Application Note

Food Testing

Agilent

Trusted Answers

A comparison study of the analysis of volatile organic acids and fatty acids

Using DB-FATWAX Ultra Inert and other WAX GC columns

Author

Yun Zou
Agilent Technologies
(Shanghai) Co., Ltd,

Abstract

This Application Note evaluates the performance of the Agilent J&W DB-FATWAX Ultra Inert GC column for the analysis of aqueous C2–C7 free fatty acids, C2–C18 organic acids, and Agilent WAX UI test mixtures. The DB-FATWAX Ultra Inert GC column has excellent stability to repeated injections of aqueous matrices. Compared with acid-modified wax columns such as the Agilent J&W DB-FFAP GC column, DB-FATWAX UI columns provide comparable or better peak shape for short-chain volatile organic acids. The results indicate that DB-FATWAX UI provides superior inertness, thermal stability, and retention time reproducibility compared to other commercially available WAX columns for the analysis of underivatized volatile organic acids and free fatty acids.

Introduction

Monitoring types of volatile organic acids and fatty acids is a common analysis required in food, flavors, alcoholic beverages, and other industries. In life science research, laboratories routinely analyze the profiles of the fatty acids extracted from bacterial culture media to identify the bacteria[1]. Short-chain organic acids (C2 to C7) are also regularly monitored for anaerobic or aerobic digestion in fermentations. It can be done using a combination of headspace/GC or liquid-liquid extraction followed by GC analysis. The most common method is by direct injection of the acids in water[2]. Underivatized volatile organic acids are difficult to quantify by GC because these highly polar compounds interact strongly with any active sites on the column, resulting in tailing or poorly resolved peaks that can make quantitation difficult at low levels. For some acids, adsorption can become irreversible. The direct injection method requires the use of GC column stationary phases, which do not decompose in strong acids and water.

Normally, fatty acids are derivatized to methyl esters for analysis. To identify and quantify consumer products in the food industry, fatty acid esters are routinely analyzed by determining the ratio of various saturated and unsaturated fatty acids in fat and oil[3]. These pretreatment procedures are tedious and time-consuming. They carry the potential for incomplete conversions of acids to esters and the loss of short-chain fatty acid esters in the extraction process. Therefore, to eliminate the problems associated with derivatization, extraction, and cleanup procedures, analysis of underivatized organic acids and free fatty acids is recommended. These fatty acids are typically analyzed in their free form using two types of GC columns: one is acid-modified WAX columns, such as the FFAP columns; another is ultra-inert WAX columns.

Previous articles have detailed the GC analysis of FAMEs using DB-FATWAX UI columns[4,5]. This Application Note discusses the analysis of free fatty acids using DB-FATWAX UI, DB-FFAP, and other WAX columns.

Experimental

Chemicals and standards

All standard compounds and reagents in the test mixtures were purchased from ANPEL Scientific Instrument Co. Ltd (Shanghai, China). The purity of each standard compound and solvent is more than 98 %. The standard solution mixtures were prepared from individual pure compounds. The WAX ultra-inert test mixture[6] consisted of the 12 compounds listed in Table 1, which were analyzed using the chromatographic conditions in Table 4.

Table 1. WAX UI test mixture, in dichloromethane.

Peak no.	Compound	Amount on-column (ng)
1	2-Nonanone	3.3
2	Decanal	3.3
3	Propionic acid	3.3
4	Ethylene glycol	3.3
5	Heptadecane	1.65
6	Aniline	3.3
7	Methyl dodecanoate	3.3
8	2-chlorophenol	3.3
9	1-Undecanol	3.3
10	Nonadecane	1.65
11	2-Ethylhexanoic acid	6.6
12	Ethyl maltol	6.6

Instrumentation

The analyses were performed using an Agilent 7890B GC equipped with a flame ionization detector (FID). Sample introduction was achieved using an Agilent 7683B automatic liquid sampler with a 5 µL syringe (p/n G4513-80213), and a split/splitless injection port. Tables 2–4 summarize the instrumental configuration and analytical conditions. Table 5 lists the other supplies used in this study.

Table 2. Method 1 experimental conditions (C2–C7 free fatty acids in water).

