Corona® ultra™ Charged Aerosol Detector

Application note

Lipid Analysis by Reversed-Phase HPLC and Corona CAD: Natural Oils

Natural oils are used in cooking (e.g., olive oil) and to flavor foods (e.g., sesame, walnut oil, etc.). These oils contain a complex array of fatty acids (saturated, unsaturated, and polyunsaturated), not only in different molar ratios, but also occurring both free and as part of glycerolipids. Natural oils are a dietary source of free fatty acids, triglycerides and some essential nutrients.

A number of polyunsaturated fatty acids are essential nutrients only obtained from the diet. These include the omega-3 fatty acids series (alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) and the omega-6 fatty acids that are derived from linoleic acid. Omega-3 fatty acids, contained in fatty fish such as mackerel, herring, sardines, and salmon, and in flax oil, play a crucial role in brain function as well as normal growth and development. Evidence also suggests that omega-3 fatty acids can reduce inflammation and help lower risk factors associated with chronic diseases such as heart disease, cancer, and arthritis. Conversely, omega-6 fatty acids, abundant in meat, tend to promote inflammation. For individuals that do not regularly consume fatty fish, levels of omega-3 fatty acid levels can be increased by consuming fish oil supplements. These may also provide essential fat soluble vitamins such as A, D and E.

Characterization of these oils for quality, adulteration, or for basic research requires the use of sensitive, reproducible, and accurate analytical equipment and methods. The Corona *ultra* charged aerosol detector (CAD®), a sensitive mass-based detector, is

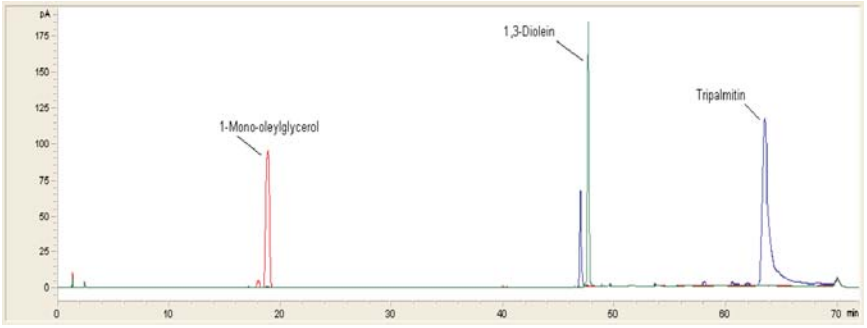

Figure 1. Acylated glycerols (1 mg/mL) In methanol/chloroform diluent (1:1 (v/v) for the mono and diglyceride; 1:3 (v/v) for tripalmitin).

Method Parameters

Column:	*Halo C8, 150 x 4.6 mm, 2.7 µm, 40 °C
Nebulizer Heater:	30 °C
Filter:	None
Mobile Phase A:	Methanol/Water/Acetic Acid (750:250:4)
Mobile Phase B:	Acetonitrile/Methanol/THF/Acetic Acid (500:375:125:4)
Gradient Profile:	Table 1
Flow Rate:	0.8 mL/min
Run Time:	72 minutes
Injection Volume:	10µL at 10 °C

Time	%A	%B	Flow Rate
0.0	100	0	0.8
40.0	30	70	0.8
60.0	10	90	0.8
65.0	10	90	0.8
65.1	100	0	0.8
72.0	100	0	0.8

Table 1. Gradient and Flow Profile.

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especially well-suited for the determination of lipids. As presented in this application note, when combined with reversed-phase chromatography, this detector enables the quantitation of low-level analytes covering a wide range of molecules, from free fatty acids to fat soluble vitamins, while maintaining sufficient specificity to resolve impurities down to low-nanogram levels.

Results and Discussion

To qualitatively define the regions of the chromatogram, examples of different lipid classes were analyzed. In Figure 1, samples of mono-, di-, and triacylglycerides, are overlayed to define the glyceride regions of the chromatogram. With this gradient, tripalmitin is the heaviest triglyceride that can be chromatographed.

