Corona® Charged Aerosol Detector

Application note

Lipid Analysis by Reversed-Phase HPLC and Corona CAD: Algal Oil

The interest in biofuels as a renewable source of liquid fuel has increased due to concerns over petroleum supplies and accessibility. Along with biodiesel, which is synthesized from vegetable oil, oil from algae is being developed as an alternate fuel source. Algal oil can be transformed into multiple fuel types, including diesel, kerosene, and gasoline. Currently (2008), corn and soy crops are used to produce ethanol and biodiesel, and unlike algal oil, have not been made into gasoline. Successful development of this potential energy source could provide a complete transformation of America's energy sources: unlike soy and corn-based fuels, algal fuels do not require the use of food crops, algae is easy to grow, algae does not require vast amounts of land to produce useful quantities of oil reducing deforestation and soil depletion, and the little solid waste that is produced can be used in other products. Most of the carbon dioxide that is recovered from the atmosphere during algal growth is turned into oil rather than silage produced from vegetable sources.

To aid in the development of this resource, analytical tools such as HPLC play an important part. Described herein is a method that can provide useful information about the components that are found within crude algae oil, as well as useful information that can assist in the development of processing technology. This method uses gradient reversed phase HPLC, to separate crude algae oil into its various components, combined with a universal mass detector, the Corona® Charged Aerosol Detector (CAD®), which can detect all non-volatile analytes down to low nanograms on column.

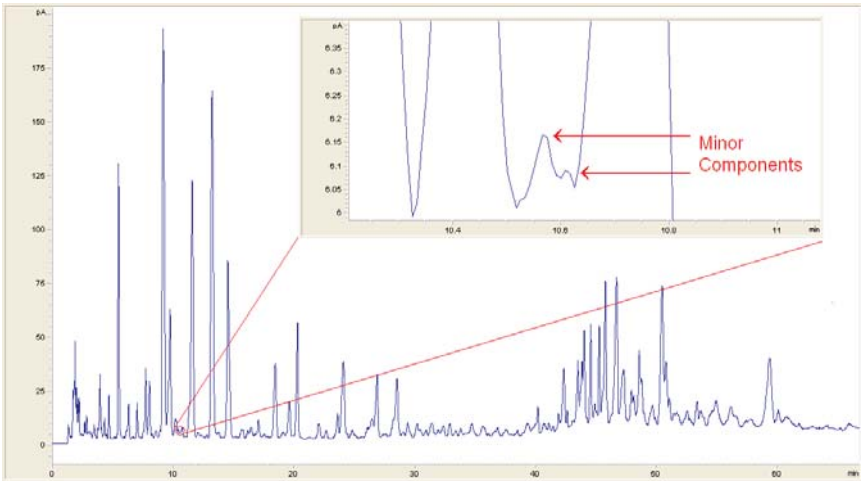

Figure 1. Analysis of Crude Algae Oil Extract* (10µg o.c.) in Methanol/chloroform (1:1) with Two Minor Analytes Highlighted in Inset.

In a different aspect, the sensitivity of the Corona CAD may also enable the identification of new compounds found during Drug Discovery, using this or other complex biological samples. Minor components that have been found in such samples, if they are pharmacologically active, can also be highly potent. An example of the CAD's sensitivity for minor components, typically in low-nanograms on column for non-volatile compounds, can be seen in Figure 1. Since the mobile phase is fully compatible with mass spectrometers, the CAD can also be easily coupled with an MS system to allow for quick identification of these compounds of interest.

The method described in this application note offers a simple but rapid procedure to help characterize the process for algae oil production using standard HPLC equipment and the Corona CAD.

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Method Parameters

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

Sample Concentration: 1mg/mL in Methanol/chloroform (1:1)

Time	%A	%B
0.0	100	0
40.0	60	70
60.0	10	90
65.0	10	90
65.1	100	0
72.0	100	0

Table 1. Gradient and Flow Profile.

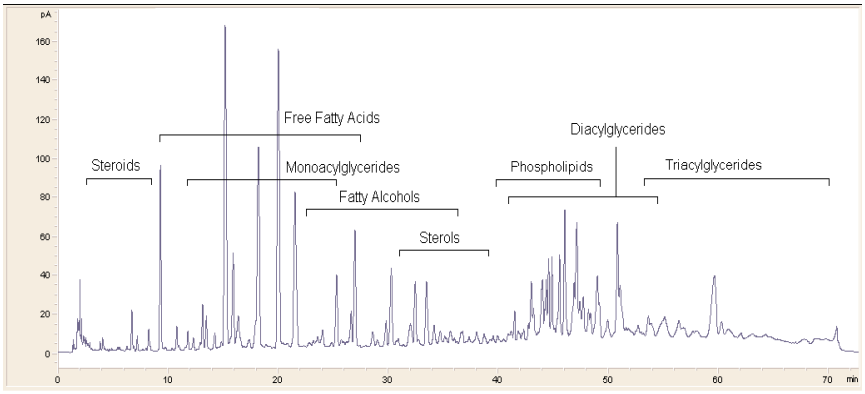

Figure 2. Analysis of Crude Algae Oil Extract* (10µg o.c.) in Methanol/chloroform (1:1) with General Lipid Classes Outlined.

