

IDEA Meeting of the Hydroperoxides Task Force

June 15th, 2015 from 10:30 to 16:00

Château du Lac
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Final Minutes

Participants: Hans Bender (HB, Moderator of the IDEA Workshops), Hugues Brévard (HB, Robertet), Alain Chaintreau (AC, Firmenich, chair), Elise Corbi (EC, Chanel), André Düsterloh (AD, DSM), Cécile González (IDEA Management Team), Ann-Therese Karlberg (ATK, University of Gothenburg), Hans Leijs (HL, IFF), Clémentine Marteau (CM, IFF), Andreas Natsch (AN, Givaudan), Ulrika Nilsson (UN, University of Stockholm), Neil Owen (NO, Givaudan), Christophe Perès (CP, Chanel), David Roberts (DR, Liverpool John Moores University), Matthias Vey (IDEA Management Team).

1. Antitrust statement

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

2. Minutes of the last meeting

The minutes of the last meeting of January 28, 2015 were adopted electronically and already uploaded on the IDEA website.

3. Global results of the ring test

AC presented the global results from the ring test for Linalool (Lin-HP) and Limonene (Lim-HP) hydroperoxides generated in the orange oil and the Lily matrixes. Overall, important differences between methodologies exist, and as occurred in the first ring test, it is too soon to conclude on the appropriate methodology to measure the hydroperoxide levels in the analysed matrixes. In this current exercise, AC used two bias cut-offs, 25 and 80% for the validation and invalidation of the results.

4. Presentation of individual results and methods

4.1. Andreas Natsch (Givaudan)

Givaudan worked with GC-MS (in combination with prior reduction of the HP) and LC-MS. Internal and external calibration provide reliable results, however both techniques provide an underestimation of the measure of Lin-HP and Lim-HP esp. in Orange oil samples and reveal the different behaviour of the isomers Limonene-1 and Limonene-2 hydroperoxide during the analysis. Results confirm the previous experience that LC-MS does not seem adapted to analyze Limonene-2-OOH in complex fragrance mixtures, while both methods work for linalool-OOH. Overall the GC-MS method, with reduction and external calibration is most versatile and robust (works for different hydroperoxides).

4.2. Alain Chaintreau (Firmenich)

The Flame Ionization Detection (FID) technique relies on the correlation of the FID response of materials and their combustion enthalpy. It is thus easy to calculate the response factors from the combustion enthalpies values. Such enthalpies are well predicted from *ab-initio* studies (density functional theory) or simply from the molecular formula.

AC applied the FID predicting factors to the samples of the ring test. When compared to the RMN measurements, the predictions are slightly lower than the measured levels. Although this method shows a good repeatability, it remains not satisfactory on the ROOH measurement as it underestimate the level of hydroperoxides in the samples compared to the NMR reference. There is a suspicion of Lim-1-HP degradation even at -18°C which would not allow a direct comparison of the results to the NMR reference.

4.3. Michael Calandra (Firmenich – presented by Alain Chaintreau)

AC explained to the participants that the samples arrived by error to Switzerland and therefore the analysis was delayed and a degradation of samples might have occurred. The results suggest that reactivity seems fundamental in this ring test and potentially affects all the chromatographic techniques employed, as a lot of chemistry seems to take place. AC provided data obtained with the Chemiluminescence detection, but this technique does not provide an information on the molecular

structure. Furthermore, this method only detects products that are highly oxidized and cannot differentiate the products in case of coelutions.

However, the method showed the occurrence of new, late eluting peaks in spiked oils, indicating they are reaction product between hydroperoxide and the oil. From his results, MC suggests a dimerization of Lim-HP and he proposed potential structures. AN went back into the LC-MS data and could confirm the late eluting peaks, and their fragmentation pattern would be consistent with the proposed Dimer. UN added that this dimerization is formed through a covalent union and that the mechanism and impact of this union in the equilibrium is unknown. The quantification for the purpose of assessing the sensitizing potential would require both groups of peaks to be included in the calculation. Similar results are also observed for the Linalool.

Further analysis show that such dimerization is polarity-dependent. Indeed, when the sample was placed back in polar solvent and analyzed, the monomer peaks where regenerated. Thus, citrus oils (principally composed of Limonene) could act as non polar solvent. However, nothing is known on the mechanisms of dimerization, the reversibility of the reaction and its occurrence during the measurements. Nevertheless, the reactivity of the hydroperoxides could explain why the standard addition approach, which should be the most reliable technique to validate a quantitative method, did not demonstrate to be the most suitable way in this case.

5. Conclusions

Under the guidance of HB, moderator of the IDEA Workshops, the participants agreed on the conclusions of the meeting. Such conclusions were used to draft the slides on the update on the work of the TF presented by the chairman during the pre- and pro-haptens workshop in June 16 to the rest of participants. The slides are attached to the minutes (Att. 01). Such slides are also available in the IDEA website under the link:

<http://www.ideaproject.info/uploads/Modules/Documents/idea-pre-and-prohaptens-ws-june-2015---hydroperoxides-tf---alain-chaintreau.pdf>

For this second ring test, 4 different isomers were produced for the first time commercially in almost pure form, 3 of them sufficiently stable that they can be shipped and used as reference standards.

Participants agreed that it was still premature to conclude on the methodology to enable the quantification of the amount of hydroperoxides in a fragrance oil sample. The uncertainty revealed during the second ring test suggesting complex chemical reactions and/or interaction within the sample and during the measurement brings up to front the need to a better understanding of the chemistry of hydroperoxides, in particular Limonene and Linalool. However, such reactivity could be



an indication that the standard addition might not be the suitable way to define the method to quantify the level of hydroperoxides in fragrance oil samples.

Therefore, the participants suggested to look closer in to the stored samples (repeatability, repeated NMR analysis) and in the impact of the polarity of the samples. A detailed action plan (Att. 02) including the next steps will be elaborated by the participants.

Minutes: C. Gonzalez

First review: July 9th, 2015 (AC, AN)

Review by the Hydroperoxides TF: July 15th, 2015

Final: September 2nd, 2015