MATRINE

Collaborative Study

Full Scale Collaborative Study for the Determination of
Matrine, by Reversed Phase HPLC

Report to CIPAC
By
Chinese Pesticide Analytical Committee（CHIPAC）

Method Developed by CHENGDU NEWSUN CROP SCIENCE CO., LTD

May 2022

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1. Participants

By mid of May 2022, 17 of the 18 laboratories provided their results on the determination of Matrine according to CIPAC Information Sheet No. 331.

One participant (Laboratory 18) could not complete the test in time due to the failure receipt of samples caused by COVID-19 pandemic.

The results for the 17 participants are presented in the following section.

Participating laboratories are listed in the table below, and lab numbers in all tables were assigned according to the chronological order of enrollment.

Javier García-Hierro Navas	Laboratorio Arbitral Agroalimentario Aguarón, 13. E28023 Madrid, Spain
Kaiwei Shi	Institute for the Control of Agrochemicals, Ministry of Agricultrue and Rural Affairs Maizidian street 22, Chaoyang District, Beijing, P. R. China
Olga Novakova	UKZUZ (Central Institute for Supervising and Testing in Agriculture), Czech Republic Zemedelska 1a, 613 00 Brno Czech Republic
Florentina Ciotea	Head of laboratory for quality control of pesticides National Phytosanitary Authority 11 Voluntari Bvld.,VOLUNTARI, Romania
Mirror Chen	GreenTech Laboratory CO. Ltd 2th Building, 650 Shunqing Road, Songjiang, Shanghai 201612, China
Chunqing Hou, Haixia Wang	Shenyang SYRICI Testing Co., Ltd. No.8, Shenliao East Road, Tiexi District Shenyang 110021, P.R. China
Cornel Grecu	Alchimex quality control laboratory ALCHIMEXSA, 63-ALEXANDRU CONSTANTINESCU–011472, BUCHAREST-1, ROMANIA.
Hongxia Li	Nutrichem Laboratory Co., Ltd. No. 27, Life Science Park Road, Changping Dist., Beijing 102206, P. R. China
Elen Karasali, Anna Marousopoulou	Benaki Phytopathological Institute 8 Stefanou Delta Street, Kifissia, Athens, 14561 Greece.
Jim Garvey	Department of Agriculture, Food and The Marine,Ireland The Food Chemistry Laboratories, Backweston Laboratory Complex, Backweston, Celbridge W23 X3PH, Co. Kildare, Ireland
Agus Salim	PT Agriculture Construction (AGRICON) Jl. Siliwangi No. 68 Bogor 16134 West Java Indonesia
Aiping Xu	Laprode (Zhejiang) analysis Co., Ltd 4/F, Building 6, No.503 Xingguo Road, Yuhang District, Hangzhou, Zhejiang P.R. China

Vanessa Lecocq	Walloon Agricultural Research Centre (CRA-W) Knowledge and Valorization of Products Department (D4) Protection, control products and residues Unit (U10) Carson Building Rue du Bordia, 115030 GEMBLOUX BELGIUM
Judy Dong	BNS GLP TEST FACILITY, China 199 Fenghuang 8th Road, Bincheng District, Binzhou City, Shandong Province, China
Lily Yan	Jiangsu Rotam Chemistry Co., Ltd No.88 Rotam Road, ETDZ, Kunshan, Jiangsu, 215301, China
Bo Zhang	Central Research Institute of China Chemical Science and Technology Co., Ltd 309, Beijiao building, No. 20 Xueyuan Road, Haidian District, Beijing, China
Jianzhong Yu	Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences 1-5040, New Area of Zhejiang Academy of Agricultural Sciences, No. 198, Shiqiao Road, Hangzhou, Zhejiang, China
Lu Huang	Hunan Research Institute of Chemical Industry Testing Technology Co., Ltd. No. 550, Changsha Avenue, Lituo street, Yuhua District, Changsha City, Hunan Province, China

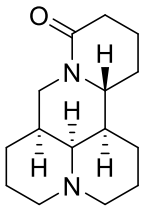
2. Matrine, General Information

Chemical name: (1R,2R,9S,17S)-7,13-diazatetracyclo [7.7.1.02,7.013,17] heptadecan -6-one

Common name: Matrine

CAS-Number: 519-02-8

Structure:



Molecular mass: 248.36 g/mol

Empirical formula: $C_{15}H_{24}N_2O$

3. Samples

In February 2022, Information Sheet No. 332 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study on the determination of Matrine by reversed phase HPLC.

