and residues Unit (U10)	Belgium

2. Gibberellic acid, General Information

Chemical name: (1α,2β,4aα,4bβ,10β)-2,4a,7-trihydroxy-1 -methyl-8-methylenegibb-3-ene-1,10-

dicarboxylic acid 1,4a-lactone

Common name: Gibberellic acid

CAS-Number: 77-06-5

Structure:
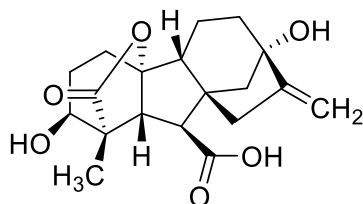
Molecular mass: 346.4 g/mol

Empirical formula: $C_{19}H_{22}O_6$

3. Samples

In October 2023, Information Sheet No. 344 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Gibberellic acid by HPLC.

Three test samples (described below), including the Gibberellic acid analytical reference standard were shipped to the participants:

A) Gibberellic acid TC-1
B) Gibberellic acid TC-2
C) Gibberellic acid TC-3

Gibberellic acid analytical reference standard (99.4% purity)

All participants sent back their results in time.

4. Method

4.1 Scope

The content of Gibberellic acid in technical materials was determined.

4.2 Principle

The content of Gibberellic acid in the sample is determined by high performance liquid chromatography on a reversed phase column (C_{18}) with UV detection at 210 nm and external standardization.

4.3 Procedure

Samples should be analyzed in duplicate at two different days resulting in a total of four individual test results for each sample. All test solutions should be prepared freshly on Day 2.

5. Remarks of the Participants

Participants made comments about the performance of the method and noted deviations from the method. Below is a summary of specific method conditions provided by the participating laboratories. No remark was feedback from participants.

Lab Number	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (µL)	Mobile phase (v/v)
1	Waters, Alliance	Agilent Zorbax Extend-C$_{18}$ (150×4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
2	Thermo Vanquish Flex	Zorbax Eclipse-C$_{18}$ (150× 0.46 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
3	Agilent 1100 Series	Agilent Zorbax SB-C$_{18}$ (150× 4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
4	Perkin Elmer FLEXAR	Zorbax SB-C$_{18}$ (250×4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
5	Agilent 1260 Infinity II	Agilent Zorbax SB-C$_{18}$ (150× 4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
6	Agilent 1100	Agilent Zorbax SB-C$_{18}$ (150× 4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
7	Agilent 1260	Agilent Zorbax SB-C$_{18}$ (150× 4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
8	Agilent 1260	Agilent SB-C$_{18}$ (150×4.6 mm, 5 µm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)

Lab Number	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (μL)	Mobile phase (v/v)
9	Agilent 1260 infinity Ⅱ	Agilent SB-C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
10	Agilent 1260	Zorbax SB-C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
11	Agilent 1260 infinity Ⅱ	Agilent SB-C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
12	Agilent 1260 Ⅱ	Agilent Zorbax Eclipse Plus C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
13	Shimadzu 20AT	Thermo hypersyl ODS (150× 4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
14	Agilent 1260 infinity Ⅱ	Intertsustain C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
15	LC-20AD	Zorbax SB-C_{18} (150×4.6 mm, 5 μm)	1	30	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
16	Agilent 1260 infinity	Agilent SB-C_{18} (150×4.6 mm, 5 μm)	1	30	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
17	Agilent 1260 Ⅱ	Zorbax SB-C_{18} (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
18	Agilent 1260 Ⅱ	Waters ODS2 (150×4.6 mm, 5 μm)	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)
19	Agilent 1200	Venusil XBP C_{18} 150mm×4.6mm，5μm	1	33	210	5	MeOH : 0.05 % phosphoric acid aqueous solution 33 : 67 (v/v)

6. Evaluation and Discussion

6.1 Evaluation of the Quality of Data and Chromatograms

The data obtained from each of the laboratories were reviewed to determine if there were any significant deviations regarding the chromatography which might affect the analysis results.
Visual examination of the chromatograms showed no evidence for invalid data.
All other changes and observations noted by the 19 participants were not expected to affect the analysis results significantly.