Parameter	Value
GC system	7890B/FID
Column	J&W DB-FATWAX Ultra Inert, 30 m, 0.25 mm, 0.25 µm (p/n G3903-63008) J&W DB-FFAP, 30 m, 0.25 mm, 0.25 µm (p/n 122-3232) Commercially available wax columns from suppliers 1 and 2, 30 m, 0.25 mm, 0.25 µm
Carrier gas	Helium, 42 cm/s, constant flow mode
Inlet	Split/splitless, 250 °C, split ratio 30:1
Oven	162 °C isothermal
FID	250 °C, Hydrogen: 40 mL/min; Air: 400 mL/min; make-up gas: 25 mL/min
Sample	0.06–0.13 % each acid in water
Injection	0.1 µL

Table 3. Method 2 experimental conditions (C2–C18 organic acids).

Parameter	Value
GC system	7890B/FID
Column	J&W DB-FATWAX Ultra Inert, 30 m, 0.25 mm, 0.25 µm (p/n G3903-63008) J&W DB-FFAP, 30 m, 0.25 mm, 0.25 µm (p/n 122-3232) Wax columns from suppliers 1 and 2, 30 m, 0.25 mm, 0.25 µm
Carrier gas	Helium, 42 cm/s, constant flow mode
Inlet	Split/splitless, 280 °C, split ratio 50:1
Oven	120 °C (2 minutes), 5 °C/min to 140 °C (3 minutes); 20 °C/min to 250 °C (10 minutes)
FID	280 °C, Hydrogen: 40 mL/min; Air: 400 mL/min; make-up gas: 25 mL/min
Sample	0.05–0.1 % each component in dichloromethane
Injection	1 µL

Table 4. Method 3 experimental conditions (WAX UI test mixture).

Parameter	Value
GC system	7890B/FID
Column	J&W DB-FATWAX Ultra Inert, 30 m, 0.25 mm, 0.25 µm (p/n G3903-63008) J&W DB-FFAP, 30 m, 0.25 mm, 0.25 µm (p/n 122-3232) Wax columns from suppliers 1 and 2, 30 m, 0.25 mm, 0.25 µm
Carrier gas	Helium, 40 cm/s, constant flow mode
Inlet	Split/splitless, 250 °C, split ratio 75:1
Oven	130 °C isothermal
FID	250 °C, Hydrogen: 40 mL/min; Air: 400 mL/min; make-up gas: 25 mL/min
Injection	0.5 µL

Table 5. Flowpath supplies.

Supply	Description
Vials	Amber, write-on spot, certified, 2 mL, screw top vial packs (p/n 5182-0554)
Septa	Nonstick BTO septa (p/n 5183-4757)
Column nut	Self Tightening, inlet/detector (p/n 5190-6194)
Ferrules	15 % graphite: 85 % Vespel, short, 0.4 mm id, for 0.1 to 0.25 mm columns (10/pk, p/n 5181-3323)
Liner	Agilent Ultra Inert split liner with glass wool (p/n 5190-2295)
Inlet seal	Ultra Inert, gold-plated, with washer (p/n 5190-6144)

Results and discussion

Aqueous C2–C7 free fatty acid test mixture

Direct injection of free fatty acids dissolved in water for GC analysis is quite challenging. Due to the presence of strong acids and water, conventional WAX-type phases have been unstable and active, resulting in poor peak shapes and reproducibility, as well as decreased lifetime. To prevent vapor volume overloading of the liner, the injection volume for aqueous samples should be less than 1 µL.

Figure 1 shows a GC/FID chromatogram of a mixture of C2–C7 free fatty acids in water on a J&W DB-FATWAX Ultra Inert (UI) column. Table 2 lists the analysis conditions. Due to the Ultra Inert performance of the DB-FATWAX UI GC column, all acids were well resolved with sharp and symmetrical peaks.

Repeatability of the analysis and column performance stability were tested by 15 injections of aqueous C2–C7 free fatty acids sample. Figure 2 shows that there is no retention time stability drift resulting from repeated injections of aqueous samples. Peak shapes were also maintained over the course of this study. Relative standard deviations (RSDs) for retention times were less than 0.03 %, and absolute peak areas were within 2 % for all free fatty acids.

