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The Separation of Saturated and Unsaturated Acids and FAMEs Using HP-FFAP and HP-INNOWax Columns

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Columns and Supplies

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Keywords

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Abstract

Both HP-FFAP and HP-INNOWax fused silica capillary columns were used with the HP 6890 GC for the analysis of free fatty acids and their derivatives in edible oils. Peak area reproducibility and peak symmetry were the primary criteria used to evaluate method and column suitability.

Introduction

Monitoring of the short chain organic acids (C2-C7) is a common analysis required for industrial fermentation processes. It can be done using a combination of headspace/GC or liquid-liquid extraction followed by GC analysis. However, the most common method is by direct injection of the acids in water. The direct injection method requires the use of non-siloxane containing stationary phases which are not decomposed in the presence of strong acids and water. Volatile organic acids can be difficult to quantify even with the proper choice of stationary phase.

The polar carboxylic acid group will interact strongly with any active sites on the column and this results in tailing peaks that can make quantitation difficult at low levels. The two requirements for successfully completing this analysis are proper phase selectivity and the use of very inert columns. Several publications have detailed this problem (1,2,3). This work will show that both columns provide adequate resolution of all components, **but peak area reproducibility and peak symmetry dictate the use of the HP-FFAP fused silica capillary column as the choice for volatile free acids.**

It is also desirable to analyze the higher molecular weight organic acids between C8 and C24. Unfortunately, the free acids are difficult to analyze because the temperatures required to elute all acids from the column can be in excess of the upper temperature limit of some stationary phases. For this reason, the fatty acids are derivatized to the methyl ester prior to analysis. Although troublesome and time consuming, the derivatized acids are more volatile and more compatible with the stationary phase chemistry.

The separation by carbon number and degree of unsaturation also requires a very polar stationary phase. Of primary importance is the separation of steric (C18:0), oleic (C18:1) linoleic (C18:2) and linolenic (C18:3) acid. Cross-linked polyethylene glycol (PEG) type phases are the preferred choice for this analysis. In this application both the HP-FFAP and HP-INNOWax columns accomplished the desired separation.

In other work (4,5) reviewing the use of HP-FFAP and HP-INNOWax columns for suitability in the analysis of FAMEs, both columns exhibited the desired selectivity and were highly inert. **The HP-INNOWax column had the advantage of lower column bleed at the high temperatures required to elute the highest FAMEs and faster analysis time because of the availability of thinner films.**

Experimental

Analytical Conditions

The GC analysis of free volatile organic acids and FAMES's was completed using an HP- 6890 Series GC equipped with a 150 psi inlet manifold and a flame ionization detector (FID). An HP G1513A autosampler and tray were used to automate the injection. The GC separations were done using an HP-FFAP 25 m x 0.32 mm x 0.5 um (Part Number 19091F-112) and an HP-INNOWax 30 m x 0.32 mm x 0.25 um (Part Number 19091N-133) columns.

Table 1. Reproducibility study on different columns

Compound Name	Retention Time	%RSD	Peak Area	%RSD	Peak Symmetry	%RSD
FFAP Column, Helium Carrier Gas						
Acetic acid	4.61	0.02	18.76	0.7	0.79	2.48
Proprionic acid	5.47	0.02	35.58	1.00	0.85	1.17
Iso-Butyric acid	5.77	0.09	51.32	0.91	0.87	1.23
Innowax Column, Helium Carrier Gas						
Acetic acid	4.32	0.04	18.36	5.94	0.34	1.50
Proprionic acid	5.00	0.02	37.49	0.89	0.50	2.58
Iso-Butyric acid	5.24	0.02	55.19	0.95	0.65	1.08

The injection volume for all free fatty acids dissolved in water was 0.5 ul to prevent vapor volume overloading of the liner (HP part number 19251-60540). A silanized glass wool plug was used to ensure complete vaporization of the sample. All injections of olive oil samples for FAME analyses were 1.5 µl.

