

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

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

Report to CIPAC
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1. Participants

	Name of responsible person	Lab Name	City, Country
1	Helen Karasali	Benaki Phytopathological Institute	Greece
2	Denis Carr	Department of Agriculture, Food and the Marine	Ireland
3	Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.	China
4	Cornel Grecu	Laboratory of Alchimex	Romania
5	Angela Santilio	National Institute of Health – Department of Environment and Health – ECASS Section	Italy
6	Huang Liang	Anhui Fengle Agrochemical Co., Ltd. Product Testing Center	Auhui, China
7	UENO Takanori	Agricultural Chemicals Inspection Station, Food and Agricultural Materials Inspection Center	Japan
8	Vanessa Lecocq	Walloon Agricultural Research Centre (CRA-W) Knowledge and Valorization of Products	Belgium
9	Zhang Juntao	Helm China Co., Ltd.	China
10	Xu Aiping	Laprobe (Zhejiang) Analysis Co., Ltd	Zhejiang, China
11	Agus Salim	Laboratorium PT Agriculture Construction (AGRICON)	Indonesia
12	He Zhiyu	Guizhou JAD technology co., LTD	Guizhou, China
13	Chang Feng	Shandong Huihan Biotechnology Co., Ltd. - analysis room	Shandong, China
14	Sun Fengying	Kaifeng Yitian Biotechnology Co., Ltd. - quality control department	Henan, China
15	Liu Xinsheng	Guangdong Zhongxun Agricultural Technology Co., Ltd. - PTD Center	Guangdong, China
16	Liu ShuZhen	Jiangxi Windeal Biotechnology Co., Ltd. - Quality Department	Jiangxi, China

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

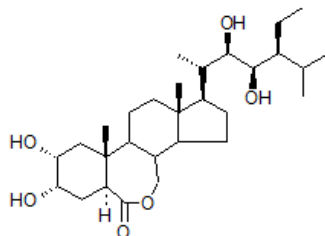
2. Active Ingredient, General Information

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: $C_{29}H_{50}O_6$

3. Samples

In Nov. 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 19/20.02.2021 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, Luna 5µm C18, 250 x 4.6 mm Phenomenex S/N: H19-029626	Acetonitrile + water = 80 + 20 (v/v)	1.3	25	10
2	SHIMADZU, Inertsil ODS-C18, 250mm x 4.6mm, 3µm	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
3	Agilent 250mm*4.6mm (id), packed with ZORBAX SB-C18, 5µm	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
4	AkzoNobel, Kromasil 100-5C18 250 x 4.6mm, batch no. E95557	Acetonitrile + water = 80 + 20 (v/v)	1	30	10
5	RESTEK Roc C18 5 um; 250 x 4.6 mm	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
6	ODS-3 (Inertsil) 4.6mm*250mm*5µm C/N.5020-01732 S/N.1A7184863	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
7	NUCLEOSIL 5C18 (250 mm x 4.6 mm, particle size 5 µm, Column No. L7A44)	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
8	Agilent Zorbax SB-C18, 5 µm, 250 mm x 4.6 mm. Batch # B07021	Acetonitrile + water = 80 + 20 (v/v)	1.5	25	10
9	SHIMADZU Shim-pack VP-ODS C18 250 mm x 4.6 mm	Acetonitrile + water = 80 + 20 (v/v)	1	30	10
10	Agilent Eclipse Plus C18 (4.6mm*250mm*5µm)	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
11	Phenomenex Gemini NX-C18 110 Å, 250 x 4.6 mm (i.d) , particle size 5 µm S/No : H19-280905 Part No : OOG-4454-E0	Acetonitrile + water = 80 + 20 (v/v)	1.4	25	10
12	GL Sciences Inertsil ODS-SP,250 mm*4.6 mm,5 µm	Acetonitrile + water = 80 + 20 (v/v)	1	30	10