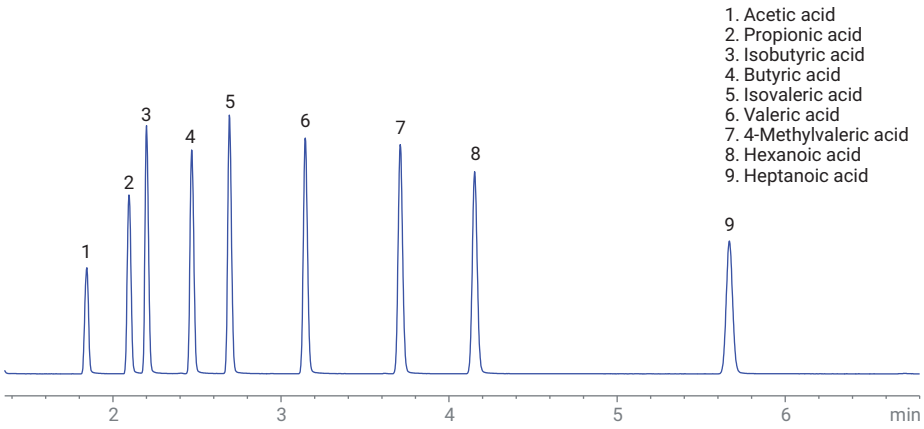

Figure 1. GC/FID chromatogram of a mixture of C2–C7 free fatty acids in water on a 30 m × 0.25 mm id, 0.25 µm J&W DB-FATWAX Ultra Inert column (conditions listed in Table 2).

