CORONATINE

Collaborative Study

Full Scale Collaborative Study for the Determination of Coronatine, by HPLC

Report to CIPAC
By
Chinese Pesticide Analytical Committee（CHIPAC）

Method Developed by Chengdu Newsun Crop Science Co., Ltd.

May 2024

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1. Participants

By mid of February 2024, all of the 18 laboratories provided their results on the determination of Coronatine according to CIPAC Information Sheet No. 342.

The results for the 18 participants are presented in the following section.

Participating laboratories are listed in the table below.

Contact person	Participating Laboratory	Country
Rana Ouf, Moustafa Khalifa	Al - Sharhan Laboratory for Chemical Testing , Al Sharhan Industries	Kuwait
Cornel Grecu	Biochem SRL– Quality Control Laboratory	Romania
Yanqin Yang	Chengdu Newsun Crop Science Co., Ltd.	China
Lixia Liu, Chunqing Hou	China National Pesticide Quality Inspection and Testing Center (Shenyang)	China
Lisha Zhang, Bing Li, Mi Ren	Chongqing Chemical Pesticide Quality Supervision and Inspection Station	China
Hongcai Xu, Rongmei Chen	GreenTech Laboratory Co., Ltd.	China
Lu Huang	Hunan Research Institute of Chemical Industry Testing Technology Co., Ltd. China	China
Peize Li	Institute for the Control of Agrochemicals, Ministry of Agricultrue and Rural Affairs (Beijing)	China
Yanru Li, Ke Cao, Jianzhong Yu	Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences	China
Lucy Zhou, Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.	China
Yiyang Miao, Xuxi Fang, Rong Xie	Jiangsu Authority Testing Co., Ltd.	China
Tinghua Chen, Yily Yan	Jiangsu Rotam Chemistry Co., Ltd	China
Volodymyr Mykhaylov	L.I. Medved's Reseach Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health, Ukraine	Ukraine
Huali Chen, Hongxia Li	Nutrichem Laboratory Co.,Ltd.	China
Shizhong Li, Yuying Wang, Daifeng Wang	Pesticide Quality Supervision, Inspection and Testing Center in Shenyang, MOA	China

Moch. Ramadhan, Agus Salim	PT Agriculture Construction (AGRICON)	Indonesia
Kai Zhang, Lele Li, Yongfei Guo	Testing Technology Center of Sino-Agri Leading Bioscience Co., Ltd.	China
I. Goffin, D. Lagasse, Marie BAES	Walloon Agricultural Research Centre (CRA-W)	Belgium

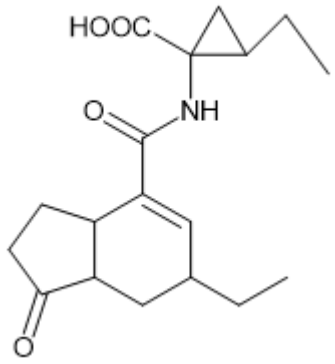
2. Coronatine, General Information

Chemical name: 2-Ethyl-1-{[(6-ethyl-1-oxo-2,3,3a,6,7,7a-hexahydro-1H-inden-4-yl)(hydroxy)methylidene] amino} cyclopropane-1-carboxylic acid;

Common name: Coronatine

CAS Number: 73366-39-9

Structure:



Molecular mass: 319.4 g/mol

Empirical formula: $C_{18}H_{25}NO_4$

3. Samples

In October 2023, Information Sheet No. 342 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Coronatine by HPLC.

Five test samples (described below), including the Coronatine analytical reference standard were shipped to the participants:

A) Coronatine TC-1
B) Coronatine TC-2
C) Coronatine SL-1
D) Coronatine SL-2
E) Coronatine SL-3

Coronatine analytical reference standard (99.2% purity)

4. Method

4.1 Scope

The contents of Coronatine in technical materials and in soluble concentrates were determined.

4.2 Principle

The content of Coronatine in the sample is determined by reversed phase HPLC on a 250 x 4.6 mm (i.d.), 5 µm C_{18} column, with mobile phase composed of acetonitrile/0.1% phosphoric acid aqueous solution, (30:70 v/v) and UV detection at 220 nm. Quantitation is done by 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.

