Detection of Squalene and Squalane Origin with Flash Elemental Analyzer and Delta V Isotope Ratio Mass Spectrometer

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Application Note 30276

Introduction
Squalane (C₃₀H₆₂) is widely used in cosmetic products due to its high skin hydrating capability. On an industrial scale, squalane is obtained by hydrogenation under high pressure of its natural precursor molecule squalene, which is also used in personal care products such as skin moisturizers. Squalene is derived from two main natural sources, shark liver oil and vegetable oils, e.g. olive oil. The production of squalene and squalane from shark liver oil is less complex and cheaper than from olive oil. Hence, the vast majority of squalene and squalane used in the cosmetics industry during the past decades originated from shark liver oil. However, shark liver oil does not represent a renewable raw material and the populations of some shark species are endangered. As a consequence of such ecological awareness and ethical arguments, many consumers are now requesting animal product free cosmetics. Therefore, the cosmetics industry is urged to use olive oil as a renewable and ethically acceptable source for the production of squalene and squalane.

The two source materials for squalene and squalane, shark liver oil and olive oil, exhibit significantly distinct carbon isotopic compositions (δ¹³C). Shark liver oil derived squalene is characterised by δ¹³C values between -19.9 to -20.9‰ whereas olive oil derived squalene shows noticeably lower δ¹³C values between -27.8 to -28.4‰. This systematic difference in δ¹³C between the two sources can be employed to detect (a) the origin of squalane and (b) an adulteration of olive oil derived squalane by admixtures of shark liver oil derived squalane¹. Analysis of δ¹³C of squalene and squalane can be performed by means of an Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS).

Analytical Method
Carbon isotope compositions (δ¹³C) of squalene and squalane from shark liver oil, olive oil, and mixtures of both oils were measured by a Thermo Scientific™ Flash™ 1112 EA for IRMS coupled to a Thermo Scientific Delta™ V Series IRMS via a Thermo Scientific ConFlo™ IV interface.

Results and Discussion
Figure 1 shows a typical chromatogram of a carbon isotope analysis of squalane. Flat top reference gas pulses of CO₂ are followed by the chromatographically separated CO₂ peak from the combusted sample.

Key Words
Delta V, Flash 1112 EA, Origin Control, Squalene, Squalane, Cosmetics
Table 1 summarizes the results and precisions of the carbon isotope ($\delta^{13}C$) analyses of squalene and squalane derived from olive oil and shark liver oil and a 50:50 squalane mixture of squalane from these two sources. These $\delta^{13}C$ data are based on a two-point calibration of the VPDB-scale using international reference materials IAEA-CH-6 and IAEA-CH-7.

<table>
<thead>
<tr>
<th>Sample Identifier</th>
<th>Mean $\delta^{13}C$ [% vs. VPDB; n = 5]</th>
<th>S.D. [%; n = 5]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil squalene</td>
<td>-28.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Olive oil squalane</td>
<td>-27.99</td>
<td>0.12</td>
</tr>
<tr>
<td>Shark liver oil squalene</td>
<td>-20.56</td>
<td>0.07</td>
</tr>
<tr>
<td>Shark liver oil squalane</td>
<td>-20.28</td>
<td>0.15</td>
</tr>
<tr>
<td>Mixture 50:50 shark-olive oil</td>
<td>-24.25</td>
<td>0.11</td>
</tr>
</tbody>
</table>

TABLE 1. Results and precisions of $\delta^{13}C$ of replicate analyses (n = 5).

In order to validate the detection and characterization of possible mixtures of squalane from the two sources (shark liver oil, olive oil) by means of $\delta^{13}C$ analysis, measurements of mixtures of squalane from shark liver oil and olive oil, respectively, with defined proportions were performed. The results were used to determine the correlation between the $\delta^{13}C$ of the squalane mixtures and the percentage of squalane from olive oil (Figure 2).

As shown in Figure 2, results of analyses of mixtures of squalane derived from shark liver oil and olive oil, respectively. The linear regression through the data of these analyses provides a correlation that can be used to calculate the percentage of squalane from olive oil in unknown mixtures of the two potential sources of squalane.

For example, the correlation shown in Figure 2 predicts a $\delta^{13}C$ of -23.86‰ for a 50:50 mixture of shark and olive oil derived squalane. Replicate analyses (n = 5) of another 50:50 mixture of squalane from shark and olive oil, respectively, resulted in a mean measured $\delta^{13}C$ of -24.25‰ (Table 1). Applying the correlation from Figure 2, this measured $\delta^{13}C$ of the 50:50 mixture corresponds to a content of 54.9% squalane derived from olive oil. This demonstrates the applicability of the described methodology and an associated uncertainty of ±10% or better.

Conclusion

The significant difference in $\delta^{13}C$ between the two main sources of squalane (plant versus animal) and the high precision of the well-established analytical technique of EA-IRMS facilitate reliable and conclusive quantifications of the contents of squalane derived from olive oil versus shark liver oil in mixtures and hence the verification of the origin(s) of squalane contained in cosmetic products.

Acknowledgements

The authors would like to thank Hervé Casabianca, Vanessa Salomon and Patrick Goetinck from CNRS for their involvement in isotopic ratio mass spectrometry and Richard Watts from CRM International for his inputs to this project, providing standards and technical assistance.

References