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Highly selective and sensitive 2D-LC/MS/MS for fragrance HP analysis

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Introduction

Accurate quantification of fragrance HPs in essential oils and commercial products is demanding due to:

- The low levels of HPs compared to other constituents in essential oils and commercial products.
- Analysis interference due to the complex matrix (other autoxidation products, compounds from the matrix)

One-dimensional HPLC usually does not yield sufficient separation selectivity, leading to matrix effects like ion suppression in the ESI-MS/MS analysis and low accuracy.

Either a selective clean-up or a separation with high peak capacity is needed prior to the MS detection, such as a two-dimensional chromatographic separation

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Two-dimensional LC: Principles

- **2D-LC increases the selectivity, expands peak capacity, especially when fully orthogonal retention mechanisms**
- **2D-LC gives better opportunities for separation from interfering oxidation products**
- **In 2D-LC, the sample is subjected to two different separation mechanisms, which are practically achieved by using two columns with different stationary phases.**
- **There are two modes of 2D-LC :**

- **Comprehensive 2D-LC (LCxLC):** The whole eluent of the 1st D column is transferred in several fractions to be analyzed by the 2nd D.
- **Heart-cut 2D-LC (LC-LC):** The fractions of interest are analyzed by the 2nd D.

Quantification of limonene and linalool HPs in perfumes

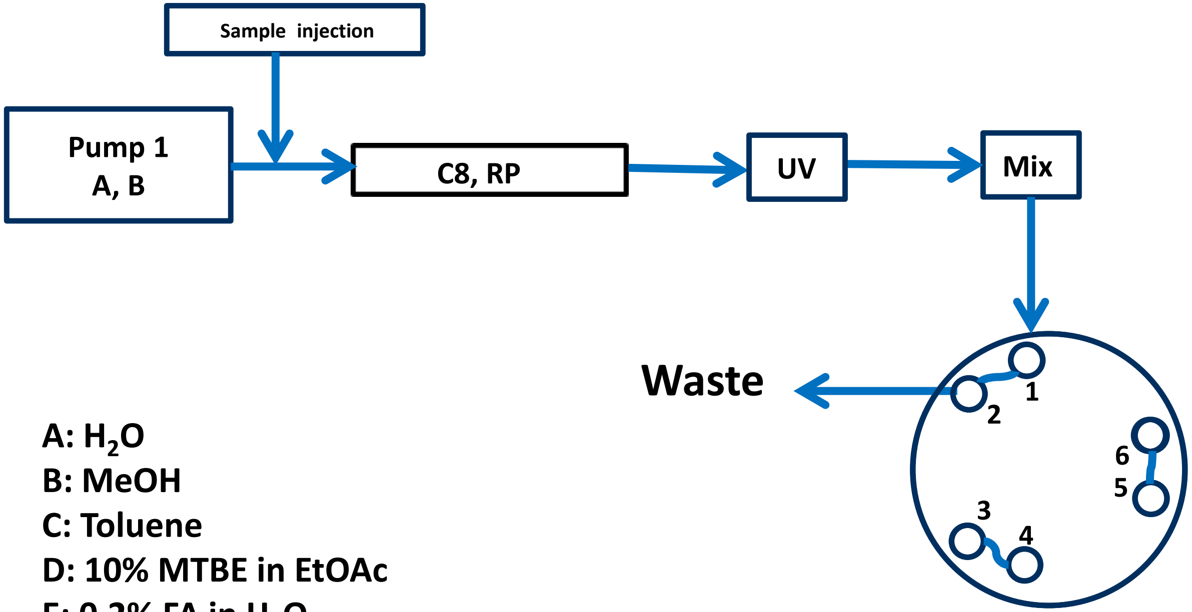
Heart-cut 2D-LC/MS/MS system was developed by a successful coupling of reversed phase in the 1st dimension with normal phase in the 2nd dimension, despite of solvents immiscibility.

A porous graphitized carbon (PGC) column trap was used for analyte focusing, solvent exchange and inter-system transfer

In order to couple normal phase to the electrospray ion source an assistant solvent was used to promote the ionization.

