

Detection of terpene hydroperoxides

Results of ring study and method comparison

11.5.2016



Givaudan

engage your senses

Agenda

- 1. Reminder: Original action standards**
- 2. Ring study setup**
- 3. Toxicological relevance of different isomers**
- 4. Reduction method: Ring trial results**
- 5. Alternative methods: Results ring trial**
- 6. Hemiacetal detection**
- 7. Conclusions ring trial**
- 8. Testing in alternative matrix: First results with reduction method (Givaudan)**
- 9. Alternative method proposed by Waters**

Reminder: Level of detection needed as defined in 2014

- *Target set: "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:
 - Linalool-OOH 16'000 ppm in LLNA [1]
 - Limonene-2-OOH 8300 ppm in LLNA / 15'000 ppm in guinea pigs [2,3]
 - **5000 ppm** maybe taken as a default induction level
- Estimated elicitation level:
 - Linalool: Lowest elicitation level in humans = **560 ppm** * [4]
 - No data on limonene in humans, Lowest elicitation level in guinea pigs = 3000 – 10'000 ppm [3]

* Patients in elicitation study were exposed simultaneously over three weeks twice daily to a 5640 ppm dose on same arm, LOEL for elicitation may thus be clearly higher under realistic application of single low dose.
- Note: Above levels are final levels in a preparation. If a fragrance oil or raw material is considered, dilution factor in product must be taken into account

[1] M. Sköld, A. Börje, E. Harambasic, A. T. Karlberg, *Chemical Research in Toxicology* **2004**, *17*, 1697-1705.

[2] S. Johansson, E. Gimenez-Arnau, M. Grotli, A. T. Karlberg, A. Börje, *Chem Res Toxicol* **2008**, *21*, 1536-47.

[3] A. T. Karlberg, L. P. Shao, U. Nilsson, E. Gafvert, J. L. Nilsson, *Arch Dermatol Res* **1994**, *286*, 97-103.

[4] Y. Andersch Bjorkman, L. Hagvall, C. Siwmark, B. Niklasson, A.T. Karlberg, J. Brared Christensson, *Contact Dermatitis* **2014**, *70*, 129-38.

Set-up of Ring study in fine fragrance

- During the discussions at the Prehaptent-workshops, suspicion was raised that even lower levels of Hydroperoxides could be critical
- If we would come up with methods with LOD of 500 ppm, our results in products would be reported as < 500 ppm
- **500 ppm would then be viewed as default value contained in products**
- **Hence we decided to test lower levels in Ring trial**

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

Reminder: Toxicological relevance of different isomers

- Limonene-1-OOH:
 - Minor isomer (1%) generated in autoxidation of limonene, stronger sensitizer (EC3 0.33%)
- Limonene-2-OOH
 - Main isomer (6%) generated upon autoxidation, weaker sensitizer (EC3 0.83%)
- Linalool-6-OOH:
 - Minor isomer generated upon autoxidation, 40% in classical synthetic preparations
- Linalool-7-OOH:
 - Major isomer generated upon autoxidation, 60% in classical synthetic preparations
- Linalool toxicological information: LLNA on synthetic 3:5 mixture; patch tests on oxidized linalool (Linalool-7-OOH more prominent)

⇒ For the isomers of limonene we have differentiated toxicological information

- **Contents of these isomers can be related to toxicological information**

⇒ For the isomers of Linalool-OOH we have only toxicological information for the mixtures (currently)

- **So any analytical results on the real products shall be on the sum of isomers, and the sum of isomers can be related to the tox information (currently)**

Redution method: Key results

- On average, very accurate determination of the spike levels added by Greenpharma!
- Most z-scores < 1 or close to 1 indicating high interlab precision
- Highest variation seen for unspiked samples – here we are close to experimental noise as levels in unspiked perfumes are very low (as also confirmed by the alternative methods; see below)
- Somewhat higher variance for Limonene-1-OOH and Linalool-6-OOH vs. Limonene-2-OOH and Linalool-7-OOH, **but the latter are also the main isomers formed by autoxidation!**
 - **Hence we can most accurately determine the quantitatively most isomers**
- The standard addition experiments also indicate high recovery of spikes performed within the labs

Reduction method: Results ring trial – Limonene-1-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	25.8	61.0	193.5	11.6	55.8	166.5
FIRMENICH GC/MS	3.5	32.1	144.2	0.0	29.8	115.4
IFF-FRANCE GC/MS	4.7	35.9	156.8	8.1	33.9	111.2
CHANEL GC/MS	16.3	49.3	164.4	0.0	57.4	166.7
DSM GC/MS	6.3	49.8	188.2	119.5	161.6	262.1
Average	11.3	45.6	169.4	4.9	44.2	140.0
Standard deviation	9.6	11.7	20.9	5.8	14.4	30.8
RSD (%)	84.6	25.6	12.3	119.1	32.5	22.0
spike level detected		34.3	158.1		39.3	135.1
spike level added		26.0	140.0		32.0	128.0
	Outlier, not included in calculation of average and standard deviation					
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	1.5	1.3	1.1	1.1	0.8	0.9
FIRMENICH GC/MS	-0.8	-1.2	-1.2	-0.8	-1.0	-0.8
IFF-FRANCE GC/MS	-0.7	-0.8	-0.6	0.5	-0.7	-0.9
CHANEL GC/MS	0.5	0.3	-0.2	-0.8	0.9	0.9
DSM GC/MS	-0.5	0.4	0.9	19.6	8.2	4.0

