HP analytical task force meeting

12.12.16

Givaudan

engage your senses

Agenda

1. **First ring trial: Decision on journal for re-submission**
2. **Analysis in complex products: Review method development**
3. **Extrelut method – experience**
4. **Stability in product bases for ring test**
5. **Extraction method for LC-MS analysis**
6. **Set-up and timing ring test**
7. **Discussion: Contract lab or taskforce for routine analysis**
8. **Discussion: Best approach to test market samples, aged products and patientssamples**
9. **Discussion: What products to test in a market overview / samples from consumers and patients**
10. **Review tomorrows presentation**

Re-submission paper

1. **Three options**
2. **A) Journal of Analytical Toxicology**
3. **B) Flavour and Fargrance journal**
4. **C) Contact Dermatitis**

Review method development: Analysis in complex bases

- Three standard bases distributed to all labs
- Spiking performed by individual labs
- Different methods tested by different labs

- LC-MS: Difficulty for ion dupression
  - Best method proposed By Michael Calandras's Lab

- GC-MS: Different dilute and shoot methods tested

- Extrelut extraction method adapted from allergen detection work
  - Appears to give best recoveries
  - Below results on repeated tests are summarized

Givaudan Method 3 – Extrelut adsorption with MTBE extraction - previous data

- Less hydrophobic solvent combined with Extrelut
- No longer loss of Linalool-OH
- Good recovery **> 80% for all four isomers**
- **Experiment so far only conducted once on one creme / one spike level**
- **Needs to be repeated on all samples**

		trans-Limonene-1-OH	trans-Carveol	7-OH-Linalool	6-OH-Linalool
Creme 1 + 200 ppm HP	MTBE	**96**	**94**	**83**	**89**
Creme 1 + 200 ppm HP	Extrelut afte	20	20	23	22
Creme 1 + 200 ppm HP	Pentane	88	85	8	13
Creme 1 + 200 ppm HP	Extrelut afte	31	29	80	74

Extrelut adsorption with MTBE extraction

- All samples of the method development study were tested now
- Overall 80 – 95% recovery

	trans-Limonene-1-OH		trans-Carveol	
	ppm	% recovery	ppm	% recovery
7) Creme 1 + 0 ppm HP	NF		NF	
8) Creme 1 + 50 ppm HP	44.5	89.0	46.3	92.6
9) Creme 1 + 200 ppm HP	172.8	86.4	189.1	94.5
10) Creme 2 + 0 ppm HP	NF		NF	
11) Creme 2 + 50 ppm HP	46.9	93.8	55.3	110.6
12) Creme 2 + 200 ppm HP	192.9	96.4	214.4	107.2
13) Deodorant liquid 3 + 0 ppm HP	NF		NF	
14) Deodorant liquid 3 + 50 ppm HP	41.4	82.9	40.3	80.5
15) Deodorant liquid 3 + 200 ppm HP	168.6	84.3	145.0	72.5
Average		*88.8*		*93.0*

Extrelut adsorption with MTBE extraction (II)

- All samples of the method development study were tested now
- Overall 80 – 95% recovery

	7-OH-Linalool		6-OH-Linalool	
	ppm	% recovery	ppm	% recovery
7) Creme 1 + 0 ppm HP	NF		NF	
8) Creme 1 + 50 ppm HP	40.2	80.4	40.8	81.7
9) Creme 1 + 200 ppm HP	174.6	87.3	154.8	77.4
10) Creme 2 + 0 ppm HP	NF		NF	
11) Creme 2 + 50 ppm HP	51.2	102.4	41.3	82.6
12) Creme 2 + 200 ppm HP	196.0	98.0	155.6	77.8
13) Deodorant liquid 3 + 0 ppm HP	NF		NF	
14) Deodorant liquid 3 + 50 ppm HP	46.8	93.6	39.2	78.4
15) Deodorant liquid 3 + 200 ppm HP	191.8	95.9	160.4	80.2
Average		*92.9*		*79.7*