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

A) Matrine TK-1
B) Matrine TK-2
C) Matrine SL-1
D) Matrine SL-2
E) Matrine SL-3

Matrine analytical reference standard (95.2% purity)

All participants except for Laboratory 18 (total of 17 laboratories) sent back their results in time.

4. Method

4.1 Scope

The content of Matrine in technical concentrate and in formulated products (soluble liquid) was determined.

4.2 Principle

The content of Matrine in the in technical concentrate and the formulation (SL) is determined by reversed phase HPLC on Inertsustain 150 x 4.6 mm (i.d) columns, C18 packed with octadecyl silane filler (5 µm), or equivalent, with mobile phase composed of acetonitrile / water (0.02% Ammonium acetate + 0.02% Triethylamine) = 23 / 77 (v/v) and UV detection at 215 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:	Column	wavelength	Injection volume:	Column Temp:	Remarks
Laboratory 1	Shimadzu-20 AT	Acetonitrile : 0.02 % ammonium Acetate + 0.02 % triethylamine in water = 23 : 77 (v/v)	1.0 mL/min	Thermo scientific ODS Hypersil, particle size 5 µm, 150 x 4.6 mm id	215 nm	10 µL	30 °C	
Laboratory 2	Agilent 1200	Acetonitrile : Water(0.02% Ammonium acetate+0.02% Triethylamine)=23:77(v/v)	1.0 mL/min	Inertsil ODS-3 150*4.6mm 5 Micron with Column ID,150mm*4.6mm	215 nm	10 µL	30 °C	
Laboratory 3	Agilent 1100 Series	Acetonitrile - Water (0.02 % Ammonium acetate + 0.02 % Triethylamine) (23-77 v/v)	1.0 mL/min	Phenomenex Prodigy ODS-3.5 µm,150 x 4.6 mm i.d.	215 nm	10 µL	30 °C	
Laboratory 4	Agilent 1260 Infinity Ⅱ	Acetonitrile : Water (0.02% Ammonium acetate + 0.02% Triethylamine) = 23 : 77(v/v)	1.0 mL/min	Agilent ZORBAX Eclipse Plus C18 5 µm, octadecyl silane filler,150 mm, 4.6 mm	215 nm	10 µL	30 °C	Day 1: the last Standard Cc was forgotten, and the result 1240.93878 was the mean value of the last two Standards Cc before it.
Laboratory 5	SHIMADZU LC20A	Acetonitrile / Water (0.02% Ammonium acetate + 0.02% Triethylamine)) = 30/ 70(v/v)	0.85 mL/min	Shim pack GIST C18, 250*4.6mm, 5µm	215 nm	10 µL	30 °C	
Laboratory 6	Agilent 1200	Acetonitrile / Water (0.02% Ammonium acetate + 0.02% Triethylamine) =	1.0 mL/min	InertSustain AQ-C18, 5 µm, 4.6 mm × 150 mm	215 nm	10 µL	30 °C	

		23 / 77 (v / v)						
Laboratory 7	Shimadzu LC-30A	Acetonitrile / Water（0.02% Ammonium acetate + 0.02% Triethylamine）=23/77（v/v）	1.0 mL/min	XTERRA MS C18 5 µm, Column,150 mm, 4.6 mm	215 nm	10 µL	30 °C	retention time: approximately 9.9 min
Laboratory 8	Agilent 1260	Acetonitrile/Water（0.02%Ammonia acetate+0.02%Triethylamine）=23/77(v/v)	1.0 mL/min	InertSustain C18 packed with octadecyl silane filler (5 µm), 250mm×4.6 mm (i.d.)	215 nm	10 µL	30 °C	
Laboratory 9	Agilent 1100 series - DAD	Acetonitrile/ Water (0,02% ammonium acetate + 0,02% triethylamine) (23:77)	1 mL/min	Phenomenex Gemini® 5 µm, C18 110 Å,150 mm x 3.0 mm	215 nm	20 µL	30 °C	We have prepared two standard solutions each day. Therefore Ca-Cc and Cb-Cd have the same weight
Laboratory 10	Agilent 1200	Acetonitrile/Water(0.02% Ammonium acetate+0.02% Triethylamine)=23/77 (v/v)	1.0 mL/min	InertSustain C18, 150 mm×4.6 mm (i.d.), 5 µm	215 nm	10 µL	30 °C	
Laboratory 11	Waters Alliance e2695, PDA detector 2998	77%Water (0.02% AmAc+0.02% TEA) 23%Acetonitrile mixed on-line	1 mL/min	Zorbax Extend C18 5 um, C18 ,150 x 4.6 mm	215 nm	10 µL	30 °C	software: Empower 3 MF was mixed by instrument pH of buffer (0.02% AmAc+0.02% TEA) was 9.7
Laboratory 12	Dionex	77 %Water (0.02 % ammonium acetate, 0.02 % triethyl amine) 23 % acetonitrile	1 mL/min	Phenomenex Gemini NX C18 3 microns,150x4.6 mm	215 nm	10 µL	30 °C	TK and SL formulation tend to evaporate, stoppered flasks were needed. Analysis is far to be an optimum