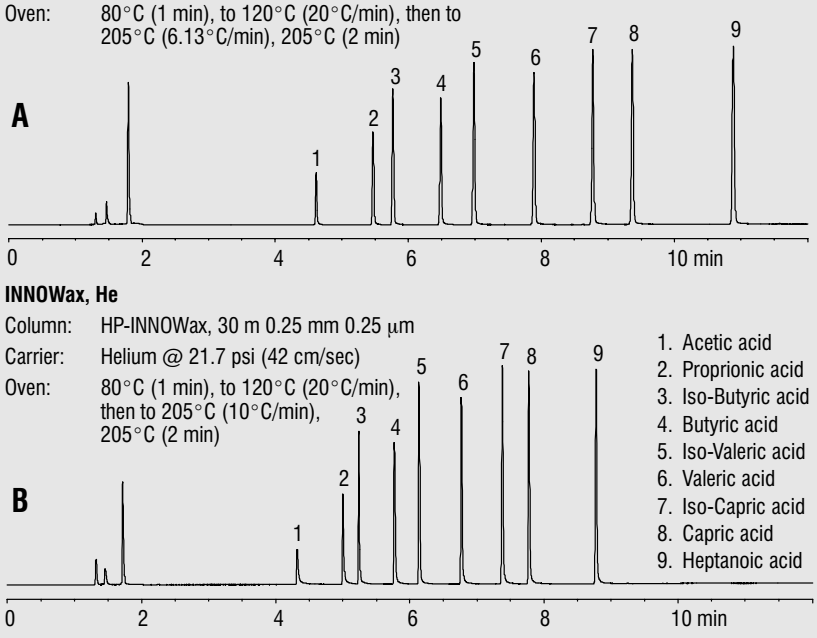

Figure 1. Free fatty acids (C2–C7) on different polar columns

Sample Preparation

A mixture of C2-C7 free acids in water was obtained from Supelco. The olive oil samples were purchased from a local food store.

The olive oils were derivatized for the FAMEs analyses using the following procedure (6).

1. Mix 1 µl of olive oil with 1 ml of a 3.75 M NaOH/Methanol solution and heat to 100 degrees C for 25 minutes.
2. Add 2 ml of 3.25 N HCl/ Methanol and mix. Heat to 80 degrees C for 10 minutes.
3. Add 1.25 ml of a 1/1 mixture of Hexane and MTBE and mix. Discard the bottom layer.
4. Add 3 ml of 0.3 M NaOH/ water and mix. Transfer top layer to GC vial for injection. Volume was reduced by 1/2 with a nitrogen purge.

Results and Discussion

Figure 1 shows a typical analysis of the free organic acids in water on both an HP-FFAP column (chromatogram A) and an HP- INNOWax column (chromatogram B) with helium as the carrier gas. An initial oven temperature of 80 degrees was needed with the agueous solvent to refocus the components on the column and prevent peak splitting. All of the acids were completely resolved from each other and the analysis was completed in approximately 11 minutes on the HP-FFAP column and 8 minutes on the IHP-INNOWax column.

Table 1 summarizes the results of the retention times, peak areas, and peak symmetry of ten repetitive injections for the three earliest eluting acids. Both columns showed excellent retention time reproducibility as a result of the automated sample introduction and the accurate carrier gas flow control. The HP-FFAP column showed better peak area reproducibility for the acetic acid and better peak symmetry than the Innowax column (a peak symmetry value of less than one indicates the peak is tailing). Similar results were obtained using hydrogen as a carrier gas with the added benefit that the analysis time was reduced by about 30 percent. Although both columns are suitable for this analysis, the HP-FFAP column is the column of choice because the peak areas for acetic acid are more reproducible and the peaks are much more symmetrical permitting quantitation at lower levels.

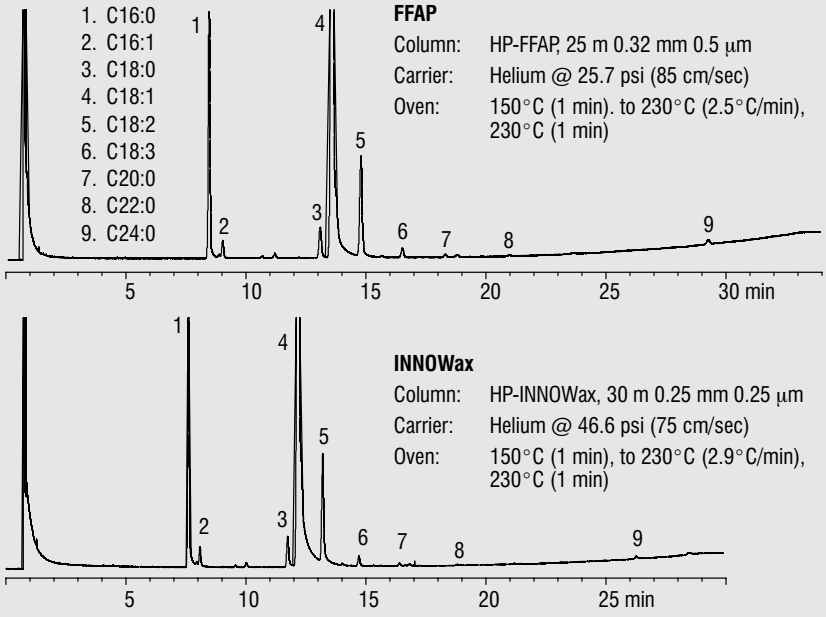

Figure 2. Derivatized olive oil (FAMEs) on two different columns

Table 2. Fatty acid methyl ester of different olive oils (area %)