13	Agilent 5 TC-C18(2) 250x4.6mm	Acetonitrile + water = 80 + 20 (v/v)	1	25	5
14	Elliot, 4.6mmx250mm E2616571	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
15	XTERRA MS c18, 4.60mm*250cm, 03273926014075	Acetonitrile + water = 80 + 20 (v/v)	1	25	10
16	SHIMADZU, 4.6x250mm, 8FR98171	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, Luna 5µm C18, 250 x 4.6 mm, Phenomenex S/N: H19-029626 Remarks: Flow rate was increased from 1ml/min to 1.3 ml/min.
Laboratory 2	Column: SHIMADZU, Inertsil ODS-C18, 250mm x 4.6mm, 3µm Remarks: The particle size of the HPLC column used was 3µm.
Laboratory 3	Column: Agilent 250mm*4.6mm (id), packed with ZORBAX SB-C18, 5µm Remarks: None
Laboratory 4	Column: AkzoNobel, Kromasil 100-5C18 250 x 4.6mm, batch no. E95557 Remarks: None
Laboratory 5	Column: RESTEK Roc C18 5 µm; 250 x 4.6 mm Remarks: None
Laboratory 6	Column: ODS-3 (Inertsil) 4.6mm*250mm*5µm, C/N.5020-01732 S/N.1A7184863 Remarks: None
Laboratory 7	Column: NUCLEOSIL 5C18 (250 mm X 4.6 mm, particle size 5 µm, Column No. L7A44) Remarks: None
Laboratory 8	Column: Agilent Zorbax SB-C18, 5 µm, 250 mm x 4.6 mm, Batch # B07021 Remarks: Flow rate was increased from 1ml/min to 1.5 ml/min.
Laboratory 9	Column: SHIMADZU Shim-pack VP-ODS C18 250 mm x 4.6 mm Remarks: None
Laboratory 10	Column: Agilent Eclipse Plus C18 (4.6mm*250mm*5µm) Remarks: None
Laboratory 11	Column: Phenomenex Gemini NX-C18 110 Å, 250 x 4.6 mm (i.d) , particle size 5 µm, S/No : H19-280905, Part No : OOG-4454-E0 Remarks: Flow rate was increased from 1ml/min to 1.4 ml/min.
Laboratory 12	Column: GL Sciences Inertsil ODS-SP,250 mmx4.6 mm,5 µm Remarks: Change the column temperature to 30°C.
Laboratory 13	Column: Agilent 5 TC-C18(2) 250x4.6mm Remarks: None
Laboratory 14	Column: Elliot, 4.6mmx250mm E2616571 Remarks: None
Laboratory 15	Column: XTERRA MS c18, 4.60mm*250cm, 03273926014075 Remarks: None
Laboratory 16	Column: SHIMADZU, 4.6x250mm, 8FR98171 Remarks: None

7. Evaluation and Discussion

The full results of 16 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 material (marked with +/+ in Table 1).

All results reported by the 16 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 TC samples, the values of RSD_R (reproducibility relative standard deviation) were less than Horwitz's value. As a reference, the HorRat values for TC were not greater than 1.0. All HorRat values for SL and EC were not greater than 2.0. No more than one value has been removed per sample (Table 4-5). The validity of the results and the suitability of the analytical method are shown. This collaborative trial is acceptable.

9. Appendix A

Tables and Figures for 28-Homobrassinolide.

