CIPAC STATUS REPORT

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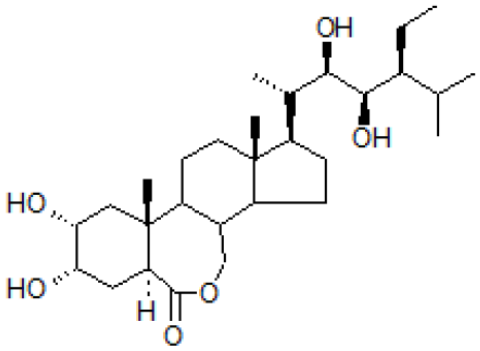

1006 28-Homobrassinolide

Allocated to CHPAC

CIPAC methods published in :

CIPAC

CIPAC 65th meeting, June 2021 virtual

28-homobrassinolide by Mr. Jason Zhang (5269, 5270): Mr Jason Zhang presented the results of a full scale collaborative trial for 28-homobrassinolide as a follow up of the small scale trial in the previous year. 16 laboratories originating from Europe, China, Japan, and Indonesia participated with a method based on HPLC (C18) with UV detection (220 nm) after phenylboronic acid derivatization and external standardization. The deviations from the original method as applied by the participants were assessed as minor.
Two technical materials, two soluble concentrates, and one emulsifiable concentrate were investigated. The TCs fulfilled the statistical criteria (RSDR < RSDR(Horwitz) and HorRat ≤1.0) however both SCs and the EC showed RSDR > RSDR(Horwitz) and HorRat 1.0<x≤2.0, even after removal of stragglers and/or outliers. This would be acceptable if appropriate reasoning was presented. However this remained unclear.
Mr Zhang proposed adoption as a full method.

The following comments were received from the meeting:
Mr de Rijk made a remark concerning the traceability of the participating laboratories. This should not be possible.
Mr Garvey asked whether the optimization of the derivatization was also carried out, and if yes, how. Mr Zhang answered that 25 mg of phenylboronic acid was used and in 30 min the derivatization was complete. Mr Garvey asked for some data concerning the derivatization including the derivatization reaction products. The answer will be given in written after the meeting.
Mr Perez Albela questioned whether there is no underestimation using the derivatization CIPAC/5294/P2 process in comparison with the determination by DAD or MS/MS?

Closed Meeting:
Questions were raised about the completeness of the derivatization. It appeared that Mr Zhang had reported that the derivatisation was >99% complete but no correlation with another method without derivatisation (e.g. LC-MSMS) was performed. Furthermore more information about the structure of the derivatised molecule is needed.
A further explanation will be requested from Mr Zhang about the HorRat values >1.0. It was mentioned that the very low concentrations of the active might have contributed to the somewhat larger RSDR. It was mentioned that the analytical method as described at 4.2 (4-hydroxylated brassinosteroid) was more or less similar but resulted in much lower HorRat values, suggesting that better results would be possible.

Questions were also raised about the availability of representative chromatograms in the method. Two trial participants reported the presence of an interfering compound eluting close to 28-homobrassinolide which resulted in complicated peak integration. They will send examples ofthe chromatograms to the chairman and secretary
Finally a request will be send to Mr Zhang to explain why derivatization was deemed to be necessary.
The meeting decided to promote the method to **provisional CIPAC method**, pending on the above clarifications.

CIPAC 66th meeting, June 2022 virtual

Closed Meeting:

At the previous meeting, the method was accepted as provisional. No further comments were received. The clarifications requested last year were submitted and considered acceptable. The method can be promoted to a **full CIPAC method**.