Determination of 14-Hydroxylated brassinosteroid Active in TK and SL

Small Scale Collaborative Study for the Determination of 14-Hydroxylated brassinosteroid Active in TK and SL by High Performance Liquid Chromatography

Report to CIPAC
by
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Content page

1. Participants

Name of responsible person	Lab Name	City, Country
Yang Yan Qin	CHENGDU NEWSUN CROP SCIENCE CO., LTD	Sichuan, China
Yu Chunxin	Laboratory of Molecular Physiology and Chemical Regulation of Ground Covers, Beijing University of Agriculture	Beijing, 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.

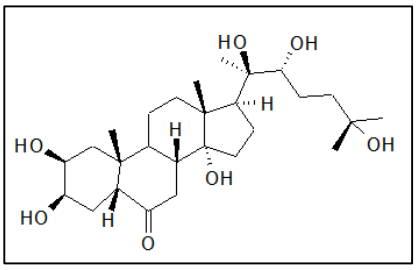
2. Active Ingredient, General Information

IUPAC name: (2β,3β,5β,22R)-2,3,14,20,22,25-hexahydroxy-Cholestan-6-one

Common name: 14-Hydroxylated brassinosteroid

CAS-Nr.: 457603-63-3

Structure:



Molecular mass: 482.7

Empirical formula: $C_{27}H_{46}O_7$

3. Samples

In Feb. 2021 the following samples were sent to the participants:
Describe sample:
TK: light-yellow powder
SL: colorless homogeneous liquid without visible suspended solids

In 19/20.04.2021 results were obtained.

4. Method

4.1 Scope

The content of 14-Hydroxylated brassinosteroid is determined in technical concentrate and soluble liquid products.

4.2 Principle

The 14-Hydroxylated brassinosteroid content of the samples is determined by high performance liquid chromatography on ODS-C18 film stainless column with UV detector at 222 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	Eclipse plus C18 4.6mm*100mm*3.5um	Acetonitrile + water = 45 + 55 (v/v)	1	30	10
2	Eclipse plus C18 4.6mm*100mm*3.5um	Acetonitrile + water = 45 + 55 (v/v)	1	30	10
3	Angilient 4.6x100mm，2.7 Micron with Column ID,USCFS09415	Acetonitrile + water = 45 + 55 (v/v)	1	30	10
4	Eclipse plus C18 4.6mm*100mm*3.5um	Acetonitrile + water = 45 + 55 (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: Eclipse plus C18 4.6mm*100mm*3.5um Remarks: None
Laboratory 2	Column: Eclipse plus C18 4.6mm*100mm*3.5um Remarks: None
Laboratory 3	Column: Angilient 4.6x100mm，2.7 Micron with Column ID,USCFS09415 Remarks: None
Laboratory 4	Column: Eclipse plus C18 4.6mm*100mm*3.5um 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 1-3.

The testing for outliers/stragglers of the laboratory mean values were performed according to Grubbs test on a 1%/5% significance level, respectively. There was no stragglers and outliers for the SL formulations as well as for the technical concentrate identified by the Grubbs test.

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.

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 14-Hydroxylated brassinosteroid in technical concentrate and SL formulation. We proposes to proceed with a large scale collaborative study.

9. Appendix A

Tables and Figures for 14-Hydroxylated brassinosteroid.

Table 1-1: 14-Hydroxylated brassinosteroid assay in TK and SL (g/kg); results for each laboratory on day 1 and day 2

	14-Hydroxylated brassinosteroid SAMPLE A				14-Hydroxylated brassinosteroid SAMPLE B				14-Hydroxylated brassinosteroid SAMPLE C	
	Day1		Day2		Day1		Day2		Day1	
Laboratory 1	759.4	762.6	757.4	762.7	765.3	762.9	757.9	767.9	0.081	0.079
Laboratory 2	775.7	766.3	776.4	767.5	782.5	767.2	778.2	764.9	0.078	0.078
Laboratory 3	774.0	777.9	772.5	775.0	753.3	775.1	769.7	763.4	0.077	0.078
Laboratory 4	792.6	798.4	787.6	790.2	787.4	781.6	787.2	773.7	0.077	0.077

Table 1-2: 14-Hydroxylated brassinosteroid assay in TK and SL (g/kg); results for each laboratory on day 1 and day 2