Two-dimensional LC/ESI-MS/MS

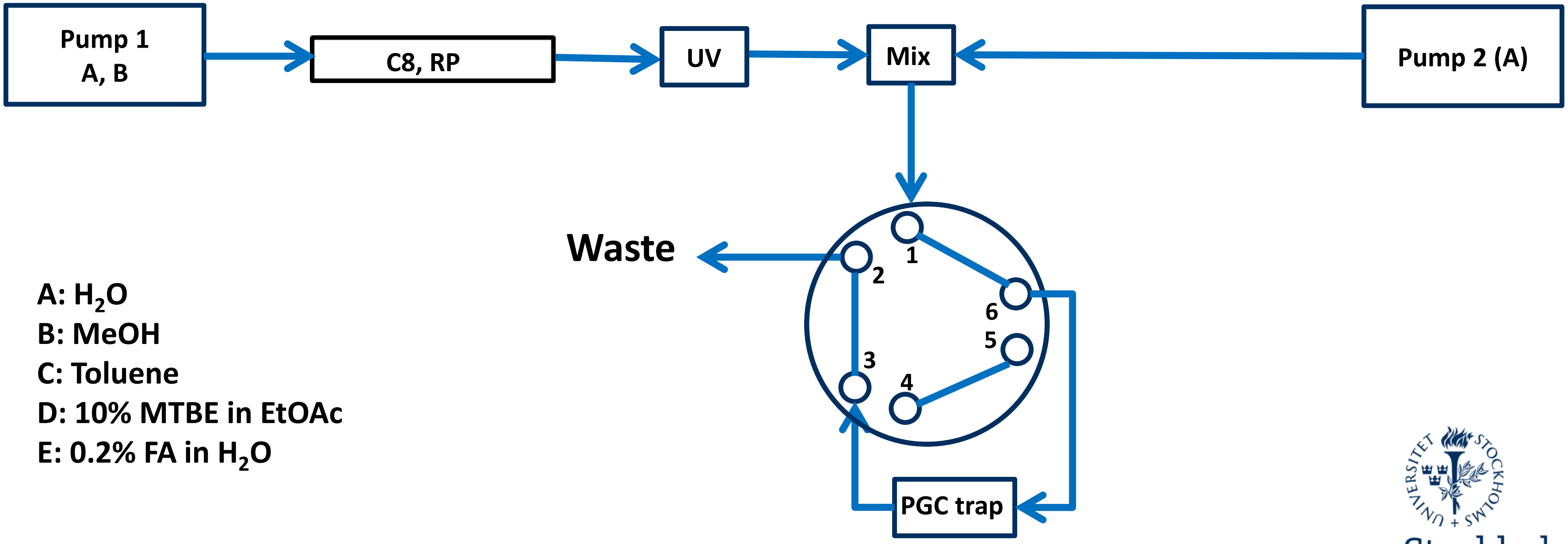
Separation in the first dimension (valve position 1-2)



A: H_2O
B: MeOH
C: Toluene
D: 10% MTBE in EtOAc
E: 0.2% FA in H_2O

Two-dimensional LC/ESI-MS/MS

Heart cutting and analyte trapping (valve position 1-6)

