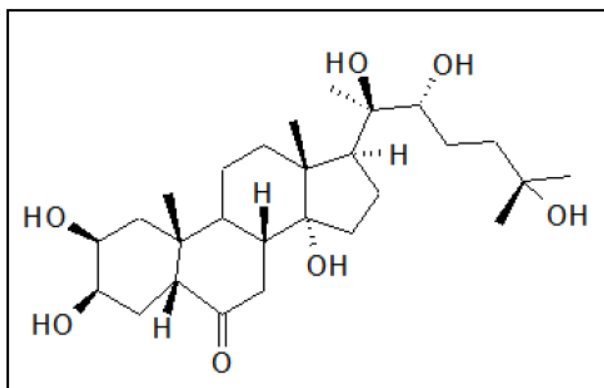


# CIPAC STATUS REPORT

29.08.2023



## 1023 14-hydroxylated brassinosteroid

Allocated to CHPAC

CIPAC methods published in :

CIPAC

**CIPAC** 65<sup>th</sup> meeting, June 2021 virtual

**14-hydroxylated brassinosteroid by Mr. Jason Zhang (5271, 5272).** Mr Jason Zhang presented the results of a small scale collaborative trial for 14-hydroxylated brassinosteroid with four participants from China. The method was based on HPLC (C18) with UV detection (222 nm) after phenylboronic acid derivatization and external standardization. The method included a dry-down step and a triple washing step with methanol. The deviations of the original method as applied by the four participants were assessed as minor.

Two technical materials, and three soluble concentrates were investigated. All samples fulfilled the statistical criteria (RSDR < RSDR(Horwitz) and HorRat ≤1.0).

Mr Zhang proposed to proceed to a full scale trial.

The following comments were received from the meeting:

**Mr Garvey** asked the same question as for the previous method and why the derivatization is necessary. Mr Zhang answered that without derivatization the peak is not detected and that 20 min was enough for the derivatization reaction, however they requested 30 min, to be sure that the reaction is complete.

**Mr Mink** asked if DAD or MS detection was also tried. Ø Mr Perez Albela remarked that no control for complete derivatization or comparison with another method (e.g. LC-MSMS) was performed. Mr Zhang answered that derivatization was performed over a prolonged period however that after 0.5 hours no higher UV signal was obtained indicating the completeness of the derivatization.

**Ms Vinke** asked what is the product after the derivatization step. Furthermore an explanation has to be added why the dry-down and triple washing steps are necessary. The answer will be given in written after the meeting.

### **Closed Meeting:**

A small scale trial was presented and the method was proposed for a large scale collaborative trial with similar comments concerning the derivatization as under 4.1.

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CIPAC 66<sup>th</sup> meeting, June 2022 virtual

## 14-hydroxylated brassinosteroid by Ms Yue Wang (5311, 5312)

Ms Wang presented the results of a full scale collaborative trial for the determination of 14-hydroxylated brassinosteroid, by reversed phase HPLC and 222 nm UV-detection after phenylboronic acid derivatization. Quantitation was performed by external standardization. Two TKs and three SL formulations were sent to 18 laboratories of which 17 returned their results in time. The remarks of the participants were discussed however they were regarded as minor. The data were evaluated according to “Guidelines for CIPAC collaborative study procedures for assessment of performance of analytical methods”, and were subjected to Mandel’s k-statistics on the within-lab variance, followed by Mandel’s h-statistics on the lab means. Including the data of all participants the HorRat values ranged from 1.37-2.12. However, after elimination of two-three stragglers and outliers per sample the HorRat values were in the range of 0.66-0.99.

Ms Wang recommended this method to be accepted as a provisional CIPAC method.

The following comments were received from the meeting:

- Ms Vinke remarked that an outlier in table 1 might have been overlooked. This was not the case.
- Mr Garvey asked whether the relative high evaporation temperature of 90°C might have influenced the high number of stragglers and outliers. Ms Yue Wang responded that this was not to be expected as the derivatization was tested across the range of 70-90°C and was proven to be complete.

### Closed Meeting:

Mr Garvey questioned whether the extensive use of removing outliers was necessary. Mr Hänel replied that this is allowed and agreed upon when the HorRat values are not met.

The method for the determination of 14-hydroxylated brassinosteroid in TK and SL formulations can be promoted to a **provisional CIPAC method** with additional justification for the Horrat >1 and for the eliminations.

CIPAC 67<sup>th</sup> meeting, June 2023 Braunschweig

### Decision

It appeared that a question from Mr Watanabe has not been answered yet, however the Company clarified the issues raised in the question, but the answer was not forwarded in time by the secretary to Mr Watanabe.

The reversed phase HPLC method (CIPAC/5311) for the determination of 14-hydroxylated brassinosteroid in TK and SL formulations was accepted as **full** CIPAC method after additional justification for the Horrat >1 and for the eliminations.