Determination of Matrine Active in TK and SL

Small Scale Collaborative Study for the Determination of Matrine Active in TK and SL by High Performance Liquid Chromatography

Report to CIPAC
by
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1. Participants

Name of responsible person	Lab Name	City, Country
Shan Qian	School of Food and Bioengineering, Xihua University	Sichuan, China
Yang Yan Qin	CHENGDU NEWSUN CROP SCIENCE CO., LTD	Sichuan, China
Pan Yue	The Ministry of Education Key Laboratory of Standardization of Chinese Herbal Medicine, Chengdu University of Traditional Chinese Medicine	Sichuan, China
Wu Liang	Jinhua Boyue Agricultural Development Co., Ltd	Zhejiang, China

Laboratories were identified by a confidential number prior to the trial commencing.

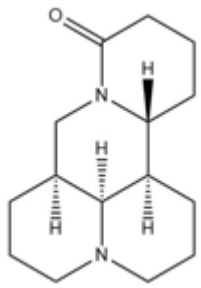
2. Active Ingredient, General Information

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

Common name: Matrine

CAS-Nr.: 519-02-8

Structure:



Molecular mass: 248.36

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

3. Samples

In Feb. 2021 the following samples were sent to the participants:
Describe sample:
TK: brown liquid without visible suspended solids
SL: homogeneous liquid without visible suspended solids

In 29/30.03.2021 results were obtained.

4. Method

4.1 Scope

The content of Matrine is determined in technical concentrate and soluble liquid products.

4.2 Principle

The Matrine content of the samples is determined by high performance liquid chromatography on InertSustain C18 column with UV detector at 215 nm, quantified by external standard method.

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 No	Column	Mobil phase	Flow rate ml/min	Column temp. (℃)	Injection vol. (μl)
1	Inertsuatain 150mm*4.6mm C18（5um）	Acetonitrile / Water (0.02% Ammonium acetate + 0.02%Triethylamine) = 23/ 77(v/v)	1	30	10
2	Inertsuatain 150mm*4.6mm C18（5um）	Acetonitrile / Water (0.02% Ammonium acetate + 0.02%Triethylamine) = 23/ 77(v/v)	1	30	10
3	Agilient，4.6x100mm，2.7 Micron with Column ID,USCFS09415	Acetonitrile / Water (0.02% Ammonium acetate + 0.02%Triethylamine) = 23/ 77(v/v)	1	30	10
4	Inertsuatain 150mm*4.6mm C18（5um）	Acetonitrile / Water (0.02% Ammonium acetate + 0.02%Triethylamine) = 23/ 77(v/v)	1	30	10

6. Remarks of the Participants

Several participants made comments about the performance of the method and noted deviations from the method:

Laboratory 1	Column: Inertsuatain 150mm*4.6mm C18（5um） Remarks: None
Laboratory 2	Column: Inertsuatain 150mm*4.6mm C18（5um） Remarks: None
Laboratory 3	Column: Agilient，4.6x100mm，2.7 Micron with Column ID,USCFS09415 Remarks: None
Laboratory 4	Column: Inertsuatain 150mm*4.6mm C18（5um） Remarks: None

7. Evaluation and Discussion

The full results of 4 labs were included within the statistical assessment. 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 assay results obtained by the collaborators and the statistical evaluation are reported in Table 1-4.

The testing for outliers/stragglers of the laboratory mean values were performed according to Grubbs test on a 1%/5% significance level, respectively. The Grubbs test identified stragglers and outliers for the SL formulations as well as for the technical concentrate (marked with + in Table 1).

All results reported by the 4 laboratories are reported and the statistical evaluation of these are listed in Tables 1-3 and displayed in Figures 1-5. These results are reported without any exclusion of outliers and/or stragglers. In addition, a separate evaluation, listed in Table 4, display the results with the exclusion of stragglers.

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 Matrine in technical concentrate and SL formulation.

CHIPAC proposes to proceed with a large scale collaborative study.

9. Appendix A

Tables and Figures for Matrine.

Table 1-1: Matrine assay in TK and SL (g/kg); results for each laboratory on day 1 and day 2

	Matrine SAMPLE A				Matrine SAMPLE B				Matrine SAMPLE C	
	Day1		Day2		Day1		Day2		Day1	
Laboratory 1	117.3	121.8	113.6	119.7	120.3	122.8	118.6	119.3	2.99	2.99
Laboratory 2	111.2	112.7	113.1	112.6	115.4	116.6	116.1	116.5	2.94	2.93
Laboratory 3	113.9	114.5	114.8	111.5	114.3	114.3	120.6	121.9	2.89	2.89
Laboratory 4	112.5	113.0	113.0	112.0	116.6	116.5	115.9	115.7	2.92	2.90

