

## Determination of 28-Homobrassinolide Active in TC, SL and EC

### Small Scale Collaborative Study for the Determination of 28-Homobrassinolide Active in TC, SL and EC by High Performance Liquid Chromatography

Report to CIPAC  
by  
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Content	page
<b>1. PARTICIPANTS</b>	<b>3</b>
<b>2. ACTIVE INGREDIENT, GENERAL INFORMATION</b>	<b>3</b>
<b>3. SAMPLES</b>	<b>4</b>
<b>4. METHOD</b>	<b>4</b>
<b>4.1 PRINCIPLE</b>	<b>4</b>
<b>4.2 PROCEDURE FOR THE COLLABORATIVE TRIAL</b>	<b>4</b>
<b>5. ANALYTICAL CONDITIONS</b>	<b>4</b>
<b>6. REMARKS OF THE PARTICIPANTS</b>	<b>4</b>
<b>7. EVALUATION AND DISCUSSION</b>	<b>5</b>
<b>8. CONCLUSIONS</b>	<b>5</b>
<b>9. APPENDIX A TABLES AND FIGURES</b>	<b>6</b>

## 1. Participants

Name of responsible person	Lab Name	City, Country
Liu ShuZhen	Jiangxi Windeal Biotechnology Co., Ltd. - Quality Department	Jiangxi, China
Sun Fengying	Kaifeng Yitian Biotechnology Co., Ltd. - Quality Control Department	Henan, China
Chen Mirror	GreenTech Laboratory Co., Ltd.	Shanghai, China
Mei Quanfu	Jiangxi Buffett Chemical Co., Ltd. - Quality Control Department	Jiangxi, China
Chang Feng	Shandong Huihan Biotechnology Co., Ltd. - Analysis Room	Shandong, China
Liu Xinsheng	Guangdong Zhongxun Agricultural Technology Co., Ltd. - PTD Center	Guangdong, China

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

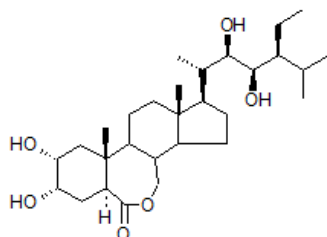
## 2. Active Ingredient, General Information

IUPAC name: (5S,6R)-10-((2S,3R,4R,5S)-5-ethyl-3,4-dihydroxy-6-methylheptan-2-yl)-5,6-dihydroxy-7a,9a-dimethyltetradecahydro-1H-benzo[c]indeno[5,4-e]oxepin-3(12bH)-one

Common name: 28-Homobrassinolide

CAS-Nr.: 80483-89-2

Structure:



Molecular mass: 494.8

Empirical formula: C<sub>29</sub>H<sub>50</sub>O<sub>6</sub>

## 3. Samples

In Feb. 2020 the following samples were sent to the participants:

Describe sample:

TC: white powdery or crystalline solid

SL: colorless or light yellow homogeneous liquid

EC: homogeneous liquid without visible suspended solids

In 19/20.04.2020 results were obtained.

## 4. Method

### 4.1 Scope

The content of 28-Homobrassinolide is determined in technical material, soluble liquid and emulsifiable concentrate products.

