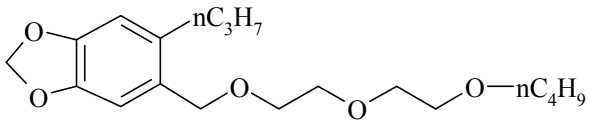
CIPAC STATUS REPORT

28.08.2023



0033 Piperonylbutoxide

Allocated to AOAC

CIPAC methods published in :

CIPAC 1C, p. 2190 (GLC)
CIPAC H, p. 239
CIPAC M, p.164

CIPAC 12th meeting, June 1968 in Braunschweig

Dr. Weinmann said that there was a GLC method (1291m). Scandinavia suggested the determination of piperonyl butoxide to be incorporated in the GLC method.

CIPAC 3th meeting, June 1969 in Oeiras

Dr. Weinmann reported that the proposed GLC method was now also applicable to formulated pyrethrins and in concentrations as low as 0.01 %. Furthermore, it is possible to determine the pyrethrins in combination with piperonyl butoxide and lindane (CIPAC 1461/R). Coll. work planned.

CIPAC 15th meeting, October 1971 in Washington

Decision The AOAC method was accepted as **provisional** CIPAC method.

CIPAC 16th meeting, June 1972 in Stockholm

Method 1461 is to be studied collaboratively and an Information Sheet to be sent.

CIPAC 17th meeting, June 1973 in Wageningen

Dr. Weinmann reported that he could not get enough collaborators.
New Information Sheet to be sent.

CIPAC 18th meeting, June 1974 in London

Work on extract and two formulations (pyrethrum + piperonyl butoxide) in progress in 5 laboratories. Samples sent in May 1974.
CSMA proposal method 2196M to be considered with other methods,

CIPAC 19th meeting, June 1975 in Oeiras

Samples were received broken. New samples to be distributed.
GLC method (2196M) for technical piperonyl butoxide was accepted as full AOAC-CIPAC method.

CIPAC 20th meeting, June 1976 in Wädenswil

Work on GLC method in progress.

CIPAC 21st meeting, June 1977 in Braunschweig

Allocated to AOAC
HPLC method under investigation. There is a small addition to the AOAC official method. (JAOAC 60,328,1977).

CIPAC 22nd meeting, June 1978 in Versailles

Continued study.

CIPAC 25th meeting, June 1981 in Gembloux

Mr Cassera (AOAC) was reported to conduct a coll. study with a GLC method for pyrethrum + piperonyl butoxide.

CIPAC 26th meeting, May 1982 in Rome

The GLC method for pyrethrins + piperonyl butoxide, 6.c 2225 was adopted as **provisional** AOACCIPAC method.

CIPAC 27th meeting, July 1983 in Brisbane

Decision The provisional method 6.C 2225 was adopted as **full** AOACCIPAC method (referee method, published in 1C).

CIPAC 40th meeting, May 1996 in Beijing

Mr Hanks reported the results of an AOAC study with a capillary GLC method for pyrethrins and piperonyl butoxide.There was a discussion about the internal standards having about the same surface, but being introduced for the small and the large peaks respectively. Before a decision is taken CIPAC will wait for the decision of AOAC.

CIPAC 41st meeting, June 1997 in Roskilde

Mr Hanks reported that the method for pyrethrins and pyrethrins with piperonyl butoxide and MGK 264 formulations is still under review by AOAC.
Decision The capillary GLC method for pyrethrins and pyrethrins with piperonyl butoxide and MGK 264 formulations, CIPAC/3908, will be accepted as **provisional** CIPAC method as soon as it has become a first action AOAC method.

CIPAC 42nd meeting, July 1998 in York

The capillary GLC method for pyrethrins and pyrethrins with piperonyl butoxide and MGK 264 formulations, CIPAC/3908, has become a first action AOAC method meanwhile.
Decision The conditional adoption of the capillary GLC method for pyrethrins and formulations of pyrethrins with piperonyl butoxide and MGK 264, CIPAC/3908, as **provisional** AOAC-CIPAC method was confirmed.

CIPAC 50th meeting, June 2006 in Geneva

The extension of the scope of CIPAC method 203 for the determination of bioallethrin and piperonyl butoxide**,** published in Handbook H**,** to EW formulations (CIPAC/4523) was accepted as **provisional** method, with the remark that in future, CIPAC will not accept method extension on

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

Mr Werner presented the results of the method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) using FID and an internal standard (heptadecane) for the determination of piperonyl butoxide in TK and AL formulations to EW formulations (CIPAC/ 4554). The method was found to be selective for piperonyl butoxide, with no interference from heptadecane, or any other compound. In addition, no peaks were found in the EW blank formulation. The data provided on the concentration range, selectivity, accuracy and specificity showed, that the method is also applicable for EW formulations. Mr Werner proposed that the method extension to EW formulations be accepted as a provisional CIPAC method.
Decision The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) for the determination of piperonyl butoxide in TK and AL formulations to EW formulations (CIPAC/4554) was accepted as **provisional** CIPAC method.
The method extension (CIPAC/4523, based on the packed column method) presented at the 50th CIPAC meeting in Geneva is therefore obsolete.