Lab Number	HPLC-System	Mobile Phase	Flow rate mL/min	Detector Wavelength	Column	Injection volume	Column temperature
Laboratory 1	THERMO Vanquish Flex	acetonitril/0.1% phosphoric acid aqueous solution 30/70(v/v)	1.0 mL/min	220 nm	Agilent Zorbax Eclipse XDB-C_{18}, 5 µm, 250 x 4.6mm	5 µL for TC ; 20 µL for SL	35℃
Laboratory 2	Shimadzu	acetonitrile:0.1%phosphoric acid aqueous solution, 30:70 (v/v)	1.0 mL/min	220 nm	Zorbax RX-C_{18}, 5 µm, 250 mm x 4.6 mm i.d.	5 µL for TC ; 20 µL for SL	35°C
Laboratory 3	Shimadzu LC 2050 with SPD-M20A	acetonitrile:0.1%phosphoric acid aqueous solution, 30:70 (v/v)	1.0 mL/min	220 nm	Kromasil C_{18}, 5 µm, 250 x 4.6mm	5 µL for TC ; 20 µL for SL	35°C
Laboratory 4	Agilent 1100	acetonitrile: 0.1% phosphoric acid aqueous solution, 30:70 (v/v)	1.0 mL/min	220 nm	Agilent ZORBAX SB-C_{18}, 5 µm, 250 x 4.6mm	5 µL(TC), 20 µL(SL)	35°C
Laboratory 5	Agilent, DAD, 1260 series	acetonitrile: 0.1% phosphoric acid aqueous solution, 30:70 (v/v)	1.0 mL/min	220 nm	Agilent ZORBAX SB-C_{18}, 5 µm, 250 x 4.6mm	5 µL	35°C
Laboratory 6	Shimadzu LC-20AD	acetonitrile: 0.1% phosphoric acid=30/70	1.0 mL/min	220 nm	Eclipse XDB-C_{18}, 5 µm, 250 x 4.6mm	5 µL for TC ; 20 µL for SL	35°C
Laboratory 7	Thermo U3000	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Agilent ZORBAX SB-C_{18},	5 µL(TC), 20 µL(SL)	35°C

					5 μm, 250 x 4.6mm		
Laboratory 8	Agilent 1260 Infinity II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Agilent ZORBAX Eclipse plus C_{18}, 5 μm, 250 x 4.6mm	5 μL(TC), 20 μL(SL)	35°C
Laboratory 9	Agilent 1260 II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Agilent ZORBAX SB-C_{18}, 5 μm, 250 x 4.6mm	TC: 5μL, SL: 20μL	35°C
Laboratory 10	Shimadzu 20AT	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Thermo hypersyl ODS, 5 μm, 250 x 4.6mm	5 μL	35°C
Laboratory 11	Shimadzu LC-2050C 3D	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Eclipse XDB-C_{18}, 5 μm, 250 x 4.6mm	5 μl	35°C
Laboratory 12	LC-20AD	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.2 mL/min	220 nm	InertSustain C_{18}, 5 μm, 250 x 4.6mm	5 μl（TC）, 20μl（SL）	35°C
Laboratory 13	Agilent 1260 Infinity	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Agilent SB-C_{18}, 5 μm, 250 x 4.6mm	5 μl（TC）, 5μl（SL）	30°C
Laboratory 14	Agilent 1260 Infinity II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	ZORBAX Eclipse Plus C_{18}, 5 μm, 250 x 4.6mm	TC:5 μL;SL:20 μL	35°C
Laboratory 15	Agilent 1260 Infinity II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	ODS2 (Reverse Phase) WATERS co. USA , Particle Size 5 μm, 150x4.6mm	5 μL(TC), 20 μL(SL)	35°C
Laboratory 16	Agilent 1200,DAD	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Venusil XBP C_{18}, 5 μm, 250 x 4.6mm	5μl;10μl	35°C