Table 1-2: Matrine assay in TK and SL (g/kg); results for each laboratory on day 1 and day 2

	Matrine SAMPLE C		Matrine SAMPLE D				Matrine SAMPLE E			
	Day2		Day1		Day2		Day1		Day2	
Laboratory 1	2.93	2.94	3.10	3.07	2.93	2.94	3.53	3.53	3.48	3.47
Laboratory 2	2.96	2.95	2.99	2.98	2.99	2.97	3.55	3.56	3.57	3.58
Laboratory 3	2.85	2.86	2.91	2.90	2.85	2.85	3.46	3.46	3.51	3.40
Laboratory 4	2.91	2.91	2.95	2.95	2.96	2.96	3.52	3.53	3.52	3.52

Table 2: Mean values

	Matrine SAMPLE A	Matrine SAMPLE B	Matrine SAMPLE C	Matrine SAMPLE D	Matrine SAMPLE E
Laboratory 1	118.1 [+]	120.3	2.97	3.02	3.51
Laboratory 2	112.4	116.2	2.94	2.99	3.57
Laboratory 3	113.7	117.8	2.88	2.88	3.46
Laboratory 4	112.6	116.2	2.91	2.96	3.53

[+] Gubbs Test straggler

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

	TK-1	TK-2	SL-1	SL-2	SL-3
Xm	114.2	117.6	2.923	2.956	3.512
L	4	4	4	4	4
S_r	1.971	2.250	0.02051	0.04695	0.02847
S_R	3.159	2.743	0.04362	0.07010	0.05100
r	5.519	6.300	0.05880	0.13146	0.07972
R	8.845	7.680	0.12214	0.19628	0.14280
RSD_r	1.726	1.914	0.70194	1.5881	0.81062
RSD_R	2.767	2.333	1.493	2.371	1.452
RSD_R (Hor)	2.772	2.760	4.814	4.805	4.682
HorRat Value	0.998	0.845	0.310	0.493	0.310

Xm = average

L = number of laboratories

S_r = repeatability standard deviation

S_R = reproducibility standard deviation

RSD_r = repeatability relative standard deviation

RSD_R = reproducibility relative standard deviation

r = repeatability

R = reproducibility

RSD_R (Hor) = Horwitz value calculated from: 2^(1 - 0.5log c) where c = the concentration of the analyte as a decimal fraction

Table 4: Summary of the statistical evaluation - with elimination of Gubbs Test stragglers

	TC-1	TC-2	SL-1	SL-2	EC
Xm	112.9	117.6	2.923	2.956	3.512
L	3	4	4	4	4
S_r	1.026	2.250	0.02051	0.04695	0.02847
S_R	1.119	2.743	0.04362	0.07010	0.05100
r	2.873	6.300	0.05880	0.13146	0.07972
R	3.133	7.680	0.12214	0.19628	0.14280
RSD_r	0.909	1.914	0.70194	1.5881	0.81062
RSD_R	0.991	2.333	1.493	2.371	1.452
RSD_R (Hor)	2.777	2.760	4.814	4.805	4.682
HorRat Value	0.357	0.845	0.310	0.493	0.310

Sample A Results of Lab 1 eliminated.

Fig. 1: Results of the Matrine Tk-1(see table 2 for the evaluation)

