GIBBERELLIC ACID

Collaborative Study

Small scale collaborative study for the

Determination of Gibberellic acid in Gibberellic acid TC by

HPLC

Report to CIPAC by CHIPAC

Method Developed by

Zhejiang Qianjiang Biochemical Co., Ltd.

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1. Participants

Small Scale Collaborative Study for the determination of gibberellic acid TC by HPLC was organized by CHIPAC, and participated by 4 labs. All the 4 laboratories provided their results, which are presented in the following sections.

Index	Contact	Lab	Address
Lab 1	Zhiyu He	Guizhou Jianande Technology Co., LTD	Guizhou Jiandee Technology Co., LTD Address: Baijin road No.3491, Baiyun district, Guiyang, P. R. China
Lab 2	Jianxin Zhu	Zhejiang Qianjiang Biochemistry Co., LTD	No.9, Shidai Road, Haichang Sub-district, Haining City, Jiaxing City, Zhejiang, P.R.China
Lab 3	Jianzhong Yu	Institute of Quality Safety and Nutrition of Agricultural Products, Zhejiang Academy of Agricultural Sciences	198 Shiqiao Road, Shangcheng District, Hangzhou City, Zhejiang Province, China
Lab 4	Lu Huang	Hunan Chemical Research Institute Testing Technology Co. LTD	No. 550, Changsha Avenue, Lituo street, Yuhua District, Changsha City, Hunan Province, China

2. Active Ingredient: General Information

ISO common name: Gibberellic acid
CAS No.: 77-06-5
Structure:
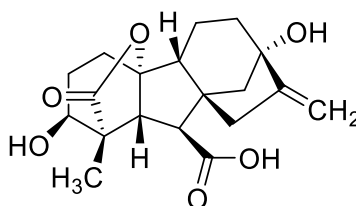
Molecular mass: 346.4
Empirical formula: $C_{19}H_{22}O_6$
Activity: Plant growth regulation

3. Samples

Three test samples and gibberellic acid analytical standard were sent to the participants:

(1) Gibberellic acid tech. sample (TC-1)

(2) Gibberellic acid tech. sample (TC-2)

(3) Gibberellic acid tech. sample (TC-3)

(4) Gibberellic acid, reference standard (purity 994 g/kg)

All participants sent back their results in time.

4. Method

4.1 Scope

The determination of gibberellic acid active ingredient content was assayed in technical material (TC).

4.2 Outline of method

Gibberellic acid is determined by high performance liquid chromatography on a reversed phase column (C18) with UV detection at 210 nm and external standardization.

4.3 Procedure for the collaborative trial

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.

5. Analytical conditions

Lab	Instrument	Column	Flow Rate (ml/min)	Column Temp. (℃)	Wavelength (nm)	Injection Volume (μl）	Mobile phase (v/v)
1	Agilent 1260	Agilent ZORBAX SB-C$_{18}$ (150×4.6 mm, 5 μm)	1.0	33	210	5	methanol: 0.05% phosphoric acid aqueous solution, 33:67
2	SHIMADZU LC-20AT	Agilent ZORBAX SB-C$_{18}$ (150×4.6 mm, 5 μm)	1.0	33	210	5	methanol: 0.05% phosphoric acid aqueous solution, 33:67
3	Agilent 1260	Waters XTERRA MS C$_{18}$ (150×4.6 mm, 5 μm)	1.0	33	210	5	methanol: 0.05% phosphoric acid aqueous solution, 33:67
4	Agilent 1260	Agilent ZORBAX SB-C$_{18}$	1.0	33	210	5	methanol: 0.05% phosphoric

		(150×4.6 mm, 5 µm)				acid aqueous solution, 33:67

6. Deviations and Remarks

because it was found that samples could not be completely dissolved by methanol and mobile phase, Lab 4 add ultrasonic steps in the preparation process of samples solution to ensure samples dissolution which was deemed not to affect the analytical results significantly.