								Results are for Matrine not for Ethephon.
Laboratory 13	Shimazu,LC-20AT	Acetonitrile/water(0.02% Ammonium acetate + 0.02% Triethylamine)= 23+77(v/v)	1 mL/min	shimadzu shim-pack GWS 5μm, C18,150 mm, 4.6μm	215 nm	10 µL	30 °C	The proportion of mobile phase Acetonitrile was increased to 80% after 10 min to flash out any potential interferences
Laboratory 14	Agilent 1260 infinity Ⅱ	Acetonitrile/Water(0.02% Ammonium acetate+0.02% Triethylamine)=23/77(v/v)	1.0 mL/min	Agilent Eclipse XDBC18 5 µm,150 mm×4.6 mm	215 nm	10 µL	30 °C	
Laboratory 15	Thermo UltiMate 3000RS	Acetonitrile / H2O (0.2% TEA/0.2% Ammonium acetate) 27/73 v/v	0.35 mL/min	Phenomenex Kinetex 2.6 µm C18 100A,100 x 3 mm	215 nm	1 µL	30 °C	
Laboratory 16	1260 System with DAD	Acetonitrile / Ultrapure Water (0.02% Ammonium acetate + 0.02% Triethylamine) = 23 / 77 (v/v)	1.0 mL/min	Acdaim TM 120 C18 5 µm,4.6mm×150mm	215 nm	10 µL	30 °C	
Laboratory 17	LC-20AB (Shimadzu)	Acetonitrile / Water (0.02% Ammonium acetate + 0.02%Triethylamine) = 23/ 77(v/v)	1.3 mL/min	Nucleodur C18 Gravity C18, 5 µm , 150 x 4.6 mm	215 nm	10 µL	35 °C	Modifications on the method: flow rate=1.3 mL/min (instead 1 mL/min) and column Temperature 35 ℃ instead 30℃)

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 17 participants were not expected to affect the analysis results significantly.

6.2 Determination of Matrine

Results reported by the laboratories and the statistical evaluation are listed in tables 1-4 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 Mandel’s k-statistics on the within-lab variance, followed by Mandel’s h-statistics on the lab means, 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 for TK-1, SL-1, SL-2 and SL-3 without elimination of stragglers and outliers (see Table 3). The relative reproducibility standard deviation (RSD_R) is below the Horwitz value for all samples (TK-1, TK-2, SL-1, SL-2 and SL-3) with elimination of stragglers and outliers and no more than three Lab results have been removed per sample (see Table 4). The Horwitz Ratio (HorRat) was found within the desired range (0.3-1.0). Due to the universal applicability of the method this collaborative trial is acceptable.

