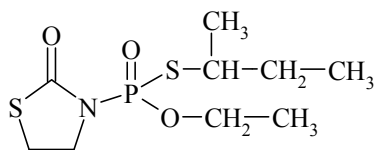


# CIPAC STATUS REPORT

09/12/2013



## 0585 Fosthiazate

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Allocated to

CIPAC methods published in:

CIPAC

**CIPAC** 54<sup>th</sup> meeting, June 2010 in Ljubljana

Mr Jim Garvey presented the results of a small scale trial on the determination of fosthiazate in TC and GR formulations using HPLC-UV at 220 nm using internal standard, organised by ESPAC in conjunction with ISK Belgium. 5 labs participated.

Comments from laboratories:

Issue of reproducibility given low injection volume (1 µl); sample weighing was difficult.

Why dissolve in acetone when mobile phase is acetonitrile/water? The Company clarified that mobile phase works just as well. Filtration was sufficient for the samples, centrifugation may not be needed; sonicate before making up to mark.

Removing the outliers all but 1 formulation met the Horwitz criteria. No obvious reasons why this was an outlier.

Comments:

As RP-HPLC is used it was proposed to use a buffer system which helps to stabilise the retention time & response of the HPLC system. Buffered on the acidic side in this instance would help to stabilise fosthiazate.

1 µl injection volume is quite low, can a higher volume be used? The company would check but they argue that the use of an internal std. is justified. Given that generally fixed loop injection is used, a higher volume would be better. The name needs to be checked against IUPAC.

It was proposed to go to full scale study, but to use as many labs as possible and without internal standard, unless it is well justified.

Decision: With the submitted data set it was not possible to make a reliable recommendation. It was proposed to have further consideration within ESPAC, however if ESPAC makes the recommendation to go for a full scale trial, this can be conducted.

**CIPAC** 55<sup>th</sup> meeting, June 2011 in Beijing

Mr Jim Garvey presented the results of a **small scale** collaborative study, on the determination of fosthiazate in technical product (TC) and granules (GR) using HPLC-UV detection at 220 nm with dimethyl phthalate as an internal standard. The trial was organised by ESPAC in conjunction with ISK Belgium. A small scale trial was presented to CIPAC in 2010 for the same method but using an internal standard, which gave unacceptable results. ESPAC re-evaluated the data without the internal standard and it became apparent that the internal standard was in fact necessary. After discussions with the company, it was proposed by the company to conduct another small scale trial.

The statistical evaluation was carried out according to the CIPAC guidelines.

For TC 1 and TC 2 Lab 2 was a Cochran's straggler. For GR 3 Lab 2 was a Cochran's straggler.

No data were excluded from the initial evaluation.

TC 1 and TC 2 meet the Horwitz criteria when all the data are included. GR 1, GR 2 and GR 3 did not meet the Horwitz criteria when all the data are included. When the results from Lab 2 (Cochran's straggler) were omitted and the statistical evaluation was repeated the Horwitz criteria were still not met. A possible explanation for this is that the sample size is not large enough to get a representative sample for extraction of GR formulations. ESPAC therefore recommends that a larger sample (at least 5g) is extracted and an aliquot is then taken for analysis.

# CIPAC STATUS REPORT

09/12/2013

ESPACE consider that with some modifications the proposed method is appropriate for the determination of fosthiazate in TC and GR and that a full scale trial can be conducted.

The following comments were received from the meeting:

- Could you clarify if the mobile phase was just acetonitrile or acetonitrile/water with a gradient programme? Mr Garvey clarified that the mobile phase was a gradient programme beginning with 100% acetonitrile/water (2:1) and ending with 100% acetonitrile.
- Was the HPLC grade water used for the mobile phase?
- The extraction procedure for the GR extraction was already improved from previous small scale trial. Could it be that acetonitrile is not a suitable extraction solvent?

A 1 µl injection for the method is very small. A higher volume would be preferable. Mr Garvey clarified that the method did actually use 10 µl but this was incorrectly stated to be 1 µl in the presentation to the meeting.

Despite a repeated small-scale trial there still appears to be a problem with the analysis of the GR. The meeting considered that a larger sample size for the GR would help with the sample preparation. If the GR issue is resolved it may not be necessary to use an internal standard, but a full scale trial using an internal standard means that there is an option to consider the results and statistical analysis with and without the internal standard.

The meeting considered that it was difficult to make a sound recommendation at this time as there are still several unknowns; the method does not appear to be optimised or robust.

Decision: A full scale trial **CANNOT** be recommend

CIPAC 56<sup>th</sup> meeting, June 2012 in Dublin

Mr Joris presented the results of a **full scale** collaborative study (4829, 4830) on the determination of fosthiazate in technical product (TC) and granule (GR) formulations using HPLC-UV, detection at 220 nm and internal standard calibration. Three samples of TC and two samples of GR were provided. 15 laboratories participated however only results for 14 labs were presented for the TC due to problems with sample shipment to 1 lab.