Analysis of olive oil is presented in Figure 2. Note the separation within each class of glyceride. Better resolution of the triglycerides can be achieved using a more specific gradient to this lipid class: see Application Note: 70-8323 - **Lipid Analysis by Reversed-Phase HPLC and Corona CAD: Triglycerides**.

Chromatograms for walnut and sesame oils, are presented in Figures 3 and 4, respectively. Note that, based on analyte retention time, the sesame oil appears to contain more triglycerides enriched with slightly larger acids, than the walnut oil.

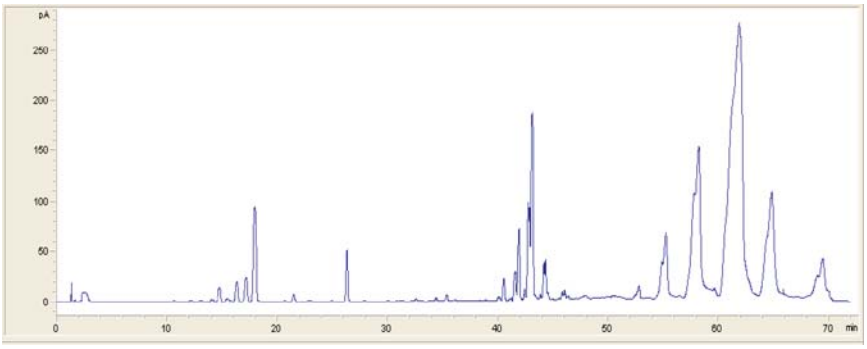

Figure 2. Olive oil 100 µL/mL in methanol/chloroform (1:1) diluent.

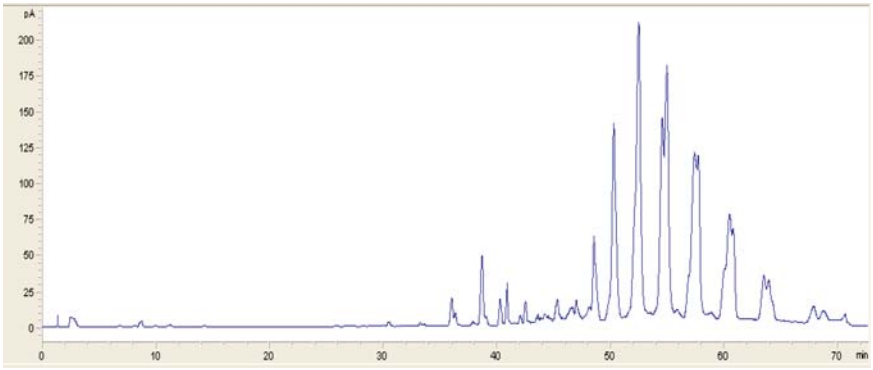

Figure 3. Walnut oil 100 µL/mL in methanol/chloroform (1:1) diluent.

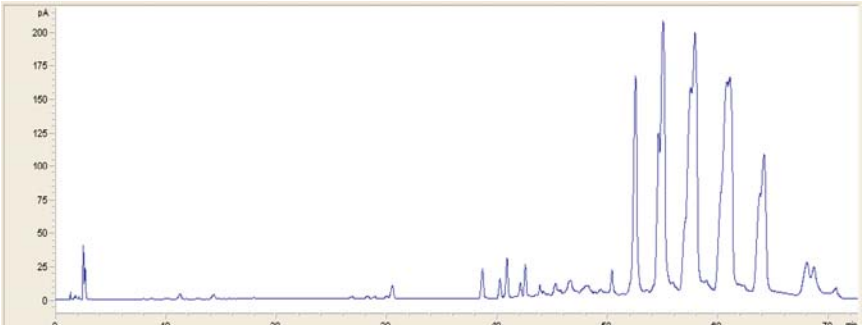

Figure 4. Sesame oil, 100 µL/mL in methanol/chloroform (1:1) diluent.