Extrelut analysis repeated: Stability in samples over time

- Different consumer bases spiked with 100 ppm
- Again good recovery with Extrelut method
- No loss over 28 days in fridge for these 5 products
- No Background hydroperoxide signal detected in these five products

% recovery of 100 ppm spike	trans-Limonene-1-OH		trans-Carveol	
	T=24 h	T=28 days	T=24 h	T=28 days
Woolwax Alcohol Creme	107.3	108.6	106.6	111.7
Deodorant Base	95.2	100.7	83.7	85.8
Bodylotion'	108.5	99.6	94.1	88.4
Anti ageing cream'	85.0	82.0	96.5	90.8
Lotion II	110.2	106.5	87.7	84.9
Average	*101.3*	*99.5*	*93.7*	*92.3*

Extrelut adsorption with MTBE extraction

- Slightly too high recovery for Linalool-7-OH
- Howver also for Linalool-OH isomers stable recoveries over time
- All analysis done by single sample, no triplicates done
- No Background hydroperoxide signal detected in these five products

% recovery of 100 ppm spike	7-OH-Linalool		6-OH-Linalool	
	T=24 h	T=28 days	T=24 h	T=28 days
Woolwax Alcohol Creme	124.7	110.6	113.1	108.3
Deodorant Base	114.4	112.8	111.2	109.4
Bodylotion'	119.0	115.5	81.2	78.8
Anti ageing cream'	126.6	104.5	85.7	80.7
Lotion II	120.0	116.8	128.4	111.7
Average	*121.0*	*112.0*	*103.9*	*97.8*

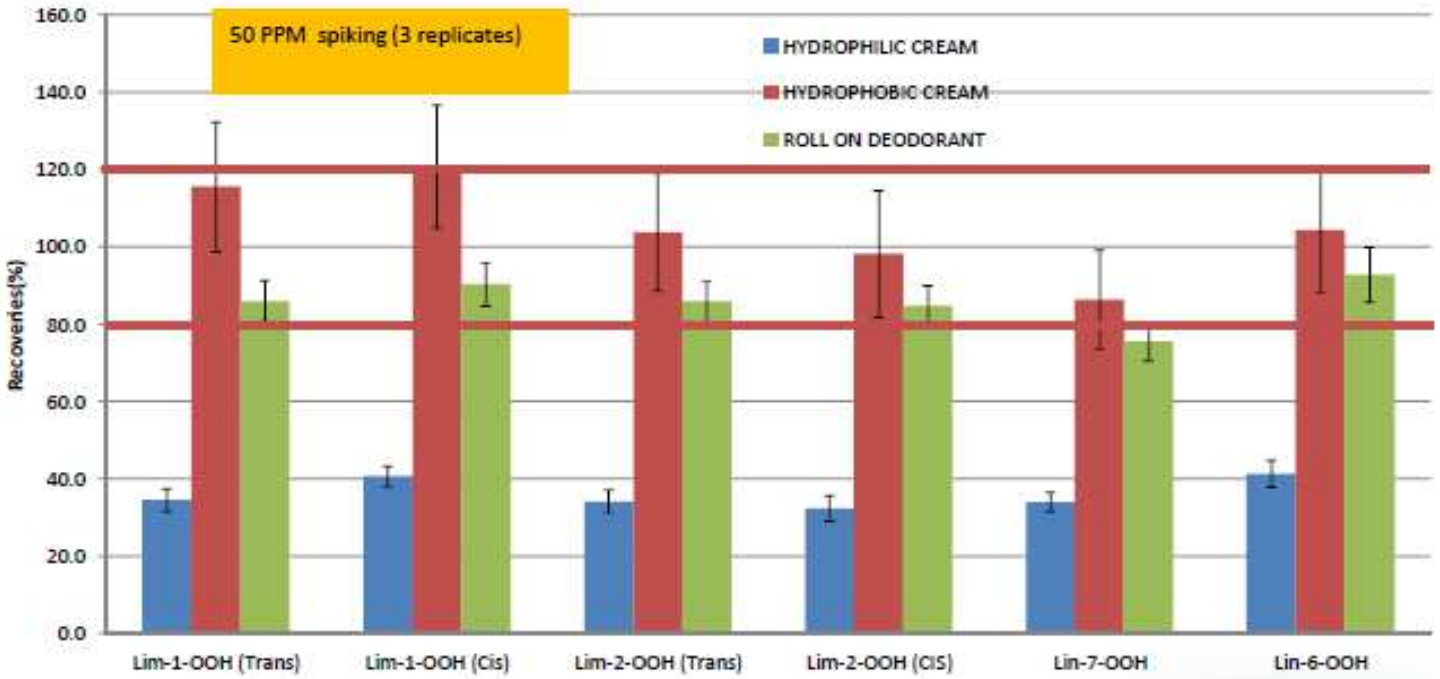
Commercial Deo base: 'All natural deodorant'

