CIPAC STATUS REPORT

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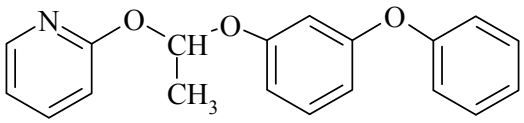

0715 Pyriproxyfen

Allocated to JAPAC

CIPAC methods published in: M

CIPAC M, p. 178

CIPAC 49th meeting, June 2005 in Utrecht

Mr Y. Asada presented a small scale collaborative study with 5 labs by JAPAC, using reversed phase HPLC with Nucleosil C18 column, acetonitrile/water as eluent and internal standardisation. Two TCs, one EC, one EW and one GR formulation were tested. Mr M. Feyerabend asked the reason of using internal standard. The answer was that for the TC and EC there is no need to use IS, but for GR is needed, as during the sample preparation the solvent used might evaporate. Mr C. Cook expressed his concerns over the long sonication time (60 min). The answer was that the granule is hard and for good extraction a min 45 minutes sonication time is needed. Mr M. Müller questioned the identity of the small peak in front of the main peak, which was confirmed to be an impurity. He suggested the possible use of an appropriate buffer in order to stabilise the retention time. Dr Asada confirmed that the retention time was sufficiently stable. The results were acceptable and Sumitomo proposed to proceed to a full scale study.
Decision It was decided to go to a full scale study with the reversed phase HPLC method for TC, EC, EW and GR formulations, with the insertion of a note that the use of the internal standard is necessary due to the possible evaporation of the solvent.

CIPAC 50th meeting, June 2006 in Geneva

Mr Y. Asada presented the results of the full collaborative study of the determination of pyriproxyfen in the TC, EC, EW, and GR formulations, using reversed-phase HPLC with UV detection at 254 nm and dicyclohexyl phthalate as internal standard. It was mentioned that companies will know if anything interferes with the internal standard, and thus this should be clarified in the method. Thirteen laboratories submitted results. All four outliers were retained. For all samples, the values of RSD_R were smaller than those calculated by Horwitz's equation. The proposed method is considered appropriate for the determination of pyriproxyfen in the TC, EC, EW and GR formulations. JAPAC proposed that the method be accepted as a provisional CIPAC method.
Decision: The reversed phase HPLC method (CIPAC/4501) for the determination of pyriproxyfen in TC, EW, GR and EC formulations was accepted as **provisional** CIPAC method.

CIPAC 51th meeting, June 2007 in Umhlanga Rocks, South Africa

Decision The reversed phase HPLC method (CIPAC/4501) for the determination of pyriproxyfen in TC, EW, GR and EC formulations was accepted as **full** CIPAC method.

CIPAC 57th meeting, June 2013 in Kyiv

Ms Mukumoto presented the results of a validation study for the **extension** of the CIPAC method 331/LN/(M2)/3 for determination of permethrin and CIPAC method 715/TC/M/3 for the determination of pyriproxyfen to include a LN formulation containing both permethrin and pyriproxyfen. The study was organised with JAPAC.
Validation data in accordance the CIPAC guideline for a method extension for permethrin were presented. The method extension met these criteria. JAPAC concluded that the method is appropriate and proposed that the permethrin method extension be adopted by CIPAC.

For pyriproxyfen some modifications to the extraction procedures were needed. A change in the extraction procedure and solvent was needed to take account of the nature of LN formulations. Heptane was used as an extraction solvent instead of acetonitrile and the samples were extracted for 45 minutes at 85-90°C.
The solvent used to prepare the internal standard solution was changed from acetonitrile to the 1-propanol. This was considered to be a minor modification.
Validation data in accordance the CIPAC guideline for a method extension for pyriproxyfen were presented. The method extension met these criteria. JAPAC concluded that the method is appropriate and proposed that the pyriproxyfen extension be adopted by CIPAC.
The following comments were received from the meeting:
Did you try to develop a single method for both a.s. in the LN? Ms Mukumoto replied that pyriproxyfen can be determined by the CIPAC permethrin method but the separation was not satisfactory and example chromatograms showed many impurities. Therefore they concluded that it was not really appropriate to determine both together.

