Analyze Dietary Fatty Acids, Sterols, and Lignans with an Agilent J&W DB-5ms UI Column

Application Note

Foods Testing & Agriculture

Authors

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Abstract

The introduction of Agilent J&W Ultra Inert (UI) capillary GC columns and the improved peak shape they afford suggested that it was possible to determine fatty acid profiles without the need to make the fatty acid methyl esters (FAMEs). The Agilent 7696A Prep WorkBench was, therefore, used to prepare canola and extra-virgin olive oils for analysis of fatty acids as their hydrolysis products. Sterols and lignans were coextracted and assayed in their native form. Completion of the reaction was monitored on an Agilent J&W DB-5ms UI GC column. Acceptable peak shape was demonstrated for unsaturated fatty acids up to docosanoic acid (C22). Resolution of oleic (C18:1) from stearic (C18:0) acid in a typical sample was achieved.

Introduction

While good peak shape for methyl esters and improved volatility relative to free fatty acids is advantageous, the ability to use tools, such as negative mode electrospray, provides a viable detection technique for organic acids in HPLC. These compounds have low wavelength UV chromophores that would present challenges if common HPLC mobile phases or buffers were used to adjust resolution. Having the ability to prepare samples for either GC or HPLC offers greater flexibility to complete analytical tasks, saving time and resources. Many modern laboratories engaged in dietary nutritional labeling are equipped with gas and liquid chromatography, and the use of LC/MS has increased dramatically. While the fatty acid profile for all consumer animal and vegetable fats is well established [1], the ability to hydrolyze these water-immiscible, highly viscous substrates presents a challenge for automated liquid handling systems. The 7696A Prep WorkBench performs all of the

steps in the sample preparation once the samples are weighed accurately and mixed in a 1:1 ratio with hexane. The hydrolysis technique that works best is saponification with NaOH in a 3:1 mole ratio, but this requires considerable time, at least 24 hours. This sample prep bottleneck is alleviated by batching as many as 20 to 30 samples and letting the automation run overnight unattended. Run times on the gas chromatography assay are approximately 40 minutes and permit the monitoring of unreacted vegetable oil triglycerides [2].

Materials and Methods

An Agilent 7890A Series GC was coupled to an Agilent 5975C Series GC/MSD System with the Inert EI 350 noncoated source. A similar setup using an FID detector was used for crude reaction monitoring.

Conditions

GC conditions

Column:	Agilent J&W DB-5ms UI, 10 m × 0.25 mm, 0.25 µm (p/n 122-5532UI)

Sample preparation

Sample:	Canola oil, extra-virgin olive oil saponified, and hexane extracted
Carrier:	MSD helium, FID hydrogen for reaction monitoring, both at 1.0 mL/min constant flow
Oven:	120 °C (hold 1 min, to 325 °C at 20 °C/min (hold 20 min)
Injection:	Split/splitless, 100 mL/min split flow with gas saver on at 5 min, 3 mL/min purge flow
Inlet temperature:	280 °C
Detector:	FID for reaction monitoring at 325 °C
MSD transfer aux temperature:	325 °C
GC:	Agilent 7890A Series GC
Sampler:	Agilent 7693 Automatic Liquid Sampler, 5 µL syringe 0.1 µL injection, split 20:1

MS conditions

MS:	Agilent 5975C Series GC/MSD System with EI inert 350 source, tandem axis detector
Solvent delay:	1 min
MS temperature:	300 °C (source); 150 °C (quad)
Scan range:	30 to 550

Flow path supplies

Vials:	Amber screw cap (p/n 5182-0716)
Caps:	Blue screw cap (p/n 5282-0723)
Vial inserts:	250 µL glass with polymer feet (p/n 5181-1270)
Syringe:	5 µL (p/n 5181-1273)
Septum:	Red Bleed Temp Optimized BTO (p/n 5183-4757)
Inlet liner:	Dual Taper Direct Connect (p/n G1544-80700)
Magnifier:	20x (p/n 430-1020)