Reduction method: Results ring trial – Limonene-2-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	9.3	32.8	140.5	7.7	33.2	128.7
FIRMENICH GC/MS	7.8	29.1	138.8	0.0	29.7	129.0
IFF-FRANCE GC/MS	3.0	26.6	135.8	0.0	24.3	116.4
CHANEL GC/MS	10.9	31.8	125.9	8.8	33.4	122.1
DSM GC/MS	2.0	39.5	154.6	0.3	39.5	141.5
Average	6.6	32.0	139.1	3.3	32.0	127.5
Standard deviation	3.9	4.9	10.3	4.5	5.6	9.4
RSD (%)	59.3	15.2	7.4	134.1	17.4	7.4
spike level detected		25.4	132.5		28.7	124.2
spike level added		28.0	154.0		32.0	142.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.7	0.2	0.1	1.0	0.2	0.1
FIRMENICH GC/MS	0.3	-0.6	0.0	-0.7	-0.4	0.2
IFF-FRANCE GC/MS	-0.9	-1.1	-0.3	-0.7	-1.4	-1.2
CHANEL GC/MS	1.1	0.0	-1.3	1.2	0.2	-0.6
DSM GC/MS	-1.2	1.6	1.5	-0.7	1.3	1.5

Reduction method: Results ring trial – Linalool-6-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.0	26.8	147.2	0.0	28.9	111.8
FIRMENICH GC/MS	0.0	34.5	134.7	0.0	33.7	98.5
IFF-FRANCE GC/MS	7.9	42.4	147.5	8.4	39.7	107.9
CHANEL GC/MS	0.0	49.1	142.9	0.0	45.3	109.3
DSM GC/MS	0.0	49.2	192.0	24.9	57.5	157.2
Average	1.6	40.4	152.9	6.7	41.0	116.9
Standard deviation	3.5	9.7	22.5	10.8	11.1	23.0
RSD (%)	223.6	24.0	14.7	162.6	27.0	19.7
spike level detected		38.8	151.3		34.3	110.3
spike level added		42.0	152.0		40.0	110.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	-0.4	-1.4	-0.3	-0.6	-1.1	-0.2
FIRMENICH GC/MS	-0.4	-0.6	-0.8	-0.6	-0.7	-0.8
IFF-FRANCE GC/MS	1.8	0.2	-0.2	0.2	-0.1	-0.4
CHANEL GC/MS	-0.4	0.9	-0.4	-0.6	0.4	-0.3
DSM GC/MS	-0.4	0.9	1.7	1.7	1.5	1.7

Reduction method: Results ring trial – Linalool-7-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	6.5	46.7	161.2	6.2	29.5	114.6
FIRMENICH GC/MS	4.3	39.6	158.5	0.0	28.2	115.5
IFF-FRANCE GC/MS	1.6	49.3	187.3	1.6	30.1	129.0
CHANEL GC/MS	9.4	50.8	156.2	9.4	34.0	116.4
DSM GC/MS	0.9	54.8	199.5	0.6	33.4	142.8
Average	4.5	48.2	172.5	3.6	31.0	123.7
Standard deviation	3.5	5.6	19.6	4.1	2.5	12.2
RSD (%)	77.4	11.7	11.4	114.3	8.1	9.9
spike level detected		43.7	168.0		27.4	120.1
spike level added		50.0	170.0		30.0	114.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.6	-0.3	-0.6	0.6	-0.6	-0.7
FIRMENICH GC/MS	-0.1	-1.5	-0.7	-0.9	-1.1	-0.7
IFF-FRANCE GC/MS	-0.8	0.2	0.8	-0.5	-0.4	0.4
CHANEL GC/MS	1.4	0.5	-0.8	1.4	1.2	-0.6
DSM GC/MS	-1.1	1.2	1.4	-0.7	0.9	1.6

Reduction method: Results ring trial – Sum Linalool-OOH isomers

	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	6.5	73.6	308.4	6.2	58.4	226.4
FIRMENICH GC/MS	4.3	74.0	293.1	0.0	61.9	214.0
IFF-FRANCE GC/MS	9.5	91.7	334.8	10.0	69.7	237.0
CHANEL GC/MS	9.4	99.9	299.1	9.4	79.3	225.6
DSM GC/MS	0.9	104.0	391.5	25.5	90.8	300.0
Average	6.1	88.6	325.4	10.2	72.0	240.6
Standard deviation	3.7	14.2	40.2	9.4	13.2	34.2
RSD (%)	59.7	16.1	12.4	92.1	18.4	14.2
spike level detected		82.5	319.3		61.8	230.3
spike level added		92.0	322.0		70.0	224.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.1	-1.1	-0.4	-0.4	-1.0	-0.4
FIRMENICH GC/MS	-0.5	-1.0	-0.8	-1.1	-0.8	-0.8
IFF-FRANCE GC/MS	0.9	0.2	0.2	0.0	-0.2	-0.1
CHANEL GC/MS	0.9	0.8	-0.7	-0.1	0.5	-0.4
DSM GC/MS	-1.4	1.1	1.6	1.6	1.4	1.7