### 4.2 Principle

The 28-Homobrassinolide content of the samples is determined by high performance liquid chromatography on ODS-C18 film stainless column with UV detector at 220 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. (°C)	Injection vol. (µl)
1	SHIMADZU, 4.6mm×250mm, 8FR98171	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
2	Elite, 4.6mm×250mm, E2616571	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
3	Shimadzu InerStain™ C18, 250mm×4.6mm, 5µm	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
4	Agilent, 4.6mm×250mm, USNH075205	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
5	Agilent, TC-C18(2) 250mm×4.6mm	Acetonitrile + water = 80 + 20 (v/v)	1	35	5
6	XTERRA MS C18, 4.6×250mm, 03273926014075	Acetonitrile + water = 80 + 20 (v/v)	1	25	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: SHIMADZU, 4.6mm×250mm, 8FR98171 Remarks: None
Laboratory 2	Column: Elite, 4.6mm×250mm, E2616571 Remarks: None
Laboratory 3	Column: Shimadzu InerStain™ C18, 250mm×4.6mm, 5µm Remarks: None
Laboratory 4	Column: Agilent, 4.6mm×250mm, USNH075205 Remarks: None
Laboratory 5	Column: Agilent, TC-C18(2), 250mm×4.6mm Remarks: Column temperature is 35°C, Injection volume is 5µL.
Laboratory 6	Column: XTERRA MS C18, 4.6mm×250mm, 03273926014075 Remarks: None

## 7. Evaluation and Discussion

The full results of 6 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 1-5.

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 and EC formulations as well as for the technical concentrate (marked with +/+ in Table 1).

All results reported by the 6 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 4-5, display the results with the exclusion of stragglers and outliers.

## 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 28-Homobrassinolide in technical material, SL and EC formulation. CHIPAC proposes to proceed with a large scale collaborative study.

## 9. Appendix A

Tables and Figures for 28-Homobrassinolide.

**Table 1-1: 28-Homobrassinolide assay in TC, SL and EC (g/kg); results for each laboratory on day 1 and day 2**

	28-Homobrassinolide SAMPLE A				28-Homobrassinolide SAMPLE B				28- Homobrassinolide SAMPLE C	
	Day1		Day2		Day1		Day2		Day1	
Laboratory 1	959.5	957.7	955.1	956.1	951.6	952.3	951.0	952.9	0.041	0.041
Laboratory 2	959.7	957.1	958.7	954.6	950.5	950.8	953.4	959.2	0.040	0.042
Laboratory 3	953.1	956.4	953.5	954.8	937.8	939.7	938.4	937.9	0.038	0.038
Laboratory 4	954.6	960.5	954.2	957.9	954.6	951.6	952.8	952.1	0.042	0.042
Laboratory 5	952.0	952.0	953.2	954.1	957.9	956.5	952.1	955.4	0.040	0.041
Laboratory 6	955.6	956.1	955.7	955.7	953.2	948.8	949.5	956.6	0.042	0.042

**Table 1-2: 28-Homobrassinolide assay in TC, SL and EC (g/kg); results for each laboratory on day 1 and day 2**

	28- Homobrassinolide SAMPLE C		28-Homobrassinolide SAMPLE D				28-Homobrassinolide SAMPLE E			
	Day2		Day1		Day2		Day1		Day2	
Laboratory 1	0.041	0.041	0.042	0.042	0.042	0.042	0.101	0.101	0.103	0.101
Laboratory 2	0.041	0.040	0.046	0.047	0.044	0.042	0.104	0.105	0.101	0.102
Laboratory 3	0.038	0.038	0.038	0.039	0.038	0.038	0.085	0.085	0.086	0.0854
Laboratory 4	0.041	0.042	0.043	0.042	0.042	0.043	0.103	0.101	0.103	0.102
Laboratory 5	0.041	0.041	0.041	0.041	0.041	0.041	0.103	0.104	0.104	0.103
Laboratory 6	0.042	0.041	0.042	0.042	0.043	0.042	0.103	0.102	0.103	0.102

**Table 2: Mean values**

	28-Homobrassinolide SAMPLE A	28-Homobrassinolide SAMPLE B	28-Homobrassinolide SAMPLE C	28-Homobrassinolide SAMPLE D	28-Homobrassinolide SAMPLE E
Laboratory 1	957.1	952.0	0.041	0.042	0.102
Laboratory 2	957.5	953.5	0.041	0.045	0.103
Laboratory 3	954.5	938.5 <sup>+/++</sup>	0.038 <sup>+</sup>	0.038	0.086 <sup>+/++</sup>
Laboratory 4	956.9	952.8	0.042	0.042	0.102
Laboratory 5	952.9	955.5	0.041	0.041	0.104
Laboratory 6	955.8	952.0	0.041	0.042	0.102