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

	Matrine TK-1		Matrine TK-2		Matrine SL-1		Matrine SL-2		Matrine SL-3	
	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2
Lab 1	102.18**	96.78**	130.08	127.44	3.1484*	3.0764*	3.0386	3.0497	3.0524	3.1080
Lab 2	101.32	103.02	132.34	132.62	3.1634	3.1764	3.0996	3.1356	3.1100	3.1091
Lab 3	101.98	102.71	131.86	132.28	3.1853	3.1831	3.1592	3.1509	3.1857	3.1602
Lab 4	102.57	102.92	134.53	134.30	3.2326	3.2357	3.1943	3.2132	3.2046	3.2141
Lab 5	101.30	100.92	101.00	100.68	3.2483*	3.1657*	3.2064	3.1520	3.2338	3.1730
Lab 6	98.75	97.90	124.46	122.42	3.0280	3.0390	3.0507	3.0274	3.0307	3.0536
Lab 7	106.39	106.13	137.94	137.25	3.2347	3.2435	3.2306	3.2269	3.2355	3.2213
Lab 8	102.98	102.99	132.59	133.25	3.2446	3.2367	3.1829	3.1801	3.2064	3.2201
Lab 9	101.90	101.96	132.71	133.04	3.2214	3.2025	3.1778	3.1718	3.1951	3.2033
Lab 10	104.74	102.68	135.23	135.20	3.2659	3.2644	3.2727	3.2103	3.2399	3.2178
Lab 11	101.39	101.25	128.68	132.65	3.1621	3.1152	3.1080	3.1275	3.1224	3.1122
Lab 12	102.54	103.08	102.67	102.88	3.1603	3.1750	3.1094	3.1747	3.1448	3.1726
Lab 13	101.23	101.25	131.26	131.25	3.1680	3.1879	3.1242	3.1550	3.1549	3.1592
Lab 14	101.72	99.89	127.60	127.85	3.1295	3.0726	3.0851	3.0606	3.1050	3.0781
Lab 15	102.44	102.43	130.54	131.73	3.1920	3.2458	3.1541	3.2434	3.2006	3.2276
Lab 16	103.40	102.31	132.85	131.27	3.0355	3.0370	3.1194	3.0733	3.0832	3.0596
Lab 17	108.63	111.04	118.71	118.43	3.0279	2.9930	3.0387**	2.9066**	3.4471*	3.5214*

* Mandel's k-statistic straggler

** Mandel's k-statistic outlier

Table 2: Mean values

	Matrine TK-1	Matrine TK-2	Matrine SL-1	Matrine SL-2	Matrine SL-3
Lab 1	99.48	128.76	3.1124	3.0442	3.0802
Lab 2	102.17	132.48	3.1699	3.1176	3.1096
Lab 3	102.35	132.07	3.1842	3.1551	3.1730
Lab 4	102.75	134.42	3.2342	3.2038	3.2094
Lab 5	101.11	100.84**	3.2070	3.1792	3.2034
Lab 6	98.33	123.44	3.0335	3.0391	3.0422
Lab 7	106.26	137.60	3.2391	3.2288	3.2284
Lab 8	102.99	132.92	3.2407	3.1815	3.2133
Lab 9	101.93	132.88	3.2120	3.1748	3.1992
Lab 10	103.71	135.22	3.2652	3.2415	3.2289
Lab 11	101.32	130.67	3.1387	3.1178	3.1173
Lab 12	102.81	102.78*	3.1677	3.1421	3.1587
Lab 13	101.24	131.26	3.1780	3.1396	3.1571
Lab 14	100.81	127.73	3.1011	3.0729	3.0916
Lab 15	102.44	131.14	3.2189	3.1988	3.2141
Lab 16	102.86	132.06	3.0363	3.0964	3.0714
Lab 17	109.84**	118.57**	3.0105*	2.9727*	3.4843**

* Mandel's h-statistic straggler

** Mandel's h-statistic outlier

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

	TK-1	TK-2	SL-1	SL-2	SL-3
xm [g/kg]	102.49	127.34	3.1617	3.1356	3.1754
xm [% w/w]	10.25	12.73	0.3162	0.3136	0.3175
L	17	17	17	17	17
S_r	1.19	0.98	0.0260	0.0356	0.0226
S_R	2.69	10.60	0.0806	0.0771	0.1011
S_L	2.41	10.56	0.0763	0.0683	0.0985
r	3.35	2.73	0.0728	0.0996	0.0634
R	7.54	29.69	0.2257	0.2157	0.2831
RSD_r	1.17	0.77	0.82	1.13	0.71
RSD_R	2.63	8.33	2.55	2.46	3.18
RSD_R (Hor)	2.82	2.73	4.76	4.76	4.75
HorRat	0.93	3.05	0.54	0.52	0.67

Table4: Summary of the statistical evaluation with elimination Mandel's h and k Statistic Stragglers /Outliers

	TK-1	TK-2	SL-1	SL-2	SL-3
xm [g/kg]	102.20	131.62	3.1728	3.1458	3.1561
xm [% w/w]	10.22	13.16	0.3173	0.3146	0.3156
L	15	14	14	16	16
S_r	0.67	1.07	0.0187	0.0283	0.0193
S_R	1.77	3.51	0.0743	0.0646	0.0637
S_L	1.63	3.34	0.0719	0.0581	0.0607
r	1.88	3.00	0.0719	0.0792	0.0540
R	4.94	9.82	0.2080	0.1809	0.1784
RSD_r	0.66	0.81	0.59	0.90	0.61
RSD_R	1.73	2.66	2.34	2.05	2.02
RSD_R (Hor)	2.82	2.71	4.75	4.76	4.76
HorRat	0.61	0.98	0.49	0.43	0.42

