ABAMECTIN

Collaborative Study

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

Report to CIPAC by
Chinese Pesticide Advisory Council（CHIPAC）

Method Developed by

Hebei Xingbai Agricultural Technology Co., Ltd.

May 2025

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1. Participants

By mid of March 2025, all of the 18 laboratories provided their results on the determination of Abamectin according to CIPAC Information Sheet No. 354. 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**
Ioana Peptanaru	Agro-Est Muntenia	Romania
Helen Karasali, Anna Marousopoulou	Benaki Phytopathological Institute	Greece
Krste Tashev	State Phytosanitary Laboratory (SPL), Ministry of Agriculture, Forestry and Water Economy (MAFWE)North Macedonia	North Macedonia
Christian Mink	Syngenta Crop Protection AG	Switzerland
Orsolya Báthory, Ágnes Nagy	National Food Chain Safety Office, Food Chain Safety Laboratory Directorate, Plant Protection and Soil Conservation National Reference Laboratory	Hungary
Estela Bonilha	Laboratório Federal de Defesa Agropecuária - LFDA/SP - Ministério da Agricultura e Pecuária	Brazil
Nam Le	Vestergaard Sarl	Vietnam
Anucha Phonswai	Ministry of Agriculture and cooperatives, Department of Agriculture, Agricultural Production Sciences Research and Development Division	Thailand
Haixia Wang	Shenyang SYRICI Testing Co., Ltd	China
Yanqin Yang	Chengdu Newsun Crop Science Co., Ltd	China
Rong Xie	Jiangsu Authority Testing Co., Ltd.	China
Daifeng Wang, Yuying Wang	Pesticide Quality Supervision, Inspection and Testing Center in Shenyang, MOA	China
Yalan Kou	The National Pesticide Quality Inspection and Testing Center(Beijing), Institute for the Control of Agrochemicals	China
Yily Yan	Jiangsu Rotam Chemistry Co., Ltd	China
Yugui Huang	Guizhou Jiandee Technology Co., Ltd	China

Jianzhong Yu	Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences	China
Ping Zhang	Hunan Safety Science And Technology Co., Ltd	China
Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd	China

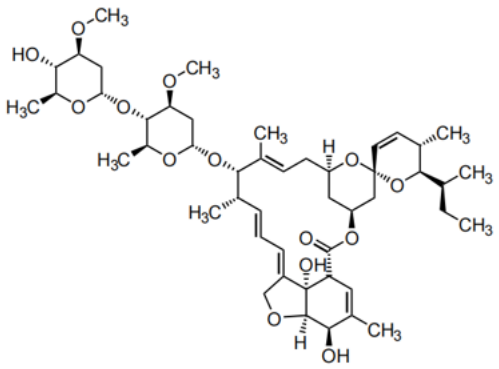
2. Abamectin, General Information

Chemical name: mixture of 80–100% (10E,14E,16E)-(1R,4S,5′S,6S,6′R,8R,12S,13S,20R,21R,24S)-6′-[(S)-sec-butyl]-21,24-dihydroxy-5′,11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.14,8.020,24]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(5′,6′-dihydro-2′H-pyran)-12-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl--Larabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside and 20–0% (10E,14E,16E)-(1R,4S,5′S,6S,6′R,8R,12S,13S,20R,21R,24S)-21,24-dihydroxy-6′-isopropyl-5′,11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.14,8.020,24]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(5′,6′-dihydro-2′H-pyran)-12-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-Larabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside

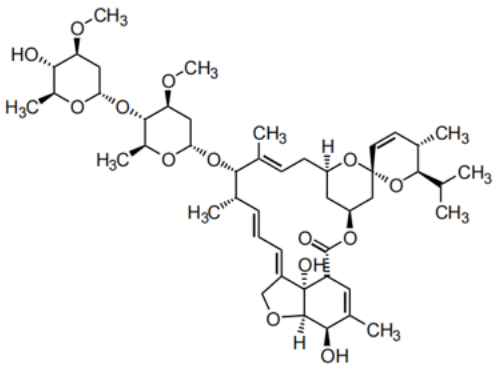
Common name: Abamectin

CAS-Number: Abamectin: 71751-41-2
Avermectin B_{1a}: 65195-55-3
Avermectin B_{1b}: 65195-56-4