<sup>+</sup> Gubbs Test straggler

<sup>++</sup> Gubbs Test outlier

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

	<b>TC-1</b>	<b>TC-2</b>	<b>SL-1</b>	<b>SL-2</b>	<b>EC</b>
<b>X<sub>m</sub></b>	955.7	950.7	0.041	0.042	0.100
<b>L</b>	6	6	6	6	6
<b>S<sub>r</sub></b>	1.862	2.535	0.00053	0.00098	0.00101
<b>S<sub>R</sub></b>	2.422	6.516	0.00146	0.00229	0.00715
<b>r</b>	5.214	7.098	0.00148	0.00274	0.00283
<b>R</b>	6.782	18.245	0.00409	0.00641	0.02002
<b>RSD<sub>r</sub></b>	0.195	0.267	1.296	2.342	1.017
<b>RSD<sub>R</sub></b>	0.253	0.685	3.585	5.485	7.173
<b>RSD<sub>R</sub> (Hor)</b>	2.014	2.015	9.160	9.123	8.004
<b>HorRat Value</b>	0.126	0.340	0.391	0.601	0.896

X<sub>m</sub> = average

L = number of laboratories

S<sub>r</sub> = repeatability standard deviation

S<sub>R</sub> = reproducibility standard deviation

RSD<sub>r</sub> = repeatability relative standard deviation

RSD<sub>R</sub> = reproducibility relative standard deviation

r = repeatability

R = reproducibility

RSD<sub>R</sub> (Hor) = Horwitz value calculated from:  $2^{(1 - 0.5 \log c)}$  where c = the concentration of the analyte as a decimal fraction