Decision:
The extension of the scope (CIPAC/4887) of CIPAC method 715/TC/M/2 for the determination of the pyriproxyfen content of a the long lasting insecticidal mosquito net (incorporated type) (LN) containing permethrin and pyriproxyfen was accepted as a **provisional** CIPAC method

CIPAC 58th meeting, June 2014 in Liège

No further comments were received.

Decision: The extension of the scope (CIPAC/4887) of CIPAC method 715/TC/M/2 for the determination of the pyriproxyfen content of a long lasting insecticidal mosquito net (LN) (incorporated type) containing permethrin and pyriproxyfen was accepted as **full** CIPAC method.

Further:
Ms Kozuki presented a brief introduction to a proposed new formulation type (matrix release formulation).
Ms Mukumoto presented information on a proposed method for determining the release rate or retention rate for the new formulation type.
The method needs to be

- Product specific
- Simple and informative method to describe release properties
- Able to distinguish a good product from a bad one
- Ms Mukumoto outlined some of the method development already undertaken and proposed two possible methods (a replenishment method and a non-replenishment method) to take forward for further development and collaborative studies.
- To evaluate AI movement of MR immersed into water in short period, testing systems using shaking in 50% ethanol/water is most appropriate.
- Two testing systems, using non-replenishment and replenishment methods, are able to distinguish good MR from a bad one
- Non-replenishment method is simpler but longer, and replenishment method is shorter but requires more steps

Ms Kozuki and Ms Mukumoto proposed that following any opinions and suggestions obtained from CIPAC, the method will be modified and then a collaborative study will be conducted.

The following comments were received from the meeting:

- Can you clarify the type of shaking that is needed if any at all? Ms Mukumoto replied that they had used horizontal shaking not rotary shaking.

The closed meeting discussed the presentation given during the open meeting and the comments received
The meeting considered that the first step would be to get confirmation from WHOPES as to whether they need release rate in the specification for the new formulation. It was noted that currently there is no specification template for matrix release formulation as it is a novel formulation. The draft specification is currently under development however it is expected that the JMPS will ask for data on release rate for this product.

The meeting was reminded of the issues with LN methods and washing methods that meant it was many years before suitable methods were available. Is the use of ethanol/water designed to be a model to mimic what would happen in the water bottles to allow a specification that can be tested quickly?
It was noted that in the presentation it was shown that the active ingredient content slowly decreases over 6 months and that the company are trying to model a test to cover this. It was questioned whether this is really determining the release rate or just the amount of active ingredient that is retained in the product over time?
The WHO explained their understanding is that once the water jug containing the formulation is emptied completely, if you add more water to the container, through the process of diffusion the active ingredient content will increase and then becomes effective again. The 6 month period is the period of efficacy that the company is claiming but this has not yet been tested by WHOPES
It was agreed that there are two ways to measure the release index you can either measure what is retained in the formulation after time or what is in the mixture (i.e. the treated water). Perhaps retention index might be a better way to refer to this test.
The company had data that showed the tests could distinguish between a good and bad product, but it's not clear what a bad product is. Does a bad product not release active ingredient rapidly into water? Or does it not contain enough active ingredient to be effective? The time taken to reach an effective concentration in the water will depend on the volume of water (i.e. 1 L will reach saturation quicker than 10 L).
The rate of release has to be controlled. It cannot be too fast or too slow. The correct amount needs to be correlated with WHOPES trials to see what is good or bad product in the field. Once this has been resolved then this product should be modelled to develop a CIPAC method.
Critical factors that need to be considered when developing this method:

- The product is intended to be used in potable water so how much of the insecticide is available is a critical factor as you cannot exceed risk assessment values.
- How fast the product is releasing active ingredient as the water is replenished.
- How can we prove the long lasting 6 month effect? This is the most challenging. This is not a " fast kill" product it's designed to regulate the growth of new larvae. All this will become clearer as WHOPES develop their testing.
- All these details need to be worked out.