Structure:



Avemectin B_{1a}



Avemectin B_{1b}

Molecular mass: Avermectin B_{1a}: 873.1
Avermectin B_{1b}: 859.1

Empirical formula: Avermectin B_{1a}: $C_{48}H_{72}O_{14}$
Avermectin B_{1b}: $C_{47}H_{70}O_{14}$

3. Samples

In January 2025, Information Sheet No. 495 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Abamectin by HPLC. Two test samples (described below), including the Abamectin analytical reference standard were shipped to the participants:

A) Abamectin TC-1

B) Abamectin TC-2

Abamectin analytical reference standard:

Purity of Abamectin: 96.3%

Purity of Avermectin B_{1a}: 95.0%; Avermectin B_{1b}: 1.3%

All participants sent back their results in time.

4. Method

4.1 Scope

The content of Abamectin in technical materials was determined.

4.2 Principle

The content of Abamectin in the technical materials is determined by high performance liquid chromatography on a reversed phase column (C18) with UV detection at 245 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. Conditions and 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	Storage Condition	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (µL)	Mobile phase (v/v)
1	Temperature:3-4 ° C Humidity:30-40%	Thermo Scientific Vanquish Flex uHPLC	XBridge C18(150×4.6 mm, 5 µm)	0.5	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
2	Temperature:25°C	Shimadzu LC-20AB	Luna C18 Phenomenex (250×4.6 mm, 5 µm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
3	Temperature: 5.2°C Humidity:77.9%	Vanquish Core, Thermo Scientific	Phenomenex BDS C18(250×4.6 mm, 130 Å)	0.8	30±0.2	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
4	Temperature: 4°C Humidity:39 %	Agilent 1260 Infinity, Quaternery Pump	Supelco Merck, RP-18 endcapped Purospher Hibar HR(100×2.1 mm,<2um)	0.5	30	245	2	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
5	Temperature: 24°C Humidity: 44%	Agilent 1260 Infinity I	Phenomenex Kinetex C18 (250×4.6 mm, 5 µm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
6	Temperature:-18 ℃ Humidity:10-13%	Thermo UltiMate3000	XDB-C18 (250×4.6 mm, 5 µm)	0.8	30±2	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)

Lab Number	Storage Condition	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (μL)	Mobile phase (v/v)
7	Temperature:4℃	Shimadzu LC-20A	Agilent Zorbax SB-C18 (250×4.6 mm, 5 μm)	1	30±2	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
8	Temperature:4℃ Humidity:45%	Agilent 1260 Infinity II	ZORBAX Eclipse plus C18(250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
9	Temperature:4°C	Agilent 1260 Infinity	XORBAX SB-C18 (250×4.6 mm, 5 μm)	0.8	30±2	245	5	Methanol:Acetonitrile:Water 45:40:15 (v/v/v)
10	Temperature: 4℃	Agilent 1260	Eclipse XDB-C18 (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
11	Temperature: 4℃	Shimadzu LC20A	Shim-pack VP-ODS (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
12	Temperature: 4-8°C	Agilent Infinity 1200	Restek Roc C18 (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
13	Temperature: 4℃	Dionex HPLC system UV/VIS	Zorbax XDB C18 (250×3 mm, 5 μm)	0.5	25	254	5	0.1% Phosphoric acid;Acetonitrile
14	Temperature: 4-8℃	Agilent 1200 Series	Agilent Extend-C18 (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
15	Temperature: 4~6℃ Humidity:40-55%	Agilent 1260 Infinity II	Agilent ZORBAX Eclipse plus C18 (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)