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

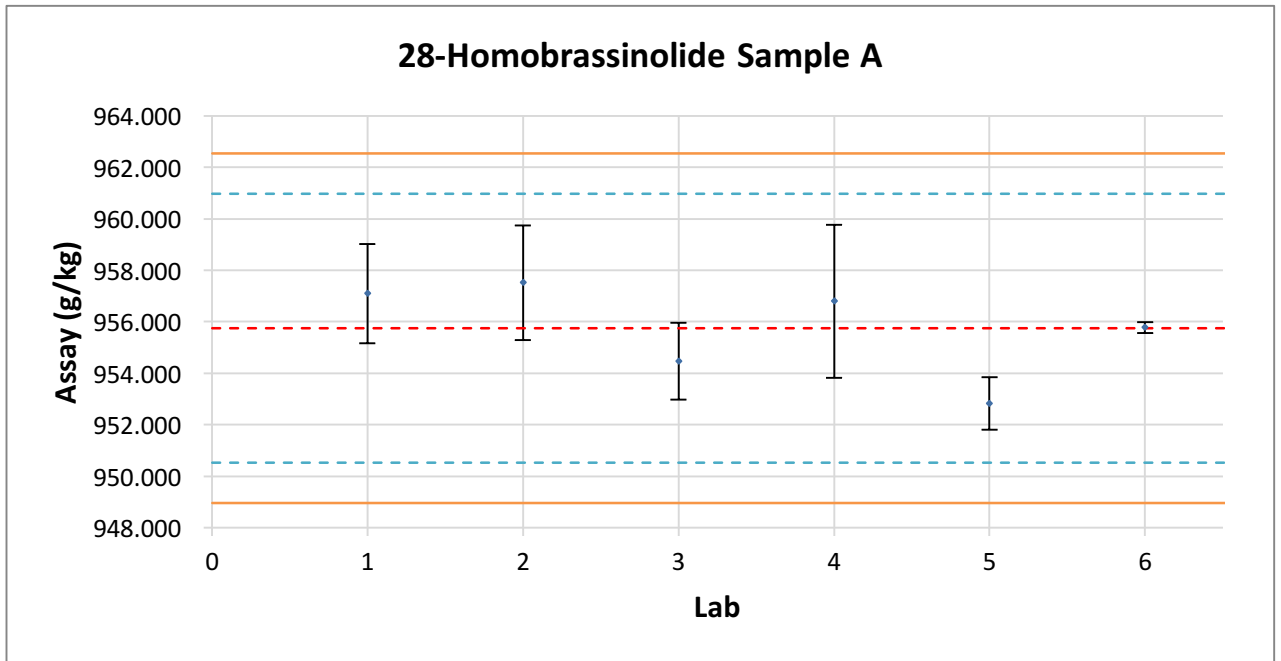
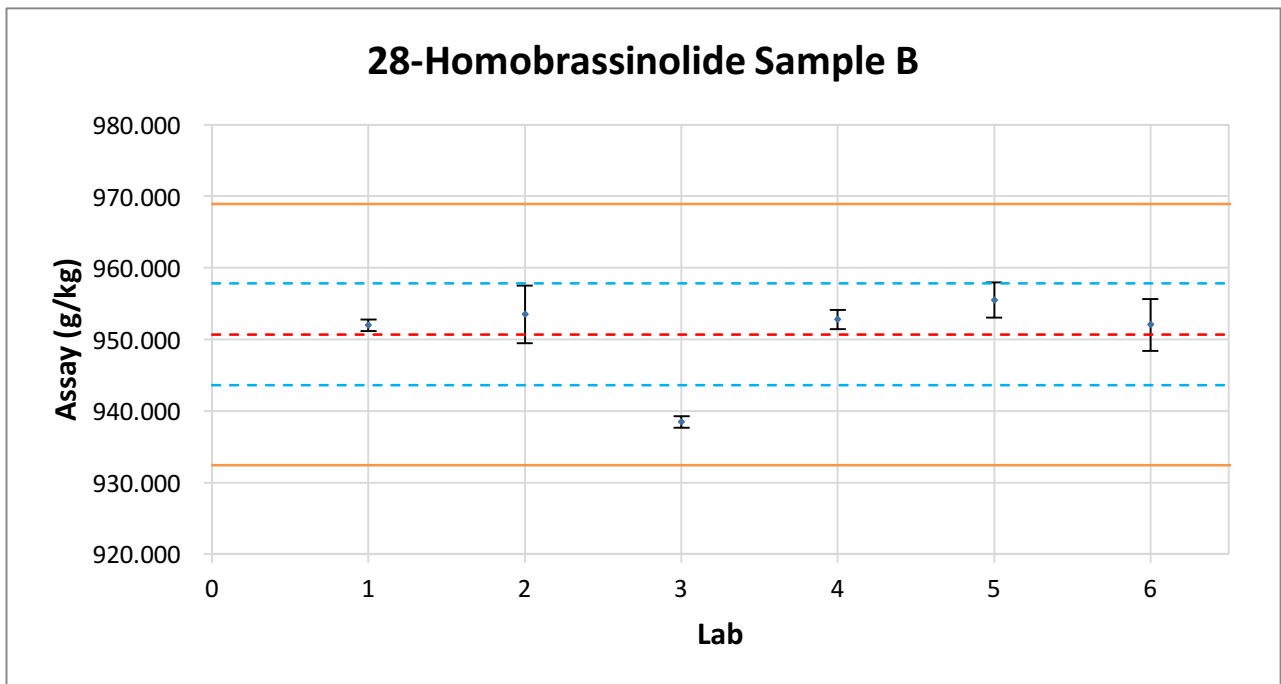
	<b>TC-1</b>	<b>TC-2</b>	<b>SL-1</b>	<b>SL-2</b>	<b>EC</b>
<b>Xm</b>	955.7	953.1	0.041	0.042	0.102
<b>L</b>	6	5	6	6	5
<b>S<sub>r</sub></b>	1.862	2.750	0.00053	0.00098	0.00109
<b>S<sub>R</sub></b>	2.422	2.786	0.00146	0.00229	0.00121
<b>r</b>	5.214	7.7	0.00148	0.00274	0.00305
<b>R</b>	6.782	7.801	0.00409	0.00641	0.00339
<b>RSD<sub>r</sub></b>	0.195	0.288	1.296	2.342	1.061
<b>RSD<sub>R</sub></b>	0.253	0.292	3.585	5.485	1.179
<b>RSD<sub>R</sub> (Hor)</b>	2.014	2.014	9.160	9.123	7.970
<b>HorRat Value</b>	0.126	0.145	0.391	0.601	0.148