Standards preparation

A six-component checkout mix at 10 ng/µL in hexane consisting of C12 through C22 was used to provide a column evaluation for peak shape up to the maximum allowable operating temperature of the column. The compounds can be purchased as pure standards in kit form from Grace (even carbon number p/n 186021) as 100 mg per compound. Pinoresinol was obtained from Sigma-Aldrich Corp. (catalog number SML-0073-1MG). Figure 1 demonstrates that chromatographic peak shape was visually acceptable for all components in the standard mix.

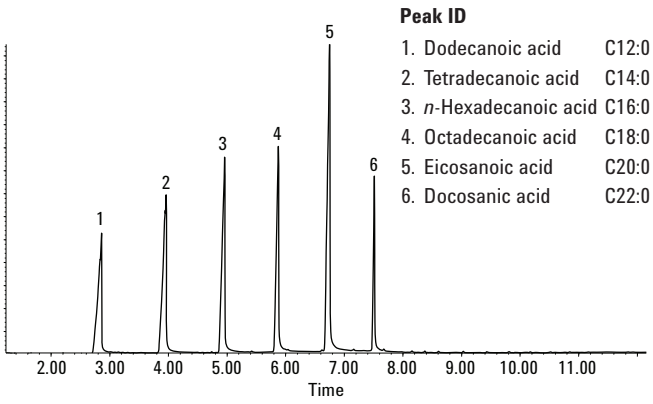

Figure 1. TIC of six-component free fatty acids evaluation mix.

Sample preparation

Vegetable oil samples for analysis were reacted/saponified and extracted using the 7696A Prep WorkBench, left to run overnight, and left unattended. Fish oil capsules high in omega-3 FAMEs were cut open with a razor. Their contents were washed with water, and hexane was extracted to remove glycerol from the formulation; no saponification was needed. Isolation of extra-virgin olive oil lignan markers was completed by multiple liquid/liquid extraction [3] in two steps with hexane:methanol and hexane:acetonitrile. Figure 2 shows a screen capture of the 7696A Prep Workbench parameters. This system allows for numerous reaction condition variations. The main problem to overcome is that the reactant oils separate from the aqueous NaOH solution. The conversion to acids can be brought to approximately 90% completion, providing sufficient quantity of the acids to allow accurate profiling as compared to reported values [4].