Lab Number	Storage Condition	Instrument	Column	Flow Rate (mL/min)	Column Temp. ℃	Wavelength (nm)	Injection Volume (μL)	Mobile phase (v/v)
16	Temperature: 4-8℃ Humidity:50-70%	Agilent 1260	Eclipes Plus C18 (250×4.6 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
17	Temperature:4.8-5.8℃	Agilent, DAD, 1260 series	Agilent ZORBAX SB-C18(250×4.6 mm, 5 μm)	1	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)
18	Temperature:5°C Humidity:30 %	Agilent 1290, DAD detector	Stainless steel ODS C18 (250×4.0 mm, 5 μm)	0.8	30	245	5	Methanol:Acetonitrile:Water 55:30:15 (v/v/v)

Remark and Comment

No.	Remark	Reply
Lab 1	ajusted flow rate because of column length	Adjust as a minor change
Lab 3	1.Analytical standard of Abamectin is stored in an ampoule without cap after opening.Therefore we weighed standard at the same time (2 weighings for "Day1" and the other two weighings for "Day 2"). --> Prepare standards (CA and CB) for "Day 1 " on 8th Apr and for "Day 2" on 10th Apr 2025. 2. Code for TC: TC1, batch no. 202502110; TC2, batch no. 202503015	-
Lab 5	At RT 9.00 min there is Emamectin benzoate B1b, at RT 10.9 Emamectin benzoate B1a, as mentioned much earlier than I would have expected it according to your method.	No impact on the result

Considering the method deviations from Lab 13 included column diameter, injection volume, wavelength, column temperature and mobile phase, the method used in Lab 13 was quite different from the proposed method.

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 that of Lab 12. After checking the chromatograms of Day 1 from Lab 12, the retention time of both peaks were varied significantly (For B1a, 22.5min ~ 26.6min) which may due to the unstable of system.

6.2 Determination of Abamectin

All laboratories provided duplicate data for two different days. As shown in tables 1-3 and figures 1-2, all results have been reported, and were taken into account by a statistical procedure after evaluating the validity of the results.

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, one straggler was identified in the TC-1 data from Lab 12 and no outliers was identified. A comparison of the RSDR of this collaborative Study with the unmodified Horwitz equation showed that the relative reproducibility standard deviation (RSDR) is below the Horwitz value in all samples (TC-1 and TC-2) and the HorRat values was between

0.3-1 even without elimination of the straggler (see in Table 3).
After eliminated the straggler (data from Lab 12 for TC-1) and the data of Lab 13 obtained from the method with major deviation, all the other data were re-analyzed (see in Table 4). The minimum number of considered results after the elimination was 16 for TC-1 and 17 for TC-2. And the results for the data after the elimination were found further improved and still within the desired range i.e. RSDR was lower than RSDR(Hor) and HorRat was in the range of 0.3~1. Due to the universal applicability of the method, this collaborative trial is acceptable.

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

	Abamectin TC-1		Abamectin TC-2	
	Day1	**Day2**	**Day1**	**Day2**
Lab 1	947.5	944.6	947.9	950.5
Lab 2	950.5	943.9	945.6	943.0
Lab 3	958.9	949.7	950.2	950.1
Lab 4	953.1	952.9	957.6	961.1
Lab 5	963.8	967.0	964.9	968.9
Lab 6	939.1	944.0	944.5	945.0
Lab 7	957.5	955.7	956.0	953.2
Lab 8	953.2	953.6	954.0	953.6
Lab 9	944.4	939.1	945.3	943.7
Lab 10	949.2	950.1	953.6	948.8
Lab 11	958.7	956.7	957.1	958.1
Lab 12	954.1	800.3	950.1	959.5
Lab 13	935.7	936.8	940.8	942.4
Lab 14	956.9	955.9	955.4	954.9
Lab 15	946.5	945.5	950.8	951.0
Lab 16	944.2	944.6	935.9	944.0
Lab 17	952.1	953.0	954.7	952.5
Lab 18	957.9	964.8	958.3	963.0