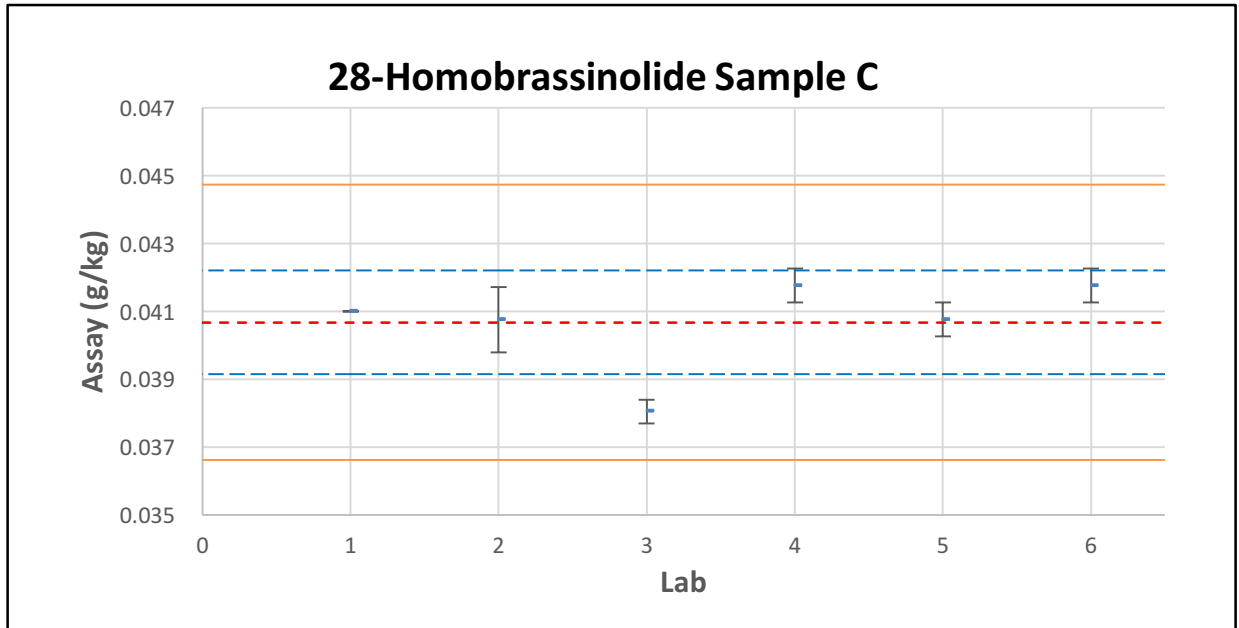
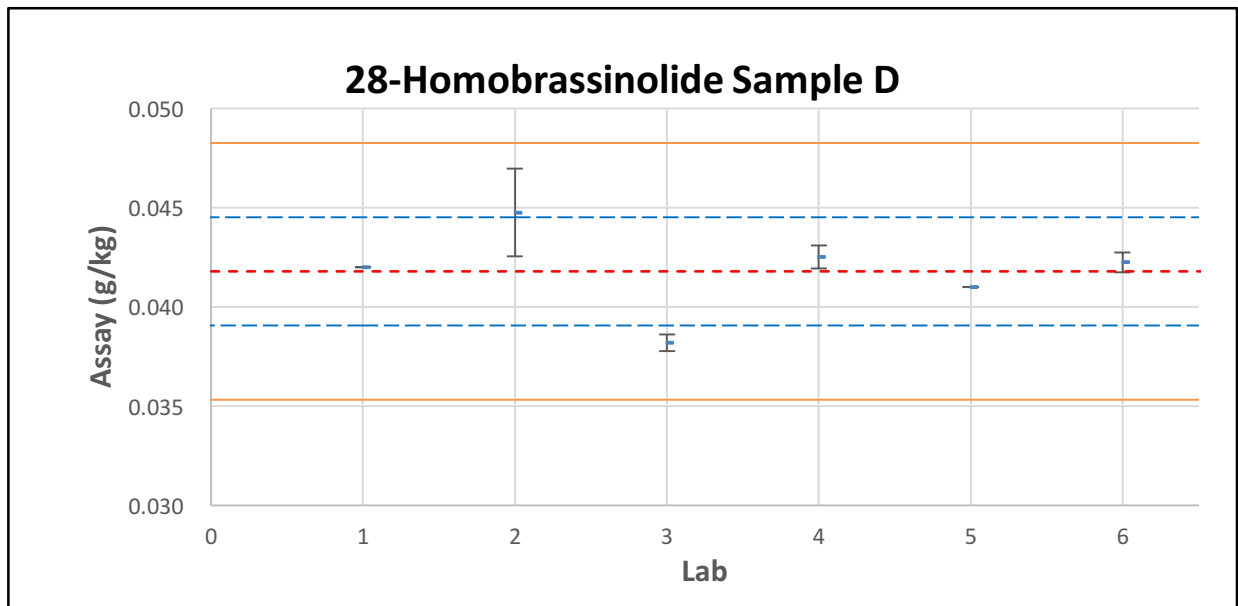
Sample B Results of Lab 3 eliminated, Sample E Results of Lab 3 eliminated.