The meeting noted that the presentation was a first proposal and that two different protocols were suggested. The company will need to choose which model will be best for a small scale trial.
The meeting agreed to **feedback to the company that the concept seems reasonable, however there are many issues to be resolved and for next steps there is a need for a close co-operation with WHOPES to ensure the method is reasonable and applicable.**

CIPAC 59th meeting, June 2015 in Athens

Mrs Mukumoto presented the results of a method extension for the determination of pyriproxyfen in a matrix release formulation (MR).
The applicability of transferring the available CIPAC method 715/TC/M/3 for pyriproxyfen TC to a pyriproxyfen containing MR product was investigated. CIPAC method 715/TC/M/3 consisted of a reversed phase C18 HPLC column, UV-detection at 254 nm and internal standardization based quantification, and was developed for TC, EC, EW and GR type products. The concentration range of the MR product was not within the scope of the existing method. Furthermore the method had to be modified with respect to the extraction solvent and extraction procedure. After consultation with CIPAC the proposed changes (replacing acetonitrile with ethyl acetate and extracting for 24 hrs at room temperature instead of 4 hrs at 40°C) the modifications were considered to as minor. Specificity tests showed no interferences whereas a company validation (two participating laboratories) showed sufficient repeatability in three different MR products (RSDr < 0.2%) and an accuracy of 100.6% (n = 3, RSDr = 0.1%). The modified CIPAC 715/TC/M/3 is considered appropriate for determination of the pyriproxyfen content in Pyriproxyfen MR.
JAPAC proposed to extend the existing CIPAC method for Pyriproxyfen MR.
The following comments were received from the meeting:

➢ It was asked if ethyl acetate is miscible with the mobile phase, which was confirmed.

Closed meeting:
The meeting discussed the comments received during the open meeting.

The two compared extraction procedures were discussed (extraction for 4 hrs at 40°C or for 24 hrs at room temperature). The meeting had a preference for the room temperature procedure as it would not involve thermostatic equipment, therefore easier to perform.
Decision: The meeting agreed that the method can be **accepted as provisional**.

CIPAC 60th meeting, June 2016 in Tokyo

Extension of 715/TC/M/ to pyriproxyfen/alpha cypermethrin Royal Guard LN (CIPAC/4886/m) by Mr Atmakuru Ramesh (5043, 5044)
Mr Ramesh presented an extension of the scope of two methods for the determination of pyriproxyfen and alpha-cypermethrin in Royal Guard LN. The content of pyriproxyfen and alpha-cypermethrin in long lasting net has been determined with reference to the method CIPAC/4887/R validated for pyriproxyfen and permethrin. The method of determination is a minor extension of the method CIPAC /4887/R.
High performance liquid chromatography with UV detector at 254 nm, a C18 HPLC column 250 mm x 4.6 mm, 5 µm and di-cyclohexyl phthalate internal standard was used for the analysis. The specificity, precision and accuracy of the method were checked. Based on the results obtained Mr Atmakuru proposed the CIPAC/4887/R extension method for LLIN, when active substances, pyriproxyfen and alpha-cypermethrin are incorporated in HDPE polymer, as there are only minor differences in the high performance liquid chromatography method using PDA detector.
The alpha-cypermetrin content was determined by method 454/LN, with the modification of using di-cyclohexyl phthalate internal standard instead of dioctyl phthalate, to use only one extraction process for both active substances.
The following comments were received from the meeting:

- It was questioned if citric acid has also been added to the calibration solutions and if the concentration range of the calibrations covered the concentration of the samples. The answer was that the calibration solutions were prepared according to the method except that acetic acid was added.
- 2 different method extension pyriproxyfen and alpha-cypermethrin
  No other comments

Closed Meeting (LN):
It was confirmed that the existing extension of the scope (CIPAC/4887) of CIPAC method 715/TC/M/2 for the determination of the pyriproxyfen content of a the long lasting insecticidal mosquito net (incorporated type) (LN) containing permethrin and pyriproxyfen is applicable for the long lasting insecticidal mosquito net containing pyriproxyfen and and alpha-cypermethrin.