6.2 Determination of Gibberellic acid

Results reported by the laboratories and the statistical evaluation are listed in tables 1-3 and displayed in figures 1-3.

The statistical evaluation of the data was done following the "Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods", according to DIN ISO 5725. The data were examined for outliers and stragglers using the Grubb's test, and the tests were performed at an alpha level of 0.01 for outlier (marked with **), and 0.05 for straggler (marked with *).

After Grubb's test, no outliers or stragglers have been found for all laboratories (19 participants). The between lab experimental Relative Reproducibility Standard Deviation (RSD_R) is below the Horwitz value for all samples (see Table 3), and the HorRat values were between 0.3 to 1.0. Due to the universal applicability of the method, this collaborative trial is acceptable.

Table 1:   Gibberellic acid (g/kg); Results for each laboratory on day 1 and day 2

	Gibberellic acid TC-1		Gibberellic acid TC-2		Gibberellic acid TC-3	
	Day1	Day2	Day1	Day2	Day1	Day2
Lab 1	913.9	910.0	915.9	913.9	914.6	918.6
Lab 2	913.4	915.2	913.4	915.4	919.2	919.7
Lab 3	912.8	913.3	915.3	916.2	915.9	917.4
Lab 4	883.7	923.7	915.8	925.2	914.4	881.1
Lab 5	914.5	916.5	916.4	916.1	922.9	917.3
Lab 6	902.0	911.6	907.9	912.5	909.9	913.6
Lab 7	911.7	912.3	912.5	909.9	915.2	914.1
Lab 8	910.7	912.9	916.4	914.7	914.8	911.8
Lab 9	914.4	915.5	915.3	915.0	916.6	917.1
Lab 10	914.8	915.0	912.7	913.0	915.4	915.5
Lab 11	904.1	904.3	911.9	911.0	910.6	908.3
Lab 12	912.7	912.4	915.6	910.9	920.2	915.4
Lab 13	907.1	901.4	905.9	901.7	906.1	903.9
Lab 14	918.2	913.4	918.6	915.8	926.9	923.5
Lab 15	908.6	911.9	909.3	911.0	913.6	913.3
Lab 16	883.8	903.7	903.8	912.4	902.9	912.5
Lab 17	909.6	908.3	910.3	908.5	910.7	907.8
Lab 18	918.0	924.5	921.0	912.1	927.8	911.1
Lab 19	924.4	917.7	917.8	919.3	919.0	921.7

Table 2: Mean values

	Gibberellic acid TC-1	Gibberellic acid TC-2	Gibberellic acid TC-3
Lab 1	911.9	914.9	916.6
Lab 2	914.3	914.4	919.4
Lab 3	913.0	915.8	916.7
Lab 4	903.7	920.5	897.7
Lab 5	915.5	916.3	920.1
Lab 6	906.8	910.2	911.7
Lab 7	912.0	911.2	914.6
Lab 8	911.8	915.6	913.3
Lab 9	914.9	915.1	916.8
Lab 10	914.9	912.8	915.5
Lab 11	904.2	911.4	909.4
Lab 12	912.5	913.2	917.8
Lab 13	904.2	903.8	905.0
Lab 14	915.8	917.2	925.2
Lab 15	910.2	910.1	913.4
Lab 16	893.7	908.1	907.7
Lab 17	908.9	909.4	909.2
Lab 18	921.3	916.5	919.4
Lab 19	921.0	918.5	920.4

Table3: Summary of the statistical evaluation - no elimination of any outliers /stragglers

	Gibberellic acid TC-1	Gibberellic acid TC-2	Gibberellic acid TC-3
xm [g/kg]	911.3	913.6	914.6
L	19	19	19
S_r	13.13	7.172	11.91
S_R	13.15	7.451	12.37
S_L	0.6770	2.021	3.356
r	36.77	20.08	33.35
R	36.82	20.86	34.65
RSD_r	1.441	0.7850	1.302
RSD_R	1.443	0.8156	1.353
RSD_R (Hor)	2.028	2.027	2.027
HorRat	0.7	0.4	0.7