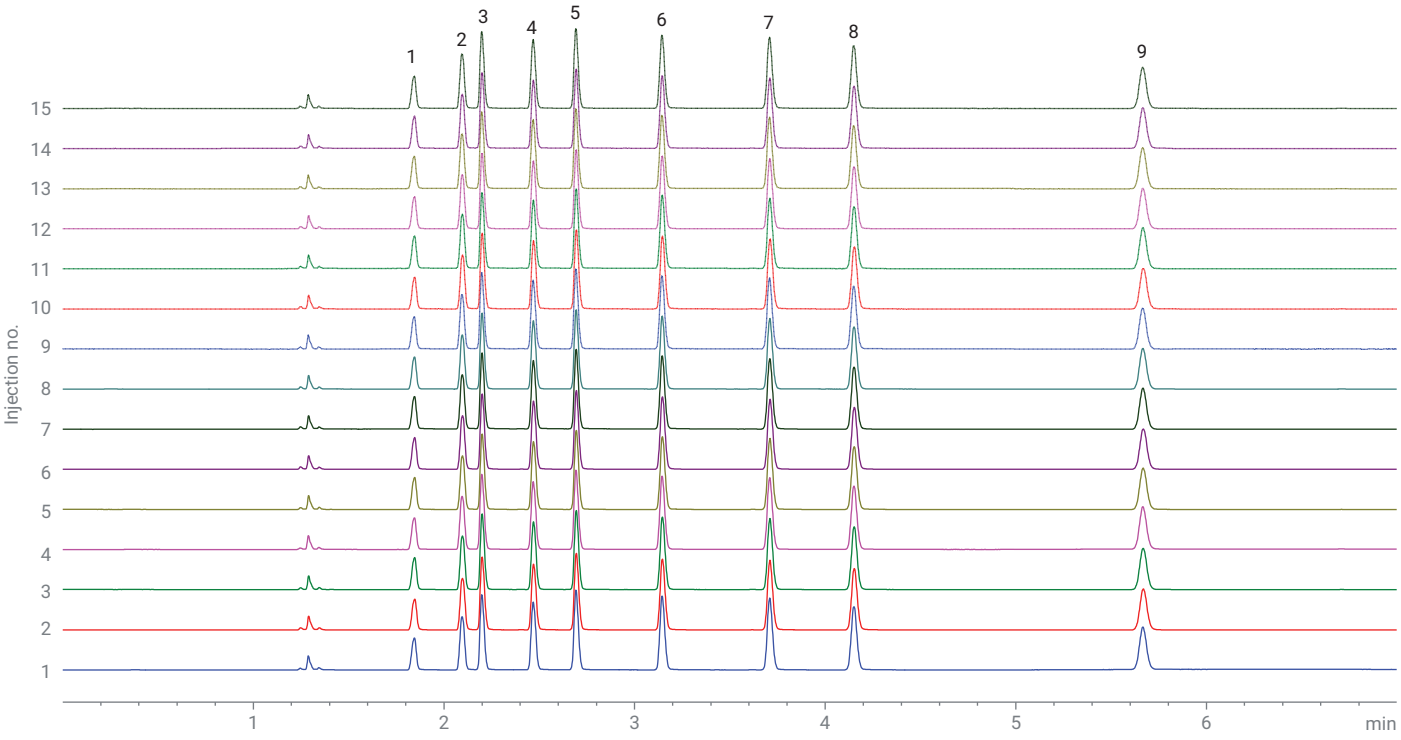

Figure 2. Overlaid GC/FID chromatograms of repeat injections of the same aqueous C2–C7 free fatty acids sample shown in Figure 1.

DB-FFAP, an acid-modified WAX column, is designed primarily for the analysis of organic acids, free fatty acids, or samples that require quantitation of acidic impurities. Figure 3 shows the chromatograms of the aqueous C2–C7 free fatty acids test mix on both a DB-FFAP column and a DB-FATWAX UI column. The analysis was completed in approximately five minutes on the DB-FFAP column, and six minutes on the DB-FATWAX UI column. Both columns provide excellent peak shape and good resolution for all these acids. Table 6 shows peak asymmetry at 10 % peak height (As.10 %) of each peak in this chromatogram; As.10 % for DB-FATWAX UI is between 0.98–1.16, and DB-FFAP is between 0.91–1.20. DB-FATWAX UI provides more symmetrical peaks of acetic acid (peak 1) and isobutyric acid (peak 3).

Two other commercially available WAX-type columns from different suppliers were used to do the same tests under the same conditions. Peak tailing was observed for the WAX columns from other suppliers using the aqueous C2–C7 free fatty acids mix (Figure 4 and Table 6). The column activity and unstable phase, especially Supplier 1's WAX column, also lead to retention time drifting, poorly resolved peaks, and loss of responses of critical analytes of interest, such as acetic acid (peak 1), propionic acid (peak 2), and isobutyric acid (peak 3). The same results are shown in Figure 6.

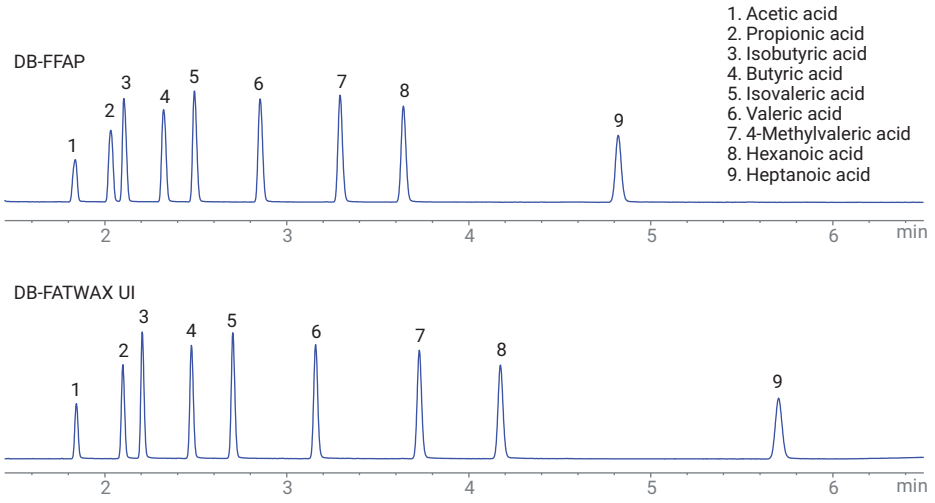

Figure 3. GC/FID chromatograms of the aqueous C2–C7 free fatty acids sample on a 30 m × 0.25 mm id, 0.25 µm J&W DB-FFAP column and a DB-FATWAX Ultra Inert column using Method 1 (see Table 2).

Table 6. Peak asymmetry at 10 % peak height (As. 10 %).

