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Hydroperoxide Task Force

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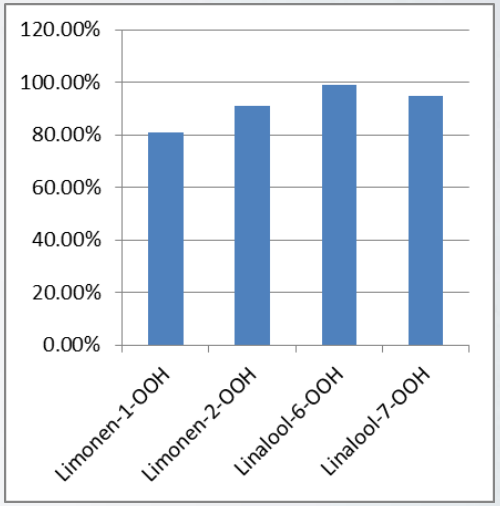
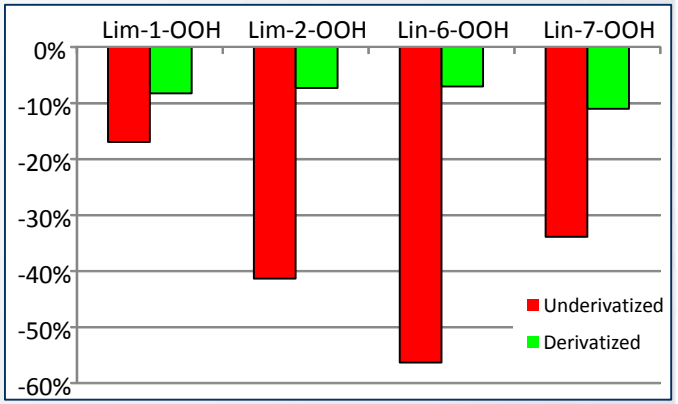
Purity of hydroperoxide standards

- NMR
  - Supplier's claim: all purities = 95%
  - With an internal standard → 80 to 99%



- Rapid purity evaluation by GC-FID
  - Underivatized → bias up to 56%
    - Thermal decomposition
  - Derivatized (trimethylsilylation)
    - Bias ≤ 11%

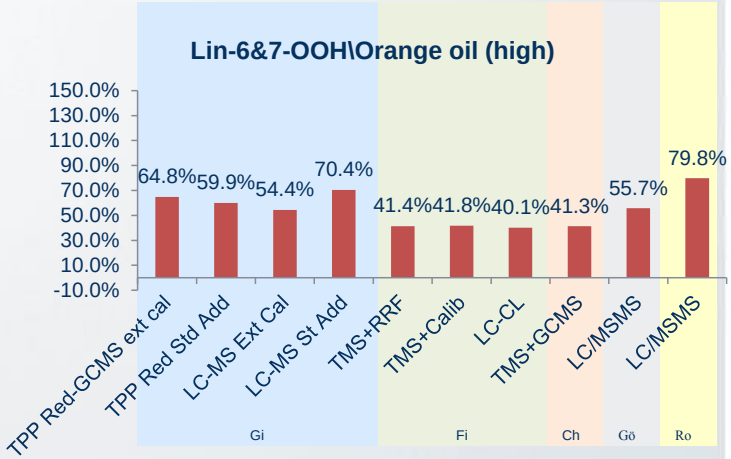


Conclusions on standards and purities

- Standards available with a good isomeric purity
- Must be stored at -80°C
- Reference method for purity measurement
  - NMR + internal standard
- Rapid purity determination without reference material
  - GC-FID + ROOH derivatization + predicted response factors
- GC of underivatized ROOH
  - = inappropriate for purity and quantification