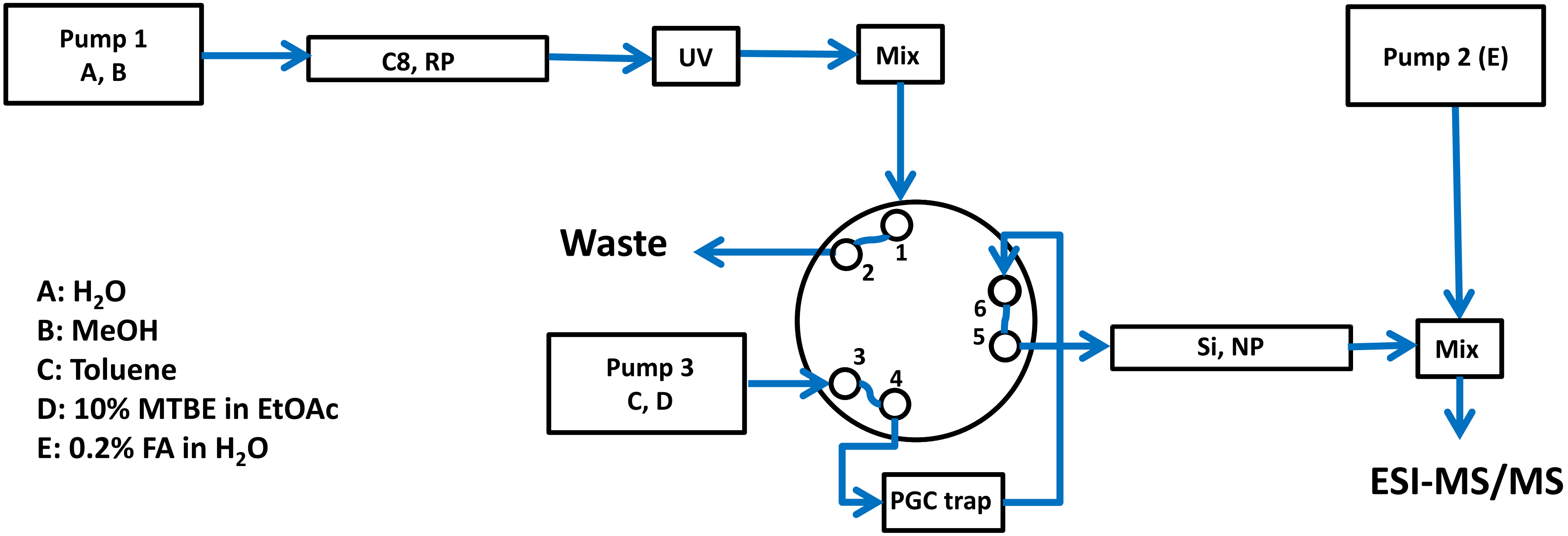


A: H_2O
B: MeOH
C: Toluene
D: 10% MTBE in EtOAc
E: 0.2% FA in H_2O

Two-dimensional LC/ESI-MS/MS

Solvent exchange, analyte transfer, and separation in the second dimension (1-2)

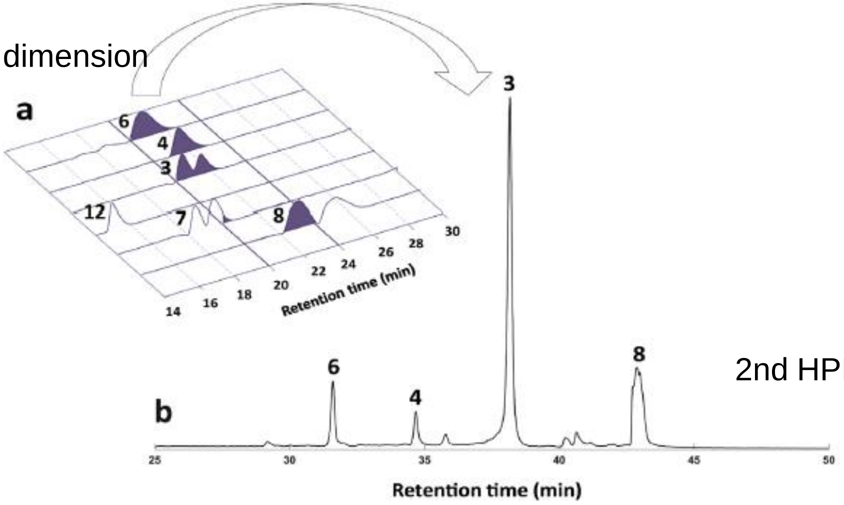
With use of the PGC trap, the immiscibility of mobile phases could be handled