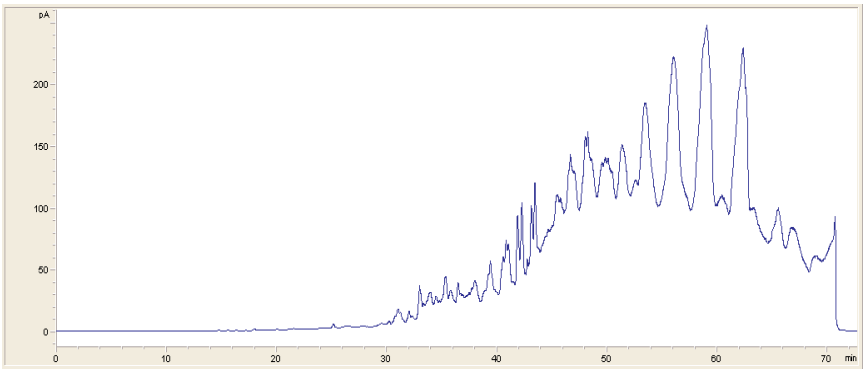

Figure 3. Analysis of Crude Algae Oil Extract* (10µg o.c.) Stripped of Hydrophilic Materials, in Methanol/chloroform (1:1).

The Corona® Charged Aerosol Detector

Results and Discussion

Several classes of lipids are identifiable using the method: free fatty acids, fatty alcohols, phospholipids, mono-, di-, and triacyl-glycerides, sterols and steroids, and fat-soluble vitamins (not shown) are all present in a single chromatogram, as shown in Figure 2. Also, many of these compounds are not visible with UV/Vis detection alone, which makes the Corona CAD the ideal detector for these samples. Another benefit lies in the relative uniformity of response of the CAD with different materials: typical response factors lie within 20% of each other, making estimates of mass-% from peak area-% more reliable and accurate than with other means of detection.

In developing a process for biofuels from algal oil, one of the first steps is to remove the hydrophilic material, leaving the more suitable, hydrophobic material for further process development. A different sample of algal oil that was stripped of hydrophilic material was chromatographed using the same conditions and is shown in Figure 3. This is algal oil is different than that depicted in Figure 2, but the load of 10 µg is the same. As can be seen, the higher amount of hydrophobic analytes causes the baseline to appear raised. A lower amount of sample injected may lessen this effect.

Once the crude material has been transformed into a biofuel, like biodiesel, the Corona CAD can also be used to further characterize the finished product. Application Note #70-8305, "Biodiesel Analysis by Normal Phase HPLC and Corona CAD," quantifies remaining free and bound glyceride contaminants that occur during base-catalyzed trans-esterification of vegetable oils.

Conclusions

This application note describes an analytical method which effectively characterizes lipid samples obtained from algae oil extracts. The Corona CAD has the sensitivity to see low-level compounds for the researcher or analytical chemist, and it has reduced chemical requirements (analytes are only required to not be volatile) to allow for a broad range of molecular species to be measured. With this analysis, it is also possible to generate important information for the process development scientist working on a robust and well-qualified method. The uniformity of response for analytes on the Corona CAD allows for a more accurate picture of analyte quantities than other methods of detection.

Ordering Information

Corona Plus Charged Aerosol Detector	70-7041
Thermal Organizer Module	70-5499TA
Nitrogen generator	70-6003
Pump, model 584 RS-232**	70-7058
Quaternary Low Pressure Gradient	70-5260
Autosampler, model 542	70-4152
EZChrom Elite™ for ESA software including PC	70-5073

*ESA wishes to thank Mac-Mod Analytical, Inc. (Chadds Ford, PA) for the use of their column and Sapphire Energy (San Diego, CA) for the algal extract.

**Modification required - contact ESA Technical Support.

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MAGELLAN BIOSCIENCES

ESA Biosciences, Inc.
22 Alpha Road
Chelmsford, MA 01824-4171
USA
+1 978.250.7000 Phone
+1 978.250.7090 Fax
+1 800.959.5095 US Toll free
Email: info@esainc.com
www.esainc.com

ESA Analytical
Brook Farm, Dorton, Aylesbury
Buckinghamshire HP18 9NH
England
+44 (0)1844 239381 Phone
+44 (0)1844 239382 Fax
Email: info@esainc.com
www.esainc.com

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