Reduction method: Results ring trial – Standard addition precision– Limonene-1-OOH

- Given is the % recovery of a 10 ppm standard addition to the analytical samples.
- In most cases recovery close to 100% is observed, indicating no significant quenching

	Eau de toilette - no spike	Eau de toilette - low spike	Eau de toilette - high spike	Eau de parfum - no spike	Eau de parfum - low spike	Eau de parfum - high spike
GIVAUDAN	94	107	102	121	105	97
FIRMENICH	74	86	102	91	76	97
IFF-FRANCE	88	106	117	61	67	93
CHANEL	104	87	78	129	105	82
DSM	136			98		
Average	99	97	100	100	88	93
Standard deviation	23	12	16	27	20	7

Reduction method: Results ring trial – Standard addition precision– Limonene-2-OOH

	Eau de toilette - no spike	Eau de toilette - low spike	Eau de toilette - high spike	Eau de parfum - no spike	Eau de parfum - low spike	Eau de parfum - high spike
GIVAUDAN	90	87	87	90	87	91
FIRMENICH	87	91	103	93	85	85
IFF-FRANCE	95	109	109	88	96	110
CHANEL	95	80	74	87	90	76
DSM	96			91		
Average	93	92	93	89	90	90
Standard deviation	4	12	16	2	5	15

Reduction method: Results ring trial – Standard addition precision– Linalool-6-OOH

	Eau de toilette - no spike	Eau de toilette - low spike	Eau de toilette - high spike	Eau de parfum - no spike	Eau de parfum - low spike	Eau de parfum - high spike
GIVAUDAN	64	88	123	92	101	92
FIRMENICH	84	83	99	90	83	94
IFF-FRANCE	83	103	117	93	105	110
CHANEL	131	82	76	114	89	78
DSM	120			79		
Average	96	89	104	94	94	94
Standard deviation	28	9	21	13	11	13

Reduction method: Results ring trial – Standard addition precision – Linalool-7-OOH

	Eau de toilette - no spike	Eau de toilette - low spike	Eau de toilette - high spike	Eau de parfum - no spike	Eau de parfum - low spike	Eau de parfum - high spike
GIVAUDAN	89	90	97	93	91	93
FIRMENICH	88	99	118	99	87	92
IFF-FRANCE	85	109	102	90	101	105
CHANEL	101	83	74	91	94	78
DSM	112			112		
Average	95	95	98	97	94	92
Standard deviation	11	11	18	9	6	11

Results with alternative methods included

- The 'methods comparison' part of the study is a separate aspects to the 'interlab reproducibility' aspect – below data still directly compared
- The IFF -LC-Q-TOF MS and the Firmenich HPLC-MHC gave reliable data for all four isomers, very promising methods
- The Givaudan HR-MS works for the sum of the linalool-isomers but is less reliable for the Limonene isomers for mass spectrometric reasons, as observed before

Results with alternative methods included: Limonene-1-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	25.8	61.0	193.5	11.6	55.8	166.5
FIRMENICH GC/MS	3.5	32.1	144.2	0.0	29.8	115.4
IFF-FRANCE GC/MS	4.7	35.9	156.8	8.1	33.9	111.2
CHANEL GC/MS	16.3	49.3	164.4	0.0	57.4	166.7
DSM GC/MS	6.3	49.8	188.2	119.5	161.6	262.1
IFF -LC-Q-TOF MS	0.0	40.0	141.0	0.0	42.0	101.0
Givaudan HR-MS	0.0	41.0	206.6	0.0	12.1	112.1
Firmenich HPLC-MHC	4.1	37.8	183.1	0.0	37.0	158.4
Average	7.6	43.4	172.2	2.8	38.3	133.0
Standard deviation	9.0	9.4	24.1	4.9	15.6	29.3
RSD (%)	118.4	21.7	14.0	174.6	40.8	22.0
spike level detected		35.8	164.7		35.5	130.2
spike level added		26.0	140.0		32.0	128.0
	Outlier, not included in calculation of average and standard deviation					
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	2.0	1.9	0.9	1.8	1.1	1.1
FIRMENICH GC/MS	-0.5	-1.2	-1.2	-0.6	-0.5	-0.6
IFF-FRANCE GC/MS	-0.3	-0.8	-0.6	1.1	-0.3	-0.7
CHANEL GC/MS	1.0	0.6	-0.3	-0.6	1.2	1.1
DSM GC/MS	-0.1	0.7	0.7	23.8	7.9	4.4
IFF -LC-Q-TOF MS	-0.8	-0.4	-1.3	-0.6	0.2	-1.1
Givaudan HR-MS	-0.8	-0.3	1.4	-0.6	-1.7	-0.7
Firmenich HPLC-MHC	-0.4	-0.6	0.4	-0.6	-0.1	0.9