TK-1: Results of Lab 1, 17 were eliminated;
TK-2: Results of Lab 5, 12, 17 were eliminated;
SL-1: Results of Lab 1, 5, 17 were eliminated;
SL-2: Results of Lab 17 were eliminated;
SL-3: Results of Lab 17 were 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 – 5 (all results)

Fig. 1:

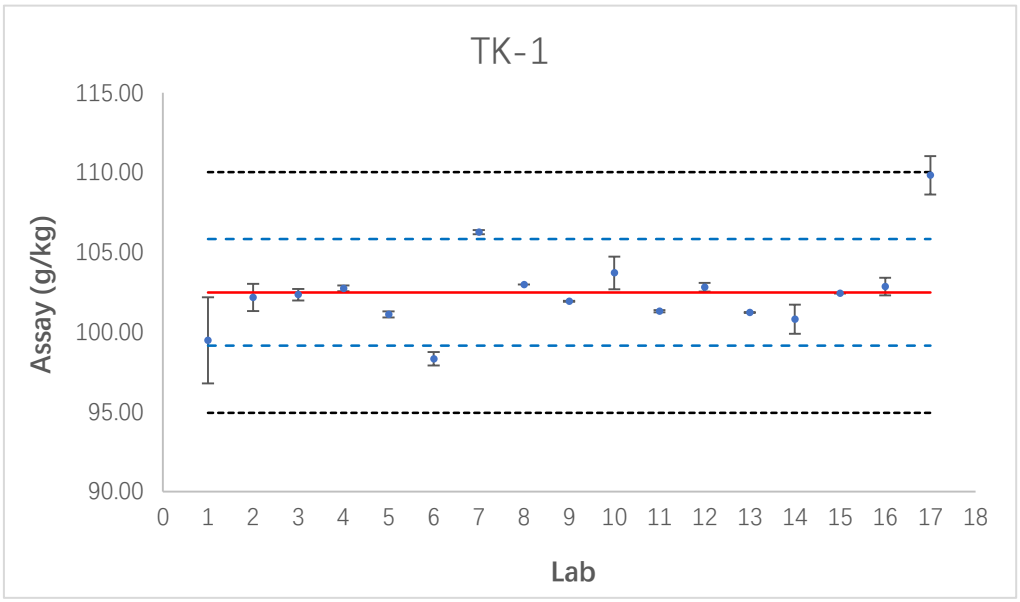


Fig. 2:

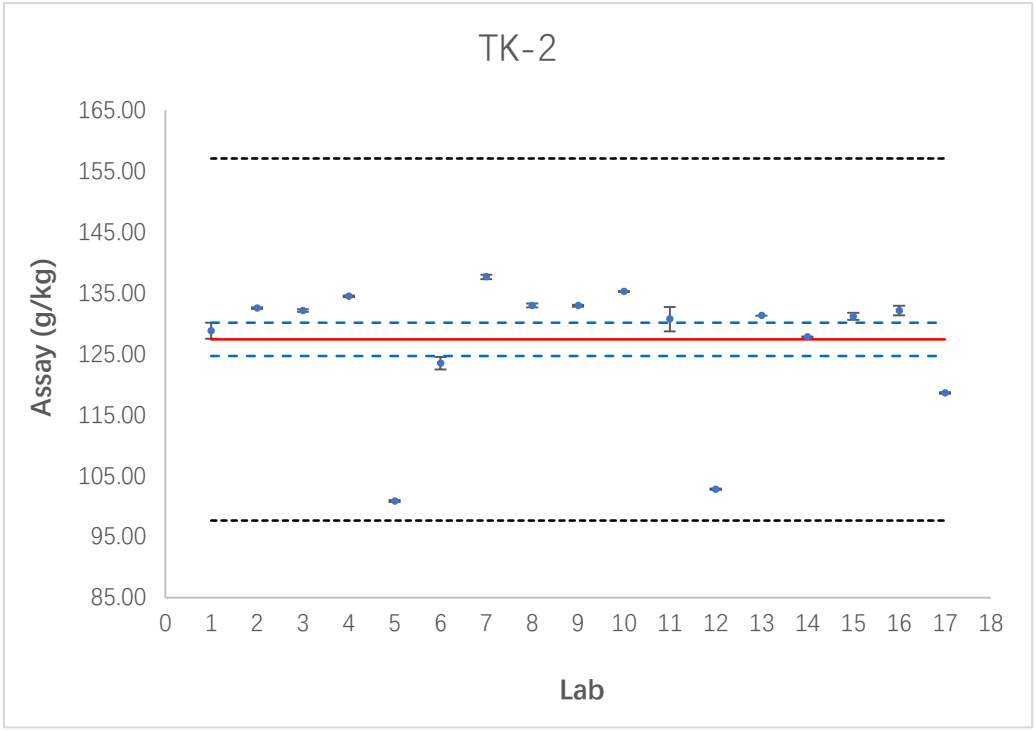


Fig. 3:

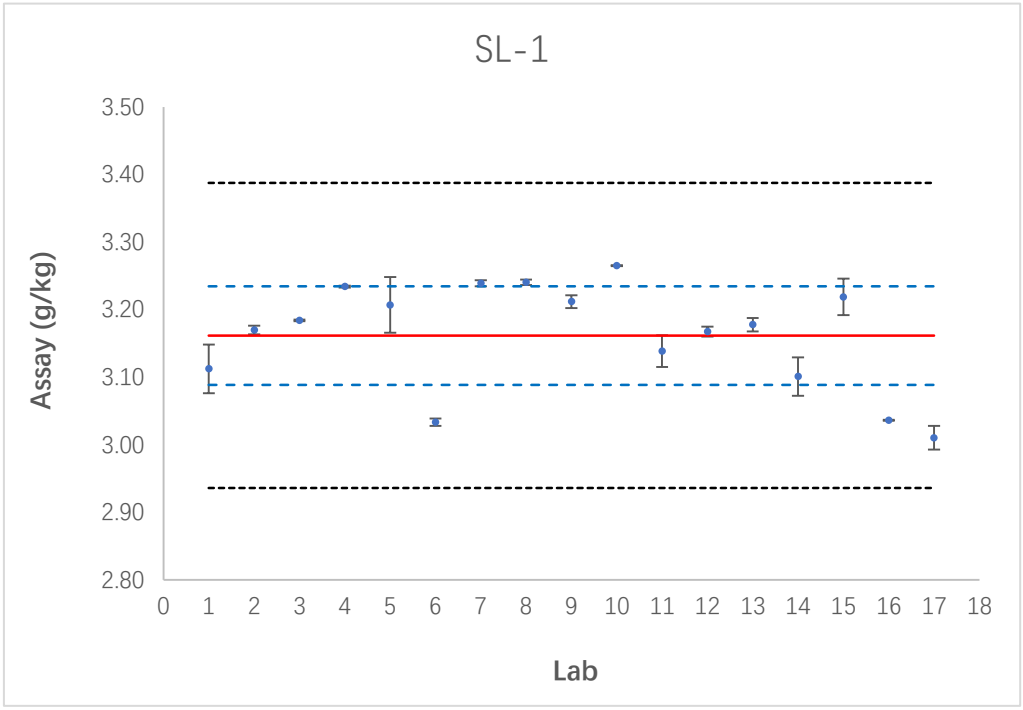

SL-1
Assay (g/kg)
3.50
3.40
3.30
3.20
3.10
3.00
2.90
2.80
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Lab

Fig. 4:

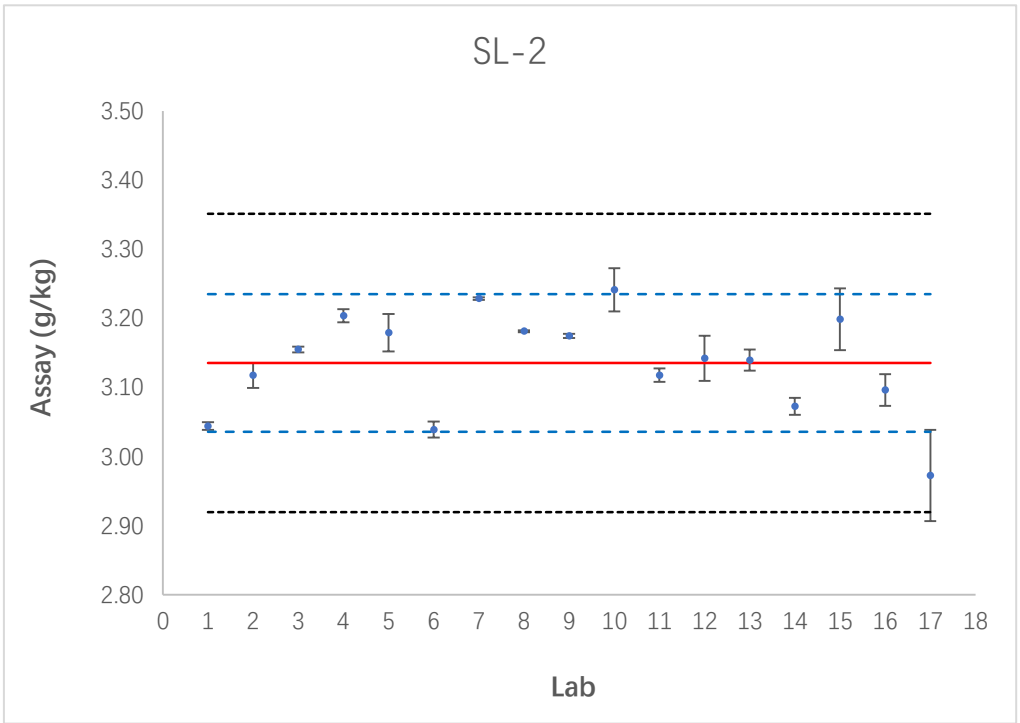
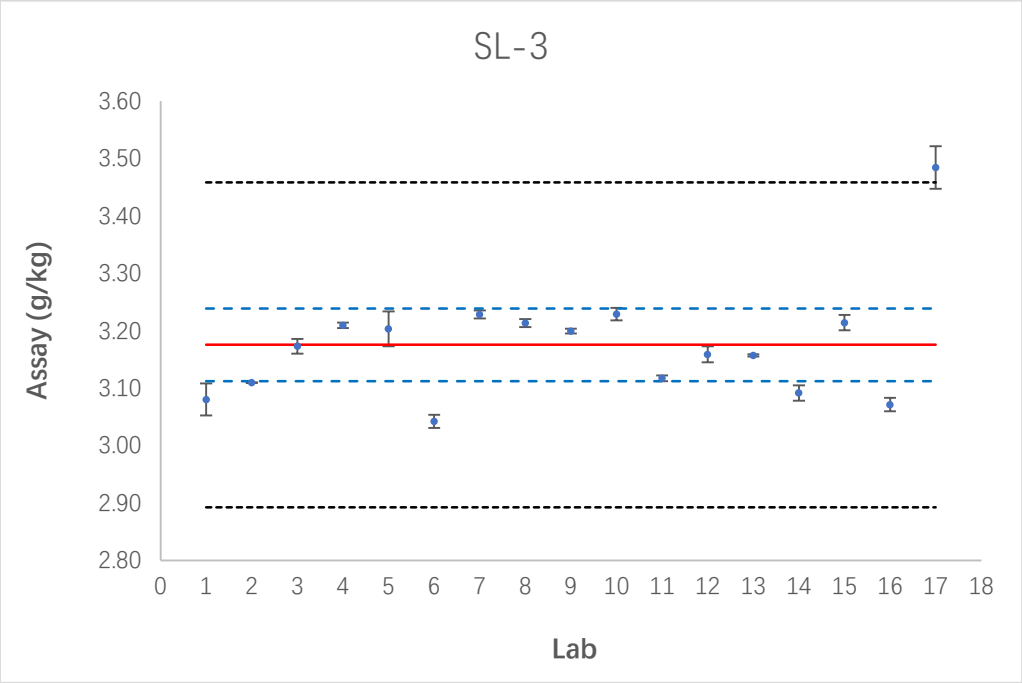

SL-2
Assay (g/kg)
3.50
3.40
3.30
3.20
3.10
3.00
2.90
2.80
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Lab

Fig. 5:


SL-3
Assay (g/kg)
3.60
3.50
3.40
3.30
3.20
3.10
3.00
2.90
2.80
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Lab

7. Conclusions

17 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 4-5. The results are illustrated in figures 1–5.

With elimination of outliers and stragglers, 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 all within the required range by employing this method. The minimum number of considered results after elimination of stragglers and outliers was 14.

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