7. Evaluation and Discussion

7.1 Evaluation of the Quality of Data and Chromatograms

The data obtained from each laboratory was visually reviewed and no significant deviation regarding the chromatography which might affect the analysis results was founded.

Therefore, all data sets were included within the statistical assessment. The report below contains statistical evaluations with the full set of 4 participating laboratories.

7.2 Determination of gibberellic acid

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 not performed.

In Tables 1-6 and Figures 1-3, respectively, the full set of all laboratories (4 participants) are reported. A comparison of the RSD_R of this collaborative study with the unmodified Horwitz equation showed that the reproducibility relative standard deviation (RSD_R) is lower than the Horwitz value for all the products (see in Table 4-6). The Horwitz Ratio (HorRat) for each sample was found within the desired range (0.3-1.0). Due to the universal applicability of the method, this collaborative trial is acceptable.

8. Conclusions

From the results shown above, the method can be considered applicable for the determination of gibberellic acid contents in TC. CHIPAC propose that a full scale collaborative trial might be conducted.

**Table 1 Results of analysis of gibberellic acid content in the TC1**

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi²	Standard Deviation Sᵢ	Sᵢ²
	1	2	1	2				
Lab 1	905.1	904.0	904.2	907.5	905.20	819387.0400	1.6062	2.5800
Lab 2	917.2	913.1	911.3	916.2	914.45	836218.8025	2.7307	7.4567
Lab 3	911.0	910.4	912.4	915.1	912.23	832154.4506	2.0918	4.3758
Lab 4	911.5	905.9	907.3	904.9	907.40	823374.7600	2.9052	8.4400

Table 2 Results of analysis of gibberellic acid content in the TC2

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi²	Standard Deviation Sᵢ	Sᵢ²
	1	2	1	2				
Lab 1	909.5	903.8	906.3	902.8	905.60	820111.3600	2.9878	8.9267
Lab 2	916.9	920.0	917.4	916.1	917.60	841989.7600	1.6872	2.8467
Lab 3	913.1	913.1	914.4	914.4	913.75	834939.0625	0.7506	0.5633
Lab 4	912.4	912.6	911.2	910.1	911.58	830968.9806	1.1615	1.3492