	14-Hydroxylated brassinosteroid SAMPLE C		14-Hydroxylated brassinosteroid SAMPLE D				14-Hydroxylated brassinosteroid SAMPLE E			
	Day2		Day1		Day2		Day1		Day2	
Laboratory 1	0.080	0.079	0.078	0.078	0.079	0.077	0.078	0.079	0.079	0.079
Laboratory 2	0.078	0.078	0.078	0.078	0.077	0.078	0.078	0.078	0.077	0.077
Laboratory 3	0.075	0.075	0.078	0.078	0.076	0.076	0.077	0.078	0.076	0.076
Laboratory 4	0.075	0.074	0.076	0.077	0.074	0.073	0.079	0.077	0.075	0.074

Table 2: Mean values

	14-Hydroxylated brassinosteroid SAMPLE A	14-Hydroxylated brassinosteroid SAMPLE B	14-Hydroxylated brassinosteroid SAMPLE C	14-Hydroxylated brassinosteroid SAMPLE D	14-Hydroxylated brassinosteroid SAMPLE E
Laboratory 1	760.5	763.5	0.080	0.078	0.079
Laboratory 2	771.5	773.2	0.078	0.078	0.078
Laboratory 3	774.8	765.4	0.076	0.077	0.077
Laboratory 4	792.2	782.5	0.076	0.076	0.077

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

	TK-1	TK-2	SL-1	SL-2	SL-3
Xm	774.8	771.1	0.077	0.077	0.077
L	4	4	4	4	4
S_r	3.917	7.405	0.00116	0.00118	0.00127
S_R	13.566	10.766	0.00208	0.00170	0.00154
r	10.968	20.734	0.00325	0.00330	0.00356
R	37.985	30.145	0.00582	0.00476	0.00431
RSD_r	0.506	0.960	1.503	1.536	1.638
RSD_R	1.751	1.396	2.685	2.212	1.998
RSD_R (Hor)	2.078	2.080	8.314	8.322	8.316
HorRat Value	0.842	0.671	0.323	0.266	0.240

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

Fig. 1: Results of the 14-Hydroxylated brassinosteroid TK-1(see table 2 for the evaluation)

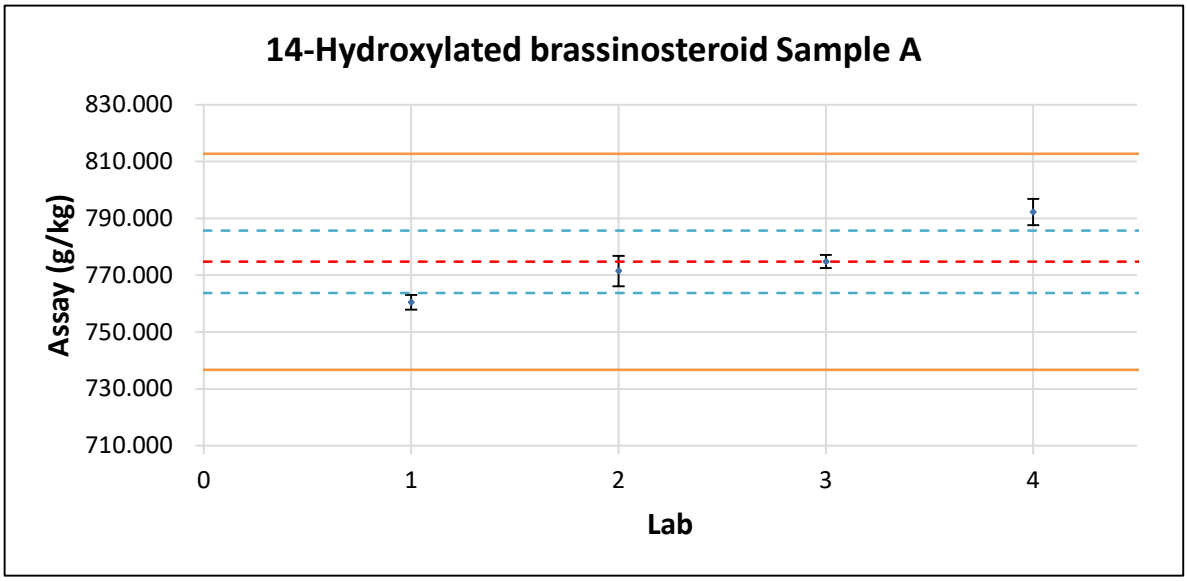


Fig. 2: Results of the 14-Hydroxylated brassinosteroid TK-2(see table 2 for the evaluation)

