Coronatine
Collaborative Study

Small scale collaborative study for the

Determination of Coronatine in Coronatine TC and SL by HPLC

Report to CIPAC by CHIPAC

Method Developed by

CHENGDU NEWSUN CROP SCIENCE CO., LTD

May 2023

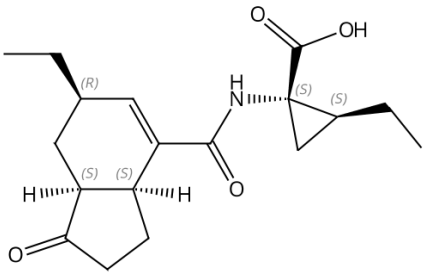
1. Participants

Small Scale Collaborative Study for the determination of Coronatine TC and SL 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	Wen Wang	Jiangsu Agrochem Laboratory Co., Ltd.	No.98, Minjiang Road, Hi-Tech Development Zone Changzhou, Jiangsu, China
Lab 2	Shujuan Chen	Jinhua Boyue Agricultural Development Co., LTD	678 Huaxi Road, Qiubin Street, Wucheng District, Jinhua City, Zhejiang Province, China.
Lab 3	Yanqin Yang	Chengdu Newsun Crop Science Co., LTD	No.35 Gongye Five Road, Heshan Town, Pujiang, Chengdu, Sichuan, China
Lab 4	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

2. Active Ingredient: General Information

Common name: Coronatine
CAS-No.: 62251-96-1
Structure:



Molecular mass: 319.4
Empirical formula: $C_{18}H_{25}NO_4$
Activity: Plant growth regulation

3. Samples

Five test samples and coronatine analytical standard were sent to the participants:

(1) Coronatine tech. sample (TC-1)
(2) Coronatine tech. sample (TC-2)
(3) Coronatine SL sample (SL-1)

(4) Coronatine SL sample (SL-2)
(5) Coronatine SL sample (SL-3)
(6) Coronatine, reference standard (purity 991.1 g/kg)

All participants sent back their results in time.

4. Method

4.1 Scope

The determination of coronatine active ingredient content was assayed in technical material (TC) and SL formulation.

4.2 Outline of method

Coronatine is determined by reversed phase high performance liquid chromatography on a reversed phase column (C18) with UV detection at 220 nm, quantified by 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} (250×4.6 mm, 5 μm)	1.0	35	220	TC: 5 SL: 20	acetonitrile: 0.1% phosphoric acid water, 30:70
2	Agilent 1100	Agilent Eclipse Plus C_{18} (250×4.6 mm, 5 μm)	1.0	35	220	TC: 5 SL: 20	acetonitrile: 0.1% phosphoric acid water, 30:70
3	Agilent 1260-II	Agilent Eclipse Plus C_{18} (250×4.6 mm, 5 μm)	1.0	35	220	TC: 5 SL: 20	acetonitrile: 0.1% phosphoric acid water, 30:70
4	Agilent 1260	Agilent ZORBAX Eclipse	1.0	35	220	TC: 5 SL: 20	acetonitrile: 0.1% phosphoric

		plus C_{18} (250×4.6 mm, 5 µm)					acid water, 30:70

6. Deviations and Remarks

For SL assay, in order to reduce the solvent effect, Lab 2 used mobile phase instead of methanol during the second dilution process of coronatine reference standard.

For TC assay, Lab 1 and Lab 3 used mobile phase instead of methanol to dissolve coronatine TC sample and reference standard for the same reason.

Considering the change is a minor change, no data was excluded from further statistical analysis. And an improvement to the solution preparation method may be considered.

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 coronatine

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 1-5 and Figures 1-5, 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 the Horwitz value for all the products (see in Table 6-10). All HorRat values were smaller than 1.0, while the TC1, SL1, SL2, and SL3 fell below 0.3, and we speculate this may be due to less participants.

8. Conclusions

For all samples, the values of RSD_R (reproducibility relative standard deviation) were less than Horwitz’s value. As a reference, all HorRat values were not greater than 1.0. The proposed method is considered to be appropriate for the determination of coronatine in TC and SL. CHIPAC proposes that a full scale collaborative trial might be conducted.

Table 1 Results of analysis of coronatine content in the TC1

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	982.5	978.2	976.6	978.3	978.9	958245.2	2.523	6.367
Lab 2	984.3	988.7	978.0	980.4	982.9	965994.1	4.685	21.95
Lab 3	978.2	986.6	980.8	978.1	980.9	962213.9	3.984	15.88
Lab 4	976.9	977.5	971.8	971.0	974.3	949260.5	3.373	11.38

Table 2 Results of analysis of coronatine content in the TC2

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	959.1	966.4	959.0	967.5	963.0	927369.0	4.583	21.01
Lab 2	947.9	953.2	952.6	956.9	952.7	907542.0	3.694	13.64
Lab 3	965.8	962.9	965.2	964.1	964.5	930260.3	1.278	1.633
Lab 4	964.1	959.9	960.6	961.6	961.6	924578.4	1.838	3.377

Table 3 Results of analysis of coronatine content in the SL1

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	0.06090	0.06102	0.06103	0.06037	0.06083	0.003700	0.00031230	9.753E-08
Lab 2	0.06035	0.06006	0.06010	0.06004	0.06014	0.003617	0.00014385	2.069E-08
Lab 3	0.05981	0.06016	0.05981	0.05989	0.05992	0.003590	0.00016601	2.756E-08
Lab 4	0.05993	0.05991	0.05989	0.06007	0.05995	0.003594	0.00008165	6.667E-09