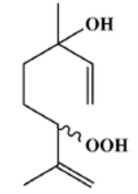
A: H_2O
B: MeOH
C: Toluene
D: 10% MTBE in EtOAc
E: 0.2% FA in H_2O

Two-dimensional HPLC/MS/MS efficiently removes interferences in the quantification

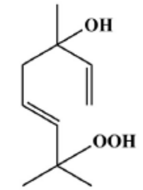
1st HPLC dimension



2nd HPLC dimension

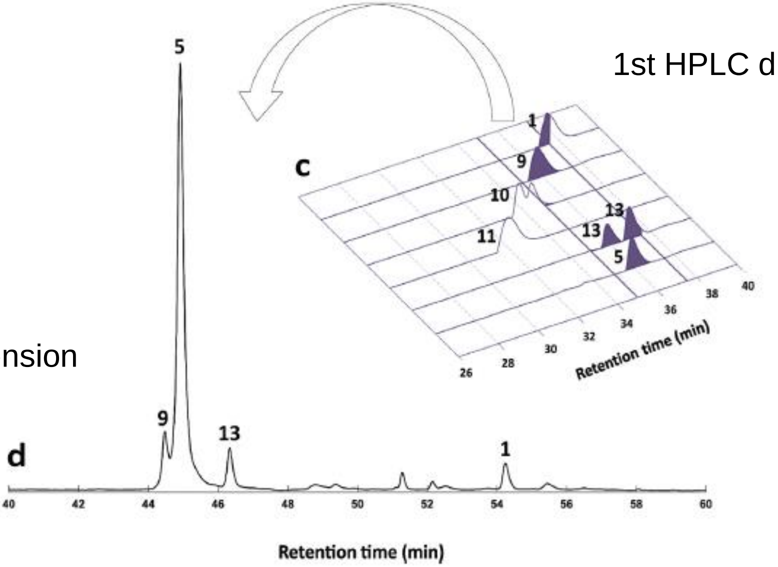


3. Lin-6-OOH, $C_{10}H_{18}O_3$

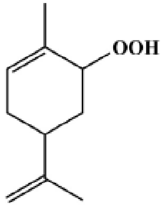


4. Lin-7-OOH, $C_{10}H_{18}O_3$

