IDEA
International Dialogue for the Evaluation of Allergens

IDEA Meeting of the

Hydroperoxides Task Force

May 11th, 2016 from 9:30 to 16:30

IFRA offices
6, Av des Arts (4th floor)
1210 Brussels (Belgium)

Final minutes

Participants:
Hans Bender (HB, Moderator of the IDEA Workshops), Hugues Brevard (HBR, Robertet), Joost Broekhans (JB, IFF), Michael Calandra (MC, Firmenich, by phone), Elise Corbi (EC, Chanel), André Düsterloh (AD, DSM), Cécile Gonzalez (CG, IDEA Management Team), Ann-Terese Karlberg (ATK, University of Gothenburg), Andreas Natsch (AN, Givaudan), Ulrika Nilsson (UN, University of Stockholm), Florian Stintzing (FS, Wala), Matthias Vey (MV, IDEA Management Team).

1. Opening of the meeting

AN opened the meeting at 9h50. Due to the presence of new members, a tour the table was organized.

2. Antitrust agreement

The participants were reminded the constraints of the antitrust law. All agreed that there should be no discussions of agreements or concerted actions that may restrain competition. This prohibition includes the exchange of information concerning individual prices, rates, coverage, market practices, claims settlement practices, or any other competitive aspect of an individual company's operation. Each participant understood the obligation to speak up immediately for the purpose of preventing any discussion falling outside these bounds.

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3. Summary of the inter-laboratory results with the PPh_3-reduction-GC method (Att. 01).

- Reduction method

Regarding the results on Lim-1-OOH from the reduction method, AN explained that there is around 5% variance which might be partly explain by the preparation process of the samples by Greenpharma. He also explained that there might also be a tendency for this isomer to be overestimated. However, the group agreed that an overestimation of 15% is still acceptable for this isomer. The average recovery is very good, close to 100%, particularly for the high spike levels. The overall variability for the spiked samples is around 10%. Similar results are observed for Lim-2-OOH, Lin-6-OOH and Lin-7-OOH. AN thanked the participants for their work.

AD reported that his team has had technical issues regarding the stability of the response factors. AD requested to the group if it was possible to have individual Standards to test the stability of the solution. EC performed additional tests with other 70 fragrances (in SIM-SCAN mode alternatively and quantification based on SIM mode), and they faced difficulties to identify the compounds, especially Lin-7-OOH due to the lack of specificity. EC reported that in SIM mode the analysis was not selective enough. EC tried a longer column and there still was still a high level of coelution. AD reported that his team tried 2D GC. The work is still ongoing as the retention time was shifted in the second column due to the presence of water in the sample. However, the separation power is promising. In EC's opinion, when an analyst will be faced to unknown samples some difficulties to identify the reduced hydroperoxides at low levels due to coelutions on polar phase and low specificity of target ions might be observed. Using a chromatographic column with different polarity and standard addition can be helpful to ascertain the identification but this methodology can be difficult to implement in a QC lab because it's time consuming.

The group discussed what was the level to which the samples should be quantified, meaning that if the hydroperoxides levels in the samples are lower than a certain amount, the samples could be considered as safe. The aim of the analytical work is to prevent induction. It is very difficult (impossible) to define secure elicitation limit. If the method is designed to avoid elicitation, the quantification level is as low as 1ppm. If the method is designed to avoid induction, the levels are higher. This is also reflected in the regulation, as only the induction is regulated. HB reminded the participants that the work on IDEA is based on the prevention of induction, as the QRA only prevents the induction but not the elicitation. Moreover, the induction and elicitation level are patient dependent, but the threshold for induction is considered to be higher than the one for elicitation. The group agreed that in SCAN mode, the limit of 15 ppm was considered acceptable.

The group was questioned on whether the matrix was specific enough and how to progress from the current matrix (alcoholic fragrance) to other product matrixes, such as creams. The reduction method can be used as such, and if issues of coelution and specificity are encountered, the group recommended to use a second column.