Table 2: Mean values

	Abamectin TC-1	Abamectin TC-2
Lab 1	948	954
Lab 2	946	951
Lab 3	952	959
Lab 4	956	969
Lab 5	966	970
Lab 6	943	957
Lab 7	956	964
Lab 8	954	955
Lab 9	943	953
Lab 10	950	964
Lab 11	958	965
Lab 12	916*	944
Lab 13	939	951
Lab 14	956	964
Lab 15	948	944
Lab 16	942	948
Lab 17	953	962
Lab 18	961	974

* Grubbs test straggler

Table 3: Summary of the statistical evaluation without elimination of any outliers /stragglers

	Abamectin TC-1	**Abamectin TC-2**
x_m [g/kg]	949	958
L	18	18
S_r	18.40	8.371
S_R	19.31	11.40
S_L	5.855	7.743
r	51.51	23.44
R	54.06	31.93
RSD_r	1.9	0.87
RSD_R	2.0	1.2
RSD_R (Hor)	2.0	2.0
HorRat	1.0	0.6

Table 4: Summary of the statistical evaluation elimination of straggler and invalid data

	Abamectin TC-1	**Abamectin TC-2**
x_m [g/kg]	952	959
L	16	17
S_r	2.755	8.566
S_R	7.182	11.57
S_L	6.632	7.784
r	7.714	23.99
R	20.11	32.41
RSD_r	0.3	0.9
RSD_R	0.8	1.2
RSD_R (Hor)	2.0	2.0
HorRat	0.4	0.6

TC-1: Results of Lab 12 and 13 were eliminated; TC-2: Result of Lab 13 was eliminated
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 – 2 (all results)