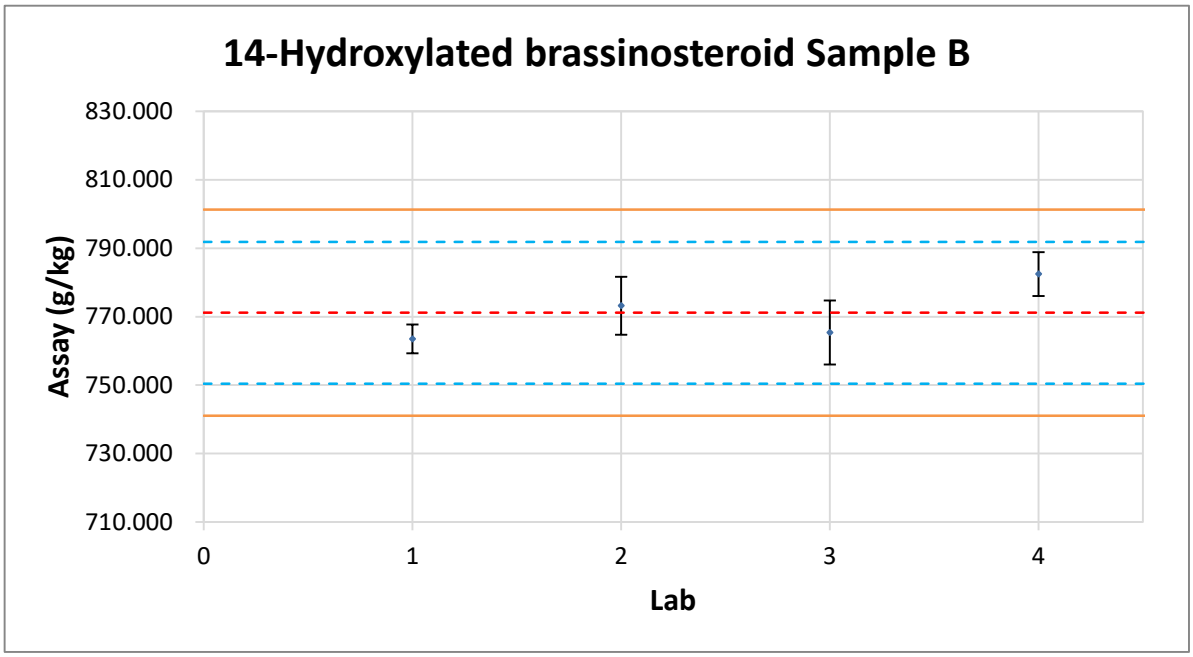


Fig. 3: Results of the 14-Hydroxylated brassinosteroid SL-1(see table 2 for the evaluation)

