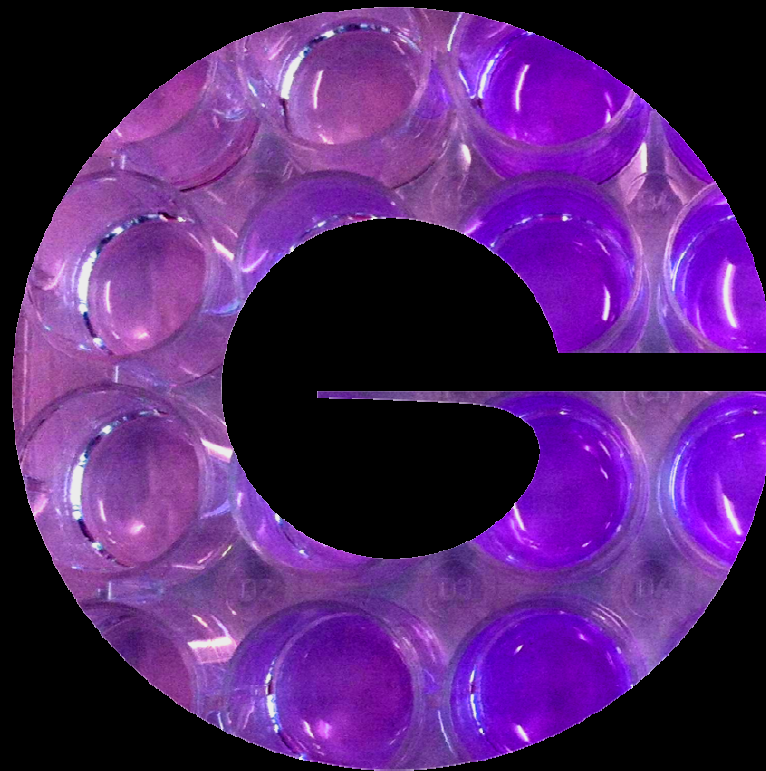


Update on the work program of the analytical task force, status, remaining activities, timelines and input to QRA2

13.12.2016, Andreas Natsch



Givaudan

engage your senses

IDEA Analytical HP task force: A multistage project

- 1. The Problem**
- 2. Scope: What are methods needed for?**
- 3. Sensitivity: Targets set for the task force**
- 4. Ring Study 1: Comparison of methods 1**
- 5. Important step: Accurate analytical standards**
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- 9. Ring Study 3: Method validation in real products – fine fragrances**
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- 14. Interpretation – how will we judge results? – Input to QRA2**

Problem definition

- Hydroperoxides (HP) are sensitizers
- Positive patch test reactions to oxidized products are reported
- Analytical detection of HP is challenging
- HP are not intentionally added to products, but
 - They could be added as impurities from raw materials
 - They may form in products if sufficient oxygen is present
- There are very little exact data on HP levels in raw materials
- There are even less data on HP level in consumer products
- Analytical data are needed to find out whether positive patch test reactions may come from use of fragranced consumer products
- Analytical methods able to detect HP in consumer products are required

Scope: What are methods needed for

- There are two different questions:
- **Quality control on raw materials:** Detection of HP in raw materials used in fragrance compounding
 - Complex essential oils from natural sources (e.g. orange oil)
 - Synthetic raw materials (e.g. synthetic linalool)
- **Detection in final consumer products**
 - Detection in general market products and aged consumer samples
 - ⇒ Presence of potentially sensitizing doses above levels considered safe by QRA?
 - Detection in products brought in by patients
 - ⇒ Presence of potentially eliciting doses which may indicate relevance of reaction to actual disease?