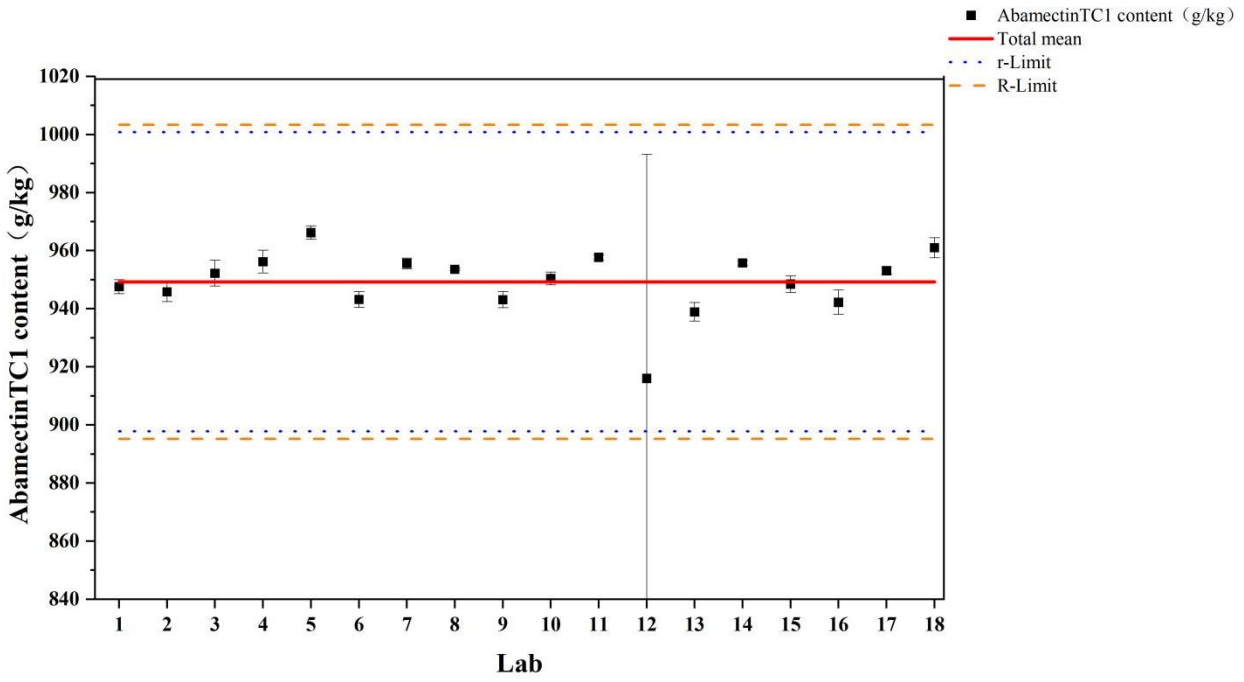


Figure 1. Graphical presentation of TC1 data

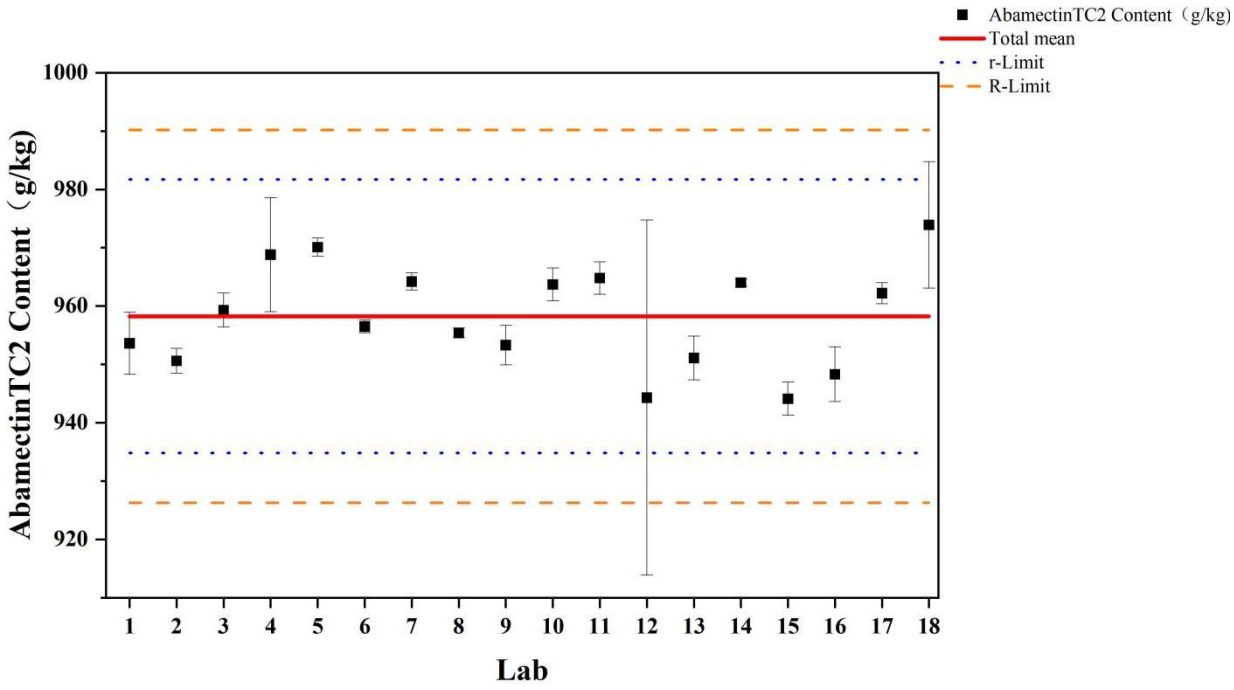


Figure 2. Graphical presentation of TC2 data

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 3-4. The results are illustrated in figures 1-2.

For TC1, the between lab experimental Relative Reproducibility Standard Deviation (%RSDR) is below the calculated acceptable value based on the Horwitz curve calculation (RSDR (Hor)) after eliminating data of Lab 12 and 13, and the HorRat values were between 0.3-1 by employing this method.

For TC2, the RSDR were below the RSDR (Hor) after eliminating data of Lab 13, and the HorRat values were between 0.3-1 by employing this method.

Taking into account the relatively high number of participating laboratories, a broad basis was given even after elimination of the straggler and invalid data. Therefore, CHIPAC considers this method to be suitable and recommend accepting it as a provisional CIPAC method for the determination of abamectinin technical materials.