CIPAC 52nd meeting, June 2008 in Braunschweig

Decision The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) for the determination of piperonyl butoxide in TK and AL formulations to EW formulations (CIPAC/4554) was accepted as **full** CIPAC method.

CIPAC 53rd meeting, June 2009 in Sonsonate/El Salvador

Mr Matthieu Zellweger presented the results of the extension of the scope of method CIPAC 32+33+345/TK/M to the determination of piperonyl butoxide content in polyethylene LNs.
The piperonyl butoxide content is determined by capillary gas chromatography using flame ionization detection and internal standard. Xylene is used under reflux to dissolve the PE. Specificity was proved, and the validation parameters i.e., linearity, accuracy and precision all met corresponding criteria of CIPAC guidelines for method validation.

Decision The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) (CIPAC/4675) for the determination of piperonyl butoxide in incorporated PE LN formulations was accepted as a **provisional** CIPAC method

CIPAC 54th meeting, June 2010 in Ljubljana

Ms Maria Cristina Zanotti presented the results of a small scale trial on the determination of piperonyl butoxide in TC using GC–FID and internal std. 4 laboratories participated.
Comments from laboratories: reduction of run-time by increasing the flow rate and temperature programme rate; to improve the description of the sample preparation.
Why such a thick film column was recommended? Lots of impurities in the TC, so the thick film is needed to separate the impurities from the main peak of PBO.
The proposed method was considered appropriate for the determination of PBO in TC and proposed to move to full scale trial.
Decision: It was recommended to go for a full scale trial. If differences from the current method, which includes also the impurity determination, are high, further consideration is needed with DAPA.

Decision: The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) (CIPAC/4675) for the determination of piperonyl butoxide in incorporated PE LN formulations was accepted as a provisional CIPAC method in 2009.
The method showed differences from the original methods – many adaptations were needed to make the method fit the standard CIPAC format. The meeting considered that the method should remain **provisional**.

CIPAC 55th meeting, June 2011 in Beijing

At the 54th meeting, 2010 in Slovenia it was agreed that the method (CIPAC 4675) should remain provisional as there were many differences from the originally proposed method. The meeting considered that if the method were to remain provisional we should provide the company with information (requirements) they need to fulfil in order to promote the method to full.
It was also considered that in practice this may not be a method extension – rather a new method but the situation was not clear. The Chairman and Secretary will compare in detail a comparison of both methods and circulate to members for further discussion. It should also be considered whether to send this to full members only or correspondents also.

Mrs Maria Cristina Zanotti presented the results of a **full scale** collaborative trial (4765, 4766) on the determination of piperonyl butoxide in technical product (TC) using GC-FID with dibutyl phthalate as internal standard (ISTD).
29 laboratories participated in the study. 3 samples of TC were provided.
Of the 29 laboratories that were sent samples 5 did not send results in time, and 3 sets of results were unusable, leaving 21 sets of data that could be used.
Several laboratories used different columns to the specified method. 6 laboratories preferred to prepare an internal standard stock solution and add it into the analysis solutions instead of weighing out the ISTD each time.
For TC 1, TC 2 and TC 3 2 laboratories were identified as Grubb's outliers. These laboratories were also identified as outliers by the "Anderson-Darling normality test".
No data were excluded from the initial evaluation.
All 3 TC samples meet the Horwitz criteria when all the data are included.
Mrs Zanotti proposed that the method was considered appropriate for the determination of PBO in TC and recommends the method is adopted as provisional.
The following comments were received from the meeting:

- The use of a stock solution of ISTD rather than adding a known weight each time would be preferable. Both approaches are possible.
- The amount of reference standard material used for the calibration curve is high. It should be possible to use a single point calibration as an alternative.