Table 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		28-Homobrassinolide Sample D		28-Homobrassinolide Sample E	
	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2	Day1	Day2
Laboratory 1	954.8	953.4	930.7	949.6	0.047	0.048	0.055	0.056	0.127	0.126
Laboratory 2	930.9	966.2	944.3	1000.7	0.051	0.053	0.051	0.054	0.097	0.111
Laboratory 3	952.0	957.0	951.9	951.5	0.039	0.039	0.042	0.042	0.097	0.096
Laboratory 4	947.0	945.8	944.4	948.7	0.043	0.042	0.049	0.048	0.105	0.105
Laboratory 5	870.9	923.6	885.9	951.0	0.050	0.050	0.050	0.060	0.120	0.120
Laboratory 6	951.7	951.1	952.3	952.5	0.033	0.032	0.040	0.041	0.088	0.087
Laboratory 7	943.8	939.1	941.8	943.0	0.034	0.033	0.039	0.038	0.086	0.083
Laboratory 8	966.6	966.7	949.7	933.4	0.038	0.038	0.048	0.045	0.096	0.093
Laboratory 9	953.4	954.9	953.1	954.3	0.035	0.035	0.042	0.042	0.089	0.090
Laboratory 10	947.9	936.7	950.1	945.2	0.033	0.033	0.040	0.040	0.085	0.085
Laboratory 11	950.0	946.3	947.2	947.3	0.039	0.038	0.046	0.043	0.091	0.090
Laboratory 12	947.7	947.0	934.8	934.1	0.032	0.032	0.040	0.038	0.070	0.073
Laboratory 13	953.0	950.7	953.1	952.1	0.040	0.040	0.040	0.040	0.100	0.100
Laboratory 14	952.4	954.2	952.0	953.9	0.042	0.040	0.042	0.040	0.101	0.101
Laboratory 15	955.7	953.8	951.7	953.9	0.040	0.041	0.040	0.041	0.102	0.102
Laboratory 16	952.7	956.6	954.0	952.6	0.040	0.041	0.040	0.041	0.101	0.101

Table 2: Mean values

	28-Homobrassinoli de SAMPLE A	28-Homobrassinoli de SAMPLE B	28-Homobrassinoli de SAMPLE C	28-Homobrassinoli de SAMPLE D	28-Homobrassinoli de SAMPLE E
Laboratory 1	954.1	940.2	0.048	0.056	0.127
Laboratory 2	948.6	972.5	0.052	0.053	0.104
Laboratory 3	954.5	951.7	0.039	0.042	0.097
Laboratory 4	946.4	946.6	0.043	0.049	0.105
Laboratory 5	897.2 ^{+/**}	918.4 ⁺	0.050	0.055	0.120
Laboratory 6	951.4	952.4	0.033	0.041	0.088
Laboratory 7	941.5	942.4	0.034	0.039	0.085
Laboratory 8	966.7	941.6	0.038	0.047	0.095
Laboratory 9	954.2	953.7	0.035	0.042	0.090
Laboratory 10	942.3	947.7	0.033	0.040	0.085
Laboratory 11	948.2	947.3	0.039	0.045	0.091
Laboratory 12	947.4	934.5	0.032	0.039	0.072
Laboratory 13	951.9	952.6	0.040	0.040	0.100
Laboratory 14	953.3	953.0	0.041	0.041	0.101
Laboratory 15	954.8	952.8	0.041	0.041	0.102
Laboratory 16	954.7	953.3	0.041	0.041	0.101

⁺ Gubbs Test straggler

^{**} Gubbs Test outlier

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

	TC-1	TC-2	SL-1	SL-2	EC
X_m	947.9	947.5	0.040	0.044	0.097
L	16	16	16	16	16
S_r	11.516	15.909	0.00069	0.00592	0.00267
S_R	16.849	16.024	0.00608	0.00592	0.01360
r	32.245	44.545	0.00193	0.01658	0.00748
R	47.177	44.867	0.01702	0.01658	0.03808
RSD_r	1.215	1.679	1.724	4.754	2.739
RSD_R	1.777	1.691	15.300	13.410	13.958
RSD_R (Hor)	2.016	2.016	9.193	9.047	8.031
HorRat Value	0.882	0.839	1.664	1.482	1.738

X_m = 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: $2^{(1 - 0.5 \log c)}$ where c = the concentration of the analyte as a decimal fraction