Sensitivity: Targets set for the task force

- **Initially set analytical Target:**

"Methods should be sensitive, specific, with target limits of quantification (LOQ) below the estimated induction levels and limits of detection (LOD) below the estimated elicitation levels"

Estimated induction levels:

- 5000 ppm taken as a default induction level
- Linalool: Lowest elicitation level in humans: 560 ppm

- **Revised analytical target** – based on improved analytical methods:

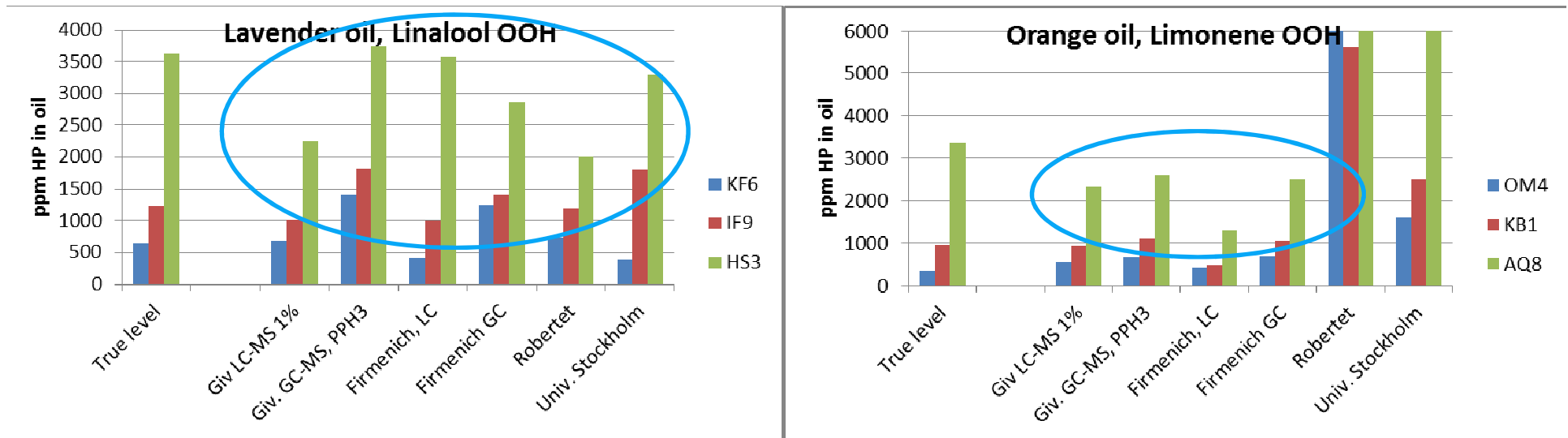
- 50 ppm in final consumer product
 - This is 100 fold below default induction level
 - 10-fold below reported elicitation level
 - Levels below 50 ppm can be considered not relevant
- **Note: This lower level is set to have a full understanding and is based on analytical feasibility: it does not mean that all levels above 50 ppm are of toxicological concern**

Study 1: Comparison of methods 1

- Lavender oil spiked with Linalool-OOH and orange oil spiked with Limonene-OOH
- Spike levels 500 (red bars) and 3000 ppm (green bars); blinded samples
 - Spike levels defined by initial target sensitivity
- 6 different methods by total 5 different laboratories

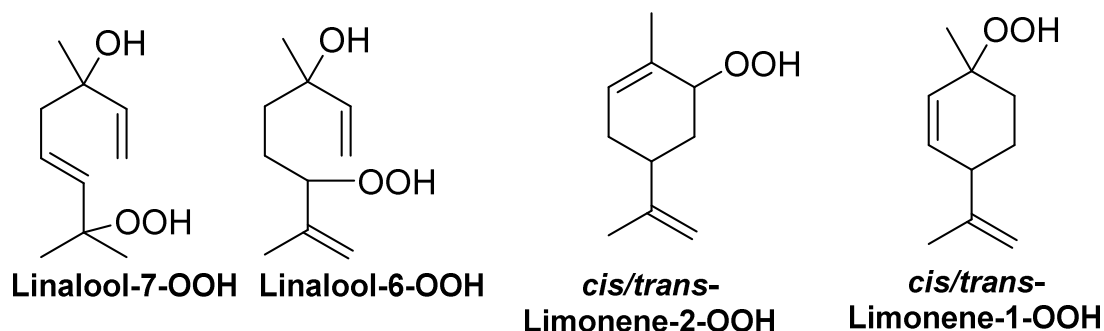
Methods can detect the HP, but significant variation from true level

Tendency for underestimation in orange oil



An important step: Accurate analytical standards

- First study was run with analytical standards containing **mixtures** of hydroperoxides, **not completely purified**
- Key to improve methods: **Highly pure reference standards**
- External company was asked to prepare 4 highly pure standards
- **These standards served to:**
 - Prepare exact spiked samples in subsequent ring tests
 - Calibrate analytical methods