Table 4 Results of analysis of coronatine content in the SL2

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	0.06028	0.06027	0.06038	0.06032	0.06031	0.003638	0.00004992	2.492E-09
Lab 2	0.06010	0.06015	0.06017	0.06018	0.06015	0.003618	0.00003559	1.267E-09
Lab 3	0.05981	0.06005	0.05999	0.05984	0.05992	0.003591	0.00011587	1.343E-08
Lab 4	0.05994	0.05986	0.05988	0.05988	0.05989	0.003587	0.00003464	1.200E-09

Table 5 Results of analysis of coronatine content in the SL3

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	0.06014	0.06016	0.06007	0.06021	0.06015	0.003617	0.00005802	3.367E-09
Lab 2	0.05997	0.06005	0.05996	0.06006	0.06001	0.003601	0.00005228	2.733E-09
Lab 3	0.05982	0.05956	0.05953	0.05953	0.05961	0.003553	0.00014071	1.980E-08
Lab 4	0.05980	0.05978	0.05982	0.05982	0.05981	0.003577	0.00001915	3.667E-10

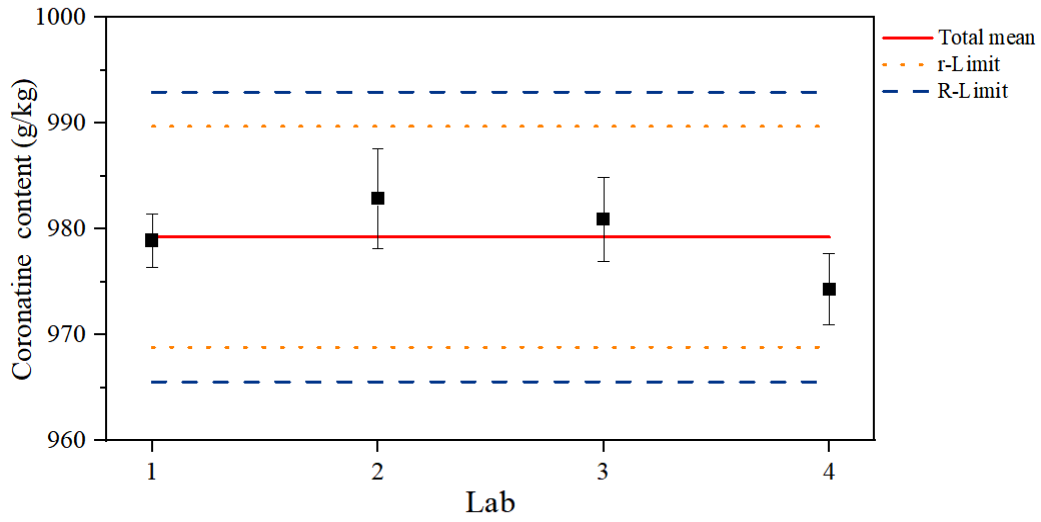


Figure 1. Graphical presentation of TC1 data

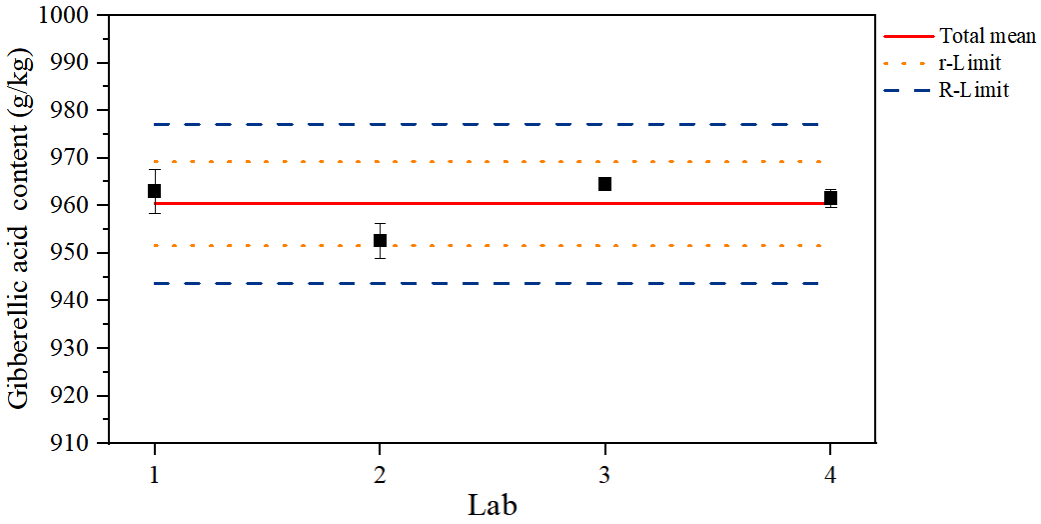


Figure 2. Graphical presentation of TC2 data

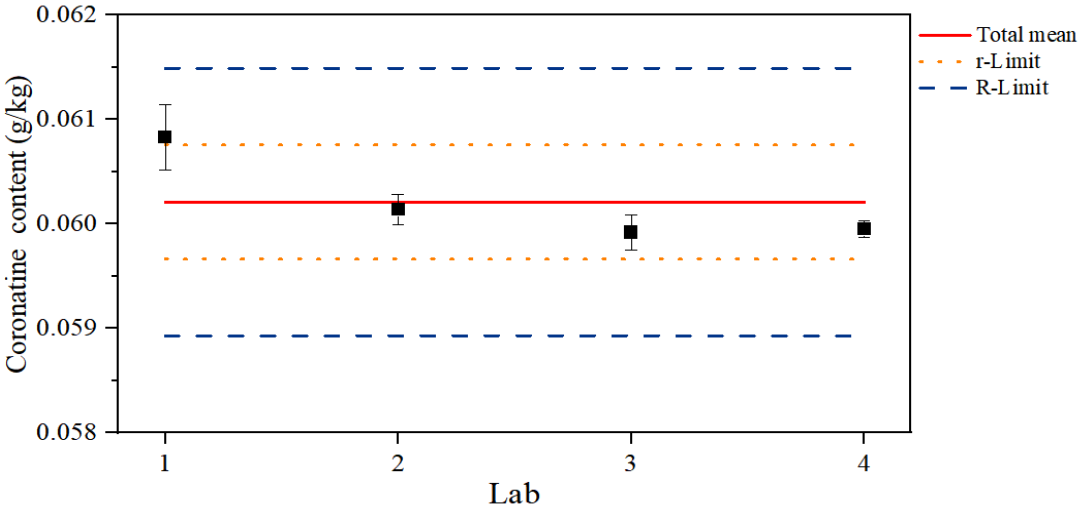


Figure 3. Graphical presentation of SL1 data

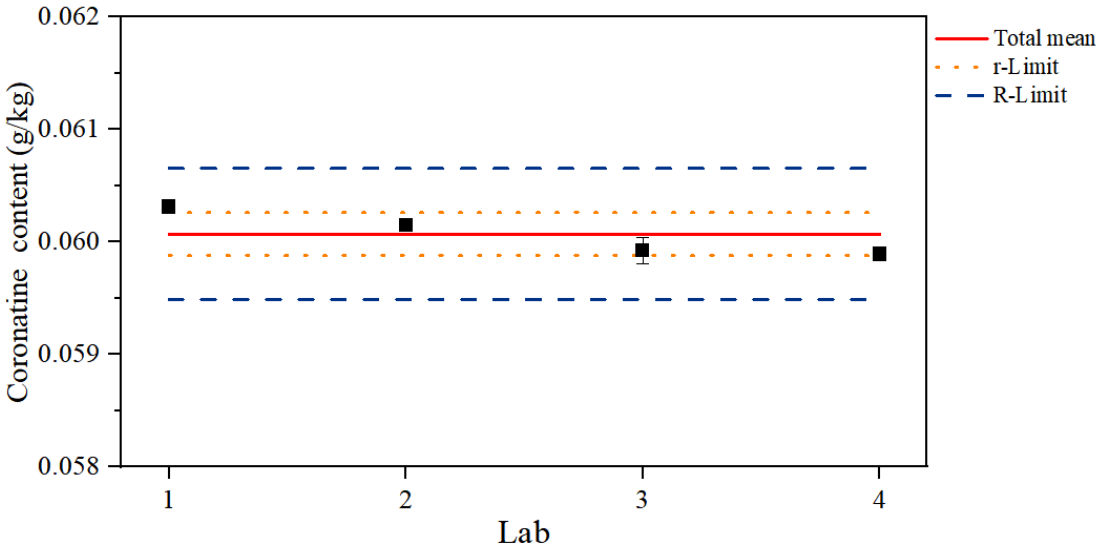


Figure 4. Graphical presentation of SL2 data

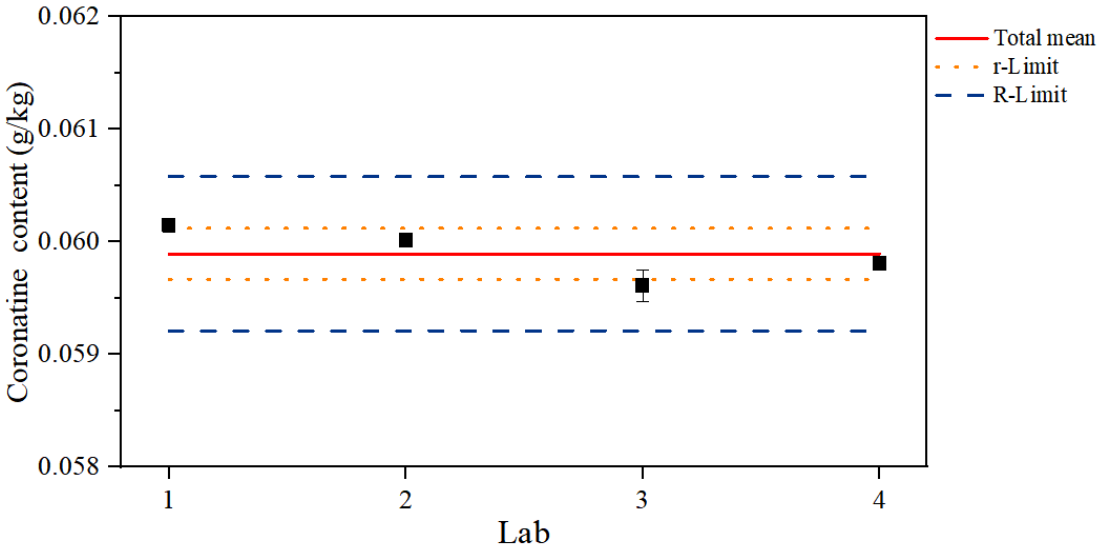


Figure 5. Graphical presentation of SL3 data

Table 6 Statistics of the results of TC1

S_1=Sum Yi	3917.0		
S_2=Sum Yi^2	3835713.7		
S_3=Sum Si^2	55.6		
No. Lab P	4		
No. Determination n	4		
Average Y=S_1/P	979.2		
Sr^2=S_3/P	13.89	S_r	3.727
S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n	9.99	S_L	3.161
S_R^2=S_r^2+S_L^2	23.88	S_R	4.887
Repeatability r=2.8*S_r	10.44		
Reproducibility R=2.8*S_R	13.68		

Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.3806
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.4991
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.006
HorRat	0.2487

Table 7 Statistics of the results of TC2

S_1=Sum Yi	3841.7		
S_2=Sum Yi^2	3689749.7		
S_3=Sum Si^2	39.66		
No. Lab P	4		
No. Determination n	4		
Average $Y=S_1/P$	960.4		
$Sr^2=S_3/P$	9.915	S_r	3.149
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	25.84	S_L	5.083
$S_R^2=S_r^2+S_L^2$	35.75	S_R	5.979
Repeatability $r=2.8*S_r$	8.817		
Reproducibility $R=2.8*S_R$	16.74		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.3279		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.6226		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.012		
HorRat	0.3094		

Table 8 Statistics of the results of SL1

S_1=Sum Yi	0.2408		
S_2=Sum Yi^2	0.01450		
S_3=Sum Si^2	1.524E-07		
No. Lab P	4		
No. Determination n	4		
Average $Y=S_1/P$	0.06021		
$Sr^2=S_3/P$	3.811E-08	S_r	0.0001952
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	1.714E-07	S_L	0.0004140
$S_R^2=S_r^2+S_L^2$	2.095E-07	S_R	0.0004577
Repeatability $r=2.8*S_r$	0.0005466		

Reproducibility $R=2.8*S_R$	0.001282
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.3242
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.7602
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	8.635
HorRat	0.08804

Table 9 Statistics of the results of SL2

S_1=Sum Yi	0.2403		
S_2=Sum Yi^2	0.01443		
S_3=Sum Si^2	1.838E-08		
No. Lab P	4		
No. Determination n	4		
Average $Y=S_1/P$	0.06007		
$Sr^2=S_3/P$	4.596E-09	S_r	0.0000678
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	3.864E-08	S_L	0.0001966
$S_R^2=S_r^2+S_L^2$	4.323E-08	S_R	0.0002079
Repeatability $r=2.8*S_r$	0.0001898		
Reproducibility $R=2.8*S_R$	0.0005822		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.1129		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.3461		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	8.638		
HorRat	0.04007		

Table 10 Statistics of the results of SL3

S_1=Sum Yi	0.2396		
S_2=Sum Yi^2	0.0143		
S_3=Sum Si^2	2.627E-08		
No. Lab P	4		
No. Determination n	4		
Average Y=S_1/P	0.05989		
Sr^2=S_3/P	6.567E-09	S_r	0.0000810
S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n	5.337E-08	S_L	0.0002310
S_R^2=S_r^2+S_L^2	5.993E-08	S_R	0.0002448
Repeatability r=2.8*S_r	0.0002269		
Reproducibility R=2.8*S_R	0.0006855		
Relative Standard Deviation of Repeatability RSD_r=S_r*100/Y	0.1353		
Relative Standard Deviation of Reproducibility RSD_R=S_R*100/Y	0.4088		
Horwitz RSD_R(Hor)=2^[1-0.5*log(Y/1000)]	8.642		
HorRat	0.04730		

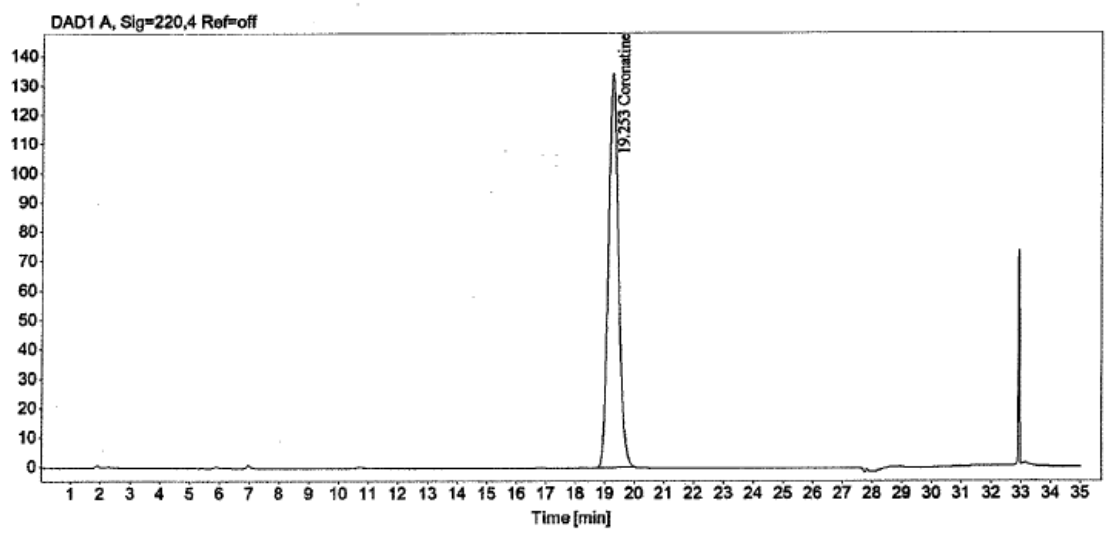


Figure 6. HPLC chromatogram of coronatine standard (Lab 1)