Table 3 Results of analysis of gibberellic acid content in the TC3

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi²	Standard Deviation Sᵢ	Sᵢ²
	1	2	1	2				
Lab 1	910.8	910.1	907.6	911.2	909.93	827963.5056	1.6153	2.6092
Lab 2	918.9	920.5	915.9	916.9	918.05	842815.8025	2.0551	4.2233
Lab 3	911.9	912.6	915.8	916.5	914.20	835761.6400	2.2876	5.2333
Lab 4	901.8	908.0	907.9	901.7	904.85	818753.5225	3.5800	12.8167
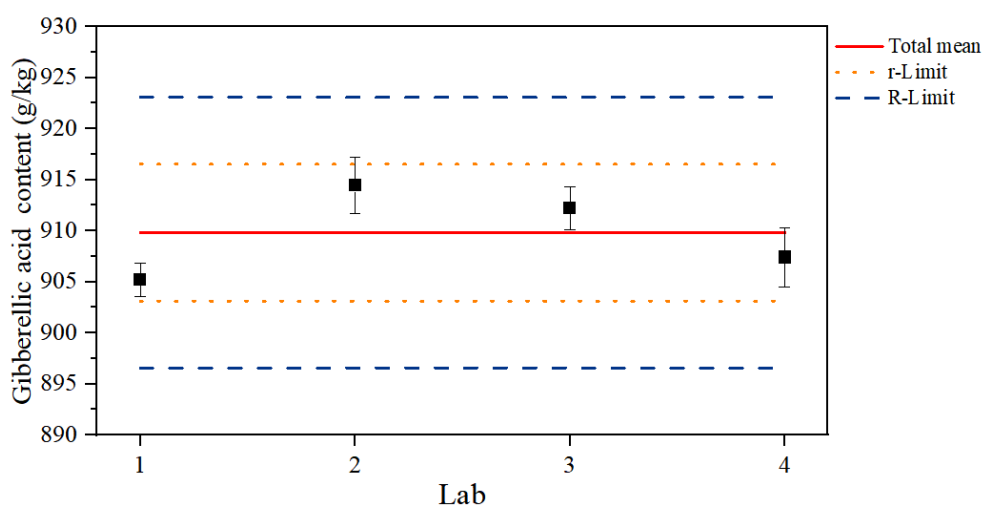
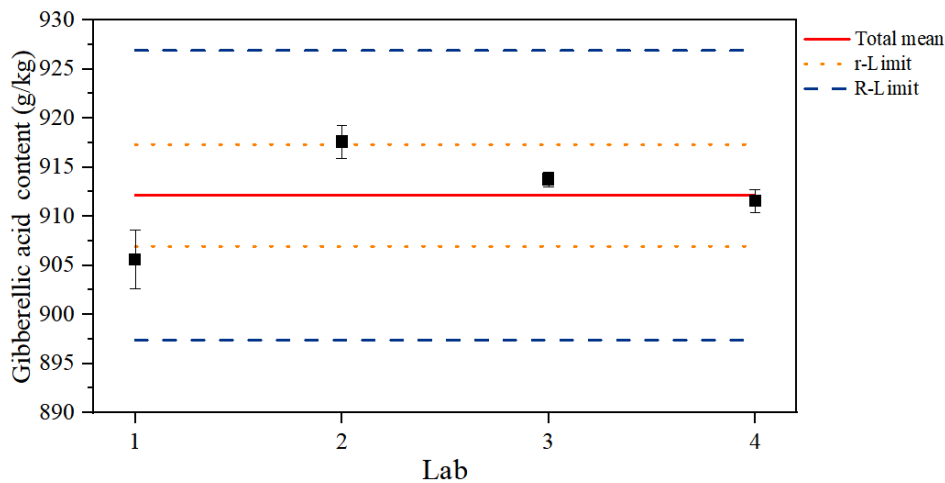**Figure 1. Graphical presentation of TC1 data**

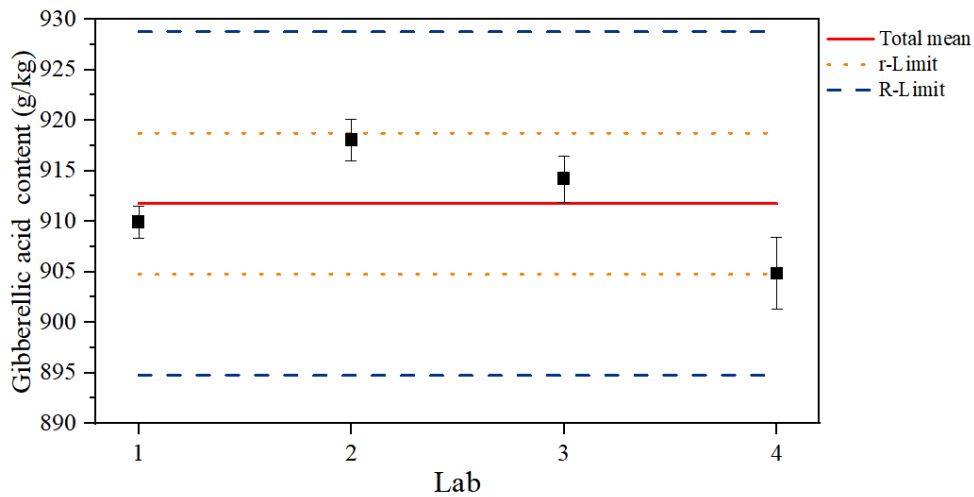**Figure 2. Graphical presentation of TC2 data**

Figure 3. Graphical presentation of TC3 data

Table 4 Statistics of the results of TC1

S_1=Sum Yi	3639.28		
S_2=Sum Yi^2	3311135.0531		
S_3=Sum Si^2	22.8525		
No. Lab P	4		
No. Determination n	4		
Average Y=S_1/P	909.82		
Sr^2=S_3/P	5.7131	S_r	2.3902
S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n	16.7123	S_L	4.0881
S_R^2=S_r^2+S_L^2	22.4254	S_R	4.7355
Repeatability r=2.8*S_r	6.6926		
Reproducibility R=2.8*S_R	13.2595		

| Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.2627
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.5205
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.0287
HorRat	0.3628