Results with alternative methods included: Limonene-2-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	9.3	32.8	140.5	7.7	33.2	128.7
FIRMENICH GC/MS	7.8	29.1	138.8	0.0	29.7	129.0
IFF-FRANCE GC/MS	3.0	26.6	135.8	0.0	24.3	116.4
CHANEL GC/MS	10.9	31.8	125.9	8.8	33.4	122.1
DSM GC/MS	2.0	39.5	154.6	0.3	39.5	141.5
IFF -LC-Q-TOF MS	0.0	35.0	132.0	0.0	27.0	111.0
Givaudan HR-MS	0.0	29.3	77.6	0.0	16.3	60.1
Firmenich HPLC-MHC	4.8	28.3	171.9	0.0	28.5	144.9
Average	4.7	31.5	134.6	2.1	29.0	119.2
Standard deviation	4.2	4.2	27.2	3.8	6.9	26.5
RSD (%)	88.9	13.3	20.2	182.1	23.8	22.2
spike level detected		26.8	129.9		26.9	117.1
spike level added		28.0	154.0		32.0	142.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	1.1	0.3	0.2	1.5	0.6	0.4
FIRMENICH GC/MS	0.7	-0.6	0.2	-0.5	0.1	0.4
IFF-FRANCE GC/MS	-0.4	-1.2	0.0	-0.5	-0.7	-0.1
CHANEL GC/MS	1.5	0.1	-0.3	1.8	0.6	0.1
DSM GC/MS	-0.6	1.9	0.7	-0.5	1.5	0.8
IFF -LC-Q-TOF MS	-1.1	0.8	-0.1	-0.5	-0.3	-0.3
Givaudan HR-MS	-1.1	-0.5	-2.1	-0.5	-1.8	-2.2
Firmenich HPLC-MHC	0.0	-0.8	1.4	-0.5	-0.1	1.0

Results with alternative methods included: Linalool-6-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.0	26.8	147.2	0.0	28.9	111.8
FIRMENICH GC/MS	0.0	34.5	134.7	0.0	33.7	98.5
IFF-FRANCE GC/MS	7.9	42.4	147.5	8.4	39.7	107.9
CHANEL GC/MS	0.0	49.1	142.9	0.0	45.3	109.3
DSM GC/MS	0.0	49.2	192.0	24.9	57.5	157.2
IFF -LC-Q-TOF MS	0.0	37.0	133.0	0.0	32.0	95.0
Firmenich HPLC-MHC	0.0	35.0	142.2	0.0	29.3	99.2
Average	1.1	39.2	148.5	4.8	38.0	111.3
Standard deviation	3.0	8.2	20.0	9.4	10.4	21.2
RSD (%)	264.6	21.0	13.5	198.0	27.3	19.0
spike level detected		38.0	147.4		33.3	106.5
spike level added		42.0	152.0		40.0	110.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	-0.4	-1.5	-0.1	-0.5	-0.9	0.0
FIRMENICH GC/MS	-0.4	-0.6	-0.7	-0.5	-0.4	-0.6
IFF-FRANCE GC/MS	2.3	0.4	-0.1	0.4	0.2	-0.2
CHANEL GC/MS	-0.4	1.2	-0.3	-0.5	0.7	-0.1
DSM GC/MS	-0.4	1.2	2.2	2.1	1.9	2.2
IFF -LC-Q-TOF MS	-0.4	-0.3	-0.8	-0.5	-0.6	-0.8
Firmenich HPLC-MHC	-0.4	-0.5	-0.3	-0.5	-0.8	-0.6

Results with alternative methods included: Linalool-7-OOH

	<i>All results in mg/l</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	6.5	46.7	161.2	6.2	29.5	114.6
FIRMENICH GC/MS	4.3	39.6	158.5	0.0	28.2	115.5
IFF-FRANCE GC/MS	1.6	49.3	187.3	1.6	30.1	129.0
CHANEL GC/MS	9.4	50.8	156.2	9.4	34.0	116.4
DSM GC/MS	0.9	54.8	199.5	0.6	33.4	142.8
IFF -LC-Q-TOF MS	0.0	53.0	146.0	0.0	27.0	105.0
Firmenich HPLC-MHC	0.0	44.5	168.5	0.0	26.9	104.5
Average	3.2	48.4	168.2	2.5	29.9	118.3
Standard deviation	3.6	5.2	18.8	3.8	2.8	13.6
RSD (%)	111.7	10.8	11.2	147.5	9.5	11.5
spike level detected		45.1	164.9		27.3	115.7
spike level added		50.0	170.0		30.0	114.0
	<i>All results expressed as z-score</i>					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.9	-0.3	-0.4	1.0	-0.1	-0.3
FIRMENICH GC/MS	0.3	-1.7	-0.5	-0.7	-0.6	-0.2
IFF-FRANCE GC/MS	-0.4	0.2	1.0	-0.2	0.1	0.8
CHANEL GC/MS	1.7	0.5	-0.6	1.8	1.4	-0.1
DSM GC/MS	-0.7	1.2	1.7	-0.5	1.2	1.8
IFF -LC-Q-TOF MS	-0.9	0.9	-1.2	-0.7	-1.0	-1.0
Firmenich HPLC-MHC	-0.9	-0.7	0.0	-0.7	-1.0	-1.0