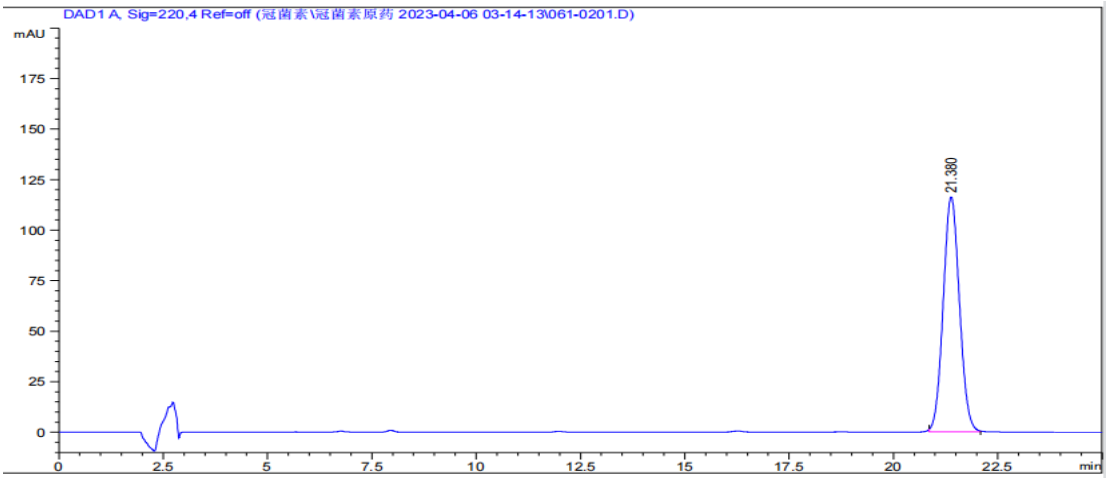


Figure 7. HPLC chromatogram of coronatine standard (Lab 2)

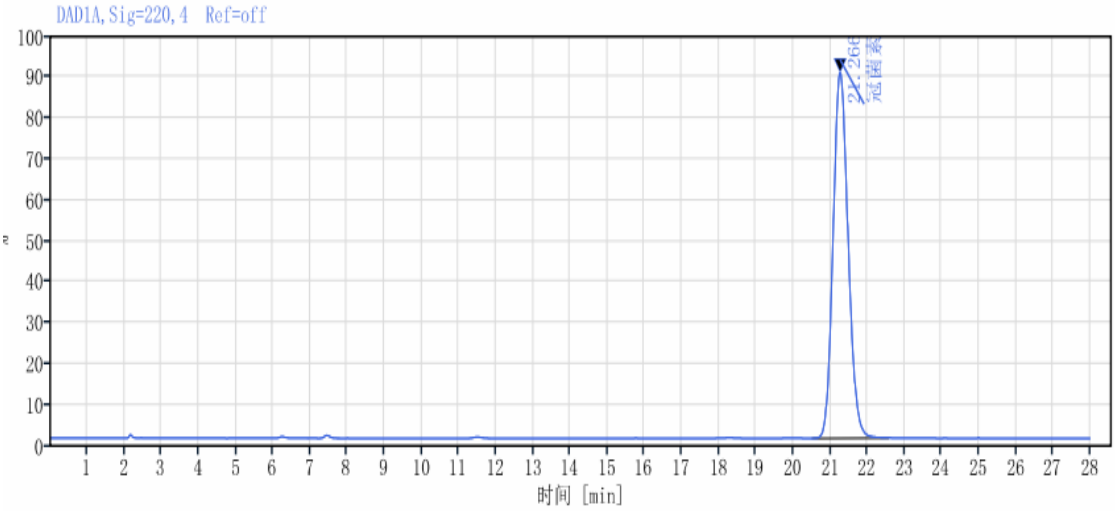


Figure 8. HPLC chromatogram of coronatine standard (Lab 3)

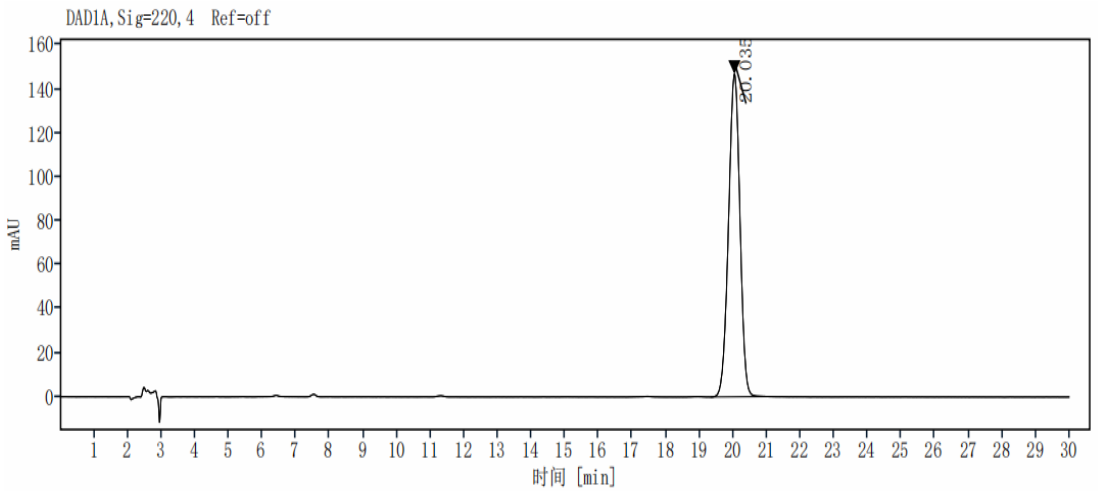


Figure 9. HPLC chromatogram of coronatine standard (Lab 4)