Laboratory 17	Agilent 1260 Infinity II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	ZORBAX Eclipse plus C_{18}, 5 μm, 250 x 4.6mm	5 μL(TC), 20 μL(SL)	30℃
Laboratory 18	Agilent 1260 Infinity II	acetonitrile:0.1%phosphoric acid aqueous solution,30:70 (v/v)	1.0 mL/min	220 nm	Agilent Eclipse plus C_{18}, 5 μm, 250 x 4.6mm	5 μL(TC), 20 μL(SL)	35°C

Lab Number	**Remarks**
Laboratory 1	The retention time of coronatine was continuous changing during the acquisition sequence.
Laboratory 2	The technical samples and analytical standard have been rather hard to homogenize and weigh as they seem to be absorbing water quite fast, which makes it flaky and sticking to the spoon.
Laboratory 10	Retention time different from reference (21.5 min), so we cut off run time from 28 to 21 min.
Laboratory 12	Calibration solution of SL. Weigh in duplicate 40 mg of Coronatine reference standard (s mg) into separate volumetric flasks (100ml). Dissolve with 5 ml methanol and place the flask in an ultrasonic bath until the sample has been dissolved completely. Allow to cool to ambient temperature and fill to the mark with methanol and mix thoroughly as stock solution. Then transfer by pipette 2.0 ml of each stock solution into separate 25 ml volumetric flask, dilute to volume with mobile phase and mix thoroughly. Filter the solution through a 0.45 μm filter before injection (calibration solutions CA and CB). Sample solution of SL. Weigh in duplicate 13 g of sample into separate 25 ml volumetric flask. Add to the mark of each flask with mobile phase and mix thoroughly. Filter the solution through a 0.45 μm filter before injection (solutions S1 and S2).
Laboratory 18	The technical samples (TC1#) is difficult to weigh and needs to be shaken while sonicated for dissolution.

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 except for Lab 1. There are obvious unknown peaks appearing on the chromatograms especially on those of day 1, which indicates that the system is contaminated.
All other changes and observations noted by the 18 participants were not expected to affect the analysis results significantly.

6.2 Determination of Coronatine

Results reported by the laboratories and the statistical evaluation are listed in tables 1-5 and displayed in figures 1-5.

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 iterating where necessary. The tests were performed at an alpha level of 0.01 for outlier (marked with **), and 0.05 for straggler (marked with *).

A comparison of the RSD_R of this collaborative Study with the unmodified Horwitz equation showed that the relative reproducibility standard deviation (RSD_R) is below the Horwitz value in all samples (TC-1, TC-2, SL-1, SL-2, SL-3) even without elimination of stragglers and outliers (see Table 3). The RSD_R further improved if stragglers and outliers are eliminated (see Table 4). No more than two Lab results have been removed per sample (Table 1). All HorRat values were smaller than 1.0. Due to the universal applicability of the method this collaborative trial is acceptable.

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

	Coronatine TC-1		Coronatine TC-2		Coronatine SL-1		Coronatine SL-2		Coronatine SL-3	
	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2
Lab 1	987.578	987.432	981.238	982.327	0.064	0.064	0.064	0.064	0.065	0.065
Lab 2	987.364	968.633	985.746	984.194	0.061	0.059	0.060	0.059	0.061	0.059
Lab 3	991.070	992.654	987.173	986.257	0.059	0.059	0.059	0.059	0.060	0.060
Lab 4	979.114	976.092	978.664	975.117	0.058	0.060	0.058	0.059	0.058	0.059
Lab 5	985.066	979.785	981.253	978.243	0.060	0.060	0.060	0.060	0.061	0.061
Lab 6	984.509	981.153	987.436	985.267	0.060	0.061	0.060	0.061	0.060	0.061
Lab 7	975.931	971.009	973.376	985.207	0.057	0.055	0.057	0.055	0.057	0.055
Lab 8	982.744	982.913	981.719	984.107	0.060	0.060	0.060	0.060	0.060	0.060
Lab 9	976.682	981.027	974.202	972.827	0.060	0.060	0.060	0.060	0.060	0.060
Lab 10	972.139	973.291	974.623	975.263	0.060	0.062	0.061	0.062	0.063	0.064
Lab 11	977.626	977.495	980.628	980.228	0.060	0.060	0.060	0.060	0.060	0.060
Lab 12	980.275	978.973	986.193	983.775	0.060	0.060	0.060	0.060	0.060	0.060
Lab 13	980.527	969.152	977.095	976.059	0.061	0.061	0.061	0.061	0.061	0.062
Lab 14	977.940	979.834	978.481	980.003	0.060	0.060	0.060	0.060	0.060	0.060
Lab 15	994.190	996.728	972.429	997.992	0.061	0.059	0.061	0.061	0.058	0.059
Lab 16	980.221	981.921	976.387	975.929	0.058	0.058	0.058	0.058	0.060	0.059
Lab 17	974.381	977.180	973.461	978.062	0.060	0.060	0.060	0.059	0.059	0.059
Lab 18	993.061	994.706	986.882	982.838	0.060	0.060	0.059	0.059	0.060	0.060

