CIPAC STATUS REPORT

11.09.2026

UVCB

1027 Eucalyptus citriodora oil, hydrated, cyclized

Allocated to -

CIPAC methods published in:

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CIPAC 69th meeting, June 2025 Galway

Eucalyptus citriodora oil by Mr George Parker (5413, 5414)

Mr George Parker presented a full scale collaborative study for the determination of three ingredients of hydrated and cyclized Eucalyptus citriodora oil (TK material) as analyzed by GC-FID. The three ingredients were *p*-menthane-3,8-diol, isopulegol and citronellol. Five samples (A to E) were sent to eight laboratories in the EU and Asia and all laboratories reported results in time. The determination was performed by capillary gas chromatography using a (5%-phenyl)-methylpolysiloxane non-polar fused silica column (such as an Agilent HP-5, Restek Rxi-5ms, Perkin Elmer Elite-5ms or equivalent, with dimensions of 30 m x 0.25 mm I.D. x 1.0µm film thickness), using hydrogen as the carrier gas and flame ionisation detection. The observed deviations of the method were assessed as non-critical. The statistical evaluation of the data was accomplished following the new CIPAC Guideline, according to DIN ISO 5725. The testing for outliers / stragglers of the laboratory mean values were performed according to Grubbs test on a 1 %/5 % significance level, respectively.

For *p*-menthane-3,8-diol (calculated as sum of four isomers) one outlier was identified and removed resulting in HorRat values ranging from 0.85 to 1.27. To explain the HorRat values >1 it was remarked that hydrated and cyclized Eucalyptus citriodora oil is difficult material showing inherent variability and potential for interference whereas one out of four isomers is prone to crystallization therefore leading to higher standard deviations. For isopulegol (calculated as sum of three isomers) one outlier was identified and removed resulting in HorRat values ranging from 1.30 to 1.54. To explain the HorRat values >1 it was remarked that hydrated and cyclized Eucalyptus citriodora oil is difficult material showing inherent variability and potential for interference. For citronellol two outliers were identified and removed resulting in HorRat values ranging from 1.56 to 2.05. To explain the HorRat values >1 and even >2 it was remarked that the method is derived from effectively identical methods that have been validated according to SANCO 3099, used to support GLP studies for submission to regulatory authorities including ECHA and as analytical enforcement methods by the EPA.

Citrefine International Ltd. considers this method to be suitable for the intended purpose, without further changes, and recommends accepting it as a provisional CIPAC method for the determination of a.i. content in technical material.

Questions and remarks from the meeting.

- Was it checked the possible isomerization during the sample preparation?
  - The isomer ration is very stable.
- FID detection is proposed, was MS detection used in identifying interfering compounds?
  - Yes, no interfering compounds present, except some below 0.1%.
- Only TC material was tested. What about formulations?
  - Formulations will follow, might be more challenging
- No method was available pre-meeting for the audience hence no proper assessment was possible.
- Sometimes only seven results were available due to removal of outliers. This below the lowest required number of eight results raising questions about the value of the statistical evaluation.
- A mistake in the calculations was identified.
- In general, in terpene analysis by GC a more polar GC column is preferred due to superior separation power compared to a non-polar column. Was a polar column tested?
  - A Carbowax column was also tested with equal results.
- An identity test is missing in the method and a GC-MS based identity test should be added to the method.
- Is the use of an internal standard really needed?
  - Including the internal standard in the calculations resulted in better results.
- When analyzing the stereo isomers, were they analysed individually or were their respective peak areas summed?
  - They were summed after which statistical evaluation was performed. Their respective FID responses were identical.

Closed Meeting:

Several comments have to be answered and further comments were given. Sample inhomogeneity should not be part of the problem but should be solved during sample pre-treatment. As a recommendation it was suggested that – instead of quantifying all isomers – only the main isomer should be selected to reduce the quantitation error of the analysis. The capillary gas chromatographic method (CIPAC/5413) for the determination *p*-menthane-3,8-diol, isopulegol and citronellol in TC formulations was accepted as **provisional** CIPAC method with the clarifications concerning the method of calculation of the active substance content.

CIPAC 70th meeting, June 2026 Krabi

Closed Meeting:

The capillary gas chromatographic method (CIPAC/5413) for the determination p-menthane-3,8-diol, isopulegol and citronellol in TC formulations with the clarifications concerning the method of calculation of the active substance content was accepted as **full** CIPAC method.