Results with alternative methods included: Sum Linalool isomers

	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	6.5	73.6	308.4	6.2	58.4	226.4
FIRMENICH GC/MS	4.3	74.0	293.1	0.0	61.9	214.0
IFF-FRANCE GC/MS	9.5	91.7	334.8	10.0	69.7	237.0
CHANEL GC/MS	9.4	99.9	299.1	9.4	79.3	225.6
DSM GC/MS	0.9	104.0	391.5	25.5	90.8	300.0
IFF -LC-Q-TOF MS	0.0	90.0	279.0	0.0	59.0	200.0
Firmenich HPLC-MHC	0.0	79.5	310.7	0.0	56.2	203.7
Givaudan HR-MS	0.2	95.7	398.7	0.0	29.1	185.4
Average	3.8	88.5	326.9	6.4	63.0	224.0
Standard deviation	4.2	11.6	45.0	8.9	18.2	34.9
RSD (%)	108.5	13.1	13.8	138.7	28.9	15.6
spike level detected		84.7	323.1		56.7	217.6
spike level added		92.0	322.0		70.0	224.0
	All results expressed as z-score					
	EdT No Spike	EdT Low Spike	EdT High Spike	EdP No Spike	EdP Low Spike	EdP High Spike
GIVAUDAN GC/MS	0.6	-1.3	-0.4	0.0	-0.3	0.1
FIRMENICH GC/MS	0.1	-1.2	-0.7	-0.7	-0.1	-0.3
IFF-FRANCE GC/MS	1.4	0.3	0.2	0.4	0.4	0.4
CHANEL GC/MS	1.3	1.0	-0.6	0.3	0.9	0.0
DSM GC/MS	-0.7	1.3	1.4	2.2	1.5	2.2
IFF -LC-Q-TOF MS	-0.9	0.1	-1.1	-0.7	-0.2	-0.7
Firmenich HPLC-MHC	-0.9	-0.8	-0.4	-0.7	-0.4	-0.6
Givaudan HR-MS	-0.9	0.6	1.6	-0.7	-1.9	-1.1

Ring trial: Level in unspiked perfume – different methods

- The two perfumes selected are commercial perfumes

⇒ **Did they contain relevant quantities of the hydroperoxides?**

- **Level Limonene isomers:**

- < 5 ppm by LC-MS methods, ca. 4 ppm by HPLC-MHC
- 3 – 25 ppm (Lim-1-OOH) and 2 – 11 ppm (Lim-2-OOH) for reduction method
- Reduction method also includes preformed alcohol!
- Content of Limonene: 4100 ppm (eau de toilette) 800 ppm (eau de parfum)
- **WoE: ≤ 1% of limonene present in hydroperoxide form at time of analysis**

- **Level Linalool isomers:**

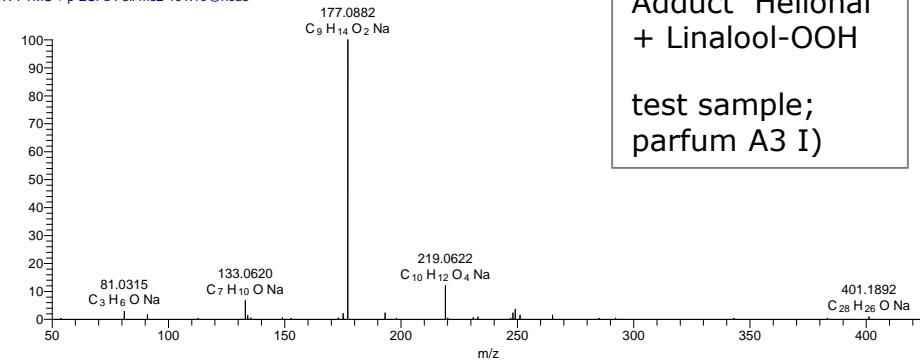
- < 5 ppm by HPLC-MHC and LC-Q-TOF; 0.2 ppm by HR-LC-MS
- 1 – 9 ppm for reduction method
- Reduction method also includes preformed alcohol!
- Content of Linalool: 6000 ppm (eau de toilette) 700 ppm (eau de parfum)
- **WoE: ≤ 1% of linalool present in hydroperoxide form at time of analysis**