Table 2: Mean values

	Coronatine TC-1	Coronatine TC-2	Coronatine SL-1	Coronatine SL-2	Coronatine SL-3
Lab 1	987.51	981.78	0.064*	0.064	0.065
Lab 2	978.00	984.97	0.060	0.060	0.060
Lab 3	991.86	986.71	0.059	0.059	0.060
Lab 4	977.60	976.89	0.059	0.059	0.058
Lab 5	982.43	979.75	0.060	0.060	0.061
Lab 6	982.83	986.35	0.061	0.061	0.061
Lab 7	973.47	979.29	0.056*	0.056	0.056
Lab 8	982.83	982.91	0.060	0.060	0.060
Lab 9	978.85	973.51	0.060	0.060	0.060
Lab 10	972.72	974.94	0.061	0.061	0.063
Lab 11	977.56	980.43	0.060	0.060	0.060
Lab 12	979.62	984.98	0.060	0.060	0.060
Lab 13	974.84	976.58	0.061	0.061	0.061
Lab 14	978.89	979.24	0.060	0.060	0.060
Lab 15	995.46	985.21	0.060	0.061	0.059
Lab 16	981.07	976.16	0.058	0.058	0.060
Lab 17	975.78	975.76	0.060	0.060	0.059
Lab 18	993.88	984.86	0.060	0.059	0.060

* Grubb's test straggler
** Grubb's test outlier

Table 3: Summary of the statistical evaluation - no elimination of any outliers /stragglers

	Coronatine TC-1	Coronatine TC-2	Coronatine SL-1	Coronatine SL-2	Coronatine SL-3
xm [g/kg]	981.4	980.6	0.05991	0.05984	0.06014
L	18	18	18	18	18
S_r	4.448	5.125	0.00056	0.00044	0.00059
S_R	6.376	3.455	0.00150	0.00156	0.00185
S_L	7.774	6.181	0.00160	0.00162	0.00194
r	12.454	14.350	0.00157	0.00122	0.00164
R	21.77	17.31	0.00447	0.00455	0.00543
RSD_r	0.453	0.523	0.937	0.728	0.974
RSD_R	0.792	0.630	2.667	2.71	3.23
RSD_R (Hor)	2.006	2.006	8.641	8.643	8.636
HorRat	0.4	0.3	0.3	0.3	0.4

Table 4: Summary of the statistical evaluation with elimination of Lab 1

	Coronatine TC-1	Coronatine TC-2	Coronatine SL-1	Coronatine SL-2	Coronatine SL-3
xm [g/kg]	981.0	980.5	0.05967	0.05959	0.05985
L	17	17	17	17	17
S_r	4.565	5.262	0.00058	0.00045	0.00060
S_R	6.385	3.555	0.00112	0.00120	0.00145
S_L	7.849	6.350	0.00126	0.00128	0.00157
r	12.781	14.733	0.00161	0.00125	0.00169
R	21.98	17.78	0.00352	0.00360	0.00438
RSD_r	0.465	0.537	0.967	0.749	1.006
RSD_R	0.800	0.648	2.110	2.156	2.616
RSD_R (Hor)	2.006	2.006	8.647	8.648	8.643
HorRat	0.4	0.3	0.2	0.2	0.3

Note：Results of Lab 1 were eliminated as the HPLC system or solvent was contaminated;