The extension of the scope (CIPAC/5043) of CIPAC method 454/LN/M/3.2 for the determina-tion of the alpha-cypermethrin content of the long lasting insecticidal mosquito net (incorporated type) (LN) containing alpha-cypermethrin and pyriproxyfen, with the modification of having di-cyclohexyl phtalathe as internal standard, instead of dioctyl phtalathe, was accepted as a tentative CIPAC method, with the need for the provision of a second data set according to the provisions of the CIPAC guideline.

Closed Meeting (MR):
The extension of the scope (CIPAC/4997) of CIPAC method 715/TC/M/3 for the determination of the pyriproxyfen content of a matrix release formulation (MR) (incorporated type) was accepted as full CIPAC method

CIPAC 61th meeting, June 2017 in Rome

Mr Atmakuru Ramesh presented a study of **method extension** of existing CIPAC methods for pyriproxyfen and alpha-cypermethrin in long lasting insecticidal nets.
Two laboratories took part in the study and each laboratory received 3 samples of Royal Guard®, 120 denier and 3 samples of Royal Guard® 150 denier, each containing five 25 cm x 25 cm pieces.
The method CIPAC 715/TC/M/3 was used to determine the pyriproxyfen content without any modifications.
The extraction method CIPAC/4887 (extension of CIPAC 715/TC/M/3) was used to determine the alpha-cypermethrin (454/LN/M/3.2) with minor modifications (CIPAC/5043) using dicyclohexyl phthalate as internal standard instead of dioctyl phthalate.
The pyriproxyfen content in LN was determined by reverse phase high performance liquid chromatography, after evaporating an aliquot of the heptane extract and dissolving it in acetonitrile, using UV detection at 254 nm with di-cyclohexyl phthalate as internal standard (CIPAC 715/TC/M/3).The same sample extracted with heptane was used for the determination of alpha-cypermethrin by GC-FID (CIPAC 454/LN/M/3.2).
The values of RSD_R were smaller than those calculated by Horwitz's equation.
The modifications were considered to be minor modifications and the method extension was proposed to be accepted.

The following comments were received from the meeting:
➢ No questions were received.

Closed Meeting:
The method was tentative. It was promoted to full CIPAC method.
The extension of the scope (CIPAC/5043) of CIPAC method 454/LN/M/3.2 for the determination of the alpha-cypermethrin content of the long lasting insecticidal mosquito net (incorporated type) containing alpha-cypermethrin and pyriproxyfen, with the modification of having di-cyclohexyl phtalathe as internal standard instead of dioctyl phtalathe, was accepted as a **full** CIPAC method.

Extension of the scope of CIPAC 454/LN/M/3.2 to LN (incorporated type) (5107)
The decision of last year was confirmed.

CIPAC 66th meeting, June 2022 virtual

Bifenthrin, pyriproxyfen and PBO by Ms Marie Baes (5299, 5300)

Ms Marie Baes presented the results of a small scale collaborative study with five participants for the determination of bifenthrin, pyriproxyfen and piperonyl butoxide (PBO) in individual TC materials and LN samples after sonification in heptane for 60 min at 80° by GC-FID on a DB-210 capillary column and internal standardization. The LN materials consisted of incorporated fibres, hence the long extraction time at an elevated temperature. The extraction time was tested and 60 min was the optimum extraction time. The resulting HorRat values were acceptable when the group of five samples was split in subgroups of three and two samples. This is a clear indication of inhomogeneous samples which is not uncommon with LN formulations.

Ms Marie Baes recommended to go forward to a full scale CIPAC collaborative trial.

The following comments were received from the meeting:

- Mr Shahabuddin asked why the extension of individual methods are discouraged. Ms Baes answered that to test each molecule with different extraction process is more expensive. Mr Pigeon mentioned that there are CPAC methods for the 3 individual actives, but not for the

LNs. One could have method extensions, but this would mean 3 different methods for the same product, doing 3 extensions. The best way for such net, containing 3 active ingredients, is to develop a new method with a single extraction and injection. This is more convenient for QC laboratories.