- **Include data on other secondary oxidation products!**

Hemiacetal formation

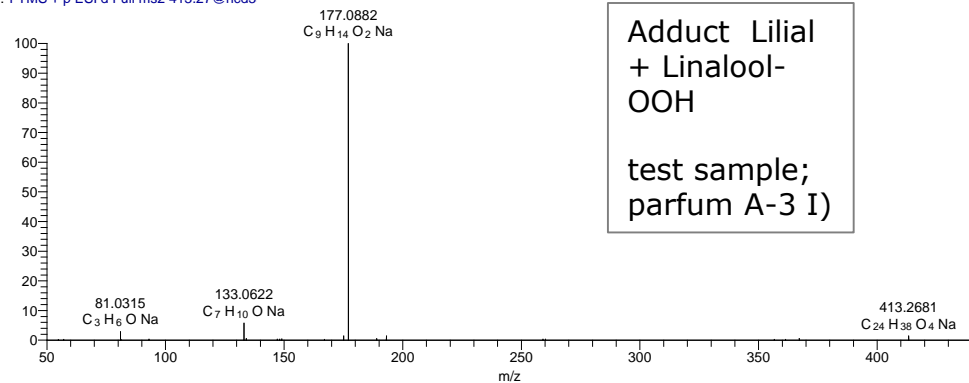
- The quantitatively most dominant aldehydes in both perfumes are Lilial and Helional
 - Lilial: 2.4% in EdT, 8.2% in EdP; Helional: 3.1% in EdT, 4.8% in EdP
- We made a specific search for the hemiacetal – adducts with Linalool ($[M+Na]^+ = 413.268$ for Lilial and 401-189 for Helional)
- MS² to confirm that the peak indeed are the Hemiacetal-adducts

L15328 #5367 RT: 13.08 AV: 1 NL: 1.83E6
T: FTMS + p ESI d Full ms2 401.19@hcd3



Adduct Helional
+ Linalool-OOH
test sample;
parfum A3 I)

L15328 #6178 RT: 14.70 AV: 1 NL: 1.12E6
T: FTMS + p ESI d Full ms2 413.27@hcd3



Adduct Lilial
+ Linalool-
OOH
test sample;
parfum A-3 I)

Hemiacetal formation

- Quantification made based on the calibration curve for Linalool-OOH
 - Initial data show that response factor of the hemiacetal is 5-times higher: Hence actual levels are probably 5-times lower as compared to below levels
- No or trace level in unspiked perfumes
- Spiked perfumes show some hemiacetal formation
 - < 1% of the hydroperoxide is in hemiacetal form
 - Caveat: How much is cleaved upon dilution / sample prep.? But as sample prep is in 80% ethanol as fine fragrance effect should be neglectable
- Based on this **very limited** data we can neglect them from toxicological point of view

Adduct Lilial + Linalool-OOH			Adduct Helional + Linalool-OOH		
	no standard addition	with standard addition		no standard addition	with standard addition
	ppm	ppm		ppm	ppm
Eau de toilette - no spike	NF	0.7	Eau de toilette - no spike	0.03	0.9
Eau de toilette - low spike	1.0	1.4	Eau de toilette - low spike	1.8	2.8
Eau de toilette - high spike	3.0	3.2	Eau de toilette - high spike	6.3	6.8
Eau de parfum - no spike	NF	NF	Eau de parfum - no spike	0.04	0.4
Eau de parfum - low spike	0.4	1.0	Eau de parfum - low spike	0.5	0.9
Eau de parfum - high spike	1.3	1.8	Eau de parfum - high spike	1.7	2.1

Key conclusions ring trial (prior to discussion at TF meeting)

Method validation part

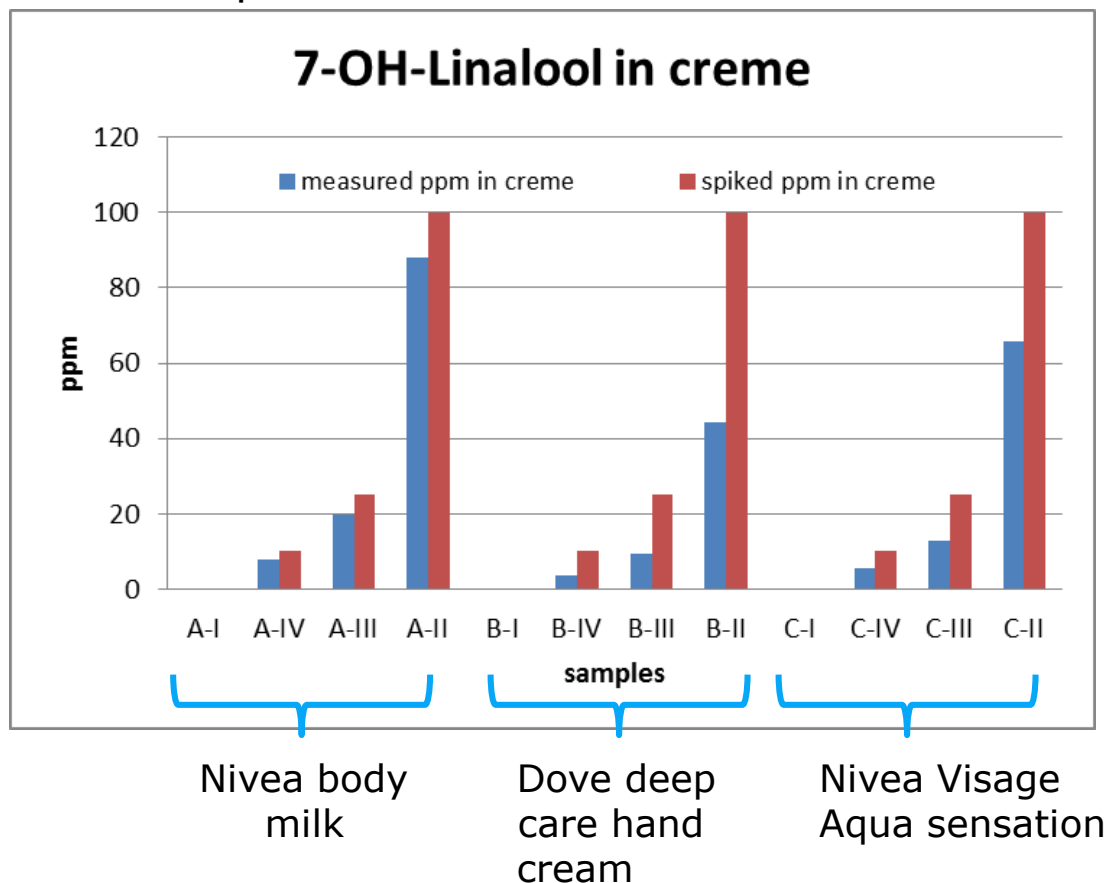
- The reduction method is a stable method which is transferable to different labs
- Allows quantification down to 50 ppm in final perfumes, much higher sensitivity than initially set target
- May pickup some background which can come from analytical noise and/or background levels of reduced alcohols present in perfume