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

	TC-1	TC-2	SL-1	SL-2	EC
X_m	947.7	947.2	0.040	0.044	0.097
L	15	15	16	16	16
S_r	11.893	16.431	0.00069	0.00592	0.00267
S_R	17.404	16.513	0.00608	0.00592	0.01360
r	33.300	46.007	0.00193	0.01658	0.00748
R	48.731	46.236	0.01702	0.01658	0.03808
RSD_r	1.255	1.735	1.724	4.754	2.739
RSD_R	1.836	1.743	15.300	13.410	13.958
RSD_R (Hor)	2.016	2.016	9.193	9.047	8.031
HorRat Value	0.911	0.865	1.664	1.482	1.738

Sample A Results of Lab 5 eliminated, Sample B Results of Lab 5 eliminated.

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

	TC-1	TC-2	SL-1	SL-2	EC
X_m	947.7	947.5	0.040	0.044	0.097
L	15	16	16	16	16
S_r	11.893	15.909	0.00069	0.00592	0.00267
S_R	17.404	16.024	0.00608	0.00592	0.01360
r	33.300	44.545	0.00193	0.01658	0.00748
R	48.731	44.867	0.01702	0.01658	0.03808
RSD_r	1.255	1.679	1.724	4.754	2.739
RSD_R	1.836	1.691	15.300	13.410	13.958
RSD_R (Hor)	2.016	2.016	9.193	9.047	8.031
HorRat Value	0.911	0.839	1.664	1.482	1.738

Sample A Results of Lab 5 eliminated.

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

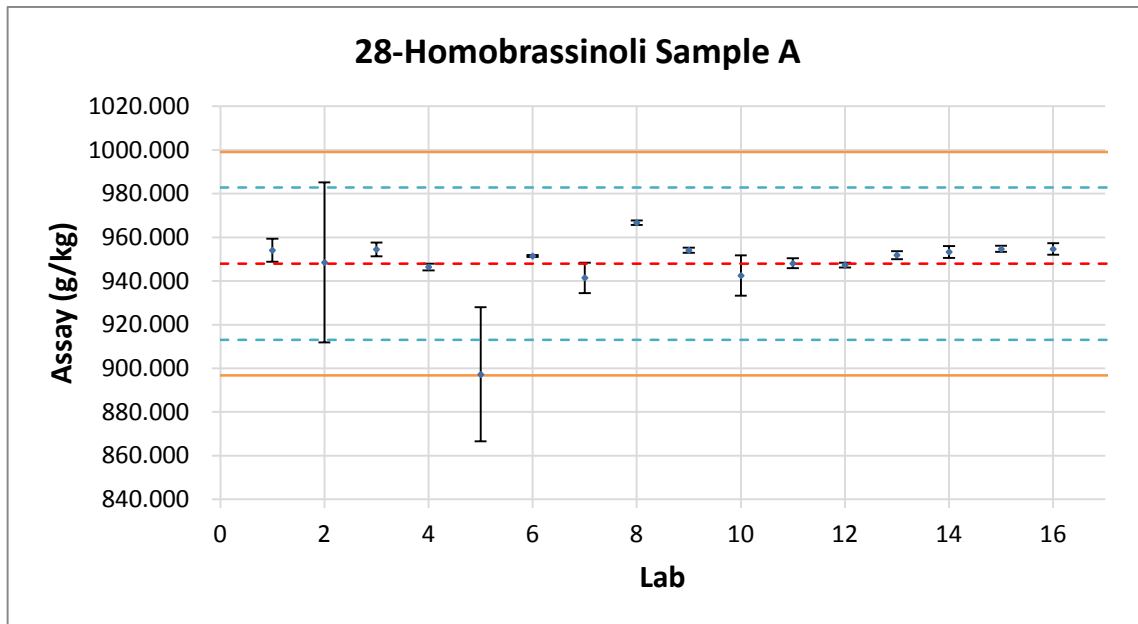


Fig. 2: Results of the 28-Homobrassinolide TC-2(see table 2 for the evaluation)