X_m = overall sample mean

L = number of laboratories

S_r = repeatability standard deviation

RSD_r = relative repeatability standard deviation

r = repeatability limit

S_R = reproducibility standard deviation

RSD_R = relative reproducibility standard deviation

R = reproducibility limit

S_L = "pure" between laboratory standard deviation

RSD_R (Hor) = relative reproducibility standard deviation (Horwitz equation)
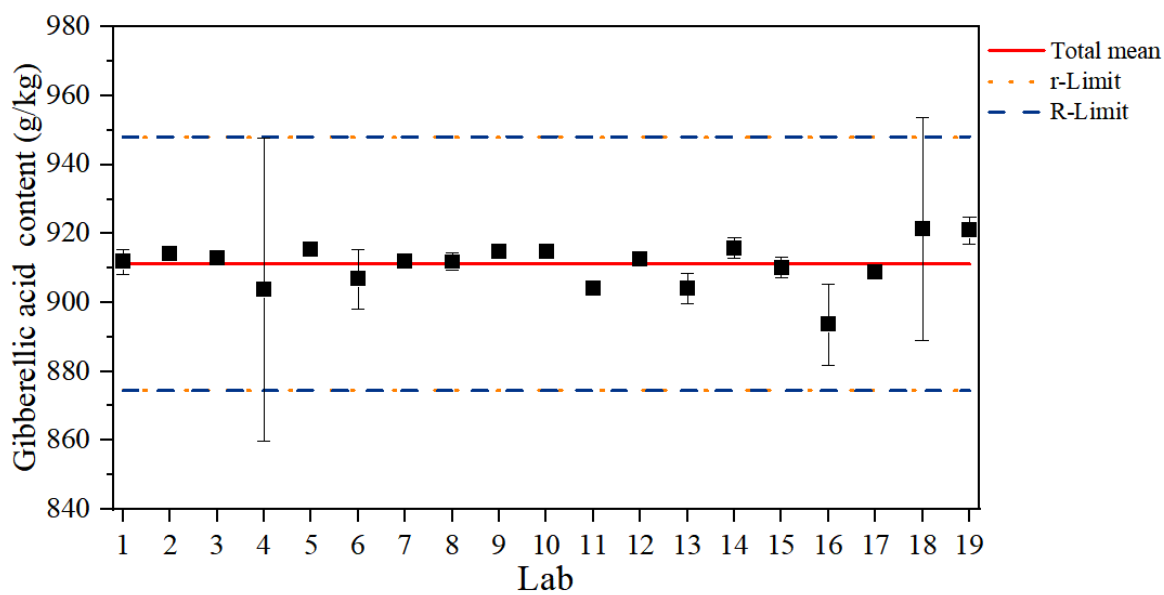
Figures 1 – 3 (all results)

