Corona® Charged Aerosol Detector

Application note

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

Fatty alcohols are aliphatic alcohols derived from natural fats and oils, found in plants, animals and algae. Fatty alcohols usually have even number of carbon atoms with the hydroxyl group attached to the terminal carbon. Due to their amphipathic nature, fatty alcohols behave as nonionic surfactants. They are also used as emollients, lubricants, emulsifiers, and also as an active pharmaceutical ingredient for anti-viral medications.

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 alcohol 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.

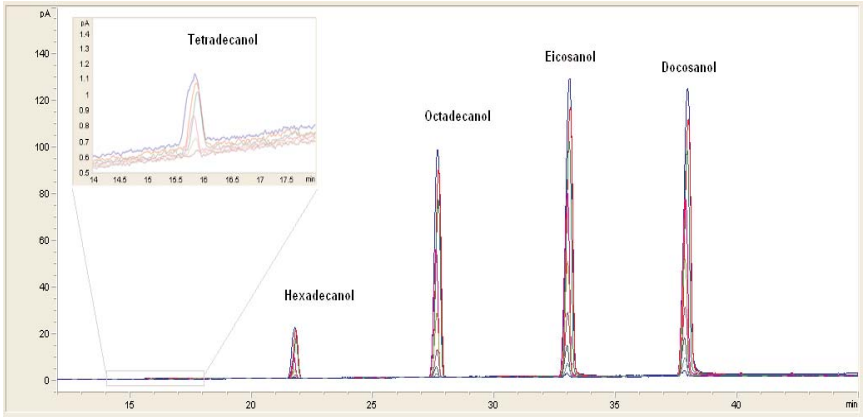

Figure 1. Overlaid Chromatograms of Different Free Fatty Alcohols (100-12,000ng on column, except for Tetradecanol at 1100-11,000ng).

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.

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: 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)

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The Corona® Charged Aerosol Detector

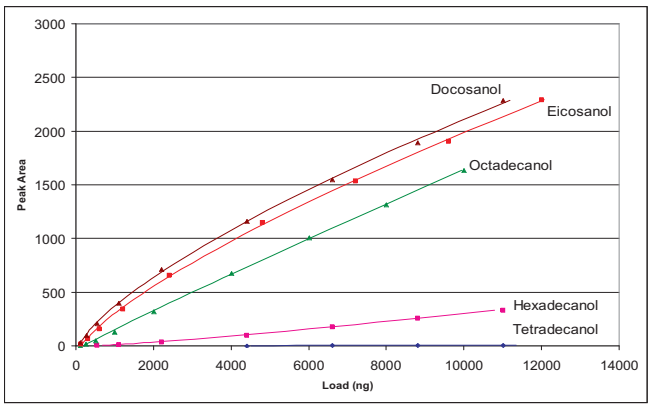

Figure 2. HPLC-CAD Standardization Curves for Free Fatty Alcohols (110-12,000ng on column).

Alcohol	Dynamic Range[1] (ng)	Recovery Range (%)	Correlation Coefficient R^2	LOQ[2] (ng)
Tetradecanol	4500-11,000+	95.7-103.2	0.9852	3000
Hexadecanol	1100-11,000+	94.4-100.5	0.9991	500
Octadecanol	250-10,000+	88.8-107.2	0.9995	60
Eicosanol	120-12,000	89.0-102.5	0.9994	30
Docosanol	110-11,000	90.5-104.7	0.9991	15

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 the LOQ upwards. [2]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 later 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., tetradecanol and hexadecanol); those that show some degree of semi-volatility (e.g., octadecanol); and those that are essentially non-volatile, and give the typical response of all non-volatile species (e.g., eicosanol and docosanol). 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 alcohols. 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

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