Table 5 Statistics of the results of TC2

S_1=Sum Yi	3648.53		
S_2=Sum Yi^2	3328009.1631		
S_3=Sum Si^2	13.6858		
No. Lab P	4		
No. Determination n	4		
Average Y=S_1/P	912.13		
Sr^2=S_3/P	3.4215	S_r	1.8497
S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n	24.3094	S_L	4.9305
S_R^2=S_r^2+S_L^2	27.7308	S_R	5.2660
Repeatability r=2.8*S_r	5.1792		
Reproducibility R=2.8*S_R	14.7448		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.2028		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.5773		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.0279		
HorRat	0.4026		

Table 6 Statistics of the results of TC3

S_1=Sum Yi	3647.03		
S_2=Sum Yi^2	3325294.4706		
S_3=Sum Si^2	24.8825		
No. Lab P	4		
No. Determination n	4		
Average Y=S_1/P	911.76		
Sr^2=S_3/P	6.2206	S_r	2.4941
S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n	30.6558	S_L	5.5368
S_R^2=S_r^2+S_L^2	36.8765	S_R	6.0726
Repeatability r=2.8*S_r	6.9835		

Reproducibility R=2.8*S$_R$	17.0033
Relative Standard Deviation of Repeatability RSD$_r$=S$_r$*100/Y	0.2736
Relative Standard Deviation of Reproducibility RSD$_R$=S$_R$*100/Y	0.6660
Horwitz RSD$_R$(Hor)=2^[1-0.5*log(Y/1000)]	2.0280
HorRat	0.4645
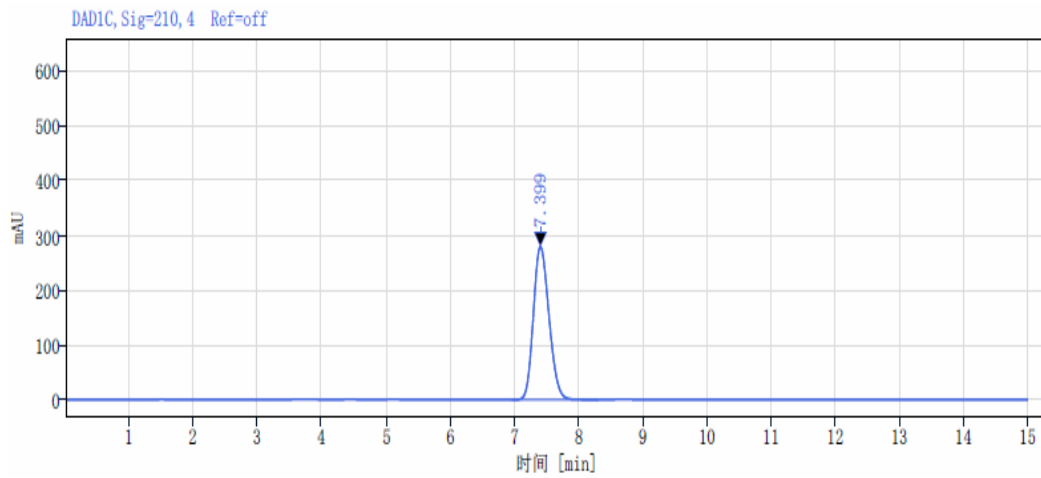
Figure 4. HPLC chromatogram of gibberellic acid standard (Lab 1)
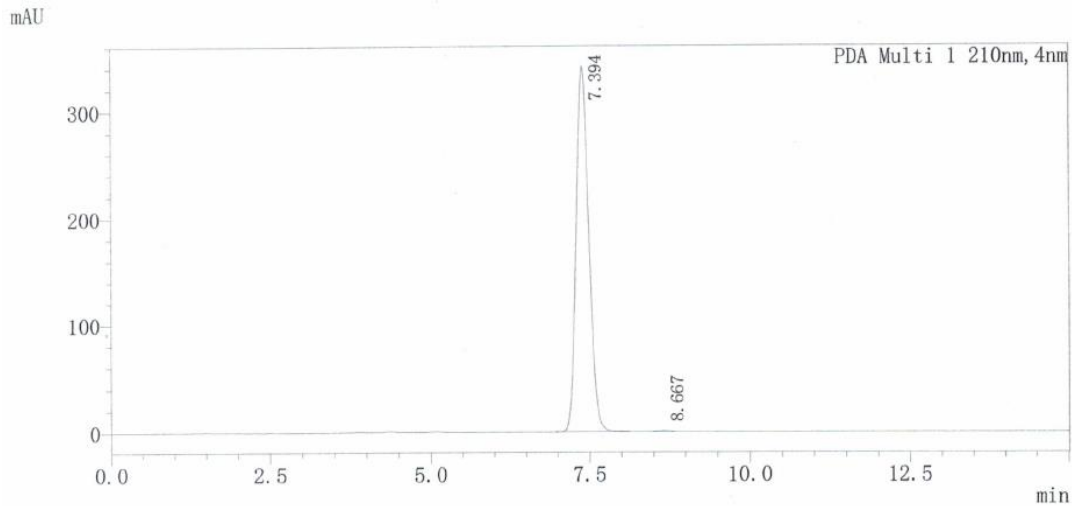
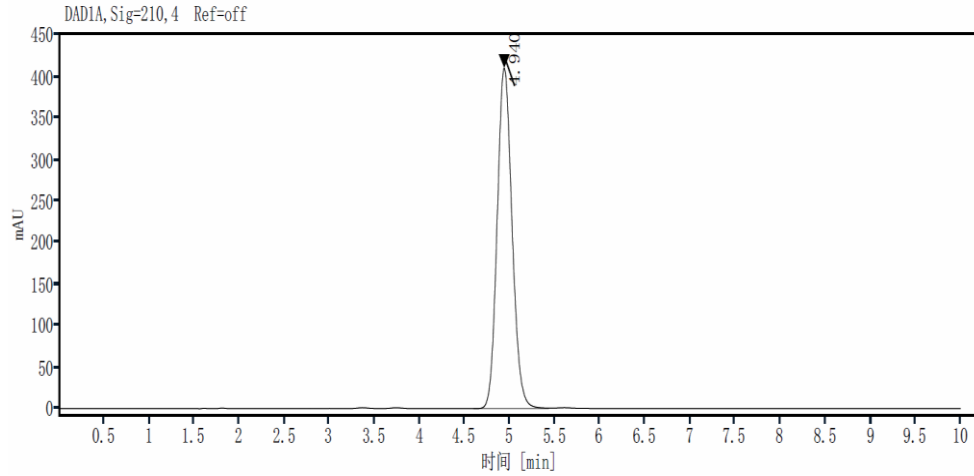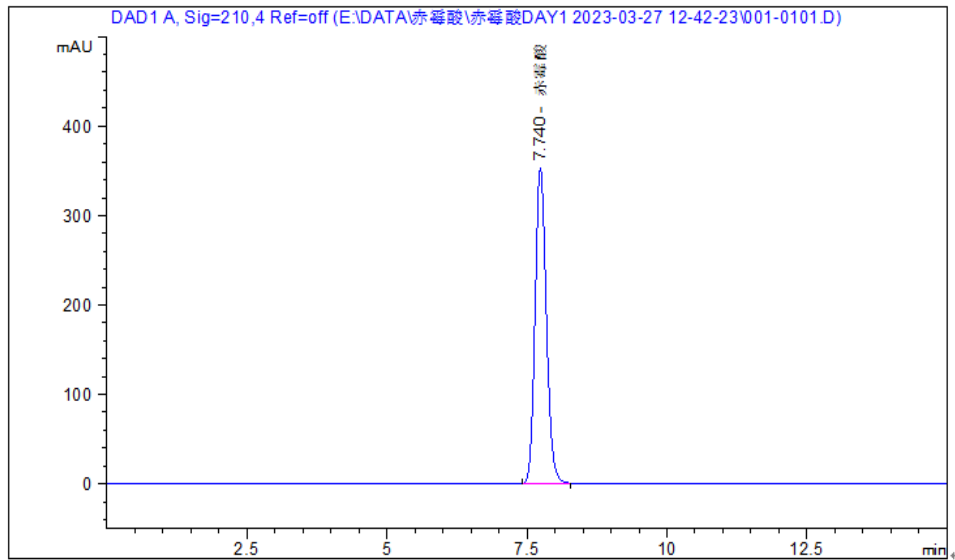**Figure 5. HPLC chromatogram of gibberellic acid standard (Lab 2)**