1st HPLC dimension



2nd HPLC dimension



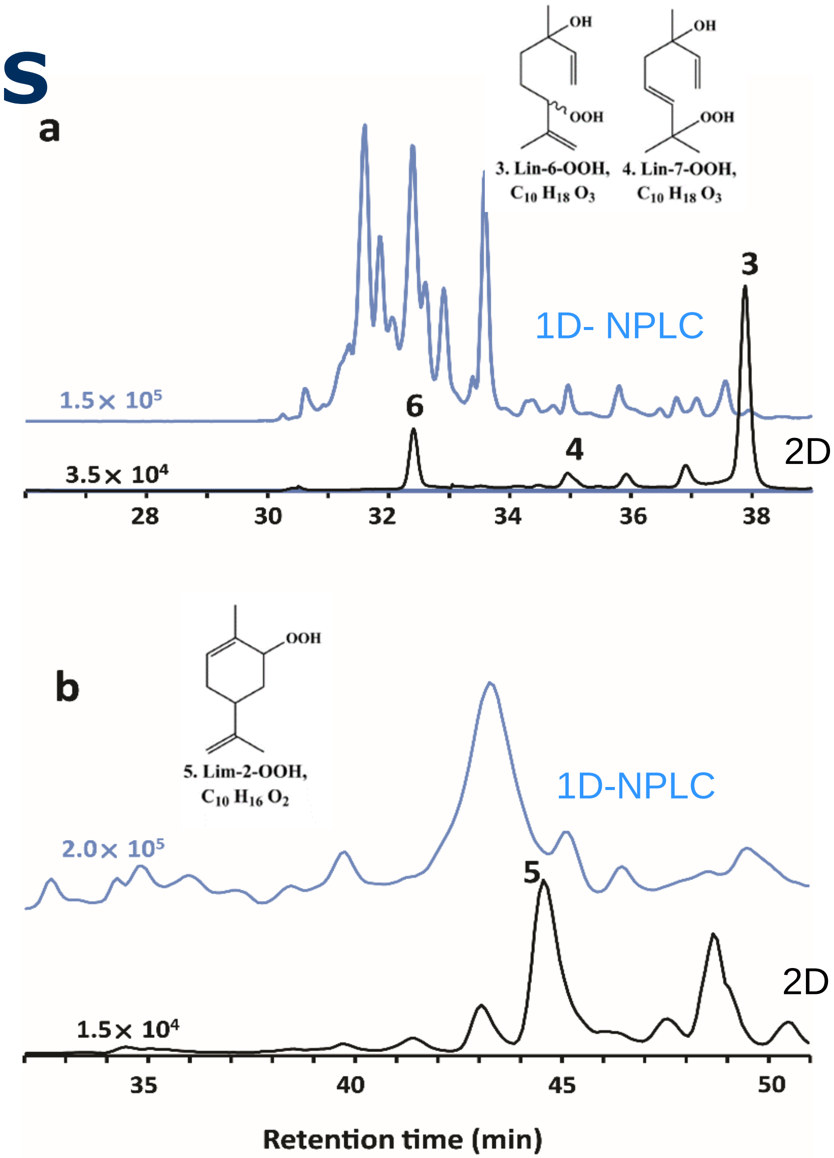
5. Lim-2-OOH, $C_{10}H_{16}O_2$

1. Linalool
2. Limonene
3. Lin-6-OOH
4. Lin-7-OOH
5. Lim-2-OOH
6. Cum-OOH (IS)
7. Linalool oxide, pyranoside
8. Linalool oxide, furanoid
9. Limonene oxide
10. L-carveol
11. L-Carvone
12. Limonene diol
13. Citral, isomers

Ramzi A, Ahmadi H, Sadiktsis I, Nilsson U. *J Chromatogr A* 2018 1566, 102-10.