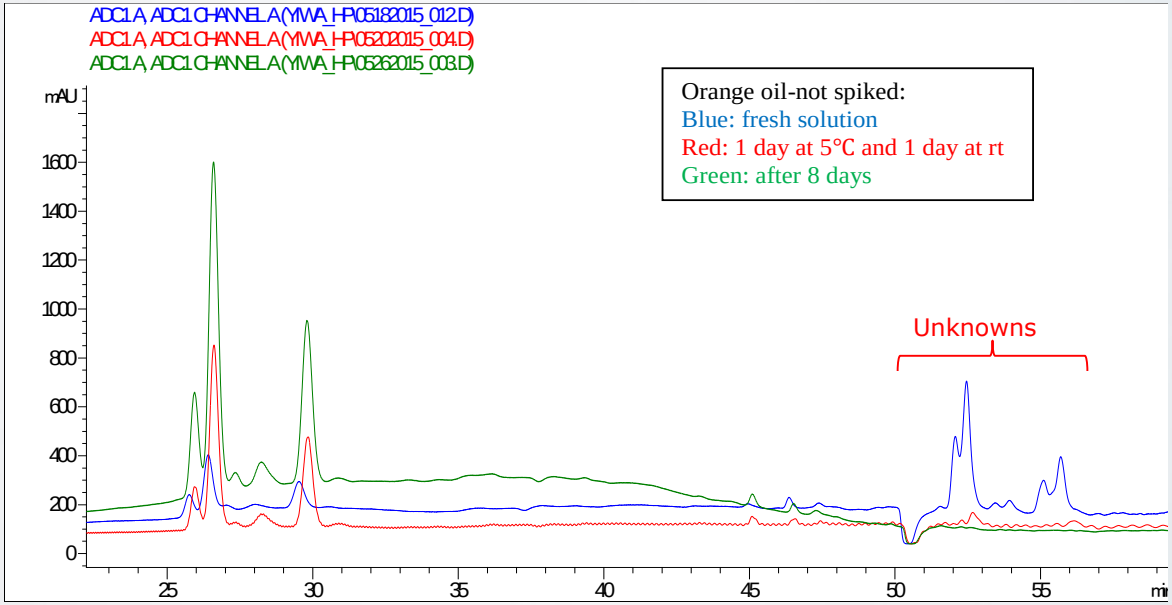
Previous ring test results (I)

- Most promising methods
  - Low level
    - LCMS-ExtCal, TMS+RRF, TMS+Calib, LC/MSMS
  - High level
    - TPP Red-ExtCal, LCMS-ExtCal, TMS-RRF, LC/MSMS
- But no fully satisfactory method
  - (max = 66% determinations with a biais of < 25%))
- Case of linalool-OOH in orange oil
  - All techniques→ bias # 50%
  - Real bias or reaction of ROOHs ?



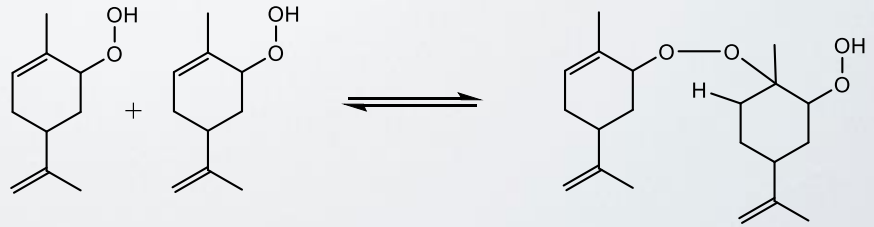
Previous ring test results (II)

› Formation of a complex (LC-CL results)



› Hypothesis

› Complex = «dimer» ?

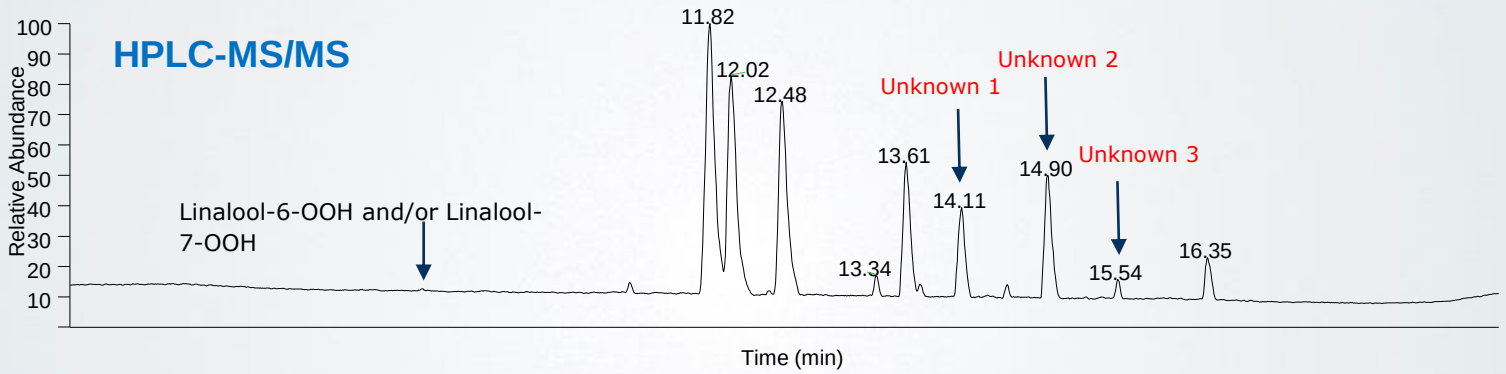


2X Limonene Hydroperoxide

Limonene Hydroperoxide "Dimer"