Figure 6. HPLC chromatogram of gibberellic acid standard (Lab 3)

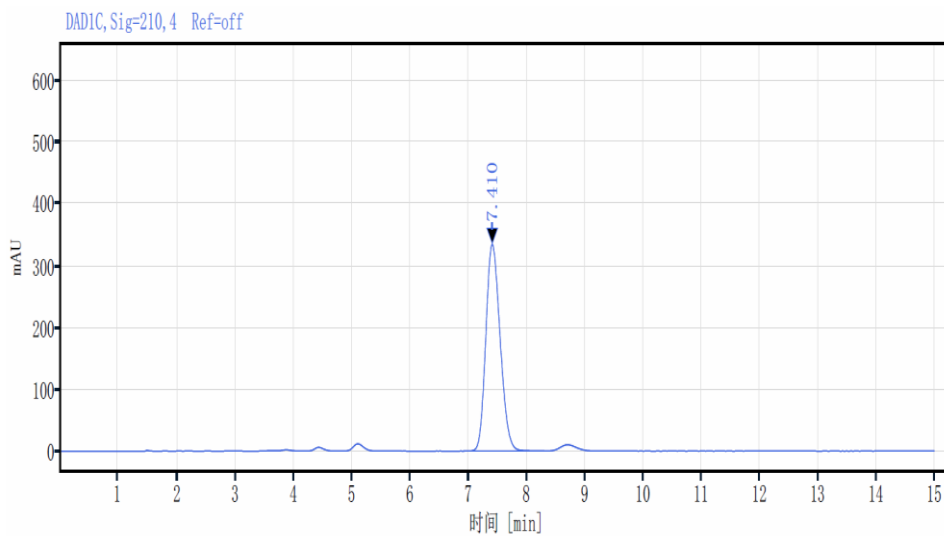**Figure 7. HPLC chromatogram of gibberellic acid standard (Lab 4)**