Peak no.	As. 10 %								
	1	2	3	4	5	6	7	8	9
DB-FATWAX UI	0.98	1.04	1.15	1.16	1.14	1.08	1.06	1.07	1.04
DB-FFAP	0.91	1.05	1.20	1.16	1.16	1.15	1.09	1.05	1.06
Supplier 1 WAX	1.56	coelution	coelution	1.97	1.65	2.04	1.96	1.96	1.87
Supplier 1 WAX	0.97	1.08	1.32	1.22	1.28	1.23	1.27	1.26	1.23

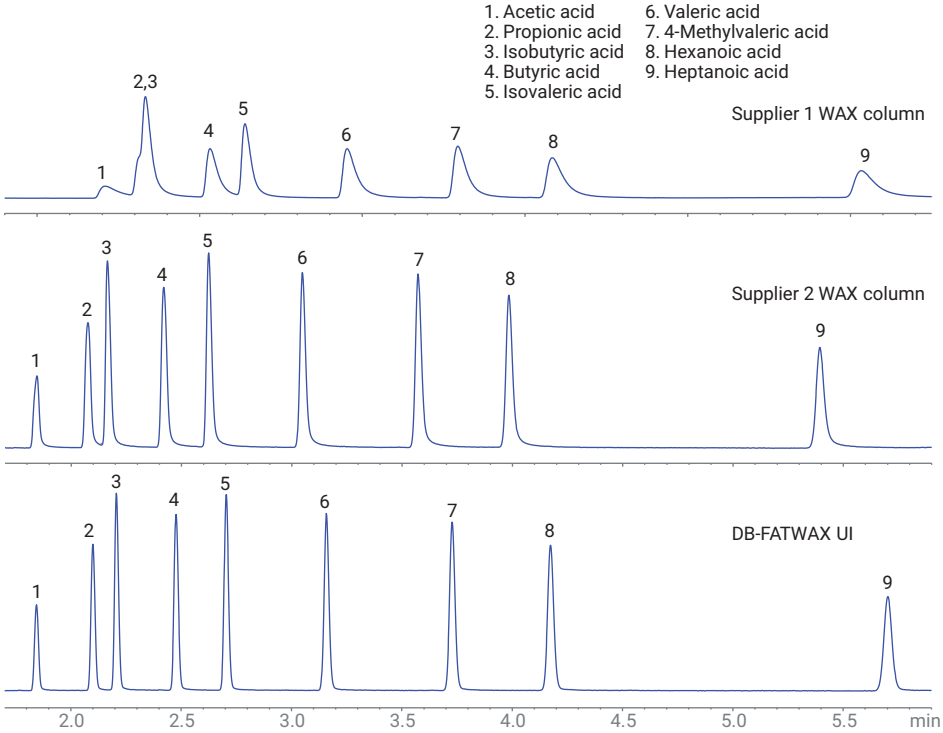

Figure 4. GC/FID chromatograms of the aqueous C2–C7 free fatty acids sample on a J&W DB-FATWAX UI column and WAX columns from Suppliers 1 and 2 using Method 1 (see Table 2).

C2–C18 organic acids test mixture

Figures 5–7 show the example GC/FID chromatograms of the C2–C18 organic acids test mixture on J&W DB-FFAP and DB-FATWAX UI columns as well as WAX columns from different suppliers. The testing was performed after the columns were conditioned for 50 hours at 250 °C. As shown in Figure 5, DB-FFAP demonstrated relatively lower column bleed at the high temperature (250 °C) and shorter analysis time to elute the higher molecular weight organic acids such as C18 fatty acids compared with DB-FATWAX UI.

However, the DB-FATWAX UI column provided comparable or even better peak shapes for C2–C12 volatile organic acids than DB-FFAP according to Figure 6, the enlarged section of Figure 5. These volatile acids significantly affect the flavor and quality of food[7], the content of these active volatile acids is an index for quality assurance in some foods. Figure 7 shows that inertness performance of WAX columns from different suppliers deteriorated differently after conditioning the column for 50 hours. The WAX column from Supplier 2 was reported as an inert wax phase column. On this column, all of the compounds, including isomers, could be separated, but there was noticeable tailing of C2–C7 free organic acids in the chromatogram. The WAX column from Supplier 1 is a typical conventional wax phase GC column. The lack of column inertness and thermal stability for the WAX column from Supplier 1 lead to severe peak tailing, and negatively affected sensitivity, resolution, and selectivity especially for active C2–C7 free fatty acids and levulinic acid.