Complex formation confirmed by LC-MS²

› Linalool-OOH added to orange EO → new compounds



› MS spectra of resulting unknowns suggest a LinOO moiety

› Direct ROOH dilution in an orange EO
→ Rapid formation of the complex

› ROOH diluted in EtOH prior to dilution in orange EO
→ No complex formation

Tentative characterization of the «dimer»

- Dilution of limonene-2-OOH and NMR monitoring
  - In deuterated DMSO
  - In deuterated CyHexane
- No change at all of the NMR spectra
- New hypotheses being tested.
  - Reporting at the TF meeting of Nov. 16th

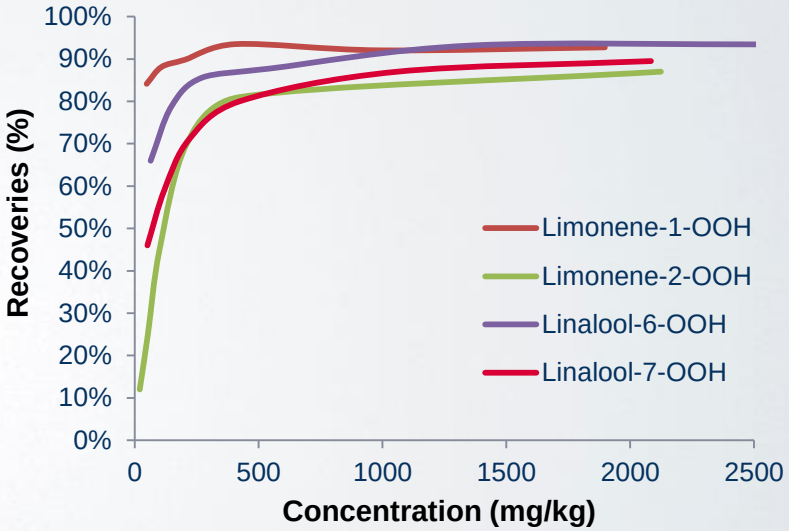
Conclusion on the complex

- Formation confirmed
  - By 2 methods
- Consumes a significant fraction of free ROOH
  - (Partially) reversible
- Structure and reactivity of the complex
  = to be studied in priority

Possible impact on the availability of ROOHs

GC+ Derivat. + Predicted RRFs approach

- Validity re-tested
  - Recoveries of derivatized hydroperoxides better than initially observed
  - Satisfactory recoveries down to 500 mg/kg



- Both, the calibration and the RRF prediction could be applied down to 500 mg/kg
  - In line with the performances observed during the ring test
  - Concentration range suitable for the elicitation level
    - But not applicable to alcoholic perfumery
    - Suitable for the QC of essential oils

Back to the last analytical ring test

- Global results could be better than it appeared to be
  - One fraction of added ROOH is consumed in the complex
    - Not quantifiable anymore
- Validating any method might be challenging
  - Validation principle of a quantitative method
    - = spiking a know amount in a matrix and retrieving this amount by quantification
  - If ROOHs spiked in a fragrance react with its ingredient: no way to retrieve the initially spiked amount…
    - Except if complexes become quantifiable
- However… analytical target = quantification of available ROOH
  - Will require a set of various methods depending on:
    - The matrix
    - The objective: free / total ROOH content

Provisional conclusion on the quantification of ROOHs

- No ring test validation until the complex is quantifiable
  - Unknown feasibility → unstable complex

- Use a different method as a function of the objective
  - Gas chromatography without derivatization → not valid
  - Reduction + GC → high levels
  - GC + derivatization → non alcoholic raw materials
    - If GC-FID and silylation: no need of ROOH standards
  - HPLC-$MS^{(2)}$ & HPLC-CL → all levels
    - If HPLC-CL: detection of new oxidants
  - Complex matrices → no method
    - Clean-up procedure to be set-up

To be confirmed once the complex is quantifiable

Impact on biological studies

- Understanding the chemistry of this complex is crucial
  - Does it contribute to allergenicity ?
  - Does it regenerate the initial hydroperoxides?
  - Does it degrade into non-sensitizing end products ?
    - If not retrieved after a chemical reaction (quantification by reduction or silylation) → not available anymore

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inspiring!

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