Figure 8. HPLC chromatogram of gibberellic acid TC (Lab 1)
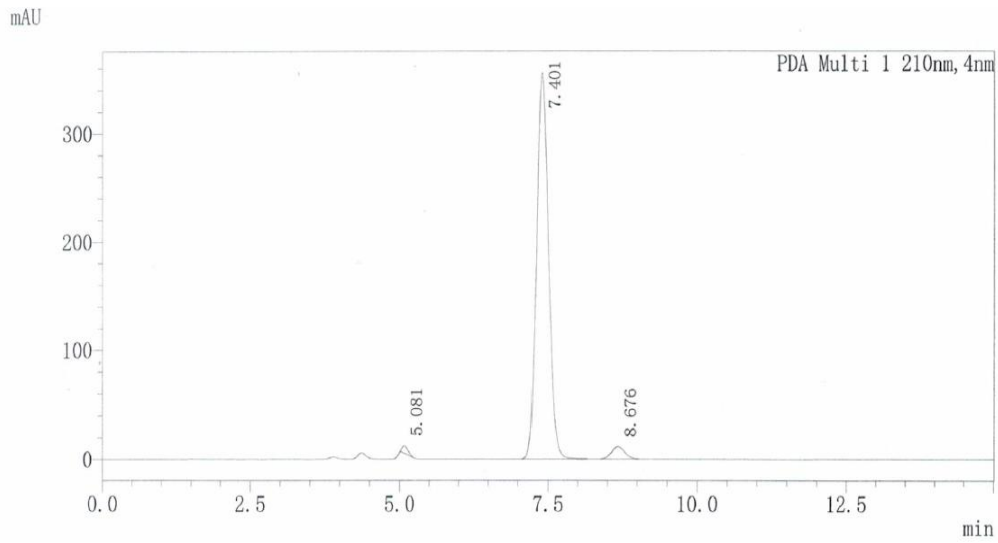
Figure 9. HPLC chromatogram of gibberellic acid TC (Lab 2)
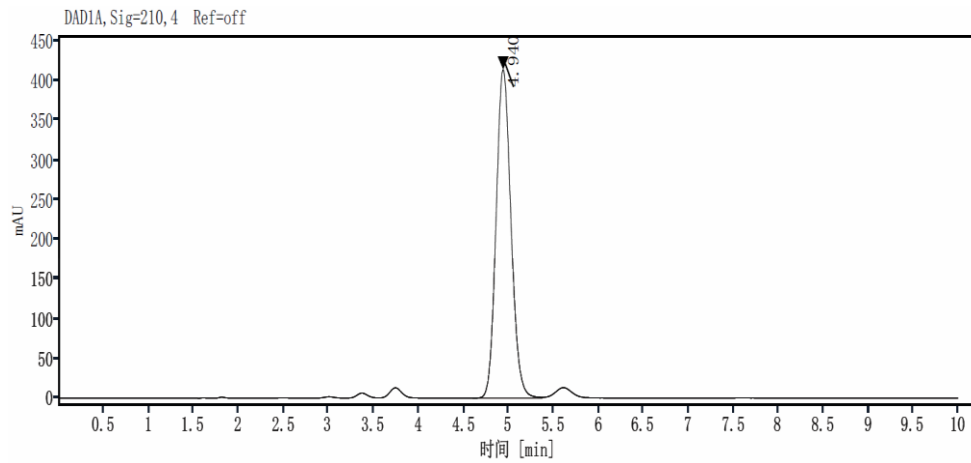

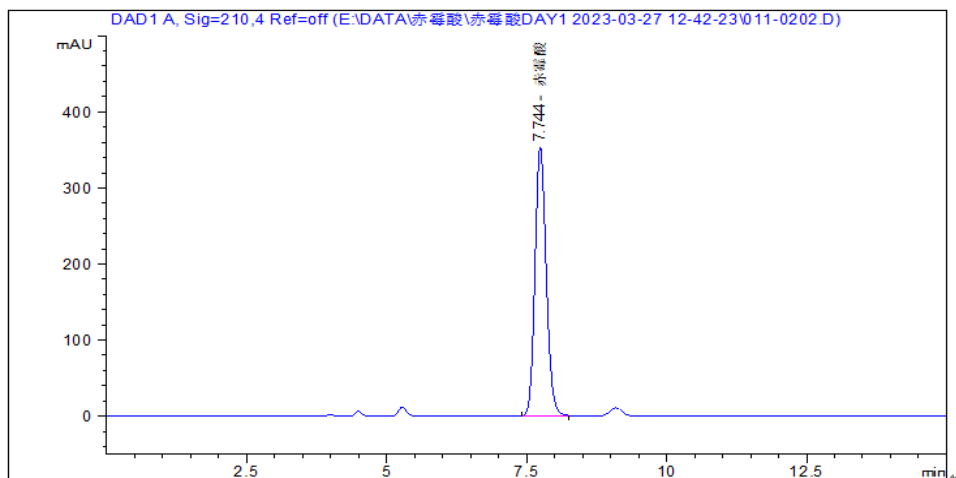**Figure 10. HPLC chromatogram of gibberellic acid TC (Lab 3)**

Figure 11. HPLC chromatogram of gibberellic acid TC (Lab 4)