- Deodorant based on all-natural ingredients, containing Linalool and Limonene
- Low background level of Linalool-hydroperoxide detected – verified by both HR-LC-MS annd GC-MS- reduction

	trans-Limonene-1-OH		trans-Carveol	
	T=24 h	T=28 days	T=24 h	T=28 days
All natural deo -unspiked	NF	NF	NF	NF
All natural deo -spiked, % recovery	101.9	104.0	92.8	98.1
	7-OH-Linalool		6-OH-Linalool	
	T=24 h	T=28 days	T=24 h	T=28 days
All natural deo -not spiked, ppm	29.7	28.2	NF (to be rechecked)	27.2
All natural deo -spiked, ppm	147.5	152.7	128.2	152.4
All natural deo -spiked, % recovery	117.8	124.6	128.2	125.2

Extrelut method: Experience Chanel

- Good recovery found by CHANEL for hydrophobic cream and deodorant base
- Poor recovery for hydrophilic cream
  - Degradation of spiked level within 24 h?



Extrelut method: Experience other Labs?

- TBD

Conclusions: Extrelut – Reduction- GC-MS method

- Reduction method efficiently reduces HP in all bases tested
  - Divergent results Givaudan / CHANEL for hydrophilic cream
- Recovery of formed alcohols from the different bases close to 100%
- GC-MS coupled to reduction and extrelut extraction method is sensitive enough to quantify in complex bases at 50 ppm
- Hydroperoxides appear stable in the bases used in fridge, so stability should no be an issue for ring study

- Note: Theoretically hydroperoxides could decay into the alcohols in the bases – this we cannot see from these results
- To test this possibility, same analysis was done with LC-MS
- 28 days samples are analyzed this week – we will report if this generates a different result, (i.e. Loss of HP in 28 days samples vs 24 h samples if analyzed with LC-MS)

Extraction for LC-MS analysis

- Results Mike
- Method Mike

Set-up and timing ring test

- Who will participate?
  - Integrate a commercial CRO ?– see below
- How many product bases
- How many replicate samples per test sample to be analysed
- Set-up as last time – low and high ranges as before?