Mrs Maria Cristina Zanotti presented the results of a **peer validation** (4812, 4813) on the determination of the relevant impurity dihydrosafrole in piperonyl butoxide technical product (TC) using GC-FID with dibutyl phthalate as internal standard. The instrumental method is the same as that for the determination of PBO in TC, but with a different sample preparation technique.
5 laboratories participated. 1 laboratory used external standard calibration.
Method validation by Endura before trial:
Calibration: at 4 levels, R2 > 0.999
Accuracy: As recovery by standard addition at concentrations of 100% and 120% of specification level. Recovery range = 104% -107%
Repeatability: 5 replicates. CV% = 1.9%.
LOQ: 30 mg /kg

Results of peer validation:
Calibration: at 4 levels, R 2> 0.999
Accuracy: Recovery range = 104% -109%
LOQ: 20 mg/kg - 30 mg/kg

Horwitz criteria were met.
Mrs Zanotti proposed that the method was considered appropriate for the determination of dihydrosafrole in PBO technical and that is should be adopted as the final CIPAC method.

No comments were received from the meeting.

Decisions:
1. The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) (CIPAC/4675) for the determination of piperonyl butoxide in incorporated PE LN formulations remains as a **provisional** CIPAC method.
2. The capillary GC method (CIPAC/4765) for the determination of the content of piperonyl butoxide in TC formulations was accepted as a **provisional** CIPAC method, with the amendments considered in the description of the method. The method should include a note that the internal standard can be added either as a known volume of solution or by weight. In addition it should be amended to note that a single point calibration can be used.
3. The capillary GC method for the determination of the relevant impurity dihydrosafrole in piperonyl butoxide TC formulations (CIPAC/4812) was noticed and adopted.

CIPAC 56th meeting, June 2012 in Dublin

Decisions:
1. The method extension to the capillary GC method (AOAC-CIPAC 32+33+345/TK(M)) (CIPAC/4675) for the determination of piperonyl butoxide in incorporated PE LN formulations was accepted as a **full** CIPAC method.
2. The capillary GC method (CIPAC/4765) for the determination of the content of piperonyl butoxide in TC formulations was accepted as a **full** CIPAC method.
3. It was confirmed (CIPAC/4843) that the existing method for the determination of piperonyl butoxide in LN formulations (33/LN/(M)/3) is applicable for the long lasting insecticidal mosquito net (incorporated type) containing permethrin and PBO.

Comments on existing methods (CIPAC 4857)

At the 54th meeting held in Ljubljana, Slovenia in 2010 and also at the 55th meeting held in Beijing, China in 2011 comments were received that the extension of the PBO AOAC method to LN formulations is not a true extension. CIPAC were tasked to compare results. Mr Bura presented a comparison to the meeting.
It became apparent that the only difference is in the extraction solvent used. As CIPAC have previously accepted extension of methods where the extraction procedure is different for LNs (e.g. deltamethrin) this similar approach should also be acceptable to the meeting.
No comments were received from the meeting

CIPAC 58th meeting, June 2014 in Liège

PBO by Mr Ramanathan Natarajan (4940, 4941)

Mr Natarajan presented the results of a method extension of CIPAC/33/LN/M for the determination of piperonyl butoxide (PBO) in the product "Veeralin" (alpha-cypermethrin and PBO incorporated LN):

- Nominal concentration of PBO in the new LN is 2 g/kg which is at the lower limit of acceptability of the current method (2 g/kg to 334 g/kg). Minor modifications were therefore proposed:
  -In the existing CIPAC method stock calibration solution of piperonyl butoxide (PBO) is prepared by dissolving 250 mg in 50 mL xylene. **Use 50 mg instead of 250 mg.**
  -LN sample of 0.5 g is digested with 23mL xylene + 2 mL octadecane internal standard solution.**Use 1 g LN sample for digestion instead of 0.5 g.**
- Linearity of the detector response in the concentration range 20 mgL^{-1} to 160 mgL^{-1} is linear with $r^2 \geq 0.99$.
- The retention times of the piperonyl butoxide and octadecane (internal standard) peaks in the sample solutions do not deviate by more than 0.5% from that of the calibration solutions.
- Analysis of blanks, calibration solutions, spiked blank formulation and VEERALIN sample solutions showed the absence of compound interference
- Accuracy (recovery) data were generated at two laboratories using a blank formulation and gave acceptable results
- Precision (repeatability) data were generated at two laboratories and gave RSD of 0.8% and 0.5% (Acceptable Horwitz value = 3.41%)
- The results demonstrated applicability and validity of method CIPAC 33/LN/M/ 3 (GC-FID) for the determination of piperonyl butoxide content in the presence of alpha-cypermethrin in Veeralin LN.

Mr Natarajan proposed that the extension of the method is accepted, with the modifications suggested

No comments were received from the meeting.

Decisions: It was confirmed (CIPAC/4941) that the existing method for the determination of PBO content in polyethylene LN (incorporated into filaments) (33/LN/(M)/3) is applicable for VEERALIN, a new LN containing alpha-cypermethrin and piperonyl butoxide with a modification in the standard weight in the stock calibration solution and sample weight.