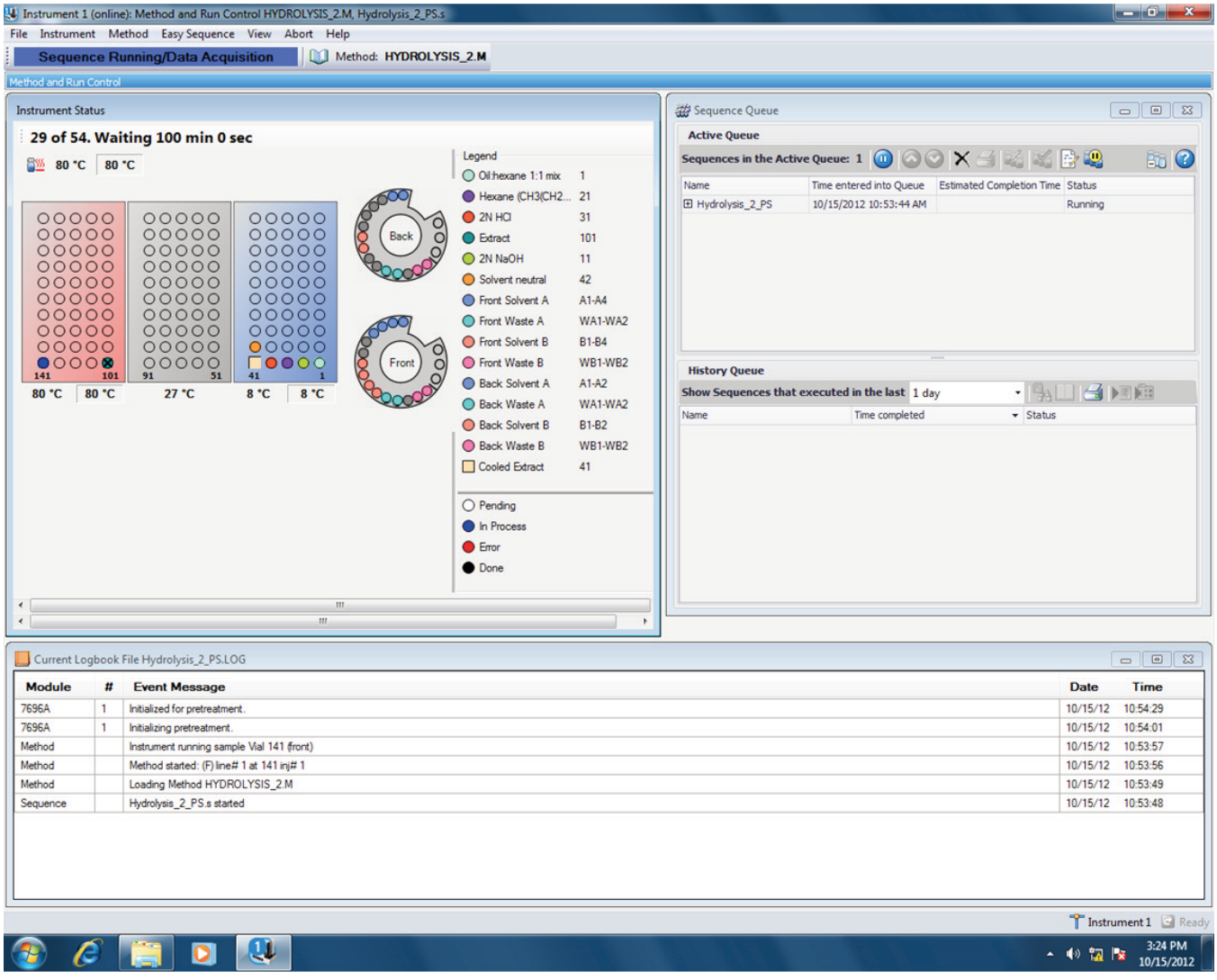

Figure 2. Agilent 7696A Prep WorkBench reagents and temperatures for hydrolysis.

Figure 3 shows the early crude reaction at 50% with elution of the unreacted mono-, di-, and triglycerides at the end of the chromatographic run.

Results and Discussion

Table 1 shows the peak width and tailing factor for the peaks shown in Figure 1, as calculated by Agilent ChemStation software. While the peak widths are considerably greater for the free acids than would be expected for the methyl esters, in the case of canola and olive oil, the separation of main constituent oleic acid (C18:1) from a trace amount of stearic acid (C18:0) is readily demonstrated in Figure 4. Separation of *cis* and *trans* isomers would require cyano functional group stationary phases, which are available in both gas and liquid chromatography columns. For C18 isomers, separation of their methyl esters can be achieved by GC using the Agilent J&W Select FAME stationary phase (p/n CP7421). Naturally occurring oils contain double bonds only in the *cis* configuration [5] but can be isomerized during processing and purification. Recovery and separation of the plant sterols, including vitamin E (tocopherol), can be seen in the TIC trace in Figure 5.