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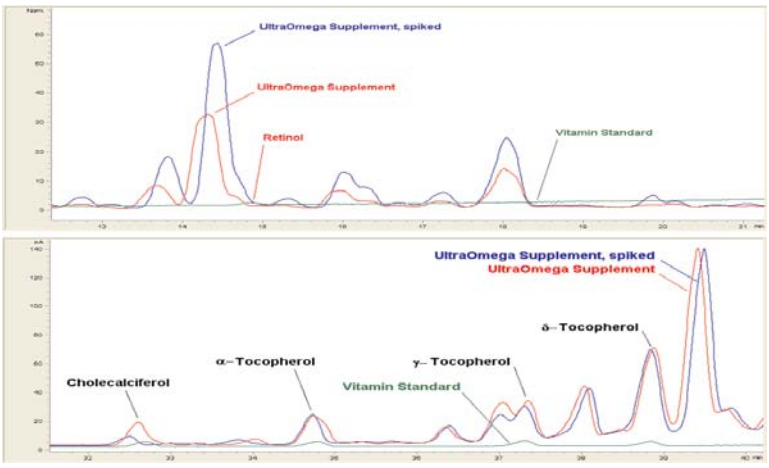

Figure 5. Analysis of an OTC omega-rich fish oil supplement. Various fat soluble vitamins and derivatives were identified by spiking external standards into the oil sample.

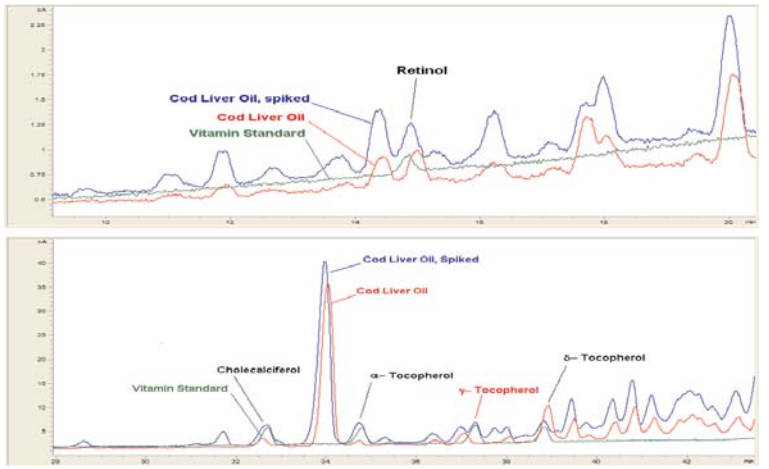

Figure 6. Analysis of a cod liver oil supplement. Various fat soluble vitamins and derivatives were identified by spiking external standards into the oil sample.

Figures 5 and 6 show the analysis of omega-fortified fish oil capsules (from anchovy and s ardine) and cod liver oil capsules, respectively. The fish oil sample, supplemented with synthetic vitamin E (d-alpha-tocopherol), was also found to contain other forms of tocopherol in addition to alpha-tocopherol, a low level of vitamin D (cholecalciferol) but no vitamin A (retinol). The coc liver oil sample contained endogenous levels of vitamins A, D and two forms of vitamin E (no gamma-tocopherol was found).

Conclusions

The general method described in this application note is capable of separating many of the lipid classes in natural oils including free fatty acids, glycerolipids and fat soluble vitamins. It can be used to generate a "lipid fingerprint" for many kinds of samples. By simple modification, the method can be tailored for a specific lipid class in order to decrease analytical run time.

Ordering Information

Corona *ultra* Charged Aerosol Detector	70-8773
Nitrogen generator	70-6003

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ESA - A Dionex Corporation
22 Alpha Road
Chelmsford, MA 01824-4171 USA
Tel: (800) 959-5095
Tel: (978) 250-7000
Fax: (978) 250-7090
Email: na_esa_info@dionex.com
www.coronaultra.com

Corporate Headquarters
Dionex Corporation
1228 Titan Way
Sunnyvale, CA 94088-3603
Tel: (408) 737-0700
Fax: (408) 730-9403
www.dionex.com

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