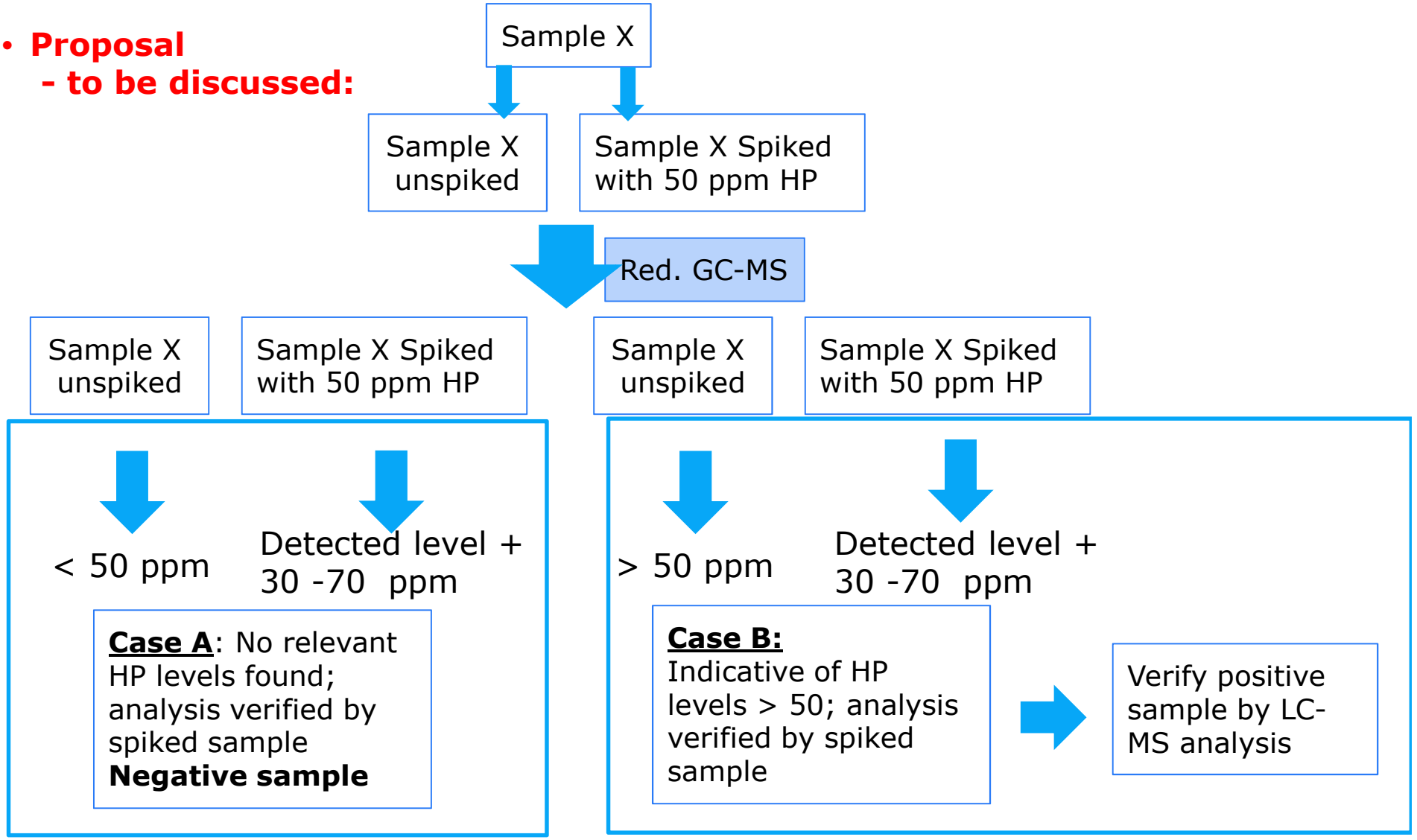
Base 1 not spiked	Base 1, low level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of **20 – 50 ppm**	Base 1, high level Spiked with different levels of Limonenen-1-OOH, Limonenen-2-OOH, Linalool-6-OOH, Linalool-7-OOH in the range of **100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

Discussion: Contract lab or taskforce for routine analysis

- Based on the method development work, we expect next ring trial to be a success
- We will then be ready to routinely test 'real' samples
- Who will perform this analysis?
  - The labs of the taskforce with a mandate from IFRA
  - An external CRO?
  - In the later case we must make sure, that the external lab is validated as we are now and performs equally well
  - **In this case the external lab should already participate in next ring trial!**

Discussion: Best approach to test market samples, aged products and patients samples

- **Proposal**
  - to be discussed:



Discussion: What products to test in a market overview / samples from consumers and patients

- Detection in final consumer products
  - Detection in general **market products**
  - Detection in **aged consumer samples**

    ⇒ Presence of potentially sensitizing doses above levels considered safe by QRA?

  - Detection in **products brought in by patients**

    ⇒ Presence of potentially elicitating doses which may indicate relevance of reaction to actual disease?
- How is such a study organized, and who will perform analysis?

Thank you

Contact