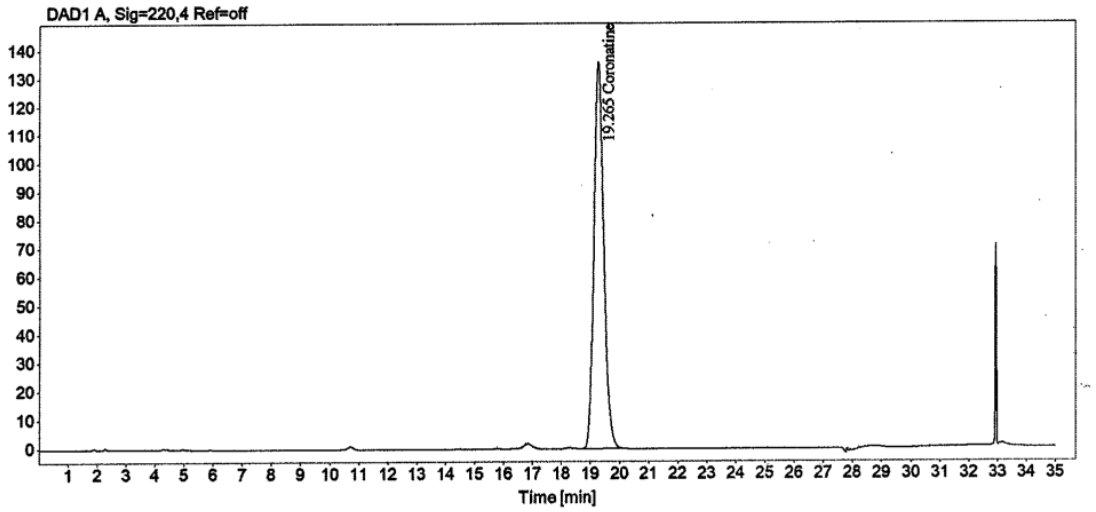


Figure 10. HPLC chromatogram of coronatine TC (Lab 1)

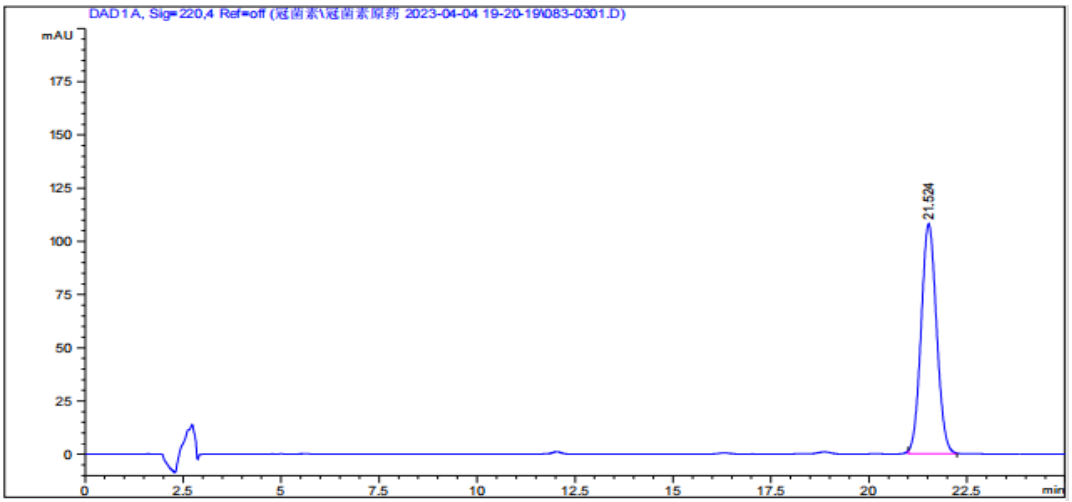


Figure 11. HPLC chromatogram of coronatine TC (Lab 2)