**Table 5: Summary of the statistical evaluation** - with elimination of Gubbs Test outliers

	<b>TC-1</b>	<b>TC-2</b>	<b>SL-1</b>	<b>SL-2</b>	<b>EC</b>
<b>Xm</b>	955.7	953.1	0.041	0.042	0.102
<b>L</b>	6	5	5	6	5
<b>S<sub>r</sub></b>	1.862	2.750	0.00058	0.00098	0.00109
<b>S<sub>R</sub></b>	2.422	2.786	0.00072	0.00229	0.00121
<b>r</b>	5.214	7.7	0.00162	0.00274	0.00305
<b>R</b>	6.782	7.801	0.00202	0.00641	0.00339
<b>RSD<sub>r</sub></b>	0.195	0.288	1.401	2.342	1.061
<b>RSD<sub>R</sub></b>	0.253	0.292	1.738	5.485	1.179
<b>RSD<sub>R</sub> (Hor)</b>	2.014	2.014	9.142	9.123	7.970
<b>HorRat Value</b>	0.126	0.145	0.190	0.601	0.148

Sample B Results of Lab 3 eliminated, Sample C Results of Lab 3 eliminated, Sample E Results of Lab 3 eliminated.

**Fig. 1: Results of the 28-Homobrassinolide TC-1(see table 2 for the evaluation)****Fig. 2: Results of the 28-Homobrassinolide TC-2(see table 2 for the evaluation)**

**Fig. 3: Results of the 28-Homobrassinolide SL-1(see table 2 for the evaluation)****Fig. 4: Results of the 28-Homobrassinolide SL-2(see table 2 for the evaluation)**

**Fig. 5: Results of the 28-Homobrassinolide EC (see table 2 for the evaluation)**