Method comparison part

- IFF -LC-Q-TOF MS and the Firmenich HPLC-MHC are two new methods with high resolution for different isomeres and very good quantification
- Givaudan HR-MS remains a method to detect the linalool-OOH.
 - Separation method from the other methods could be implemented if Linalool-6- and-7 isomers need to be separated.
 - Method can specifically detect the hemiacetals formed
- Advantage of these methods is that the hydroperoxides are directly measured
- Disadvantage is that more specific equipment is needed

Testing in alternative matrix: First results with reduction method (Givaudan)

- Reduction by triphenylphosphine works in complex cosmetic products
- Extraction of 7-OH-Linalool into hexane/MTBE followed by counterextraction into MeOH/water is possible
- Produces clean GC/MS samples without lipid load from creams
- 40% - 80% of recovery of spike
- 10 ppm spiked linalool-7-OOH reliably positively identified
- **In combination with standard addition experiments, presence in creams and lotions could be shown or disproved**



Samples were sent to Waters, who proposed to have a new method; this goes without costs and indicates whether there is an additional method available

Hydroperoxides (initial results)



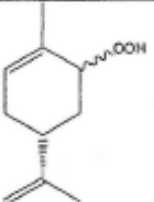
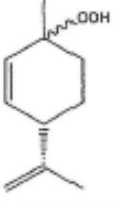
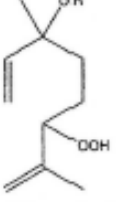
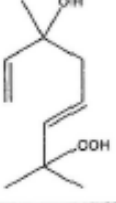
Acquity UPC² – Xevo TQD

**May 2016
Jane Cooper**



Sample Information

- Four standards were supplied:

Ref	Structure	MW
GPS008631 Batch Q8645861		168.23 g/mol
GPS008632 Batch R6383543		168.23 g/mol
GPS008633 Batch Q8185967		186.25 g/mol
GPS008634 Batch Q6691753		186.25 g/mol

Limonene-2-OOH

Limonene-1-OOH

Linalool-6-OOH

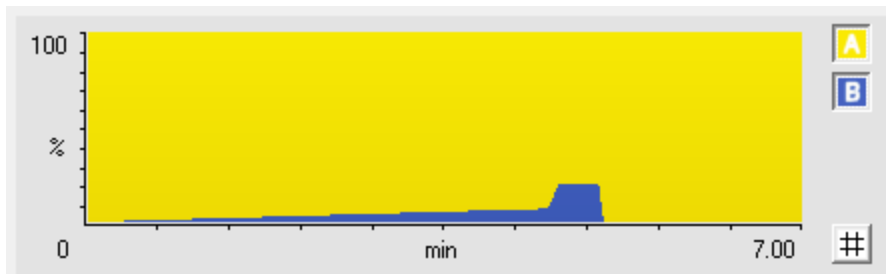
Linalool-7-OOH

Method Conditions – UPC²

System: ACQUITY UPC²
Column: ACQUITY UPC² C18 HSS, 3.0 mm x 150 mm, 1.8 μm
Mobile Phases: A: CO₂
B: Methanol (0.1% formic acid)

Gradient:

Time (min)	% CO ₂	% B
0.00	99.5	0.5
4.50	92.5	7.5
4.60	80.0	20.0
5.00	80.0	20.0
5.05	99.5	0.5
7.00	99.5	0.5



Flow Rate: 1.5 mL/min
UPC² Manager: 1500 psi
Column Temp.: 60 °C
Injection Volume: 3.0 μL

ISM solvent: methanol
ISM manager flow rate: 0.4 mL/min

ISM = Isocratic solvent manger

Method Information: MS

MS system: Xevo TQD
Ionization mode: APCI (positive)
Corona voltage : 10 μ A
Source temp.: 150 °C
APCI Probe temp.: 600 °C
Desolvation gas: 1000 L/hr
Cone gas: 15 L/hr
Acquisition: Multiple Reaction Monitoring (MRM)

