FACING THE CHALLENGE: developing and validating approaches to detect and quantify skin sensitizing hydroperoxides in consumer products

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1. Introduction

Recently, a high frequency of positive patch tests to oxidized linalool and limonene was reported from multiple clinical centers. However, there is a lack of data indicating potential sources of consumer exposure to sensitizing doses of tertiary hydroperoxides, which are the key sensitizers in preparations of these oxidized materials. To understand the frequent patch tests and define potential actions needed, this gap needs to be closed.

Within the IDEA project (International Dialogue for the Evaluation of Allergens), so far three workshops were organized to look at the clinical data, the conditions leading to hydroperoxide formation and the question of significant exposure to hydroperoxides from fragranced products. IDEA formulated a program that will drive analytical and mechanistic understanding of these potential reactions which eventually will allow to develop a framework, broadly applicable to address the PhX/PhNapler question in a meaningful approach.

As one key action, a hydroperoxide analytical taskforce was established. This group: i. reviewed the analytical challenge; ii. organized a reliable source for pure analytical standards; iii. started two method validation studies to compare different analytical methods to detect hydroperoxides in essential oils; iv. LCMS and GC-MS methods with derivatization were tested. Based on the results of these initial studies, a new study was planned with the following goals:

* Compare reproducibility in five labs of a method using reduction of hydroperoxides followed by GC-MS
* Compare on the same samples a range of different LC-based methods to directly detect hydroperoxides

Study setup

• The IDEA taskforce organized synthesis of reference standards of four key hydroperoxides at high purity. The structure of the key target analytes are shown in Figure 1
• These standards were spiked into commercial alcoholic fragrances, as shown in Table 1.
• Five labs received synthetic reference standards and the spiked samples.
• LCMS and GC-MS methods with derivatization were tested. Based on the results of these initial studies, a new study was planned with the following goals:

Table 1: Study setup – six samples received by test labs

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Spike Level</th>
<th>Spike Level</th>
<th>Spike Level</th>
<th>Spike Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eau de toilette, low level</td>
<td>Unspiked</td>
<td>20 ppm</td>
<td>50 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Eau de perfume, low level</td>
<td>Unspiked</td>
<td>20 ppm</td>
<td>50 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Eau de toilette, high level</td>
<td>Unspiked</td>
<td>20 ppm</td>
<td>50 ppm</td>
<td>100 ppm</td>
</tr>
<tr>
<td>Eau de perfume, high level</td>
<td>Unspiked</td>
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<td>100 ppm</td>
</tr>
</tbody>
</table>

Results: 1. Reproducibility / accuracy of the reduction-GC-MS method

Figure 2 shows results from five laboratories detecting four different hydroperoxides in the six samples.

• In samples spiked with 100 – 200 ppm, an average recovery of 86 – 112% with a relative standard deviation of 8.1 – 22.5% was found.
• In samples spiked with 20 – 50 ppm, the average recovery was 86 – 131% with a relative standard deviation of 8.1 – 22.5%.

• The method allows accurate detection and good quantification.
• The method is easily transferable between laboratories.

Results: 2. Method comparison of LC-based methods to directly detect the hydroperoxides

Table 2 shows results for the sum of linalool-OOH isomers detected by three different LC-based methods.

• Good recovery found with all three methods
• The results are similar to the reduction-GC-MS method
• Overall, similar results obtained for the four isomers tested (data not shown)

Table 2: Detection of Linalool-OOH (sum of isomers) by different analytical methods (data in µg/ml)

<table>
<thead>
<tr>
<th>Method</th>
<th>Eau de Toilette Low</th>
<th>Eau de Toilette High</th>
<th>Eau de Parfum Low</th>
<th>Eau de Parfum High</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-QTOF MS</td>
<td>0.0</td>
<td>90.0</td>
<td>279.0</td>
<td>0.0</td>
</tr>
<tr>
<td>HPLC/CL</td>
<td>0.0</td>
<td>79.5</td>
<td>310.7</td>
<td>0.0</td>
</tr>
<tr>
<td>LC-ORBITRAP MS</td>
<td>0.2</td>
<td>95.7</td>
<td>398.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Avg red-GCMS</td>
<td>6.1</td>
<td>88.6</td>
<td>325.4</td>
<td>10.2</td>
</tr>
<tr>
<td>Spike level added</td>
<td>0.0</td>
<td>92.0</td>
<td>332.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1. Average of the measurements with red-GCMS in Figure 3.

Fig. 2: Quantification of four different hydroperoxides spiked into a commercial Eau de Toilette (EdT) and an Eau de Parfum (EdP) by the reduction-GC-MS method.

Comparison of results for the sum of linalool-OOH isomers detected by three different LC-based methods.

Conclusions

• The reduction-GC-MS method is a simple and reproducible method
• LC-methods can directly detect the hydroperoxides
• This study offers a toolbox of methods to detect hydroperoxides in hydroalcoholic consumer products

Next steps and outlook

• In a follow up study, extraction protocols for more complex products will be developed
• The methods will be tested in creams, lotions and deodorants

Final goal will then be to:

• Quantify hydroperoxides in market products
• Quantify hydroperoxides in products used by patch-test positive patients

The methods developed by IDEA will then finally answer the question of potential consumer exposure to hydroperoxides

Acknowledgments

The authors would like to thank all colleagues who contributed to the study.

References

3. HPLC/CL and LC-ORBITRAP MS detection methods were used to quantify hydroperoxides in different fragrances.
4. The methods will be validated in different product matrices, including creams, lotions, and deodorants.
5. QTOF MS detection methods will be used to confirm the presence of hydroperoxides in various consumer products.
6. This study was supported by the IDEA (International Dialogue for the Evaluation of Allergens) project.