Corona® Charged Aerosol Detector

Application note

# Lipid Analysis by Reversed-Phase HPLC and Corona CAD: Free Fatty Acids

A fatty acid is a carboxylic acid often with a long unbranched aliphatic tail (chain). In mammals, the carbon chain is typically 4-28 in length, unbranched and even numbered. The carbon chain can be either saturated or unsaturated (containing double bonds). Saturated fatty acids can have double bonds in either the cis (naturally occurring) or trans (man made by hydrogenation of plant oils) conformation. Essential fatty acids (those that cannot be made by the human body) include a number of polyunsaturated fatty acids (PUFAs), including linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids.

Free fatty acids are produced by the hydrolysis of the ester linkages in a fat or biological oil (both of which are triglycerides). Free fatty acids act as fuel molecules (used to generate ATP during catabolism). Essential fatty acids are important in several systems *in vivo*, including the immune system and in blood pressure regulation, since they are used to make compounds such as prostaglandins. In the brain, some essential fatty acids are converted to anandamide (and other endocannabinoid derivatives), an endogenous neurotransmitter potentially involved with numerous behaviors (such as sleeping, eating) and memory. Unfortunately, PUFAs can undergo lipid peroxidation (e.g., rancidity in butter) resulting in the endogenous production of potentially cytotoxic derivatives (4-hydroxynonenal and malondialdehyde).

The Corona® Charged Aerosol Detector (CAD®), a sensitive mass-based detector, is especially well-suited for the determination of non-volatile analytes. When combined with reversed-phase chromatography, this detector enables the sensitive quantitation of a wide range of lipids, from free fatty acids to pure alkanes, at the low-nanogram level. Although only free fatty acid data are presented here, the gradient can also be adjusted to shorten the run time and/or to optimize separation of the different lipid classes.

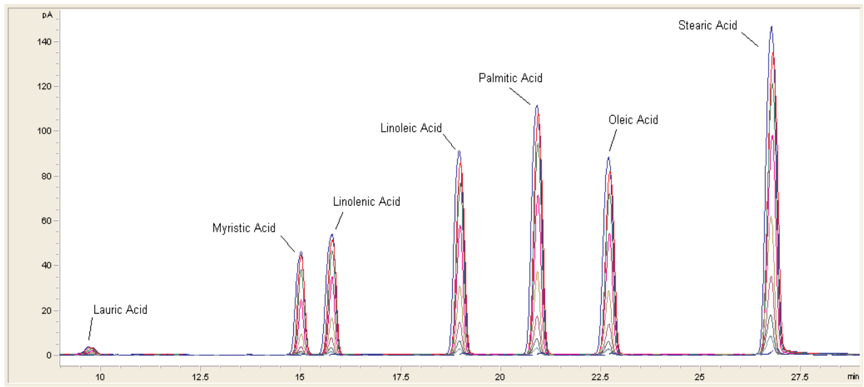


**Figure 1. Overlaid Chromatograms of Different Free Fatty Acids (110-11,000ng on column).**

## Method Parameters

Column: *Halo C8, 150 x 4.6 mm, 2.7 µm, 40°C
Detector: Corona CAD Plus
Nebulizer Heater: On
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
Sample Concentration: 1mg/mL in Methanol/Chloroform (1:1 up to 1:3).

esa MAGELLAN BIOSCIENCES

# The Corona®
# Charged Aerosol Detector


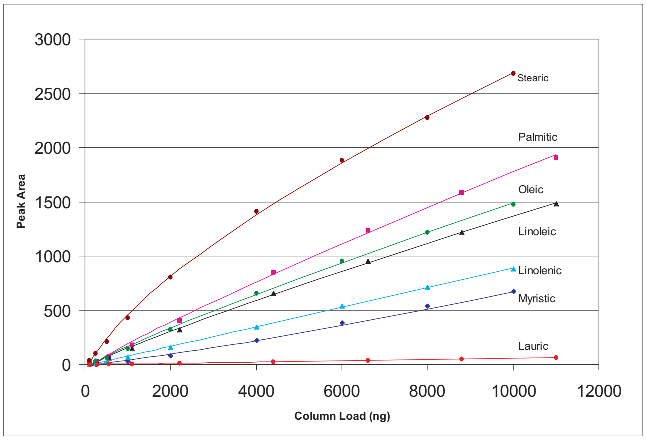


**Figure 2. HPLC-CAD Standardization Curves for Free Fatty Acids (110-11,000ng on column).**

| Time | %A | %B |
|---|---|---|
| 0.0 | 100 | 0 |
| 40.0 | 30 | 70 |
| 60.0 | 10 | 90 |
| 65.0 | 10 | 90 |
| 65.1 | 100 | 0 |
| 72.0 | 100 | 0 |

**Table 1. Gradient and Flow Profile.**

| Acid | Dynamic Range[1] (ng) | Recovery Range (%) | Correlation Coefficient $R^2$ | LOQ[1] (ng) |
|---|---|---|---|---|
| Lauric | 550-11,000+ | 94.4-108.9 | 0.9987 | 130 |
| Myristic | 1000-10,000+ | 93.0-112.2 | 0.9980 | 50 |
| Linolenic | 275-11,000+ | 89.5-107.4 | 0.9994 | 30 |
| Linoleic | 550-11,000+ | 96.1-109.8 | 0.9992 | 20 |
| Oleic | 500-10,000+ | 95.8-110.7 | 0.9991 | 20 |
| Palmitic | 550-11,000+ | 95.0-113.3 | 0.9988 | 20 |
| Stearic | 250-10,000+ | 94.4-113.8 | 0.9990 | 10 |

**Table 2. Standardization Data.**

**[1]Dynamic Range based on recovery (85.0 – 115.0%) at the highest loads. Higher loads are possible, and the dynamic range should extend from LOQ upwards. [1]LOQ values based on extrapolation of peak heights from 100 or 110 ng load to 10 x S/N.**

## Results and Discussion

Figure 1 illustrates the effects of chemical structure on retention time and response. Shorter carbon chain length and degree of unsaturation is associated with both decreased retention time and reduced response (the latter resulting from increased vapor pressure). Standardization curves for these compounds, all dissolved in methanol/chloroform (1:1), are shown in Figure 2. The response of the different analytes tend to fall roughly into three categories: those that are too volatile and show poor response (e.g., lauric and myristic acids); those that show some degree of semi-volatility (e.g., oleic and linolenic acids), and those that are essentially non-volatile (stearic acid), and give the typical response of all non-volatile species. The dynamic range, recovery range, correlation coefficient (using equation $Load = A(area)^b + C$) and estimated LOQ are presented in Table 2.

## Conclusions

The data presented here illustrate the ability of RP-HPLC-CAD to measure a variety of different fatty acids. Chemical structure plays a key role in both analyte retention and response. This method can also be adapted to measure a wide variety of different lipid classes.

## Ordering Information

| | |
|---|---|
| Corona Plus Charged Aerosol Detector | 70-7041 |
| Thermal Organizer Module | 70-5499TA |
| Nitrogen generator | 70-6003 |
| Pump, model 584** | 70-7091 |
| Quaternary Low Pressure Gradient | 70-5260 |
| Autosampler, model 542 | 70-4152 |
| EZChrom Elite(TM) for ESA software including PC | 70-5073 |

*ESA wishes to thank Mac-Mod Analytical, Inc. (Chadds Ford, PA) for the use of their column.

**Modification required - contact ESA Technical Support.

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



70-8332
Rev A