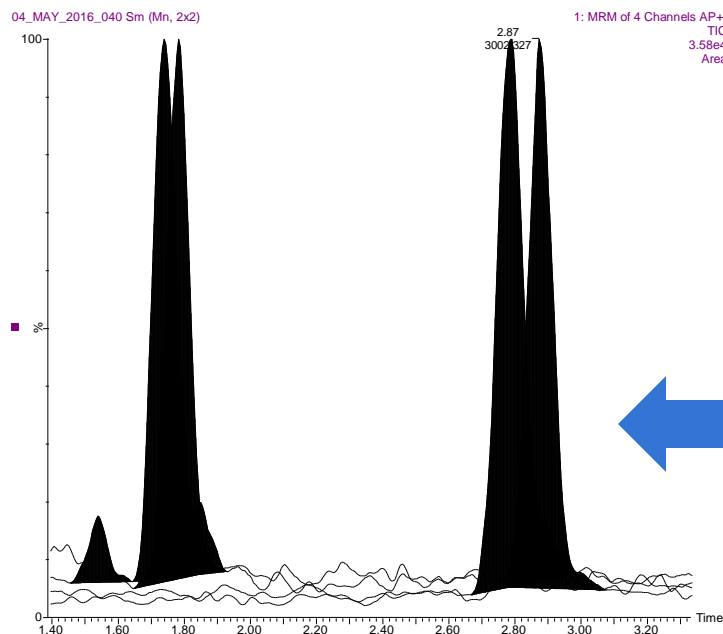
MRM conditions:

No	Chemical Substance	APCI (+/-)	Cone Voltage (V)	Transition	Collision energy
1	Limonene-2-OOH	+	14	151.20 > 57.00	10
				151.20 > 71.05	8
2	Limonene-1-OOH	+	14	151.20 > 57.00	10
				151.20 > 71.05	8
3	Linalool-6-OOH	+	16	169.10 > 57.00	10
				169.10 > 71.05	8
4	Linalool-7-OOH	+	16	169.10 > 57.00	10
				169.10 > 71.05	8



Individual standards 100 ppm

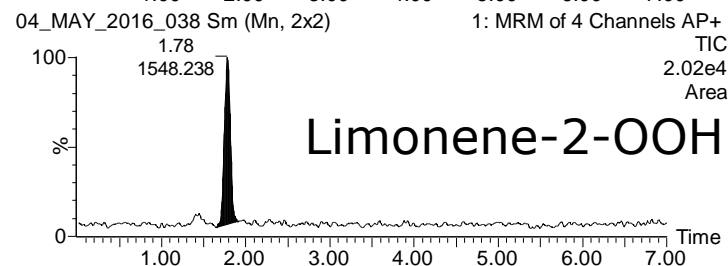
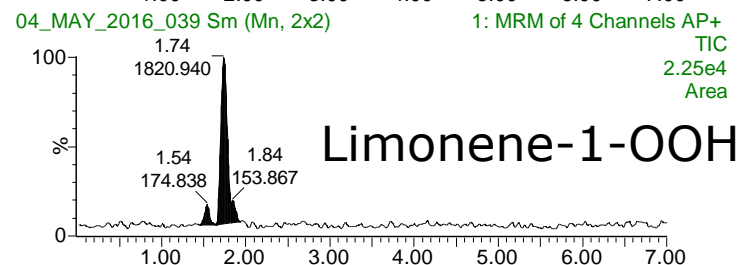
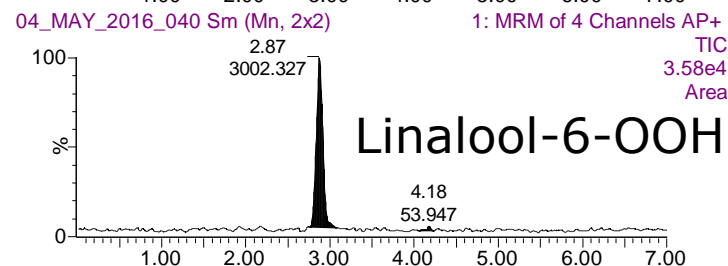
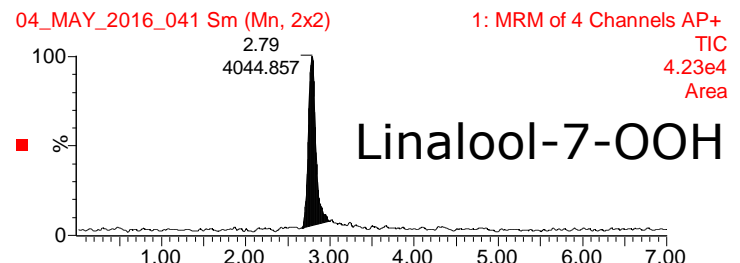
MRM chromatograms



overlaid



Further work required to separate peaks



What's next

- Re-tune the compounds, with the aim to establish more selective MRM transitions.
- Test different sample diluents, with the aim to establish more selective adduct formation.
- Further optimize separation:
 - Screen more columns
 - Screen co-solvents
 - Alter gradient conditions
 - Optimize column temperature / flow / ABPR pressure etc..
- Run sample supplied against established method.

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