Acid	Carbon Atom No.	Greece	Italy	France	Spain
Palmitic	16:0	12.5	11.5	12.2	11.5
Palmitoleic	16:1	0.8	0.9	0.8	0.8
Heptadecanoic	17:0	0.02	0.02	<0.02	0.02
Heptadecenoic	17:1	0.15	0.05	<0.02	0.2
Stearic	18:0	1.9	2.7	1.9	1.4
Oleic	18:1	76.3	73.1	73.0	74.9
Linoleic	18:2	6.7	9.2	10.0	9.6
Linolenic	18:3	0.6	0.8	0.9	0.5
Arachidic	20:0	0.05	<0.02	<0.02	0.05
Eicosenoic	20:1	0.03	0.03	<0.02	0.04
Behenic	22:0	<0.02	<0.02	<0.02	<0.02
Lignoceric	24:0	0.05	0.05	0.05	0.05

Both columns were also used for the analysis of FAMEs in olive oil. **Figure 2** shows the analysis of Greek olive oil on both the HP-FFAP and HP-INNOWax columns using helium as the carrier gas. Both columns successfully separated the saturated from the unsaturated FAMEs.

Although the olive oil shown here only separated the acids from palmitic acid (C16:0) and higher, these conditions can be used to separate all acids from caprylic acid (C8:0) to lignoceric acid (C24:0).

The oven temperature profile and the carrier gas flow rate were selected to help elute the components at as low a temperature as possible. This allows more reliable quantitation of the later eluting components because the column bleed is lower and there is less noise in the baseline. Using faster flow rates (higher linear velocity) under temperature programmed conditions does not cause loss of component resolution or excessive peak broadening even though the carrier gas velocity is twice as fast as isothermal chromatographic theory predicts for maximum efficiency.

Table 2 shows the analysis results for four different olive oils from four different regions of the world using the HP-FFAP column and showing the area percentages of the individual saturated and unsaturated FAMEs. There are noticeable differences in the area percentages for the major components C16 and C18 saturated and unsaturated peaks when using the same integration algorithm with the HP-INNOWax and the HP-FFAP columns. The faster thin- film HP-INNOWwax column provided cleaner separation of C16 to C18 components because of lower column bleed at high temperatures.

Table 3 summarizes the results of retention time and peak area reproducibility for ten replicate injections on both the HP-FFAP and HP-INNOWax columns. Both columns are well suited for the olive oil FAME analyses. Excellent peak retention time reproducibility and peak areas were obtained. Because of reduced bleed at high oven temperatures and shorter analysis times, the Innowax column probably provides the best choice for this application.

Conclusion

Both the HP-FFAP and HP-INNOWax columns are suitable for the analysis of volatile free fatty acids and fatty acid methyl esters using the HP 6890 Series GC and the HP G1530A autosampler. The better column for the volatile free fatty acid analyses is the HP-FFAP column because of better reproducible peak areas and less peak tailing especially for the most volatile components. The better column for the FAME analysis is the HP-INNOWax column because of reduced column bleed at high temperatures and shorter analysis times.

Table 3. Reproducibility study using Greek olive oil

Compound	Retention Time (min)	%RSD	Peak Area	%RSD
FFAP Column, Helium Carrier Gas				
Palmitic	8.93	0.06	12.54	1.22
Palmitoleic	9.52	0.08	0.85	7.98
Stearic	13.69	0.06	1.85	4.94
Oleic	14.19	0.06	76.34	0.42
Linoleic	15.42	0.06	6.66	1.62
Linolenic	17.19	0.05	0.65	4.41
INNOWax Column, Helium Carrier Gas				
Palmitic	8.40	0.04	12.34	1.25
Palmitoleic	8.96	0.04	0.89	2.94
Stearic	13.05	0.02	2.01	2.87
Oleic	13.54	0.02	75.71	0.74
Linoleic	14.75	0.02	6.65	1.64
Linolenic	16.49	0.02	0.64	3.57

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