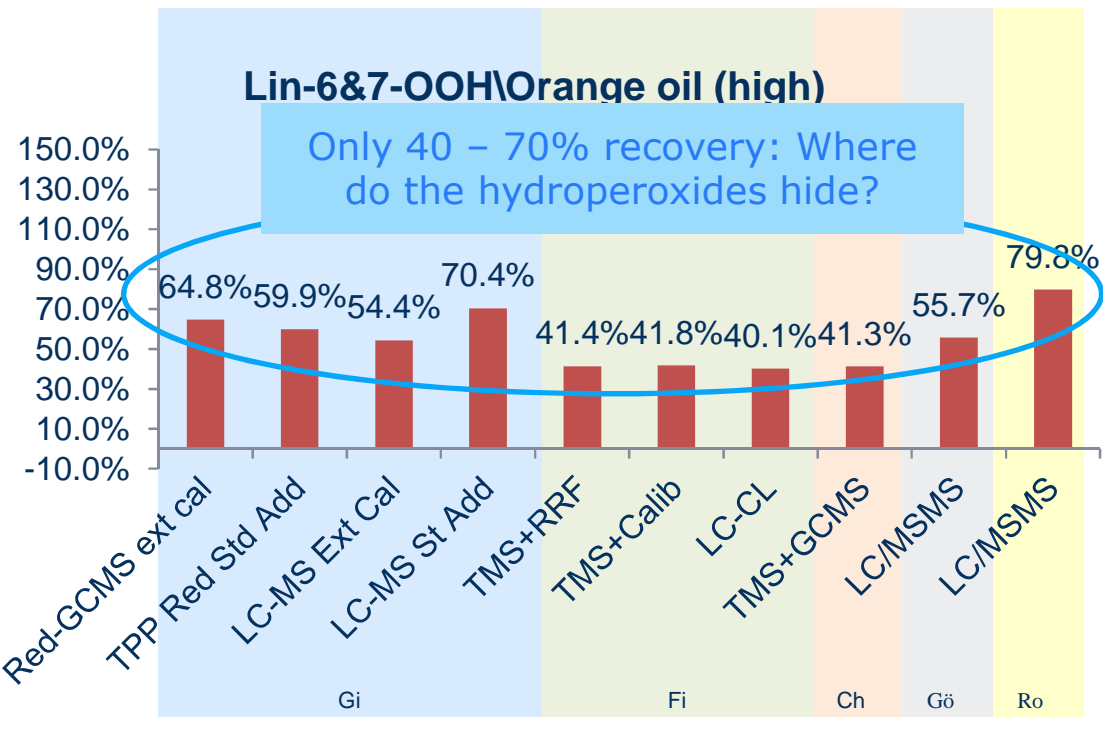
Study 2: Comparison of methods – continued

- Blind spiked samples with accurate analytical standards
- Three matrices of increasing complexity
 - Simple solvent
 - Orange oil
 - Model fragrance (Lily)
- 6 labs with a total of 10 different methods / quantification approaches

- Conclusion:

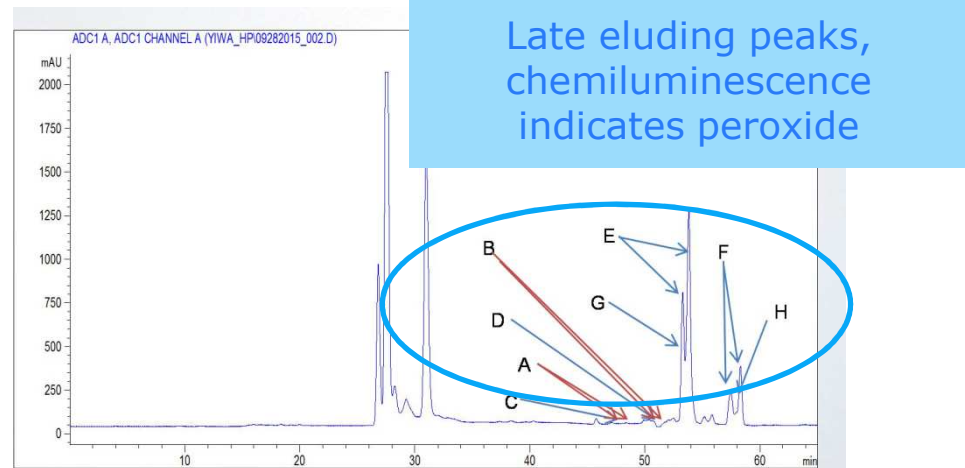
- **General underestimation in orange oil and Lily fragrance with several methods**