Table 5: Summary of the statistical evaluation with elimination Grubb's test Stragglers /Outliers

	Coronatine TC-1	Coronatine TC-2	Coronatine SL-1	Coronatine SL-2	Coronatine SL-3
x_m [g/kg]	981.4	980.6	0.05988	0.05984	0.06014
L	18	18	16	18	18
S_r	4.448	5.125	0.00055	0.00044	0.00059
S_R	6.376	3.455	0.00073	0.00156	0.00185
S_L	7.774	6.181	0.00091	0.00162	0.00194

r	12.454	14.350	0.00153	0.00122	0.00164
R	21.77	17.31	0.00256	0.00455	0.00543
RSD_r	0.4532	0.5226	0.91175	0.72746	0.97436
RSD_R	0.792	0.630	1.52	2.71	3.23
RSD_R (Hor)	2.006	2.006	8.642	8.643	8.636
HorRat	0.4	0.3	0.2	0.3	0.4

Note: For SL-1, results of Lab 1,7 were eliminated as straggler;

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 – 5 (all results)

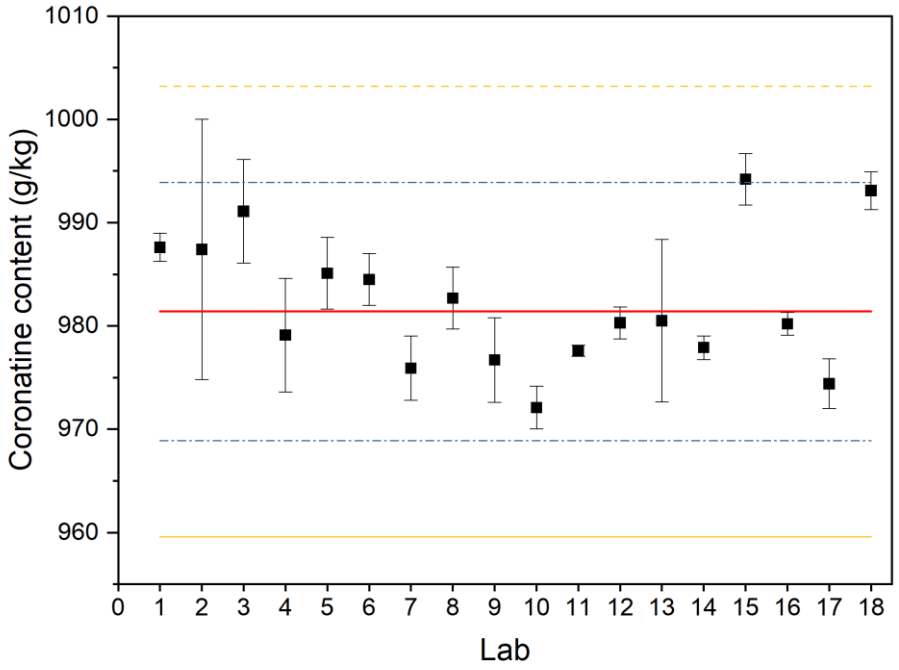