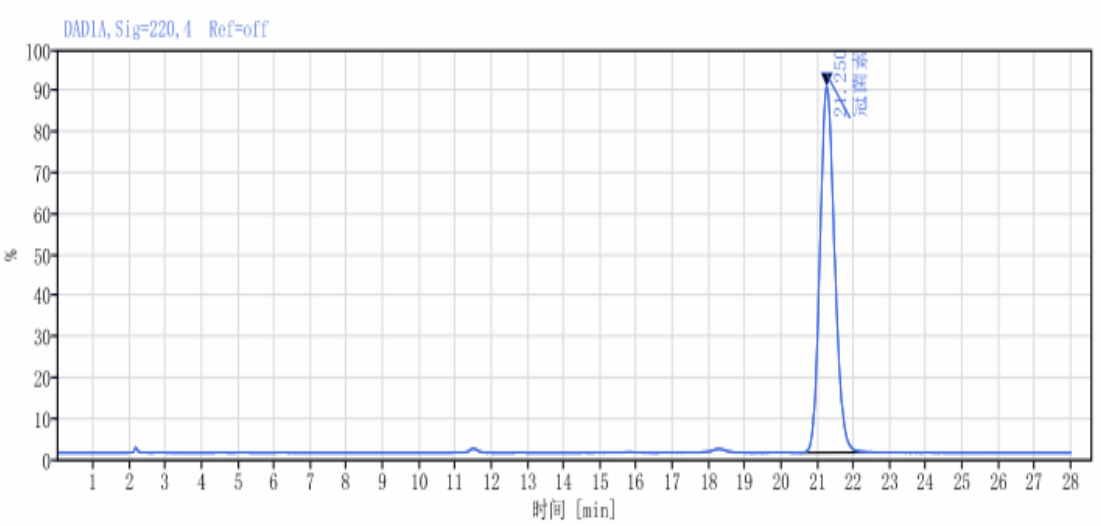


Figure 12. HPLC chromatogram of coronatine TC (Lab 3)

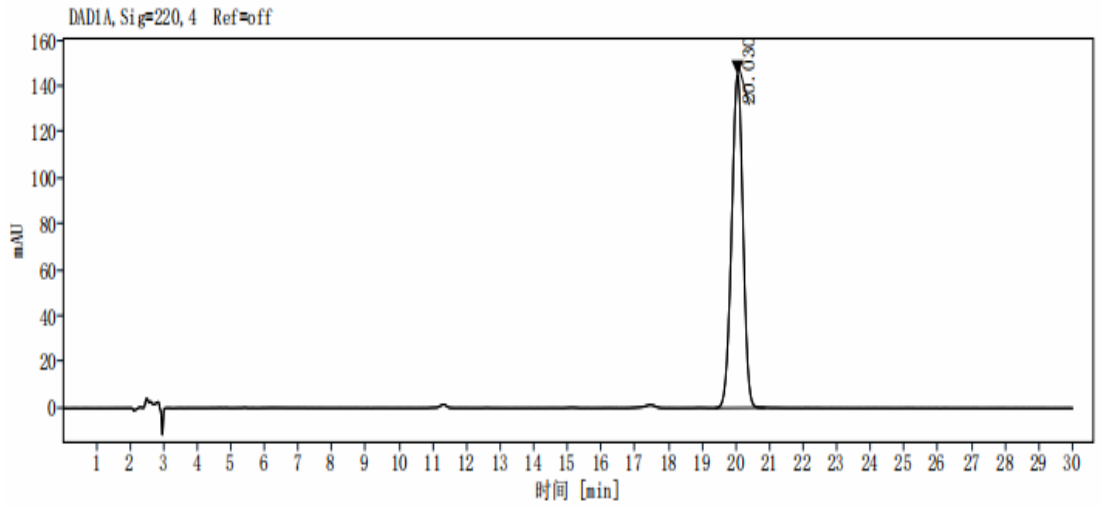


Figure 13. HPLC chromatogram of coronatine TC (Lab 4)

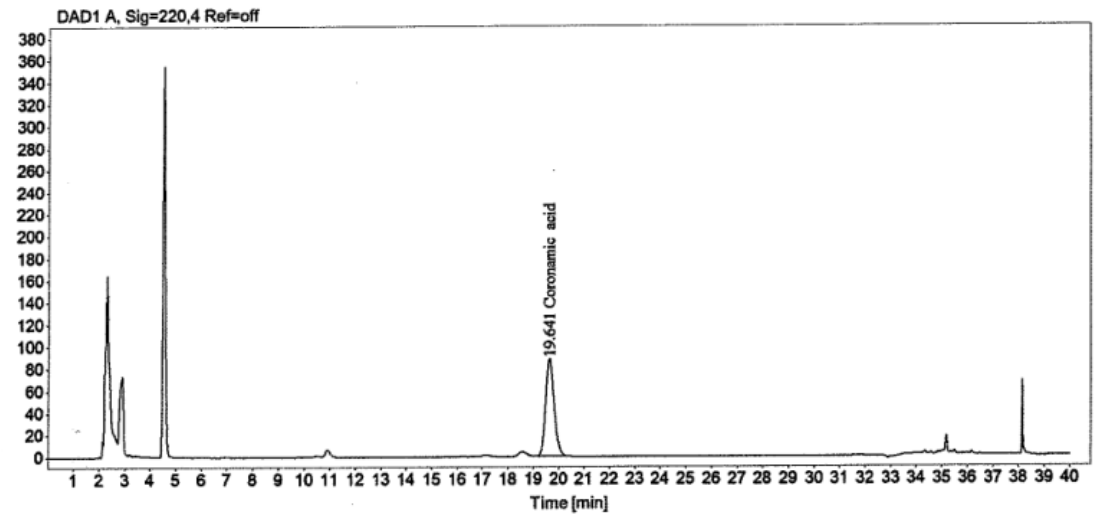


Figure 14. HPLC chromatogram of coronatine SL (Lab 1)