- Reduction / GC-MS method may be a robust method



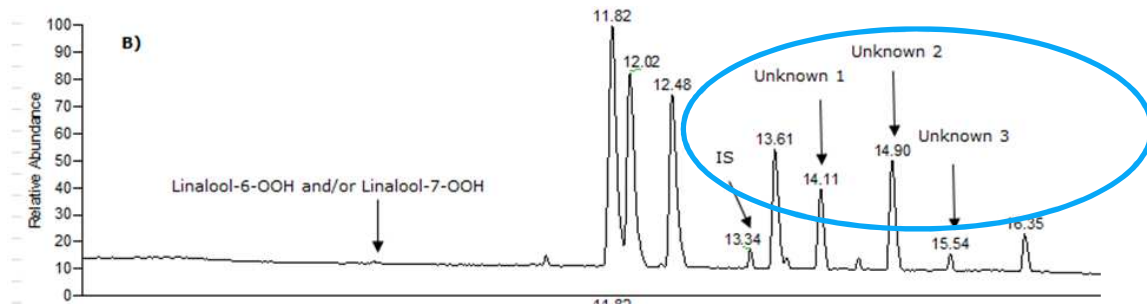
An issue encountered: Loss of hydroperoxides in essential oils

- Only 40 – 70% recovery in some oils: **Where do the hydroperoxides hide?**
- HPLC-Chemiluminescence:



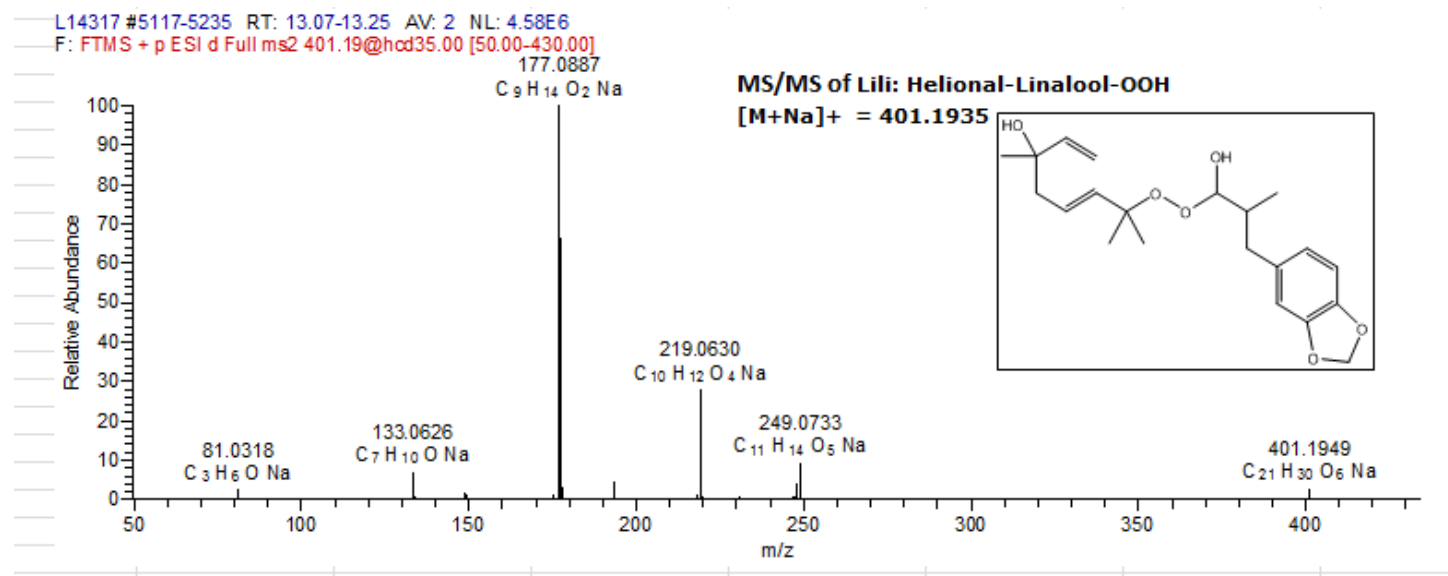
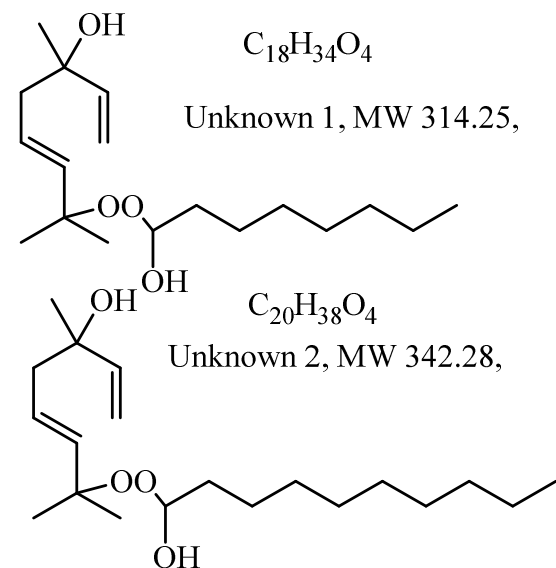
- LC-MS:

Late eluting peaks, MS indicates they contain Linalool-OOH substructure



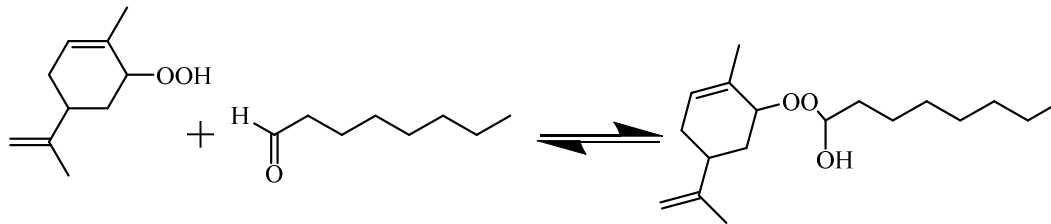
Issue resolved: Formation of Peroxy-Hemiacetals

- Two laboratories could independently resolve the issue
- Hydroperoxides form hemiacetals with aldehydes in essential oils and fragrances
 - Adduct with decanal and octanal contained as trace impurities in orange oil
 - Adducts with synthetic aldehydes such as Helional in fragrance oils



Formation of Hemiacetals – a critical hurdle?

- This research is not finished – *the following conclusions are based on current observations*
- For the time being it appears that:
- A) Hemiacetals formation is reversible



- B) Hemiacetals are mainly formed under aprotic conditions / in aprotic solvents: i.e. In neat raw materials and oils
 - May be an important interference in raw materials and ess. oils ⇒ **Quality control**
- C) Hemiacetals are present, but at low levels, in more complex mixtures with protic solvents (e.g. Fine fragrances)
 - Equilibrium is far on the left side
 - **May pose less problems in final products / when assessing consumer exposure**

Study 3: Method validation in real products – fine fragrances (2016)

