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Application Note SI-01233

Analysis of Complex Triglycerides in Starflower Oil from Borage by HPLC using ELSD

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Introduction

The Varian evaporative light scattering detector is universal and independent on the optical properties of the compound under analysis. Consequently, its good discriminating power and sensitivity is well suited to compounds, such as triglycerides, that possess weak or no UV chromophores.

Starflower oil (borage oil) is extracted from the annual herb, borage (*Borago officinalis*). The oil is rich in the fatty acid gamma linolenic acid (GLA), which is an important intermediary in the metabolic conversion of linolenic acid into prostaglandin E1 (which modulates the action of many hormones). It is claimed to be beneficial for a wide range of conditions, such as premenstrual syndrome, eczema, rheumatoid arthritis as well as reducing cholesterol and blood pressure. An optimal dose and length of treatment for borage oil has not been established, although manufacturers recommend a dose of approximately 3 g of borage oil per day, providing approximately 700 mg of GLA.

The majority of dietary supplements, such as borage oil, are not subject to review or approval by the US Food and Drug Administration (FDA). As a result, the amounts of active ingredients or contaminants which they contain, may vary between brands or between different batches of the same brand. In addition, few reliable studies into the safety and efficacy of borage oil have been done. Consequently, the side effects of long term exposure to the oil are unknown, and so there is a need for accurate, analytical methods that characterize the composition of borage oil supplements, in order to provide a means of standardizing the quality of the herbal product from batch to batch and between manufacturers.

Borage oil contains a complex mixture of triglycerides that can be analyzed using several methods. Gas chromatography (GC) of methyl ester derivatives and HPLC coupled with refractive index (RI) detection have been reasonably successful for triglyceride analysis.

However, derivatization in GC is problematic for complex mixtures, whereas refractive index lacks sensitivity and is not compatible with gradient elution. HPLC with UV detection is a better choice than refractive index, but due to the low UV wavelengths required to analyze triglycerides the choice of mobile phase solvents is limited, and baseline drift is common with gradient elution. Evaporative light scattering detection with Varian's ELSD is superior to both RI and UV detection.

Instrumentation

Column: C18 5 μ m, 250 x 4.6 mm
Detection: Varian ELSD (neb=25 °C, evap=50 °C, gas=1.4 SLM)

Materials and Reagents

Eluent A: ACN
Eluent B: DCM

Sample Preparation

Sample: 2 mg borage oil/mL

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 μ L
Gradient: 30-50 % B in 40 min, 50-90 % in 2 min, hold three min

Results and Discussion

The excellent baseline stability of the Varian ELSD is shown in Figure 1.

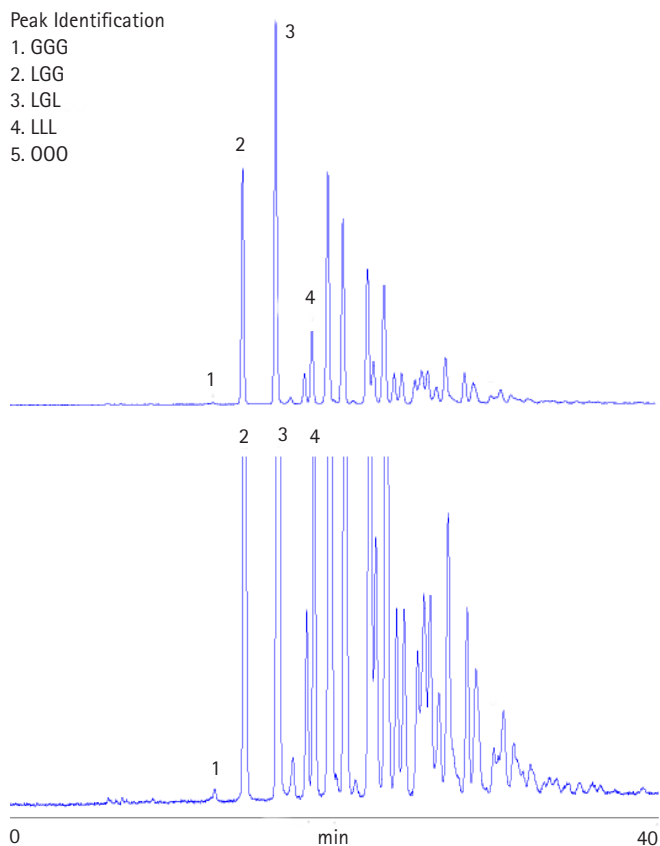


Figure 1. HPLC separation of borage oil using ELS detection (above) (with zoomed baseline (below)) showing the excellent baseline stability of the Varian ELSD.

Table 1. Abbreviations for fatty acids.

Symbol	Compound	No of Carbons
P	Palmitic acid	16
O	Oleic acid (cis-9)	18
L	Linoleic acid (cis, cis-9, 12)	18
Ln	α -Linolenic acid	18
G	γ -Linolenic acid (GLA)	18

Conclusion

The Varian ELSD revealed the true composition of triglycerides in borage oil, due to its sensitivity to compounds that possess weak or no UV chromophores. As the Varian ELSD is universal and independent of the optical properties of the compound, it detects any compound that is less volatile than the mobile phase. For samples, such as borage oil, that possess a complex triglycerides profile, gradient elution is required to provide optimum resolution and reduce run time. This is no problem for the Varian ELSD because it is compatible with a wide range of solvent gradients, displaying excellent baseline stability.

The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELSD's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELSD provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

These data represent typical results. For further information, contact your local Varian Sales Office.

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