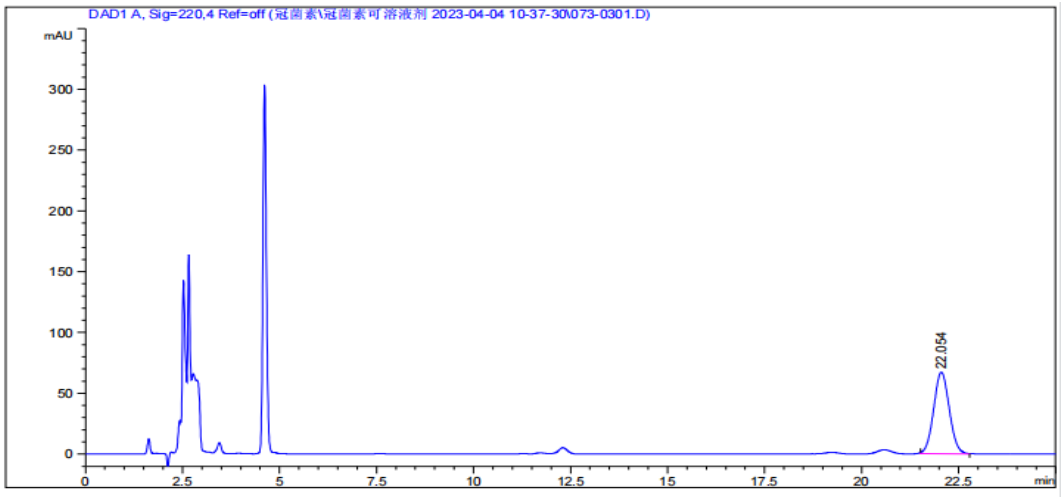


Figure 15. HPLC chromatogram of coronatine SL (Lab 2)

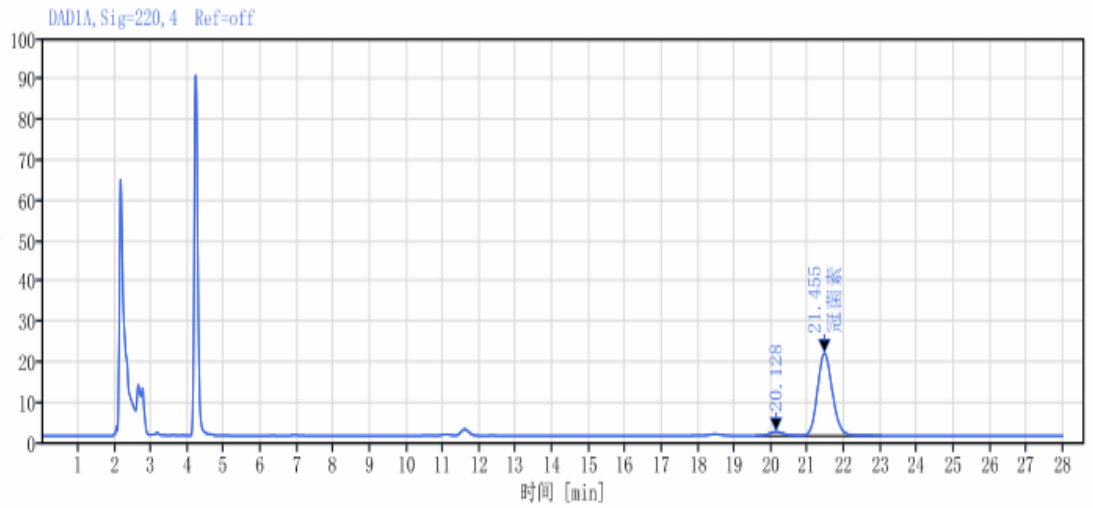


Figure 16. HPLC chromatogram of coronatine SL (Lab 3)

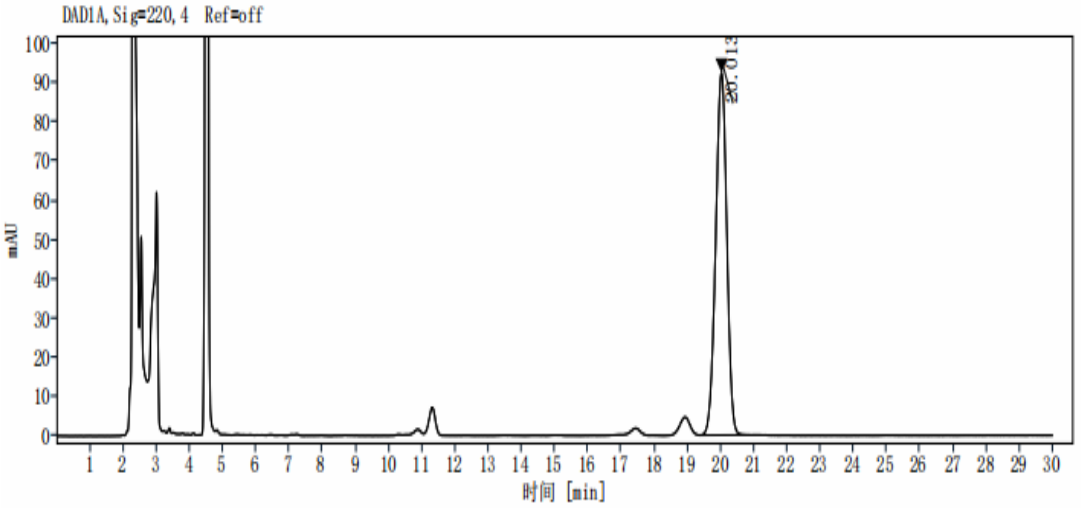


Figure 17. HPLC chromatogram of coronatine SL (Lab 4)