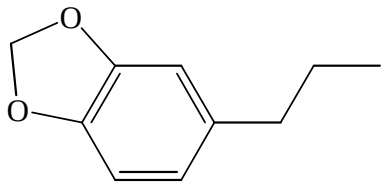
DETERMINATION OF DIHYDROSAFROLE ASSAY IN PIPERONYL BUTOXIDE TECHNICAL GRADE BY CAPILLARY GAS CHROMATOGRAPHY

1. SCOPE

This capillary gas chromatography (GC) method provides for the determination of dihydrosafrole (DHS) as relevant impurity in Piperonyl butoxide (PBO) technical grade active ingredient.



IUPAC name : 5-propyl-1,3-benzodioxole
CAS n° 94-58-6
MW=164.2

2. SUMMARY OF METHOD

The impurity assay is determined by capillary gas chromatography, using an internal standard procedure.

Significant parameters of the method include:

- thick film capillary column
- split injection
- flame ionisation detection

3. CHEMICALS

[Organic solvents for gas chromatography, Merck or equivalent]

All chemicals should be handled according to normal laboratory safety procedures, in a fume cupboard, wearing a laboratory coat, eye protection and suitable gloves.
If in any doubt about the nature and hazards of the chemicals used in this method, consult the corresponding Material Safety Data Sheet or the supplier safety manual.

n-Hexane or Acetone, organic solvent for gas chromatography.
Dibutyl phtalate (DBF), high purity (99+%)as Internal Standard,
Dihydrosafrole (DHS), Analytical Standard of known purity.

4. APPARATUS AND OPERATING CONDITIONS

The apparatus listed below is that used to establish the method. Consideration must be given to confirmation of the method on other makes of equipment, providing equivalent performance, to ensure that they are suitable.

- **Instrument**: GC system, equipped with split/splitless injector and flame ionisation detector, operating in split mode.
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- **Injection mode**: Autosampler, syringe size 10 µl.
- **Injection Liner**: Split inlet liner, single taper, glass wool, deactivated.
- **Injection Load**: 1µl.
- **Injection Port Temperature**: 250°C.
- **Split flow**: 9.9 ml min^{-1}, split ratio 10:1
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- **Gas Filtration**: all technical gases should be of high purity. The carrier gas is further purified through a triple filter cartridge containing oxygen and moisture traps.
- **Column Dimension**: length 25m, internal diameter 0.32 mm.
- **Film thickness**: 0.52 µm.
- **Stationary Phase**: Cross-linked 100% dimethylpolysiloxane.
- **Carrier gas**: High purity helium, Mode constant flow, Average velocity 22 cm s^{-1}.
- **Oven Temperature**: Initial isotherm 120°C,
  Initial time 2 min,
  Programme rate 5°C/min,
  Final temperature 285°C,
  Final time 10 min.
- **Run time:** 45.00 min

- **Detector Temperature**: 300°C
- **Detector Gas Flow Rates**: According to manufacturers recommendations.

5. CALIBRATION

The calibrations solution are prepared starting from already diluted solutions.

Preparation of AM Solution, a mother concentred solution of DHS at about 1000 mg/l:
weigh 250 ± 10 mg of DHS standard in a 250 ml volumetric flask. Dilute to volume with solvent and shake vigorously.

Preparation of A Solution, a diluted solution of DHS at about 40 mg/l:
measure with a precision volumetric pipette 2 ml of the previous prepared AM solution and put into a 50 ml volumetric flask. Dilute to volume with solvent and shake vigorously.

Preparation of the B solution: an Internal standard stock solution at about 2500 mg/l
weigh 250 ± 10 mg of Dibutyl phtalate standard in a 100 ml volumetric flask. Dilute to volume with solvent and shake vigorously.

Prepare the calibration solutions at four different concentration levels of the impurity, in presence of the Internal standard at 100 mg/l of Dibutyl phtalate

Level 1: measure with a precision volumetric pipette 1 ml of the previous prepared A diluted DHS solution and put into a 50 ml volumetric flask. Add 2 ml of the previous prepared B stock DBF solution . Dilute to volume with solvent and shake vigorously.
The final solution contains about 0.8 mg/l of DHS.

Level 2: use the same procedure of level 1 but put 1 ml into a 25 ml volumetric flask and add 1 ml of the previous prepared B stock DBF solution. The final solution contains about 1.6 mg/l of DHS.

Level 3: use the same procedure of level 1 and 2 with a 50 ml volumetric flask, 5 ml of the A diluted DHS solution and 2 ml of B stock DBF solution. The final solution contains about 4.0 mg/l of DHS.