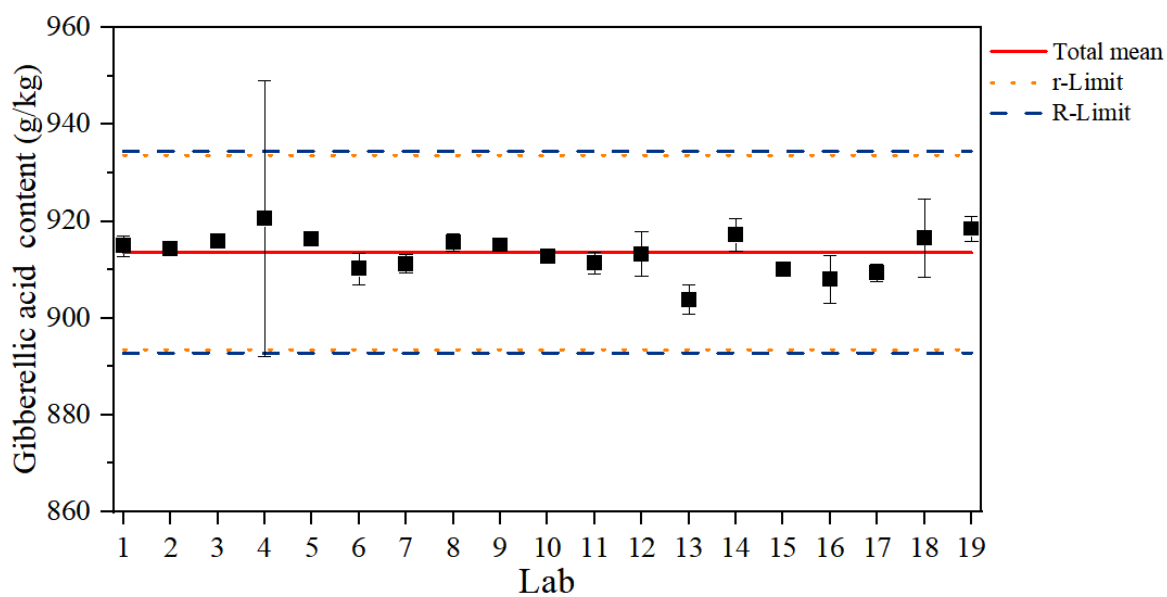Figure 1. Graphical presentation of TC1 data
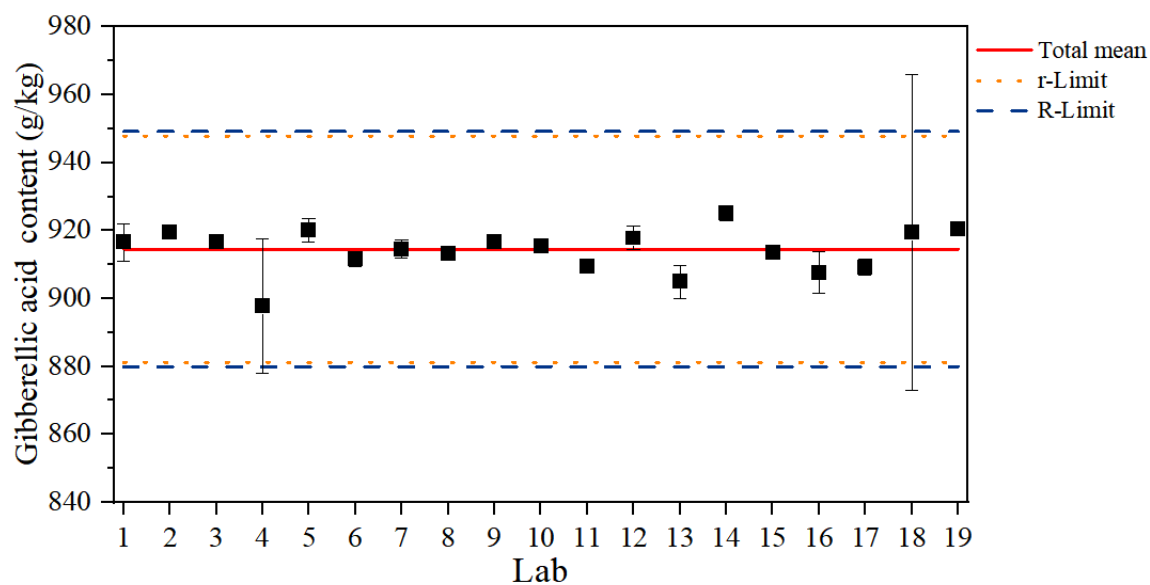

Figure 2. Graphical presentation of TC2 data

Figure 3. Graphical presentation of TC3 data

7. Conclusions

A total of 20 laboratories have participated in this full scale collaborative study, and data from 19 participants have been included in the final data statistical. The data presented in the statistical summary show that the HorRat values all within the required range. That is evidence for the fact that the present HPLC method is acceptable and suitable to produce reproducible results.

Therefore, CHIPAC considers this method to be suitable and recommend accepting it as a provisional CIPAC method for the determination of Gibberellic acid in technical materials.