



Reversed Phase HPLC of Fatty Acids

Application Note

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Introduction

The analysis of lipids is of particular importance to the food industry; a variety of lipid compounds are used commercially, particularly in processed foods. Meat and cereals contain naturally-occurring lipids ranging from polar phospholipids, fatty acids, diglycerides and cholesterol to non-polar fats and oils (triglycerides). Processed foods may also contain additional spray dried or encapsulated fats and emulsifiers. Health issues related to excessive dietary fat intake include obesity, increased risk of some forms of cancer and cholesterol deposition in cardiovascular diseases such as atherosclerosis (hardening of the arteries). Identification and quantification of the different types of lipid and their fatty acid composition is therefore required, but is complicated by the difficulty in detection due to the absence of a strong UV chromophore.

Fatty acids are long hydrocarbon chains with terminal carboxylate groups, and form a major component of triacylglycerides, phospholipids and sphingolipids. More than 1000 naturally-occurring fatty acids have been identified, but most common lipids contain only a few of this extensive group. Biological systems usually contain fatty acids with an even number of carbon atoms, between 14 and 24, the most common between 16 and 18 carbon atoms. In animals, these chains are invariably unbranched. The hydrocarbon chain can contain one or more *cis* configuration double bonds. These double bonds dramatically affect the physical properties of the fatty acids. Stearic and oleic acid are both 18 carbon atoms long, but oleic acid has one double bond and a melting point of 13.4 °C, in comparison to stearic acid which is saturated and has a melting point of 69.6 °C.

Fatty acids that contain no double bonds, when analyzed by reversed phase chromatography, are separated by chain length, the shortest eluting first.



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A PLRP-S column can be used to separate fatty acids in a variety of media. These columns are robust enough to be stable at pH 1-14 and cope with vigorous clean up procedures and aggressive eluents. The Agilent evaporative light scattering detector is an ideal detector for the analysis of fatty acids. Although these acids can be detected by UV at 210 nm, the tetrahydrofuran itself will absorb. However, the changing composition of the eluent does not present a problem for the Agilent ELSD, as it is evaporated before reaching the light scattering cell. This method of detection produces a flat, stable baseline, as illustrated in the following examples using the same experimental conditions.

Conditions

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5800)
 Eluent A: 60 mM Acetic acid
 Eluent B: ACN
 Eluent C: THF
 Gradient: 35:60:5 to 0:90:10 in 20 min
 Flow Rate: 0.5 mL/min
 Detection: Agilent ELSD (neb=80 °C, evap=70 °C, gas=1.0 SLM)

Results and Discussion

Figure 1 shows good separation of seven fatty acids and Figure 2 shows two of the fatty acids in evening primrose oil. Good baseline resolution was achieved through the use of PLRP-S reversed phase material.

Peak Identification

1. Capric acid
2. Lauric acid
3. Myristic acid
4. Linolenic acid
5. Linoleic acid
6. Palmitic acid
7. Stearic acid

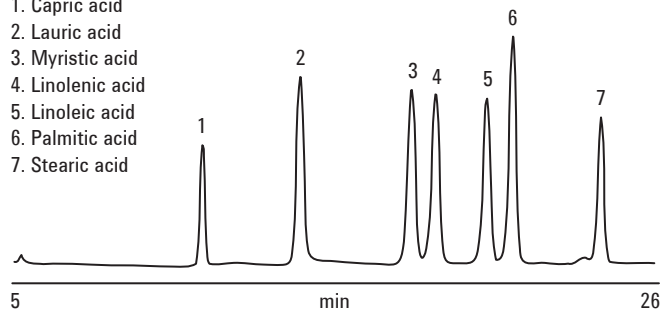


Figure 1. Separation of seven fatty acids using PLRP-S media.

Peak Identification

1. Linoleic acid
2. Palmitic acid

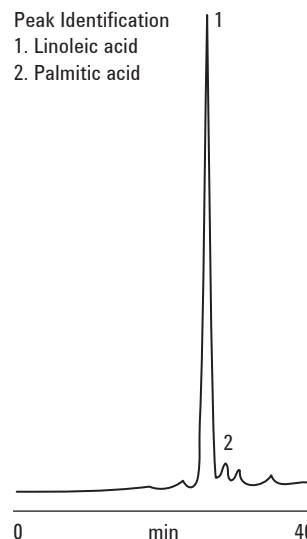


Figure 2. Fatty acid composition of evening primrose oil revealed by PLRP-S.

Conclusion

Coupling a PLRP-S column with the Agilent ELSD provides an ideal system for the quantitation of fatty acids, essential for their structural analysis and solute identification. As a single column, PLRP-S operates across the entire range of HPLC eluents. It is chemically stable and physically robust and so it is possible to switch between organic modifiers, such as ACN and tetrahydrofuran, and eluent pH 0 to 14.

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