Benefits of 2D-LC/MS/MS

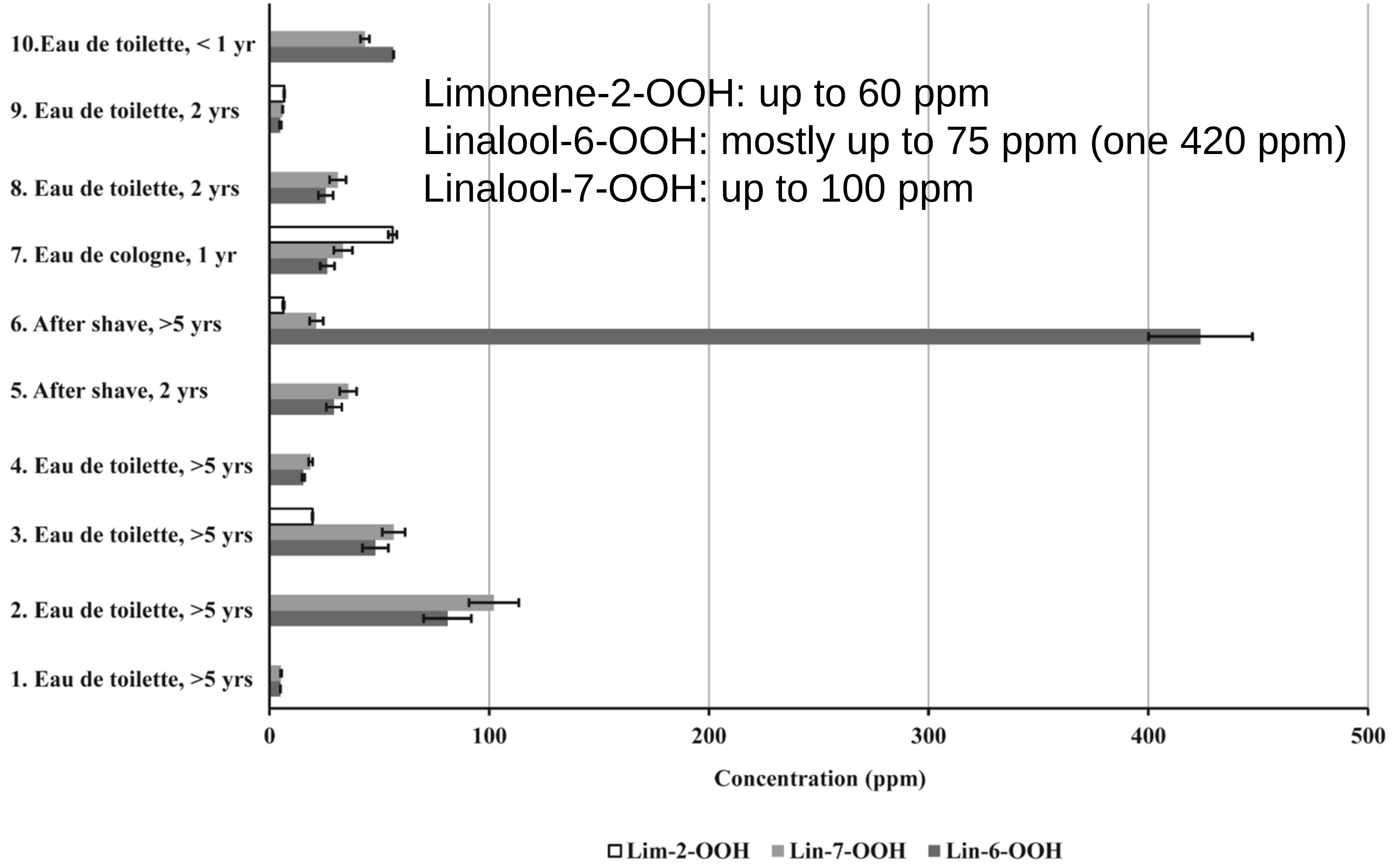
- Highly reproducible system (both responses and retention times), **minimal handling**
- Total time of analysis 60 min, “dilute and shoot”
- Limonene and linalool HPs were detected in all analyzed samples at a levels between 4 to 448 ppm
- The matrix effects in ESI were decreased (between +8% and -20%)
- MS/MS ion ratios in samples and spiked samples were reproducible and similar to in standard solutions (RSD < 9%)
- Lower limit of detection and quantification compared to previously developed methods



Some fragranced products

Analysis by two-dimensional LC/MS/MS

The compound identities confirmed by ion (m/z) ratios in the MS spectra
Relative standard deviations of ion ratios below 9%



Limonene-2-OOH: up to 60 ppm
Linalool-6-OOH: mostly up to 75 ppm (one 420 ppm)
Linalool-7-OOH: up to 100 ppm

Ramzi A, Ahmadi H, Sadiktsis I, Nilsson U. *J Chromatogr A* 2018 1566, 102-10.

Conclusions and future perspectives

- Efficient chromatographic (such as 2D-LC) separations are needed to ensure accurate quantification by minimizing interferences and matrix effects. There is also a **need for reference compounds and isotope-labeled internal standard compounds** in order to be validated.

- Our 2D-LC/MS/MS method is highly selective and sensitive for the HPs, even though it is so far evaluated ”only” for linalool HPs and limonene-2-HP, however, it can still be optimized for another HPs if the standard material is available.

- The 2D-LC/MS/MS system is possible to be transferred to other lab, as long as the modules and the software that can control all modules are available (3 binary pumps, divert valve, UV detector, autosampler, and the MS).

- In LC/MS/MS analysis we suggest that ion (m/z) ratios specific for each HP should be used to ensure a correct identification in such complex samples as commercial products

- The system was used for the determination of linalool hydroperoxides in a deodorant causing ACD (2019 Isaksson M et al)

- We suggest that also low levels of HPs should be quantified, such as <50 ppm, which might cause elicitation. Our 2D-LC/MS/MS method can detect as low as ~1 ppm

Thank You