Level 4: use the same procedure of level 1 and 2 with a 25 ml volumetric flask, 5 ml of the A diluted DHS solution and 1 ml of B stock DBF solution. The final solution contains about 8.0 mg/l of DHS.

Prepare a vial of each of the four level solutions and inject twice each solution in GC at the chromatographic condition described in the paragraph 4.
Plot the Concentration ratio *(DHS/Internal Standard, mg/l)* data versus Peak Area ratio *(DHS/Internal Standard, pA*s.*) data obtained from each chromatogram, and calculate the equation of the calibration curve, expressed as
$Y = A X + B$.

Verify the linearity of the calibration curve: the r parameter must be not less than 0.99.

The concentration at levels 1, 2, 3, 4 of the calibration range corresponds to a concentration of DHS respectively of about 40, 80, 200, 400 mg/kg_{PBO} at the method conditions.

6. DETERMINATION

6.1 Preparation of sample

Weigh (0.500 ± 0.002) g of the PBO to analyse and add 1 ml of DBF stock solution (B solution, paragraph 5) in a 25 ml volumetric flask. Dilute to volume with solvent and shake vigorously. The sample solution contains about 20000 mg/l of PBO, 100 mg/l of DBF.
Prepare a vial of the sample solution and inject twice in the gas chromatographic system at the condition described in paragraph 4.

6.2 Calculation

Calculate the content of the impurity DHS, expressed as mg/kg_{PBO}, from the equation of the calibration curve
$Y = Ax + B$, using the formula below.

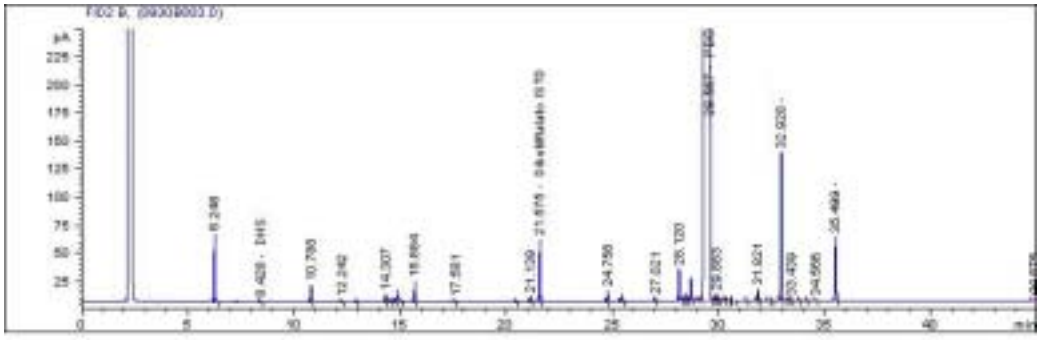
$$\textbf{DHS (mg/kg)} = \frac{(\frac{Hw}{Iq} - B) * q}{A * w} * 100 * 10000$$

Where:
Hw = mean area of the impurity DHS peak
Iq = mean area of internal standard DBF peak
w = concentration (mg/l) of PBO sample in the sample solution
q = concentration (mg/l) of internal standard DBF in the sample solution, correct for its purity
A = slope of calibration curve
B = intercept of calibration curve

7. SUITABILITY

Stored or new columns may require conditioning prior to use. Perform replicate injections of calibration solution until acceptable, repeatable chromatography is obtained.

Compare the chromatogram obtained with that of a typical PBO batch: measure the retention time of the DHS impurity, 8.43 ± 0.3 minutes; measure the retention time of the DBF Internal Standard 21.6 ± 0.3 minutes and its peak area.



If the peaks retention time is not within the quoted window, the oven temperature or the column head pressure may need to be checked or slightly adjusted.

8. ATTACHMENT

-Validation method guide to the method:
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9. REFERENCES

-CIPAC Guideline for analytical methods for the determination of relevant impurities referred to in FAO and/or WHO specifications for pesticide technical grade active ingredients and formulations CIPAC rev.7 (June 2009)

-Endura: CIPAC Method 4717/m “Determination of Piperonyl butoxide purity assay by capillary gas chromatography” (June2010)

-for the acceptance criteria: APVMA Guidelines for the validation of analytical methods for active constituent, agricultural and veterinary chemical products (October 2004)

Example chromatogram of PBO technical with elution of DHS (8.428 min), dibutylphthalate (21.575 min) and PBO (29.567 min) respectively, indicated