IDEA
Hydroperoxide task force

Brussels, March 24th 2014

A. Chaintreau

Agenda

- Antitrust statement
  - No discussions of agreements or concerted actions that may restrain competition
- Adoption of the agenda

Composition of the ROOH T-F

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Objectives

- Debates in the current literature:
  - Real kinetics of ROOH formation ?
  - Potential role of fragrances in the population allergy ?
- Vicious cycle:
  - No reliable method to assess ROOH purities
  - No (commercial) source of pure standards to calibrate the instruments
- Priorities
  - Synthesis/purification of standards
    - Ensure their availability to all teams
  - Developing/improving reliable quantitative methods

01 Synthesis

Exchanging standards: an issue

- Transportation of dangerous materials is regulated
  - Stability of pure ROOH : unknown
    - Some ROOHs commercially available at high concentration → delivered by usual transportation means
    - Standards to be sent from the synthesis lab to all partners
      - Terrestrial means can be used
      - Restrictions for air transportation

  → Are ROOH stable enough for a slow delivery system ? Under which conditions ?

- A consultant in chemical transportation has been identified
  - Address sent to Fred
  - Issue to be solved before deciding on the synthesis of standards

Hydroperoxides of concern

› All ROOHs cannot be considered
  › Too long for the IDEA time frame
  › Would require an endless budget

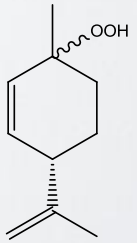
⇨ Priorities to be defined

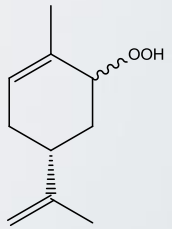
› Most frequently investigated ROOH
  › Limonene
  › Linalool
  › (Linalyl acetate)
  › Others ?

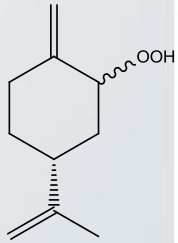
› Isomers . . . . . → next slide

Isomers of concern (I)?

- Isomers differ in allergenic activity
  - "Limonene hydroperoxide analogues differ in allergenic activity", Christensson et al, *Contact Dermatitis*, **59, 344-52**
  - "Limonene hydroperoxide analogues show specific patch test reactions"", Christensson et al, *Contact Dermatitis*, in press
- Detector response
  - Differs in MS
  - Unknown for CL
- Limonene
  - Limonene 1- and 2-hydroperoxide
    - Main ROOH in citrus oils
    - Specific synthesis only for lim-1-OOH
    - Lim-1-OOH more allergenic
  - 2-hydroperoxy-1-methylene-4-(prop-1-en-2-yl)cyclohexane
    - Absent/minor isomer in autooxidized EO
    - Generated in presence of a photosesitizer
      - P.Shieberle, HRC, 1987, *10* ,588-593

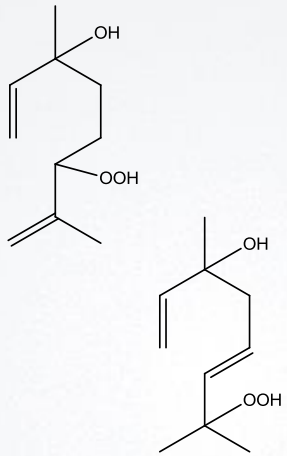






Isomers of concern (II)?

- Linalool
  - Lin-6-OOH
    - Minor
  - Lin-7-OOH
    - Major



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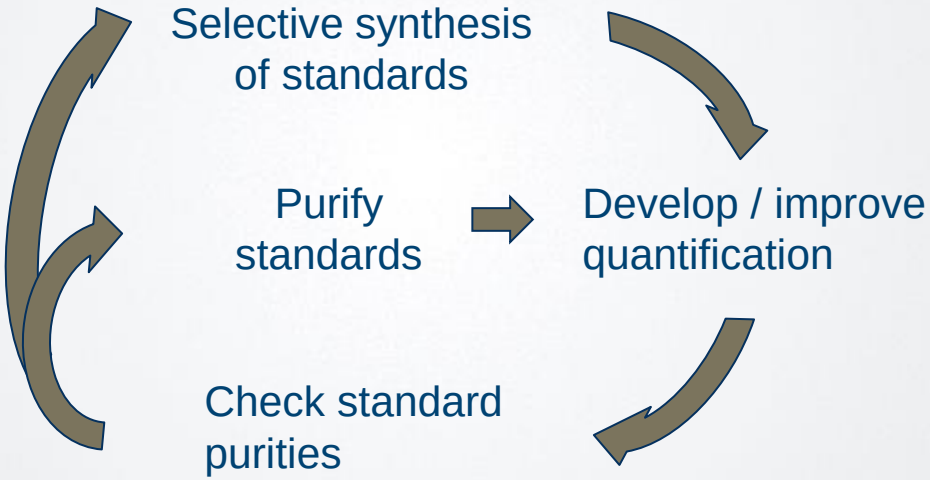
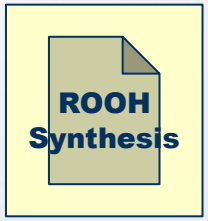
Synthesis routes