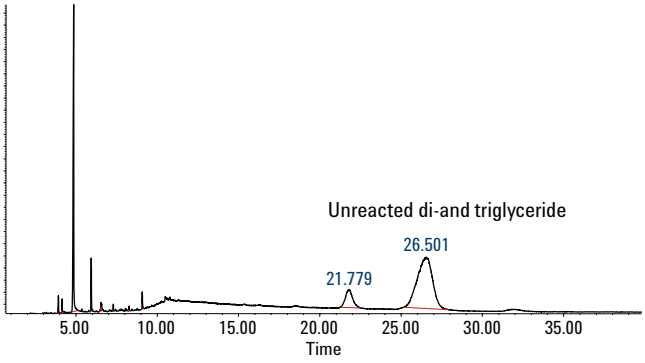

Figure 3. Agilent 7696A Prep WorkBench reaction at approximately 50% completion using GC conditions to elute all reactants.

Table 1. Calculation of peak width and tailing factor.

Component	Peak width	Tailing
C_{12} Dodecanoic acid	0.324	0.53
C_{14} Tetradecanoic acid	0.390	0.55
C_{16} *n*-Hexadecanoic acid	0.372	0.57
C_{18} Octadecanoic acid	0.238	0.61
C_{20} Eicosanoic acid	0.258	0.58
C_{22} Docosanoic acid	0.193	0.71

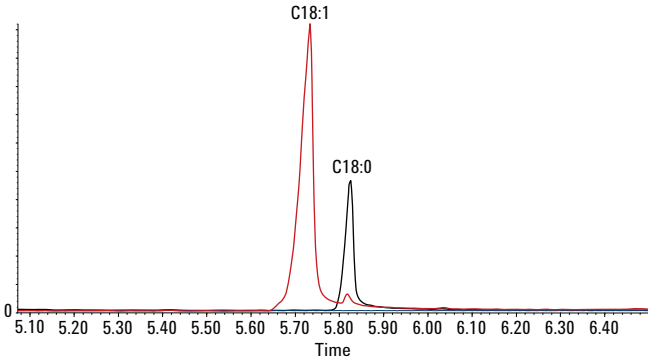

Figure 4. Separation of critical pair oleic (C18:1) from stearic (C18:0) acid.

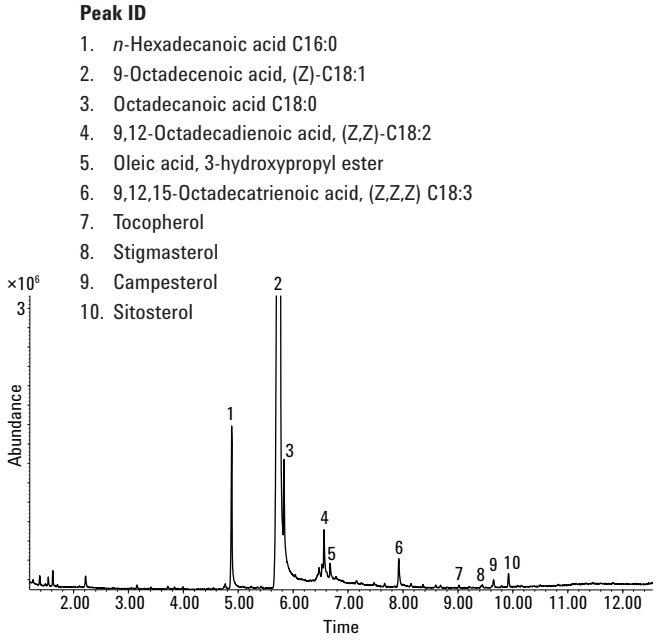

Figure 5. TIC trace of hydrolyzed canola oil showing all fatty acids and plant sterols.

The extra virgin olive oil lignan markers, pinoresinol and 1-acetoxyresinol, are shown as trace components in Figure 6, along with their EI spectra. These two compounds are not present in the recent NIST11 mass spectral library. Peak shape for the fish oil omega-3 esters of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) can be seen in Figure 7.