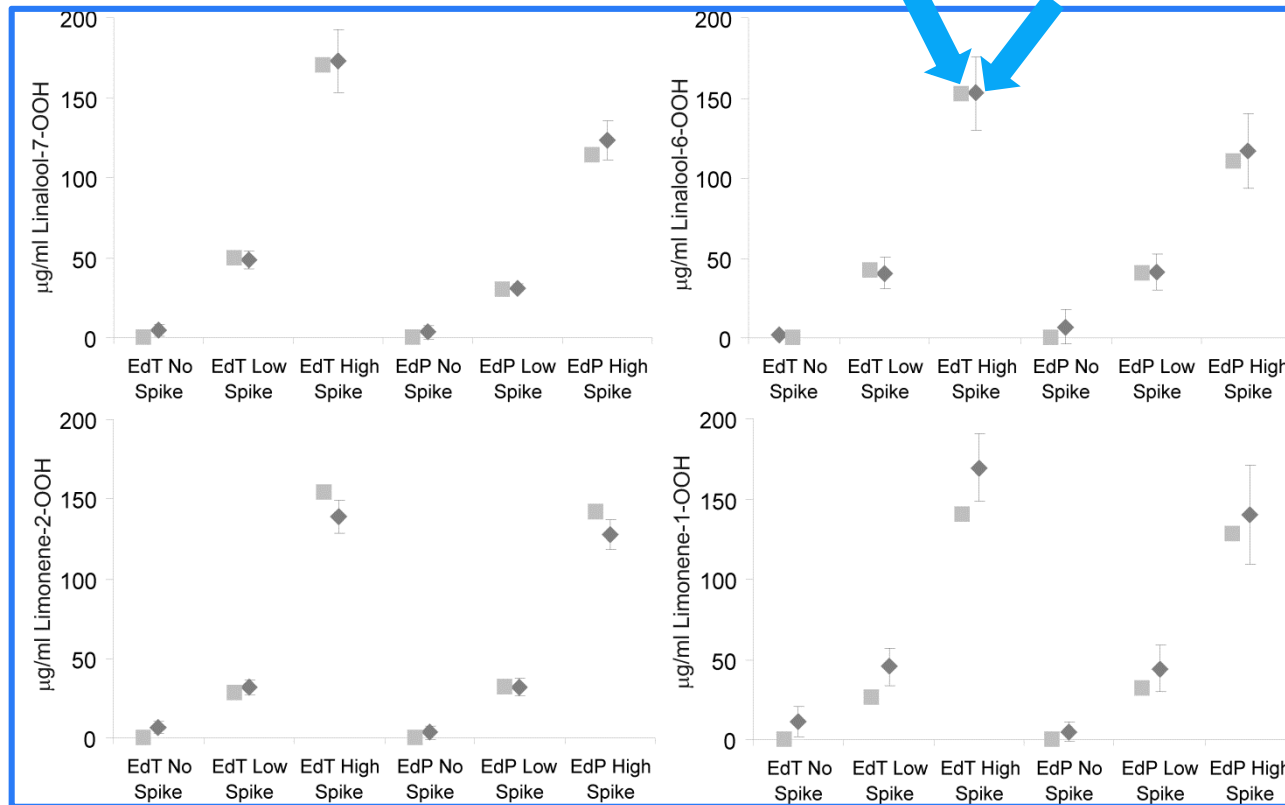
- Real market products, 2 samples with three spike levels of 4 different HP
- Blindly spiked with different levels
 - Lower analytical target levels taken
- Five labs compared same method (GC-MS reduction method to detect HP indirectly) -> **Method validation**
- Three labs tested additionally different methods (LC-methods to detecte HP directly) -> **Method comparison**

Eau de toilette, not spiked	Eau de toilette, low level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of 20 – 50 ppm	Eau de toilette, high level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of 100 – 200 ppm
Eau de parfum, not spiked	Eau de parfum, low level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of 20 – 50 ppm	Eau de parfum, high level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of 100 – 200 ppm

Study 3: Method validation in real products – fine fragrances

- Accurate detection with GC-MS reduction by all five labs
- **This method allows accurate quantification in real products**

Light grey squares: Spiked levels Dark grey diamonds: Found levels



Study 3: Method validation in real products – fine fragrances

- Three different LC-methods
- Also allow good quantification without derivatisation in most samples

Detection of Linalool-OOH (sum of isomers) by different analytical methods (data in µg/ml)

	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
LC-Q-TOF MS	0.0	90.0	279.0	0.0	59.0	200.0
HPLC-CL	0.0	79.5	310.7	0.0	56.2	203.7
LC-orbitrap-MS	0.2	95.7	398.7	0.0	29.1	185.4
spike level added	0.0	92.0	322.0	0.0	70.0	224.0

- **A Toolbox of methods is now available for analysis in fine fragrances**
- **What about more complex matrices such as creams and lotions?**

Method development work – analysis in Creams, lotions, complex matrices

- Two standard creams and a standard deodorant
- Each lab tried different methods
- Based on results promising method chosen
- Allows good recovery from different product matrices