› Preparing pure standards → an iterative process



› Possible routes →by Elena



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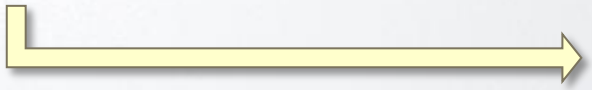
To which lab ?

- Too small niche for the fragrance industry
- University ?
  - Manpower, large scale (> 10 g), stock, certification ?
- Contract lab ?
  - Specifications to be matched



Specifications for the synthesis lab

1. Selective synthesis of isomers (without taking the chirality into account). Alternatively, a preparative isolation from a mixture of isomers could be considered, if the resulting quantities and time frame comply with items 4.and 6.
2. Structural confirmation of the compound: To be checked, notably by NMR (^{1}H and ^{13}C).
3. Purity: the highest possible purity of each isomer (>90%)
4. Quantities to be delivered: at least 10 g / isomer to be shared between the laboratories developing the quantification.
5. Stability: it should be checked in the course of the development
   a. Either by NMR
   b. And/or in partnership with a laboratory in charge of the analytical development for IDEA
6. If the stability of some isomers in pure state is too low, alternatives should be investigated so that the analytical laboratories receive samples at known concentrations.
7. Maintain the availability of most frequent standards within a reasonable time frame
8. Time frame: 6 months

The synthesis lab should work in partnership with (the) analytical lab(s)

Bond Dissociation Energies

- To be calculated for a later correlation attempt with
  - Chemical reactivity
  - Allergenic activity
  - Stability

02 Quantifications methods

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Quantification methods

- All methods are very recent:
  - HPLC-MS/MS (AT Karlberg, U. Göteborg, 2013)
    - *J. Sep. Sci. 2013, 36, 1370–1378*
  - HPLC-Chemiluminescence (M. Calandra, Firmenich)
    - Not yet published
  - GC-MS (AT Karlberg, U. Göteborg, 2013)
    - J.Sep.Sci, 2014, in press
  - $P\Phi_3$ reduction and LC-MS quantification
    - A. Natsch, submitted
  - + Another method in development (Firmenich)

Method overview

	HPLC-MS/MS	HPLC-CL	TMS+GC-MS	$P\Phi_3$ +LC-MS
Needs standards	Y	Y	Y	Y/N
Specificity to all ROOHs	N	Y Only way to detect all and only ROOHs	N	N
Specificity to known analytes	Y/Y	Y	Y/Y	Y/Y
Analyte identification	Y	n.a.	Difficult	Exact mass
Tested by spiking	Y (in EOs)	N	N	Y
Others	• Insufficient specificity in complex mixtures (Natsch)		• Structure of linalool-TMS t.b.d.	• Reduction yield t.b.d. • Pb if endogenous reduction product

No ideal method → complementary
None of these methods is really validated
→Need to be further optimized before being applicable
→ Depends on the availability of pure isomers as standards