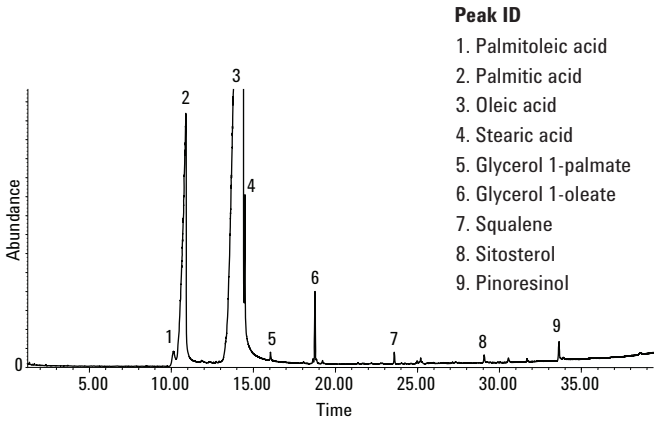

Figure 6. TIC trace of hydrolyzed extra virgin olive oil showing fatty acids and lignan markers with EI spectra.

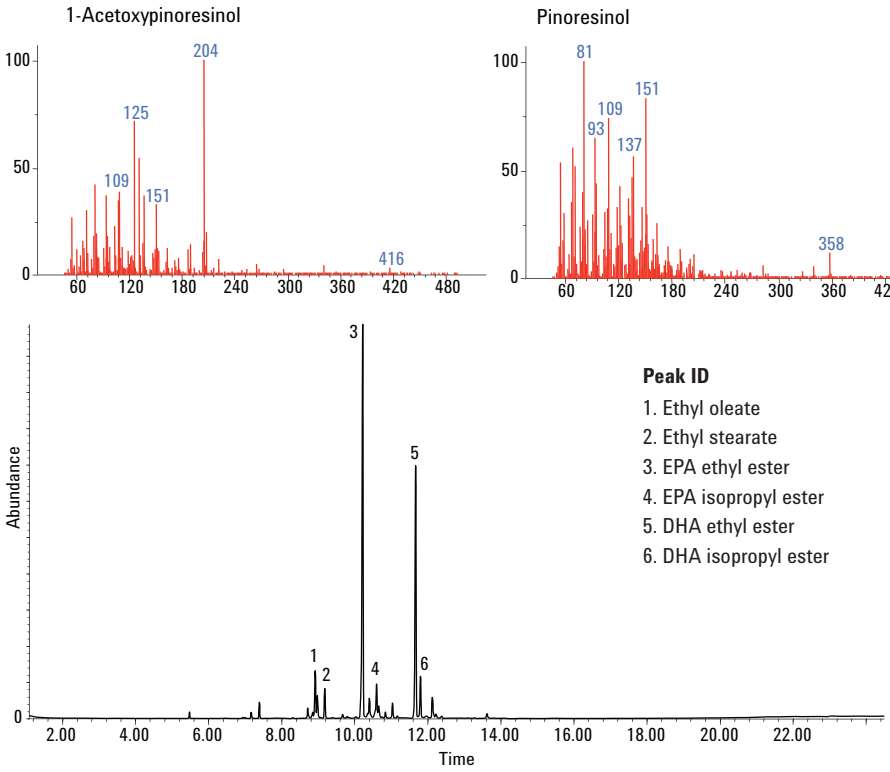

Figure 7. TIC trace of commercial fish oil omega-3 esters amenable to LC/MS with Atmospheric Pressure Chemical Ionization (APCI).

Conclusions

The Agilent J&W DB-5ms Ultra Inert GC column provides acceptable peak shape for free fatty acids. The ability to leverage both column chemistry and automated sample preparation permits flexibility to transfer the assay from GC/MS to LC/MS, with the potential to improve overall productivity and laboratory throughput by expanding instrumental resources beyond their typical application scope. In the normal evolution of analytical assays, sample preparation often becomes customized and provides numerous challenges. Removing obstacles such as cumbersome sample preparation always provides a step in the right direction toward increased laboratory productivity.

Acknowledgement

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