The reduction method is effective to retrieve the levels of –OOH that were spiked in samples. The water-ethanol samples is relatively simple and additional work is needed for more complex mixtures. A next step

for the TF would be define the performance criteria to identify the need to use a second column and improve the specificity of the analysis.

- Alternative methods:

The results obtained with other methods were presented to the participants. AN explained that IFF and Firmenich methods provided reliable results. Compared to the reduction method, the alternatives methods are able to found 0 ppm in the non-spiked samples. The specificity obtained for Lin-OOH is satisfactory. However, for Lim-OOH the coelution and the matrix effect are important and the selectivity on for these ions is low. The selection of a long method with high chromatographic resolution by both IFF and Firmenich proved to be the key to detect all four isomers by LC-MS. This comes at the cost of long analysis time, but it resolved problems encountered with ion suppression and limited chromatographic resolution in the method routinely applied by Givaudan.

Overall, the alternative methods indicate that there is less than 1% of Lim and Lin present in the sample in their hydroperoxide form. Further studies would be required to better understand how this reaction occurs in real time, before the product was purchased. The participants were reminded that this exercise is an example of a sample in the market, but it does not represent the average of what it is found in the market. AN will also include the results on the other secondary products in the conclusions.

- Further understanding of peroxyhemiacetals (PHA) formation:

MC presented three sets of results, which represent how much time past between when the samples were diluted into the HPLC preparation solution, and the actually injection/analysis. When the samples are freshly prepared, the PHAs don't have time to revert back to the parent terpene hydroperoxides. When the samples sit around for a while in the polar preparation solution, the PHAs do revert. However, we do not know exactly how long this takes for all of the different PHAs that might be formed.

The fact that the 20 h and 43 h results are larger than the fresh results indicates that there are some PHAs present in the perfumes. The 20 hour and 43 hour samples are at least in agreement with GC with TPP results, in some cases very close. One hypothetical consequence that this phenomenon may have is that, log P is higher in the PHA form, it is the PHA that has an enhanced penetration of the skin. Then, once the PHA has penetrated the skin, it may revert back to the parent –OOH form because of the polar, aqueous environment in the intracellular space, within the lower skin layers. Whether PHA's will really be more potent depends on whether the hydroperoxide reaction takes place in a lipid medium and on whether the PHA reverts to hydroperoxide and aldehyde in that medium. In this way, the PHA may act as a dermal penetration enhancer for the terpene hydroperoxide. From the relative HPLC retention times, it is clear that the PHA is more hydrophobic than the parent terpene hydroperoxide. Because the method used is a standard reverse phase type of separation, the retention time will correlate with log P. In addition, one could quite reliably predict that the addition of a C-8 or C-10 aldehyde moiety to the terpene hydroperoxide molecule would increase the log P, purely from theoretical or structural considerations.

It was suggested that a next step could be to collect toxicological information on their penetration into the skin. However, looking at the overall results on hemiacetal levels present in products it was later in the meeting decided, that at least for the time being, there is no need for an action re toxicological information on hemiacetals, as individual hemiacetals are present at so low concentration. They may however act as reservoir for the hydroperoxides, but it is then the total level of hydroperoxides which counts to make the toxicological evaluation.

- Preliminary results on the measurement of –OOH in complex matrixes.

AN presented preliminary results on the measurement of –OOH in creams and lotions which look promising. FS reminded the group that the competition effect should be taken into consideration for the analysis in complex matrixes such as deodorants or body lotions.

- The method Acquity UPC2 – Xevo TQD

NO attended the Waters open day where the technique Acquity UPC2 – Xevo TQD was presented. He presented the slides that were presented at the open day. The method seems to provide the separation of LC but keeps the speed of GC. Waters have received the blind samples and AN and NO are waiting for the results. At the moment there seems to be some issues with the ion separation, and Waters is working on potential improvements.