Several changes were made to the method based on the result of the small scale trial:

- A larger sample size was used to ensure a representative sample for analysis.
- The shaking and sonication extraction procedure is needed twice for the GR.
- Increase in injection volume

Two laboratories commented that they had used a lower sample weight for the GR than recommended.

Two laboratories used different extraction solvents to those outlined in the method (acetonitrile/water and methanol instead of the stated acetone).

No data were excluded from the initial evaluation, including the laboratories that had significantly deviated from the method (extraction solvent). With all the data included all TC samples meet the Horwitz criteria, however the criteria for the 2 GR samples were not met.

For GR 2 Lab 12 was identified as a Cochran's outlier and as a Grubb's straggler

When the outliers were omitted and the statistical evaluation was repeated the Horwitz criteria were still not met for the GR samples.

When the results from Labs 6 & 12 (those that used different solvent) and the outliers were removed the Horwitz criteria were still not met for the GR samples.

When the results from Labs 6 & 14 (those that did not use the recommended sample weight) and the outliers were removed the Horwitz criteria were met for the GR samples.

Mr Joris concluded that this indicated that the minimum sample weight of 1g is crucial for the good performance of the method. He concluded that the proposed method is appropriate for the determination of fosthiazate in TC and GR and proposed that the method be adopted by CIPAC as a provisional method.

The following comments were received from the meeting:

- Please include more detail in the method for the sample preparation and the extraction of the GR so that it's clear why this is needed.
- Is the internal standard really needed? Mr Joris replied that during the small scale test it was investigated whether or not an internal standard was needed. It was shown that the internal standard was needed from the small scale trials.
- Have you had time to evaluate the results without internal standard to see if the results show that it is not necessary? Mr Joris replied that unfortunately they had not but could do this if needed.
- GR will not dissolve completely so the internal standard could be needed to correct for any undissolved material.

# CIPAC STATUS REPORT

09/12/2013

- Why is acetone used for the extraction when the small scale study did not use acetone? Mr Joris replied that use of acetone is important. The small scale trial used acetonitrile and results were very variable.
- On lab commented that they tried several different solvents for the GR and found that acetone was the best solvent. They also noted that EPSAC had identified that acetone extraction was necessary.
- Why was Lab 6 excluded when it was not identified as an outlier by the statistical analysis? Mr Joris replied that Lab 6 was excluded as they used a different extraction procedure, but agreed that there was no statistical reason to exclude.
- If sample size is the critical criteria and not the extraction solvent then the results from Lab 6 should be included. Mr Joris replied that they had also calculated the Horwitz criteria with just Lab 14 removed and in this case Horwitz is met. But for consistency they removed both labs where different extraction solvents were used.

The meeting raised concerns about the need to analyse samples within a certain time after extraction as this may indicate instability of active substance. It was noted that the samples that were sonicated for extraction gave consistently lower results but were within the acceptable range. The meeting agrees that there were some reservations with the method but the statistical results indicate that the method is working acceptably.

The meeting considered that this method had already been through 2 small scale trials with ESPAC and that two critical issues were identified with the method – one was the sample size and the other was the extraction solvent. ESPAC specifically recommended that acetonitrile was not suitable. Of these two issues it appears that the sample size was most critical as shown by the results of the studies. It was however not made clear in the information provided with the method that these were critical steps. The method as written is not clearly defined as the critical issues are not defined. This should be clearly written in the methods.

It was noted that there is a good chemical reason to remove Lab 6 (used different solvent). But it doesn't matter as when lab 6 is included the criteria are met. It was the results from the other Lab that also changed the sample size which are not acceptable. Lab 6 commented that they had used acetonitrile/water to extract and also not used an internal standard and their results were excellent.

Considering the structure and the ISO common name fosthiazate has 2 diastereoisomers. The meeting noted that sample chromatograms of the TC show that the peak is quite broad and this may be due to partial resolution of the diastereoisomers. It may be that depending on the HPLC conditions some HPLC systems may resolve the isomers. The meeting agreed that a footnote is needed to warn future users of the method that diastereoisomers are present, the HPLC conditions may resolve them and to advise what to do if this happens.

The meeting considered whether an internal standard was needed. It was noted that the company had indicated that they are willing to re-calculate the data set without the internal standard to see what impact the internal standard has on the method. The meeting agreed that they could not conclude until this information was available. The small scale trial clearly indicates that the internal standard was needed. It may be that one lab can get robust results without the internal standard, but in order for the method to be reproducible and robust the internal standard is needed.

## Decision

The reversed phase HPLC method (CIPAC/4829) for the determination of fosthiazate in TC and GR formulations was accepted as a **provisional** CIPAC method with the necessary amendments in the description of the method and the note concerning the diastereomers.

**CIPAC** 57<sup>th</sup> meeting, June 2013 in Kyiv

At the 56<sup>th</sup> meeting, 2012 in Ireland the method was adopted as provisional subject to the additional notes needed in the method, and to the provision of the statistical analysis with and without the internal standard.

## Decision:

The reversed phase HPLC method (CIPAC/4829) for the determination of fosthiazate in TC and GR formulations was accepted as a **full** CIPAC method with the necessary amendments in the description of the method and the note concerning the diastereomers.