CIPAC 61th meeting, June 2017 in Rome

Mrs Makiko Mukumoto presented the **extension** of CIPAC/33/EW/M/3 for the determination of piperonyl butoxyde in metofluthrin/d,d-*trans*-cyphenothrin/PBO oil in water emulsion (EW).
The piperonyl butoxide content was determined by capillary gas chromatography using flame ionization detection and triphenyl phosphate as internal standard. The original method used heptadecane as internal standard, but due to separation problems it was changed to triphenyl phosphate. This modification was considered as minor change.
The data shown demonstrated that the method is appropriate for the determination of piperonyl butoxide in metofluthrin/d,d-*trans*-cyphenothrin/piperonyl butoxide EW.
JAPAC proposed the extension of CIPAC/33/EW/M/3 for metofluthrin/d,d-*trans*-cyphenothrin/ piperonyl butoxyde EW formulation.

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

Closed Meeting:
The extension of the scope (CIPAC/5084) of CIPAC method 33/EW/M/3 for the determination of the piperonyl butoxidecontent in Metofluthrin/d,d-*trans*-Cyphenothrin/Piperonyl butoxide EW formulations, with the use of triphenyl phosphate as internal standard, was accepted as a **provisional** CIPAC method.

CIPAC 62nd meeting, June 2018 in Panama City

Decision: The extension of the scope (CIPAC/5084) of CIPAC method 33/EW/M/3 for the determination of the piperonyl butoxide content in Metofluthrin/d,d-trans-Cyphenothrin/Piperonyl butoxide EW formulations, with the use of triphenyl phosphate as internal standard, was accepted as a **full** CIPAC method.

CIPAC 63rd meeting, June 2019 in Braunschweig

Applicability of 33/LN/(M)/3 to LN coated with deltamethrin SC and PBO CS by Mr Mo Lingzhi (5204)
Mr Mo Lingzhi presented the results of a study to prove that CIPAC method 33/LN/(M) was suitable for determining piperonyl butoxide (PBO) impregnated insecticidal nets in the presence of deltamethrin. Samples were extracted by refluxing for 30 min. with xylene. The PBO content was determined by capillary GC-FID on a polysiloxane, cross-linked, surface bonded stationary phase and octadecane as internal standard. Linearity was proven as the correlation coefficient was 0.999 or better. Two samples were analysed in duplicate on two days by two laboratories. The calculated RSD_R of 2.82% fulfilled the Horwitz criterion of 3.38% demonstrating that the method was fit for purpose. The method was accurate, and reproducible, and therefore CIPAC method 33/LN/(M) can be extended with the determination of PBO in coated insecticidal nets.

The following comments were received from the meeting:

- Mrs Carranza de Aguila remarked that the PBO concentration was high (3%). Mr Lingzhi replied that this was part of the formulation.
- Mr Petkar asked why no recovery or specificity was tested. Mr Bura answered that the method was not changed so there was no need for testing of the mentioned criteria.
- Mr Bascou remarked that for PBO a relevant impurity exists and asked whether the method was able to analyse this compound. Mrs Tissier answered by saying that it was not necessary at the moment because the related WHO specification was not yet made public and this is outside of the scope of this discussion. To this Mr Perez Albela Vera added that due to the dilution factor in the LN preparation there was no need for the determination of the relevant impurity.

Closed Meeting:
The applicability of 33/LN/(M)/3 to LN coated with deltamethrin SC and PBO CS 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).

PBO by Mr Molingzhi (5343, 5344)

Mr Molingzhi presented the results of the CIPAC 33/LN/(M)/3 method extension for PBO. CIPAC 33/LN/(M)/3 is suitable for PBO impregnated insecticidal nets in the presence of deltamethrin. The extension focusses on the suitability of CIPAC 33/LN/(M) for determining PBO in coated insecticidal nets in the presence of deltamethrin. Furthermore, some modifications were applied: xylene was replaced by acetone and the injection split ratio was adjusted. After reflux extraction for 30 minutes with acetone PBO is determined by capillary GC/FID and internal standardization. Five samples of coated insecticidal nets were tested by two laboratories resulting in HorRat values ranging from 0.31-0.91.
Mr Molingzhi recommends the extension CIPAC 33/LN/(M)/3 to coated insecticidal nets.

The following comments were received from the meeting:

- Mr Patrian remarked that a split ratio of 0:1 (as mentioned in the presentation) is not possible. Mr Molingzhi remarked that this is written in the Handbook. Mr Patrian suggested that this was a typo and that it should have been 10:1.
- Mr Pigeon also remarked that the replacement of xylene by acetone was a welcome improvement of the method.

Decision:
The extension of the current method can be promoted to a **provisional** CIPAC method