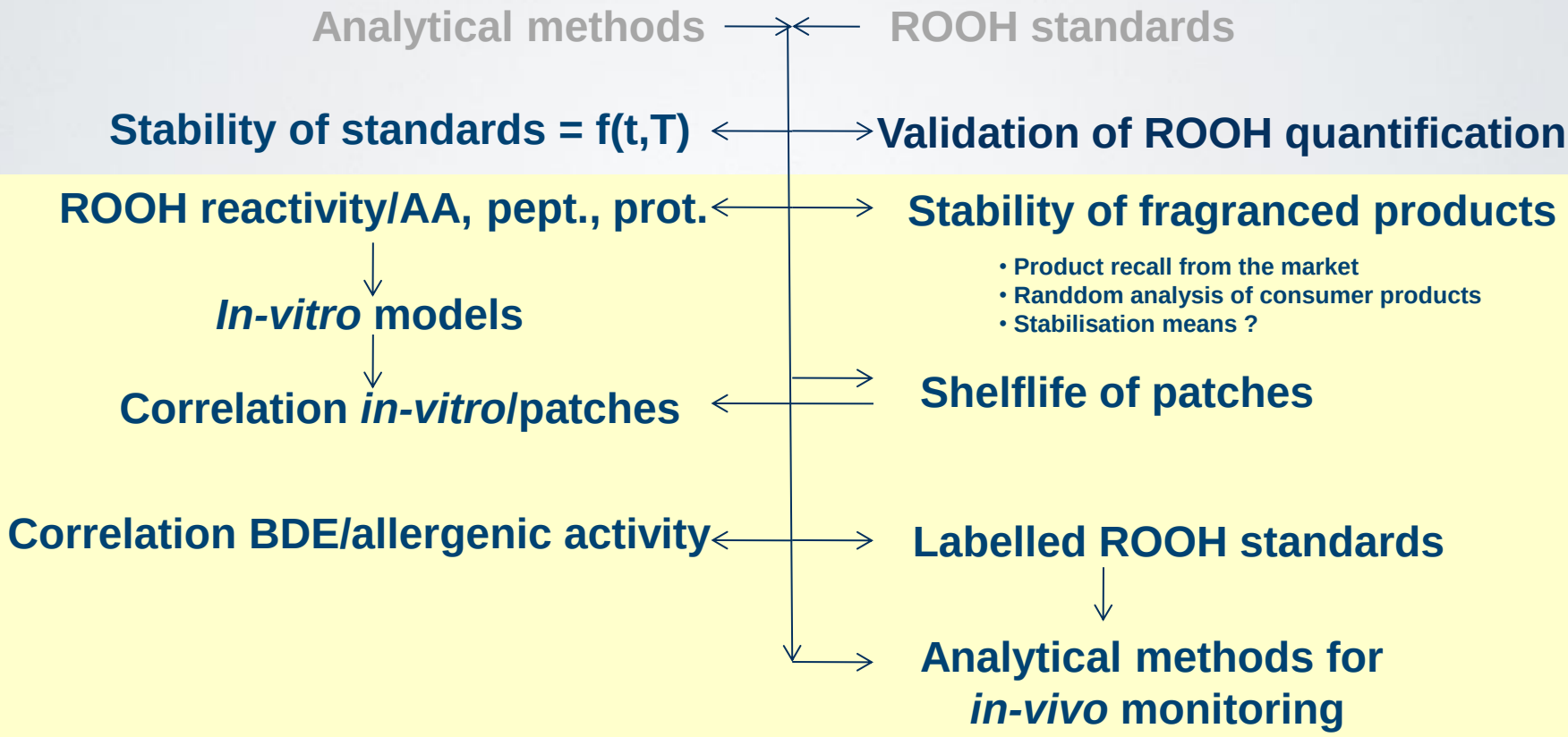
Specification for the analytical labs

- Development/improvement of a quantitative method with the following characteristics:
  - Selectivity towards the hydroperoxides, or convenient means to locate hydroperoxides in an chromatogram.
  - When the calibration of hydroperoxides has been achieved once, use of recorded (relative) responses to avoid the further use of standards
- Alternatively, if these two criteria cannot be met by a single method, several methods would be developed if each of them meets one of these criteria.
- Methods based on a spectrometric detection should comply with the state-of-the-art practices. Notably, the identity of quantified peaks should be checked to avoid analyte confusion and detect co-elutions.
- Purity and stability of standards: in partnership with the synthesis laboratory, the purity and stability of standards as a function of time will be checked, to determine possible storage conditions and selflifes.
- The proposed method should be submitted to a prevalidation (intermediate precision) by its author.
- Method delivery one year after the beginning of the synthesis project. The quantification development should start before the availability of pure standards, to support the synthesis project in the purity determination of standards.

The analytical labs should work in partnership with synthesis

03 Outlooks

Application for Horizon 2020 funding ?



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