Figure 1. Graphical presentation of TC1 data

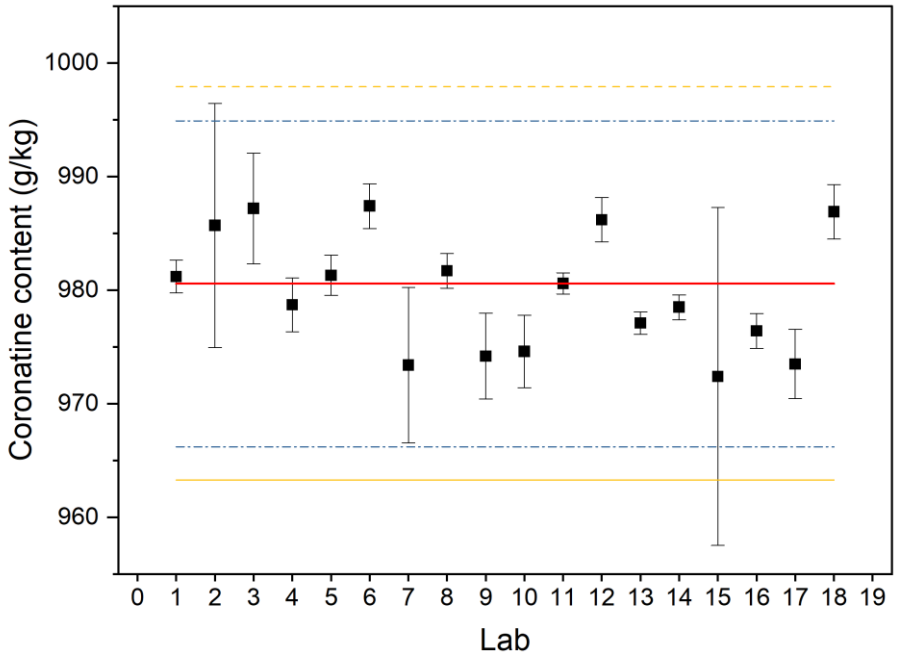


Figure 2. Graphical presentation of TC2 data

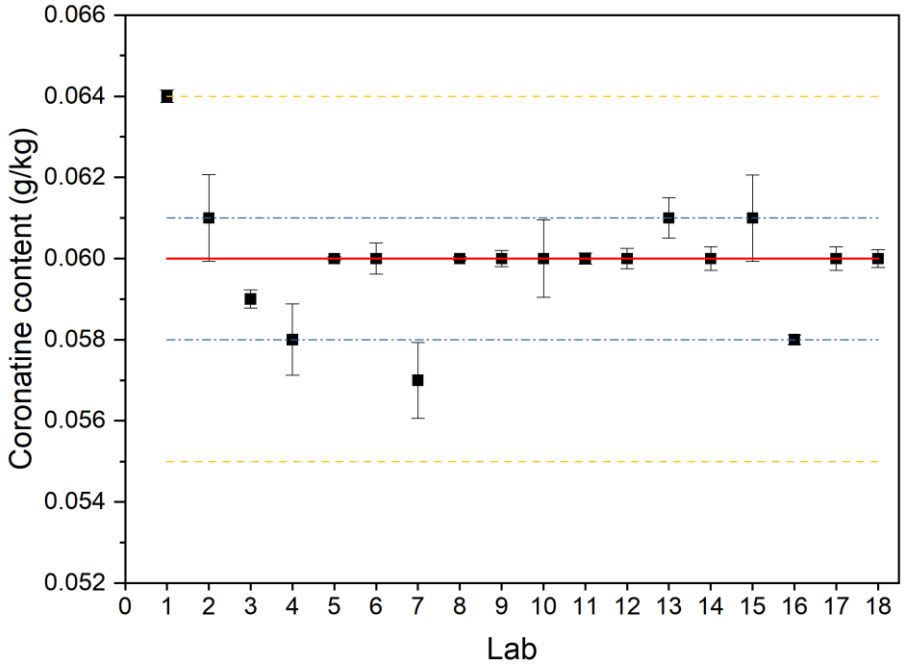


Figure 3. Graphical presentation of SL1 data

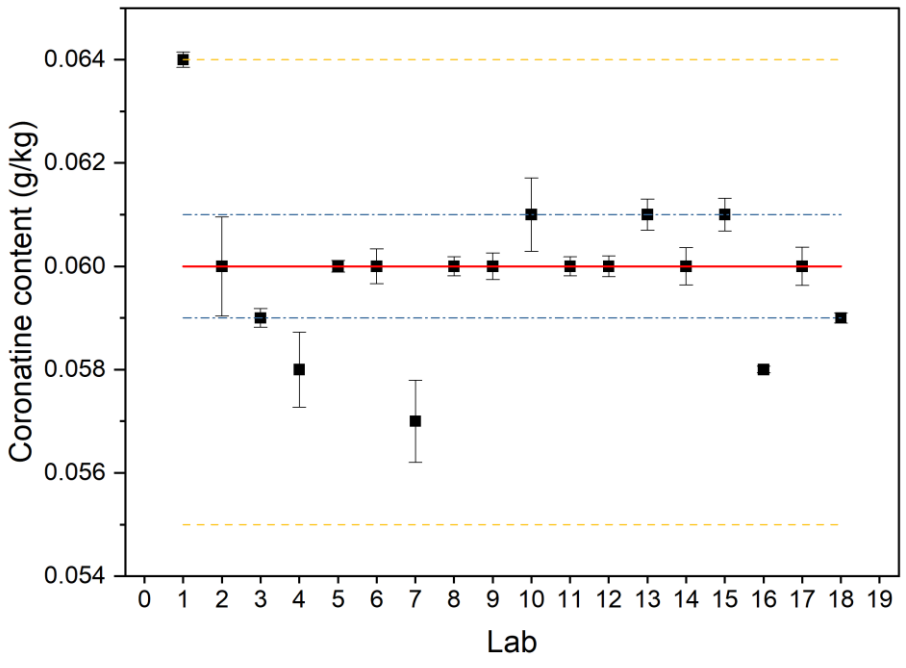


Figure 4. Graphical presentation of SL2 data

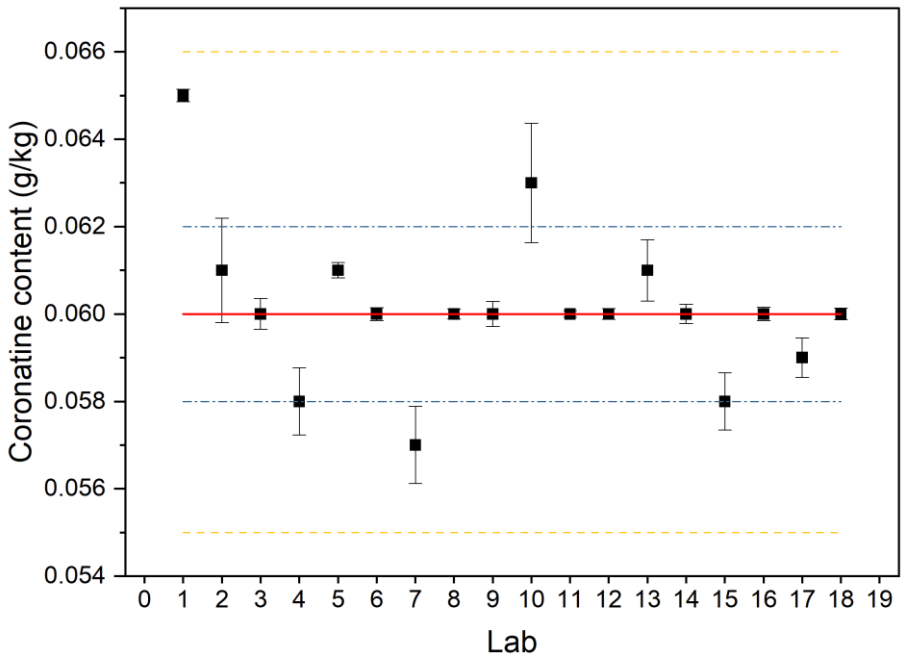


Figure 5. Graphical presentation of SL3 data

7. Conclusions

18 different laboratories participated in this collaborative study. The results of the labs are given in Table 1-2, the statistical summary is given in Table 3-5. The results are illustrated in figures 1–5.

Without elimination of stragglers or outliers, the between lab experimental Relative Reproducibility Standard Deviation (% RSD_R) is below the calculated acceptable value based on the Horwitz curve calculation (% RSD_R (Hor)) for all samples. The HorRat values were between 0.3 to 1.0 by employing this method.
The results of Lab 1 were excluded as the HPLC system or solvent was contaminated. The 2 stragglers were also eliminated during statistical evaluation for sample SL1. The minimum number of considered results after elimination of stragglers was 16. In all cases, the HorRat values were below 1.0.

Taking into account the relatively high number of participating laboratories a broad basis was given even after elimination of the Stragglers. Therefore, CHIPAC considers this method to be suitable and recommend accepting it as a provisional CIPAC method for the determination of Coronatine in both technical concentrate and its associated formulated products.