Application Note 30276

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

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Delta V, Flash 1112 EA, Origin Control, Squalene, Squalane, Cosmetics

Introduction

Squalane ($C_{30}H_{62}$) 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 ($\delta^{13}C$). Shark liver oil derived squalene is characterised by $\delta^{13}C$ values between -19.9 to -20.9‰ whereas olive oil derived squalene shows noticeably lower $\delta^{13}C$ values between -27.8 to -28.4‰. This systematic difference in $\delta^{13}C$ 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 squalane[1]. Analysis of $\delta^{13}C$ 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 ($\delta^{13}C$) 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.

The samples (≥ 200 µg) were weighed into tin capsules and dropped from a carousel-type Thermo Scientific MAS 200R™ auto sampler into a combustion reactor, which is operated at 1020 °C and packed with chromium oxide and silvered cobaltous/cobaltic oxide. Automated and timed oxygen injection ensured a complete Dumas-type flash combustion of the sample, resulting in quantitative conversion of sample-derived carbon into CO_2. All gas species generated in the combustion reactor are then carried by a continuous stream of helium carrier gas (90 ml/min) through a subsequent reduction reactor (reduced copper, 650 °C), a water trap (magnesium perchlorate), and an isothermal (40 °C) GC separation column (3 m, Porapak QS, 50/80 mesh, OD 6 mm, ID 5 mm) into the ConFlo IV interface. The ConFlo IV interface couples the elemental analyzer (EA) to the IRMS, performs referencing and dilution, and employs a dual open split design to reduce the flows of reference and sample gas, respectively, into the IRMS to ensure stable ion source conditions. The isotopic analysis of the CO_2 produced from the combustion of squalene and squalane was performed by a Delta V series IRMS. All components of the EA-IRMS system are controlled and operated by the Thermo Scientific Isodat™ software. The analysis time per squalene or squalane measurement is 400 s.

Results and Discussion

Figure 1 shows a typical chromatogram of a carbon isotope analysis of squalane. Flat top reference gas pulses of CO_2 are followed by the chromatographically separated CO_2 peak from the combusted sample.

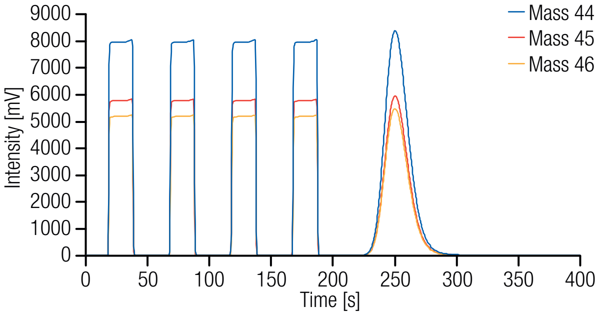

FIGURE 1. Example chromatogram of a δ^{13}C analysis with the traces for masses 44, 45, and 46. The first four peaks are pulses of CO_2 reference gas. The last peak represents the sample CO_2.

Table 1 summarizes the results and precisions of the carbon isotope (δ^{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 δ^{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.

Sample Identifier	Mean δ^{13}C [‰ vs. VPDB; n = 5]	S.D. [‰; n = 5]
Olive oil squalene	-28.06	0.06
Olive oil squalane	-27.99	0.12
Shark liver oil squalene	-20.56	0.07
Shark liver oil squalane	-20.28	0.15
Mixture 50:50 shark-olive oil squalane	-24.25	0.11

TABLE 1. Results and precisions of δ^{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 δ^{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 δ^{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.

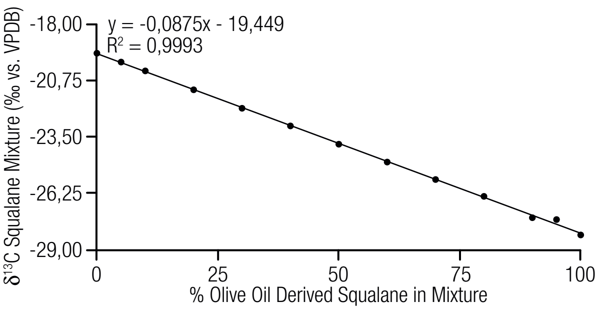

FIGURE 2. Results of analyses of mixtures of squalane derived from shark liver oil and olive oil, respectively. The linear regression through the data of these analyses provides a correlation that can be used to calculate the percentage of squalane from olive oil in unknown mixtures of the two potential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

For example, the correlation shown in Figure 2 predicts a δ^{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 δ^{13}C of -24.25‰ (Table 1). Applying the correlation from Figure 2, this measured δ^{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 δ^{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.

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