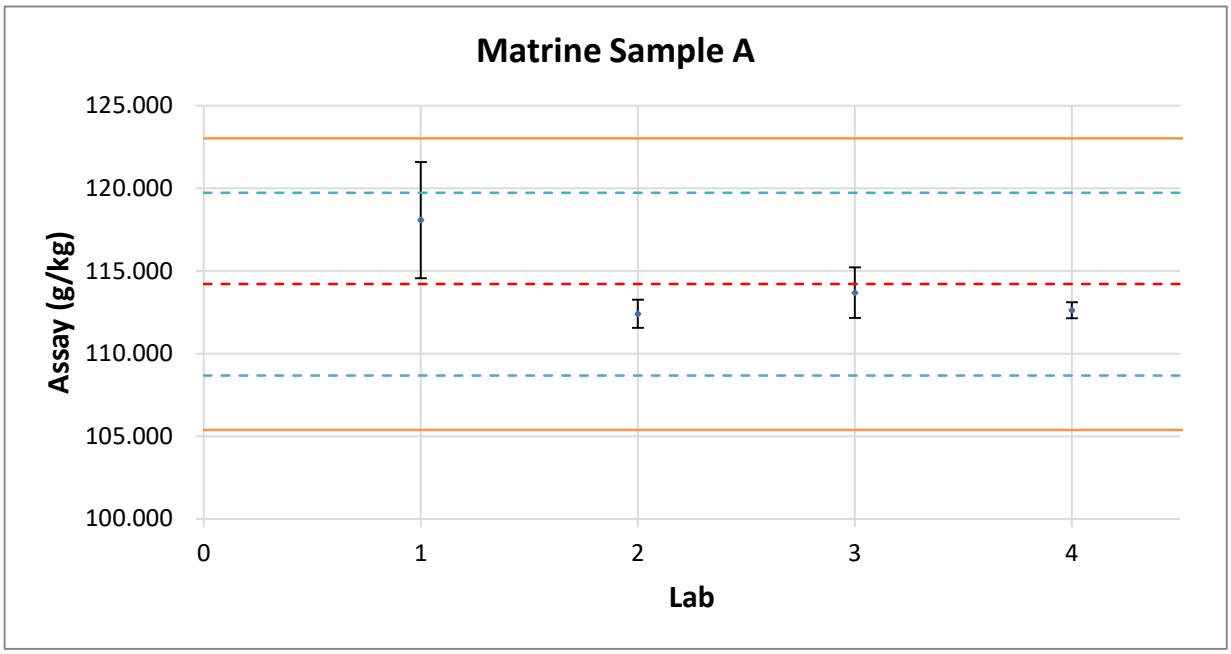


Fig. 2: Results of the Matrine Tk-2(see table 2 for the evaluation)

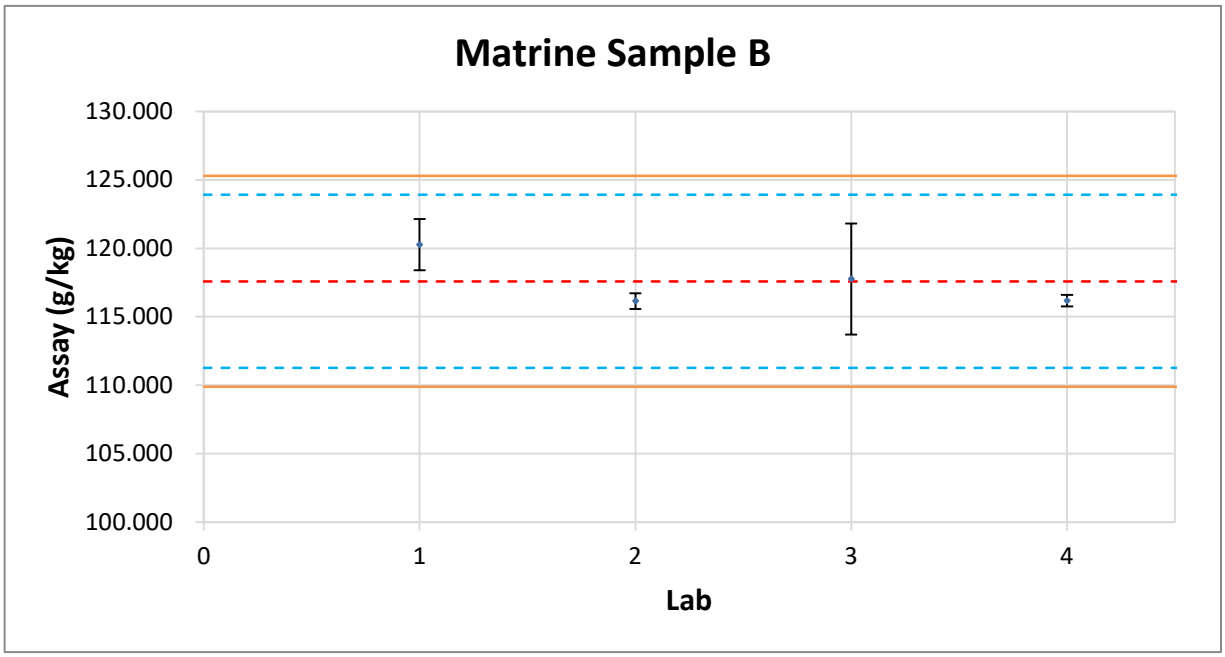


Fig. 3: Results of the Matrine SL-1(see table 2 for the evaluation)