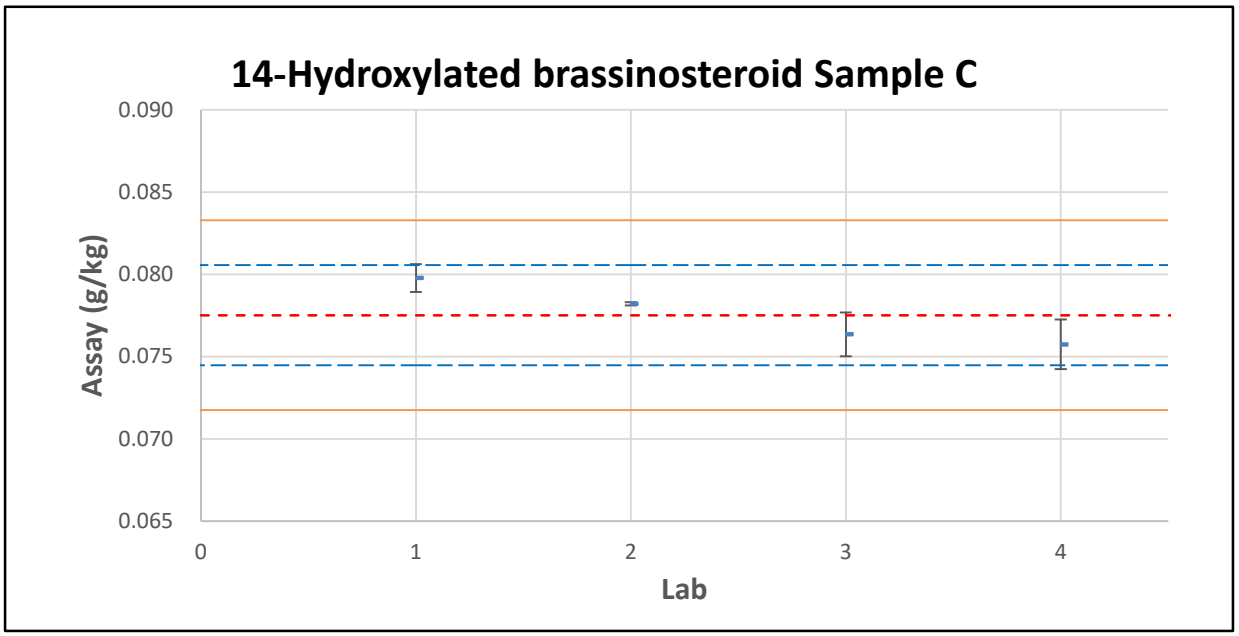


Fig. 4: Results of the 14-Hydroxylated brassinosteroid SL-2(see table 2 for the evaluation)

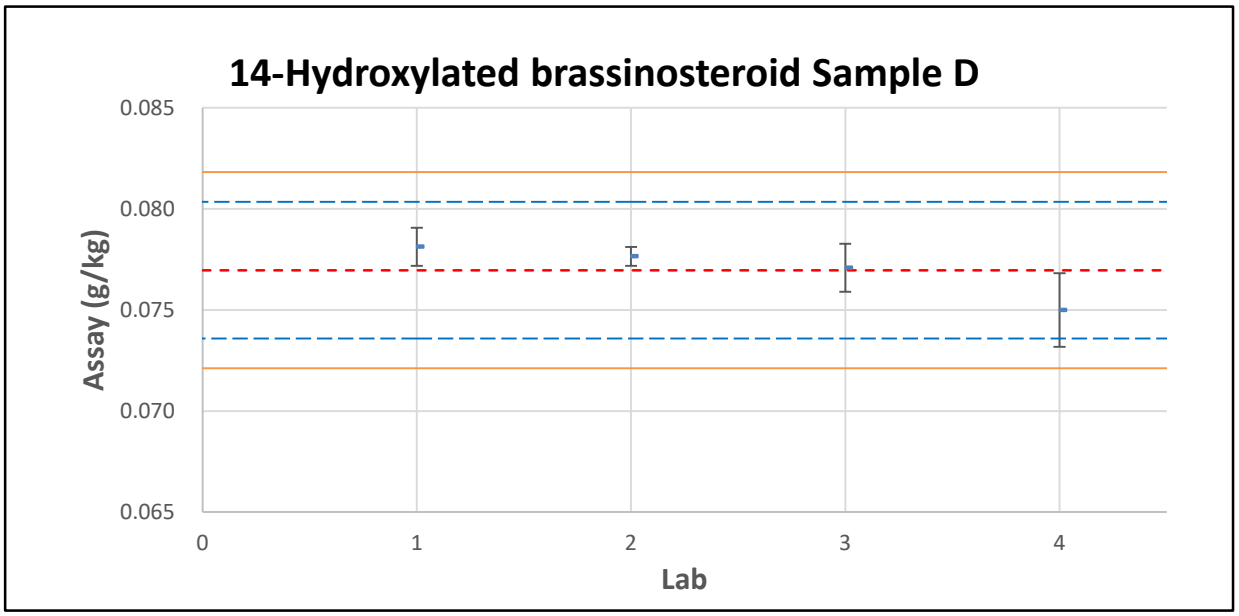


Fig. 5: Results of the 14-Hydroxylated brassinosteroid SL-3 (see table 2 for the evaluation)