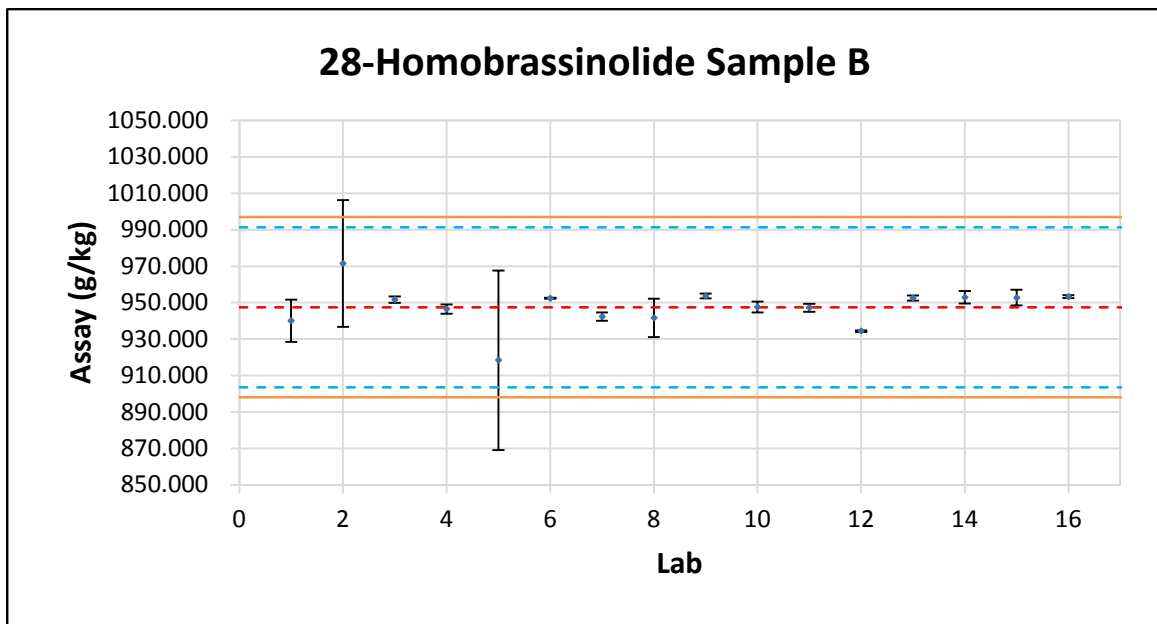


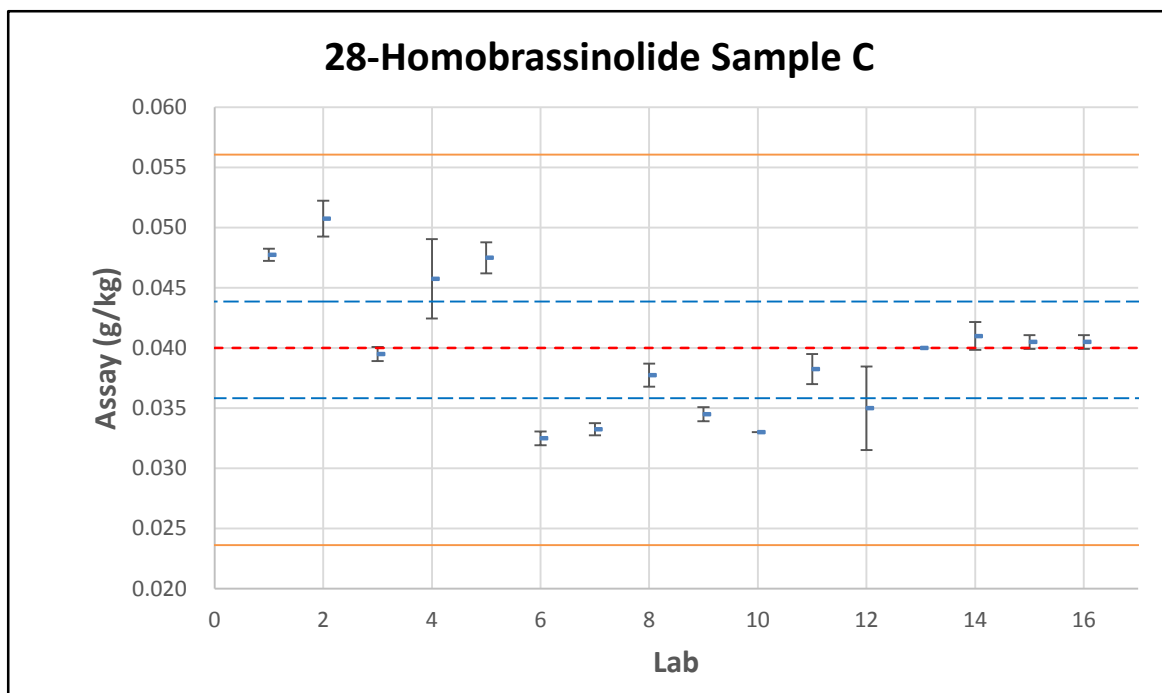
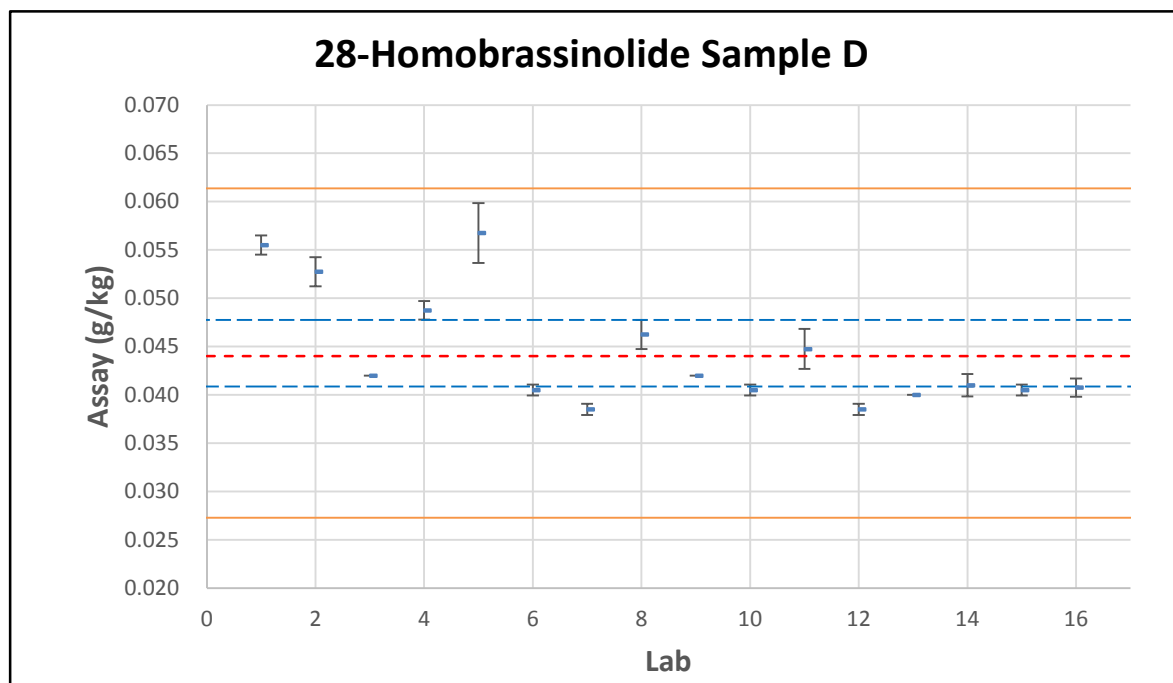
Fig. 3: Results of the 28-Homobrassinolide SL-1(see table 2 for the evaluation)**Fig. 4: Results of the 28-Homobrassinolide SL-2(see table 2 for the evaluation)**

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