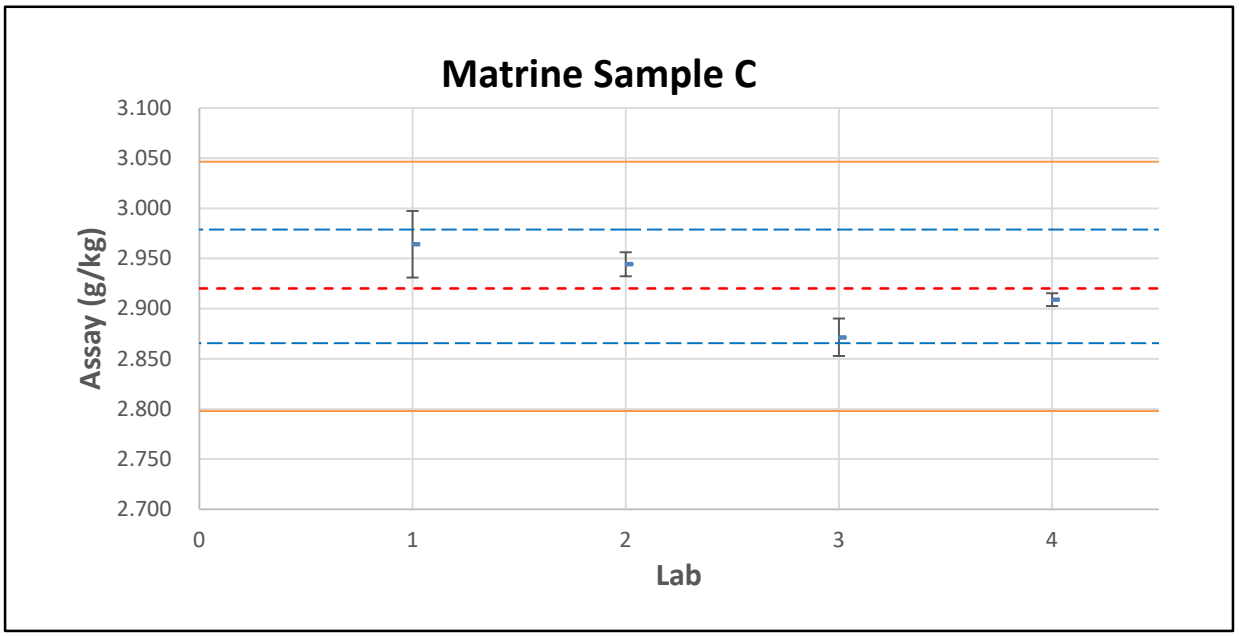


Fig. 4: Results of the Matrine SL-2(see table 2 for the evaluation)

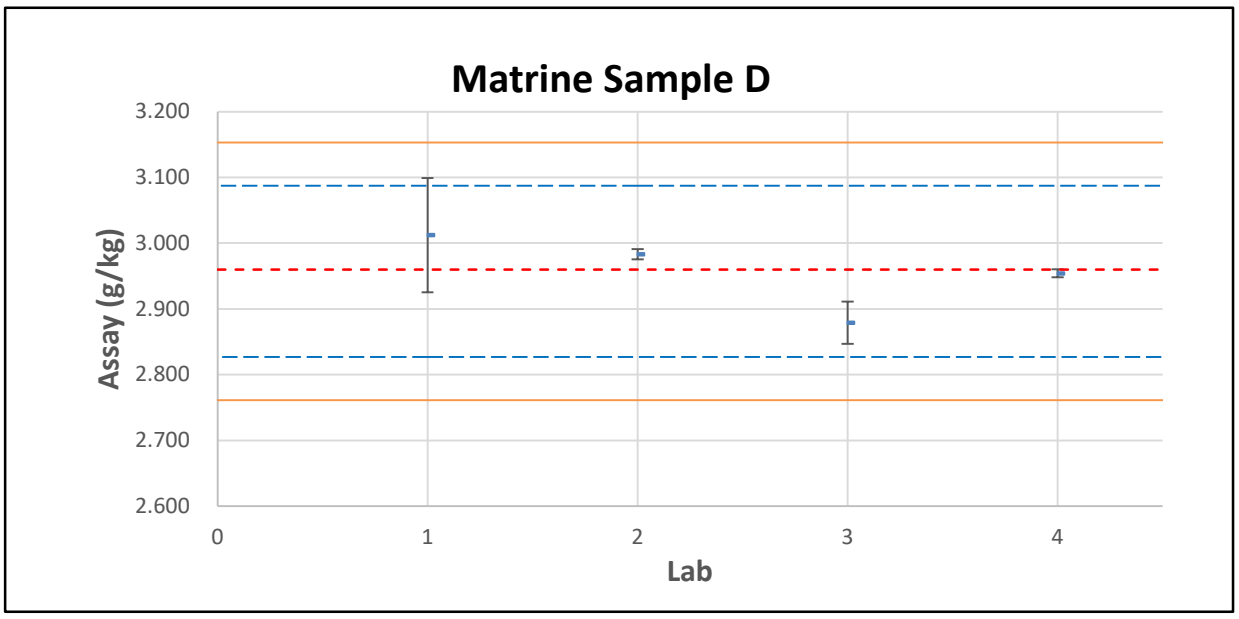


Fig. 5: Results of the Matrine SL-3 (see table 2 for the evaluation)