4. Presentation from each participant working on the action plan and discussion of the results (Participants of ring trial):

- IFF (LC-Q-TOF MS):

JB presented the work performed at IFF on LC-Q-TOF MS. They took as a starting point the method currently used at the University of Gothenburg, and tried several columns and changes of analysis parameters. The ratio between isotopes of Natrium adduct was taken as reference. UN reported that a similar separation was achieved in her laboratory, but slightly slower than IFF's study (almost 40 minutes per run).

- Firmenich (HPLC-CD) (Att. 02):

MC presented the results obtained with HPLC with Chemiluminescence Detection (HPLC-CD), which obtained a recovery of 80-100% for almost all the –OOH analysed. He also presented the impact of the formation of PHAs in the analysis. The analytical method development for hemiacetals is still beginning. Ideally, the method developed to measure –OOH should at least qualify the formation of PHAs in order to have the global information on how PHAs impact the sample. An additional optimized LC step could be appropriate for the back end.

5. Potential publication on method comparison and ring trial

The participants agreed on writing a publication on the results of the last ring test, including the lab-to-lab comparison. The previous ring tests will only be mentioned in the introduction, as data is more difficult to interpret and to publish. AN will try to prepare a draft and will circulate to the participants. The context of the IDEA project will be presented in the article. Only the participants that have participated to the ring test and performed analysis will be authors of this publication.

AN will review the data and will share with the group the outcome in order to decide whether the detection of hemiacetal by reduction method and other product methods will be included in this publication or will rather be the object of other publications.

AN, on behalf of the IDEA Hydroperoxides TF, has submitted an abstract for the ESCD Conference in Manchester in September 2016. The TF was requested to review, provide comments and agree by email.

6. Actions and next steps

The following steps and actions were agreed at the meeting:

a) In the short term:

ACTIONS in the short term:	**Who?**
To prepare a draft of the publication on ring trial	**AN**
To define performance requirements for reduction method.	**All**
To validate the POV method with analytical results for QC.	**Lead MC, may request help from other participants**
To check for detection by the reduction method of other hydroperoxides (e.g. Geraniol, Citronellol, Citral).	**All** **(Ideally a diluted reference solution of the other hydroperoxides would be provided by University of Gothenburg who have the samples in hand – this could be measured by a participant with the reduction method to generate reference chromatograms and spectra).**

b) In the mid and long term:

ACTIONS in the mid and long term:	
B.1) Market surveillance:	
Complex matrixes: Start with a defined known product matrix with critical characteristics (e.g. lipo-hydrophilic balance), e.g. skin cream and deodorant; or different cream polarities according to Pharmacopoeia.	**FS: to propose cream and cream supplier.** **AN/Givaudan: to propose deodorant formulation**

- Start with pharmaceutical creams according to Pharmacopoeia and deodorant formulation – Samples of pharmaceutical cream spiked with HP to be sent to laboratories participating to the ring test. **- Ring test laboratories to check their extraction methods for the proposed samples.** **- Check method used by Eurofins for the IFRA compliance program (Should work at 10 ppm level for the 26 allergens).**	**AN: to draft study plan** **All: to agree on parameters: pre/post addition, HP levels** **Greenpharma to prepare spiked samples** **All – extract and analyze with their method of choice** **Generate blind coded results, methodology used should not be confidential and be shared after the analysis!** **IDEA Management Team to share the Eurofins Method**
B.2) Assist dermatologists	
Once a method is also available from cream, lotions and deodorants, the methods should be applied to samples collected from consumers. **To prepare this discussion with dermatologists how to collect samples should start. Plan (how and who) samples could be collected which are suspected to cause reactions in consumers which were positively tested for the hydroperoxides. The individual clinical data (relevance, concomitant reactions) would need to be known along with the samples collected.**	**IDEA Management Team**
To assist dermatologists on patch test material (stability and real hydroperoxide content): data already available from Chemotechnique – they found best stability in fridge. **- Data could be shared by the Chemotechnique/Gothenburg University team; can be approached by the IDEA Management Team.**	**IDEA Management Team**

7. Next conference call

June 24th, 2016 – 2 to 4pm (Brussels time). The IDEA Management Team will confirm the date closer to the date of the conference call.