Closed meeting:

The method can be promoted to a to a **full scale CIPAC trial** when a clarification about sample inhomogeneity is added

CIPAC 67th meeting, June 2023 Braunschweig

Bifenthrin, chlorfenapyr, pyriproxyfen and PBO by Ms Marie Baes (5347, 5348)

Ms Baes presented the results of a full scale collaborative trial for the determination of bifenthrin, chlorfenapyr, pyriproxyfen and piperonyl butoxide (PBO) in two bifenthrin TC materials, two pyriproxyfen TC materials, two chlorfenapyr TC materials, two PBO TC materials and four LN formulations by GC/FID on a DB-210 (or equivalent) capillary column and internal standard quantification. 16 Laboratories from Europe, Asia and USA participated and 13 laboratories reported results in time. Five laboratories reported having difficulties with the ultra-sonication step (one hour at 80°), especially for not being able to reach the requested temperature. This was regarded as a major deviation. Other comments to the method were regarded as not critical. The statistical evaluation was performed according to the 'Guidelines for CIPAC Collaborative Study Procedure for Assessment of the Performance of Analytical Methods'. In all TC materials the HorRat values were good (range 0.46-0.95) or acceptable (1.3-1.8). However, in the four LN formulations the HorRat values were less satisfactory with one good result (0.87), three acceptable results (1.3-2.0) and six not acceptable results (2.1-7.1). Ms Baes therefore recommended to conduct a second full scale trial while taking into account the following adjustments: a higher analytical standard weight (25-50 mg) and to take care that the required temperature and energy dissipation of the sonication equipment is reached as the extraction of the incorporated active substances is difficult. Monitoring of the true temperature was deemed essential. Furthermore, Ms Baes suggested that the second trial should contain only the LN samples as otherwise probably not enough participants would join and that the LN samples should be prepared and homogenized in the laboratory of the organizer before dispatching to the participating laboratories.

The following comments were received from the meeting:

- Mr Hänel responded that he was not happy with the argumentation of requesting to leave out the TCs for the next full scale trial.
- Mr Benke recommended to use the graduated flask in the sample preparation phase instead of just adding exactly 1 ml of internal standard. Ms Baes replied that because of the accurate addition of the internal standard the use of a graduated cylinder was of no influence to the outcome of the trial.
- Mr Di Loreto asked whether the extraction was optimized as one hour seemed to long? Ms Baes confirmed that this was done in preparation of the interlaboratory trial and that after 45 min the full extraction was not reached, and 1 h was needed.
- Mr Treutwein suggested the use of very strong solvents which are capable of dissolving the polymer net material. Ms Baes replied that was not considered because of the toxic nature of these solvents and because of the possible occurrence of changes of the actives substances. It was demonstrated during the validation that heptane was extracting the actives.
- Mr Ramesh asked what the usefulness of this trial was as already five out of eight participants encountered major difficulties in the extraction process and suggested as a next step to perform a small scale trial focussing on the extraction only.
- Mr Bura suggested to repeat the analysis with the five laboratories who reported extraction problems. Ms Baes responded by telling that the organizers already had decided to repeat the trial because of the bad overall results.
- Ms Baes also mentioned that the bad HorRat scores could be caused by sample inhomogeneity, underperforming extraction conditions or a combination of both.

Closed meeting:

Mr Hänel proposed that both Mrs Baes and Mr Pigeon do not have to leave the room under the condition that they would not interfere with the discussion. This was accepted by the meeting. In her presentation Ms Baes mentioned that the results of the presented full scale trial were not acceptable and that a second trial should be performed. It was proposed to omit the TC materials which was accepted by the meeting. However, the second suggestion related to the sample pretreatment to be performed by the organizing laboratory instead of all individual participating laboratories encountered some opposition of the meeting as Mr Hänel, Mr De Rijk, Mr Di Loreto and Mrs Breedt remarked that the extraction was a crucial step in the method and should therefore be carried out by each individual laboratory. Mr Di Loreto also remarked that it is the duty of the organizer of the trial to deliver homogeneous sample material. Ms Nováková suggested that both intact LN nets and homogenized LN nets could be sent to all participants. Mr Hänel summarized all reactions by allowing that a second **full scale** CIPAC trial can performed under strict control of the sample pre-treatment by the individual participating laboratories and strict monitoring of the temperature and energy dissipation of the ultrasonic extraction (as suggested by Ms Baes during her presentation).