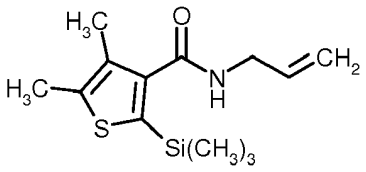
CIPAC STATUS REPORT

08/08/2016



0635 Silthiofam

Allocated to USA

CIPAC methods published in:

Not published

CIPAC 58th meeting, June 2014 in Liège

Mr Van Thuyne presented the results of a **small scale** collaborative study on the determination of silthiofam in technical material (TC) and flowable concentrate for seed treatment (FS) using reverse phase-HPLC-UV, with a C18 column, detection at 260 nm with internal standard calibration. The study was conducted in collaboration with ESPAC.
One sample of TC and one sample of FS were provided. 8 laboratories participated in the small scale study.
The following deviations from the method protocol were noted:

- Different column vendors + dimensions (comparable stationery phases used)
- Different mobile phase composition
- Different flow rate
- Different mode of addition of internal standard: Volumetric addition of a stock solution
- Different sample preparation for FS due to the formation of precipitation

The statistical evaluation was carried out according to the CIPAC guidelines. Lab 6 reported a possible bad injection on the 2nd injection of the first preparation; however the data point was not removed as final result identical to 1st injection.
All results were initially included in the evaluation. In the initial evaluation the Horwitz criteria were met for all samples when the internal standard was used.
When the results were calculated without the internal standard the interpretation of the available data showed multiple possible outliers. 3 options were evaluated:

- All results were initially included in the evaluation. In this instance the Horwitz criteria were met for the TC but failed (just) for the FS.
- Removal of Lab 1&2 + 2 data points of Lab 6 at each level. In this instance the Horwitz criteria were met for the TC and the FS.
- Selected removal of data points of Lab 1, 2 &6. In this instance the Horwitz criteria were met for the TC and the FS.

Mr Van Thuyne concluded that the method is suitable and proposed that a full scale trial is conducted using the internal standard.

The following comments were received from the meeting:

- Why did you use an HPLC column with a particle size of 10 µm? Mr Van Thuyne replied that this was the most commonly used column in his lab. He noted that other labs had used either a 10 µm or 5µm column, that there was no significant interference from the matrix and the retention time of the internal standard and active ingredient

were separated by 3.5 min for the 10µm column. From this he surmised that the particle size would not influence the results significantly.

- What temperature should be used for the HPLC column? This wasn't included in the method so different participants may have used different temperatures. Mr Van Thuyne replied that this information had not been included in the method in error and that this may explain some of the differences seen in results. He proposed that a temperature of 40°C will be added to the method.
- For sample preparation for TC why did you need to place the sample in an ultrasonic bath for 15 min? The TC dissolved very easily? Mr Van Thuyne replied that this was done to make the sample preparation the same for the TC and FS. This step is needed for the FS; however the method can be updated to reflect that less time is needed for the TC.

The closed meeting discussed the comments received during the open meeting.
The main issue was whether or not an internal standard was really needed for the method. According to the presentation of results given to the open meeting an internal standard was necessary.
One member commented that during the trial they had issues with the instrument and that the internal standard had meant that their results were still accepted so there may be some advantages to using an internal standard.
The meeting considered that if the full scale trial was conducted with the internal standard then it will be possible to consider the data both with and without the internal standard. A decision can then be made after the full scale trial as to whether the internal standard is really necessary.

The meeting agreed that a **full scale trial should be conducted with the internal standard.**

CIPAC 59th meeting, June 2015 in Athens

Mr De Groof presented the results of a **full scale** trial for the determination of silthiofam in TC and FS formulations with a reversed phase C18 HPLC column and UV-detection at 260 nm. The calibration was performed both with external and internal standardization. After presenting the history of the development of the method (company validation and small scale collaborative study) the outline of the full scale collaborative trial was presented. Eighteen laboratories have participated and reported their results. The participating laboratories received a silthiofam reference standard, an internal standard diethyl phthalate, three TC samples, and five FS samples. A detailed protocol was added and the participating laboratories reported deviations in HPLC column type and column dimensions, flow-rate, addition of internal standard (volumetric or gravimetric), and sample preparation. One laboratory did not report results for day 2 and was excluded for further data analysis. The data analysis resulted in the following remarks, conclusions:

- Instrument repeatability (expressed as % RSD) using an internal standard is statistically significant lower than without using internal standard ($p = 0.008$).
- Several Box-plot, Cochran & Grubbs outliers were detected and removed (3.8% and 7.8% of the data points for internal standardization calculation and external standardization respectively).
- The Horwitz criteria for RSD_R were met, both using internal standardization and external standardization.
- Mr De Groof also compared statistical outlier detection methods (Box-plot, Cochran, Grubbs and Hampel) and showed that the Hampel outlier detection procedure resulted in less removed results and small RSD_r and RSD_R while keeping the mean calculated content identical.
- The methodology using an internal standard is recommended for the final Silthiofam method.
- Request to recognize the presented Silthiofam analytical method as full CIPAC method.

The following comments were received from the meeting:

- Mr De Groof was thanked for introducing the improvements which were suggested after the small scale trial as presented in the 2014 CIPAC meeting.

- It was remarked that gravimetric addition (compared to volumetric addition) of internal standard was the best procedure, although it allegedly did not show up in this trial. A note can be included in the description of the method that volumetric addition of the internal standard is also possible.
- Can sonication replace shaking in sample extraction? Especially thinking of heat build-up during prolonged sonication. It was tested by a participant of the meeting and did not result in any deviations.

The report was not available, but will be made available to the participants shortly after the CIPAC meeting.

Closed meeting:
The meeting discussed the comments received during the open meeting.
Remarks were made by the meeting about the use of an internal standard which should be kept in the method, and that gravimetrical addition of the internal standard is preferred compared to volumetric addition of the internal standard. However both the gravimetric and the volumetric procedures will lead to acceptable results and both ways can be used.

Decision: The meeting agreed that with the addition of a note that the addition of the internal standard can be done also volumetrically, the method can be **accepted as provisional**.

CIPAC 60th meeting, June 2016 in Tokyo

Closed meeting:
At the 59th meeting, 2015 in Athens the method was accepted as provisional. No further comments were received.
The method can be promoted to a **full CIPAC method**.