Analysis is now also possible in complex consumer products

% recovery of 100 ppm spike	trans-Carveol ex Limonene-2-OOH	
	T=24 h	T=28 days
Woolwax Alcohol Creme	106.6	111.7
Deodorant Base	83.7	85.8
Bodylotion'	94.1	88.4
Anti ageing cream'	96.5	90.8
All natural deo	92.8	98.1
Lotion II	87.7	84.9
Average recovery	93.6	93.3

Ring Study 4: (Planned Q1 2017): Method validation in real products – Creams, lotions, and deodorants

- Last ring trial: Same setup as for fine fragrances
- Now with creams and deodorants
- 5 labs will again test reduction method
- 3 labs test different LC-methods
- Validation of the Method toolbox for more complex products

- Timeline: Sample preparation January 2017
- Data available End Q1 2017

- **With this last step – toolbox of methods to extract HP and detect them with different methods ready for Roll-out**

Application: Quality control of raw materials

- Define what next steps
- Michael
- To be discussed

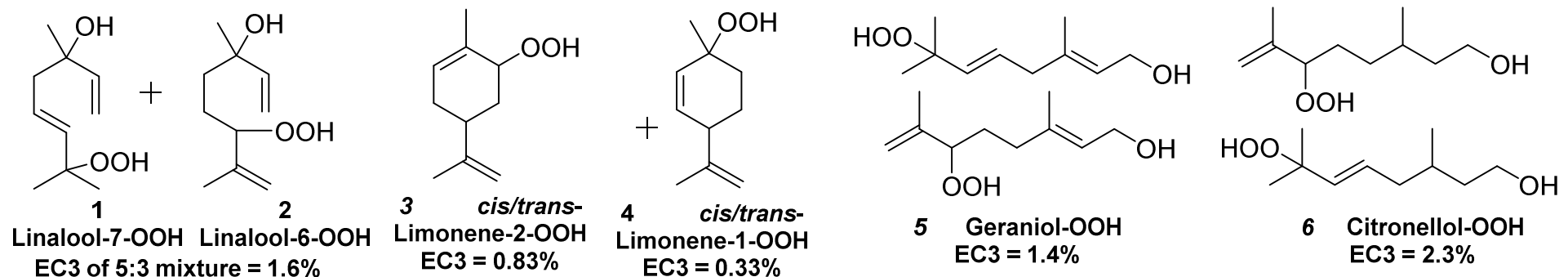
Application: Market overview and patient's products

- Application of toolbox:
- Market products from the shelf
- Aged consumer products
- Patient's samples
- Who will do analysis?
- Who will sample?

- To be discussed

Interpretation – how will we judge results? - input to QRA2

- We have good LLNA and guinea pig test data for hydroperoxides (or oxidized fractions with known hydroperoxide content)
- Based on these data we can derive NESIL values for individual HP
- Overall, potency in a similar range (EC 3 0.3 – 1.6 %)
- With a grouping / read-across approach also potency / NESIL of unknown HP can be predicted
- **Based on QRA2 we can then derive maximal levels in different product types which should not be surpassed**



Interpretation - input to QRA2: Case study

- 'All natural' deodorant (made of natural products only) was analyzed
- Contains 28 ppm Linalool-6-OOH and 27 ppm Linalool-7-OOH: Total 56 ppm
- EC3 for Linalool 6/7-OOH Mixture: 1.6% = 400 $\mu\text{g}/\text{cm}^2$
- NESIL 400 $\mu\text{g}/\text{cm}^2$

Linalool Hydroperoxides NESIL = 400 $\mu\text{g}/\text{cm}^2$			
Product Type	Proposed SAF for QRA 2	Exposure (mg/cm ² /day)	QRA2 product type upper use levels
Deodorants and antiperspirants of all types including fragranced body sprays	300	9.1	0.015% = 146 ppm

- The **analytical result** is below QRA2 level, and indicates the product is fine according QRA2
- Also the **analytical level** is 10 fold-below lowest reported elicitation level.

Expected outcome

- The analytical toolbox will be applied to market samples
- Results will indicate whether hydroperoxide levels are above QRA2 limits
- Results will indicate how frequent samples are which contain hydroperoxides above critical levels
- **Results should help to understand whether significant exposure to terpene hydroperoxides comes from IFRA regulated products**

Thank you

Contact

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