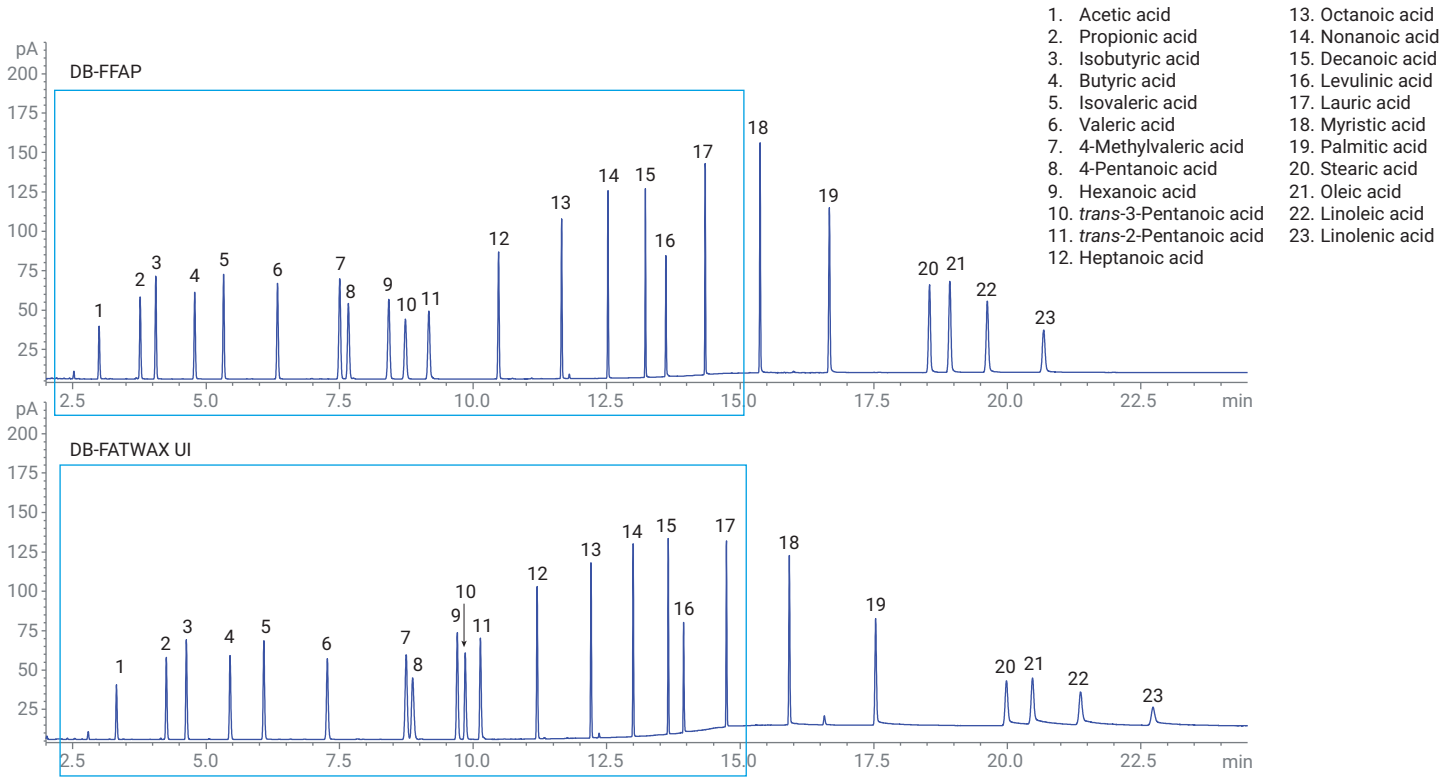

Figure 5. Example FID chromatograms of the organic acids (C2–C18) test mix on J&W DB-FFAP and DB-FATWAX UI GC columns using Method 2 (see Table 3).

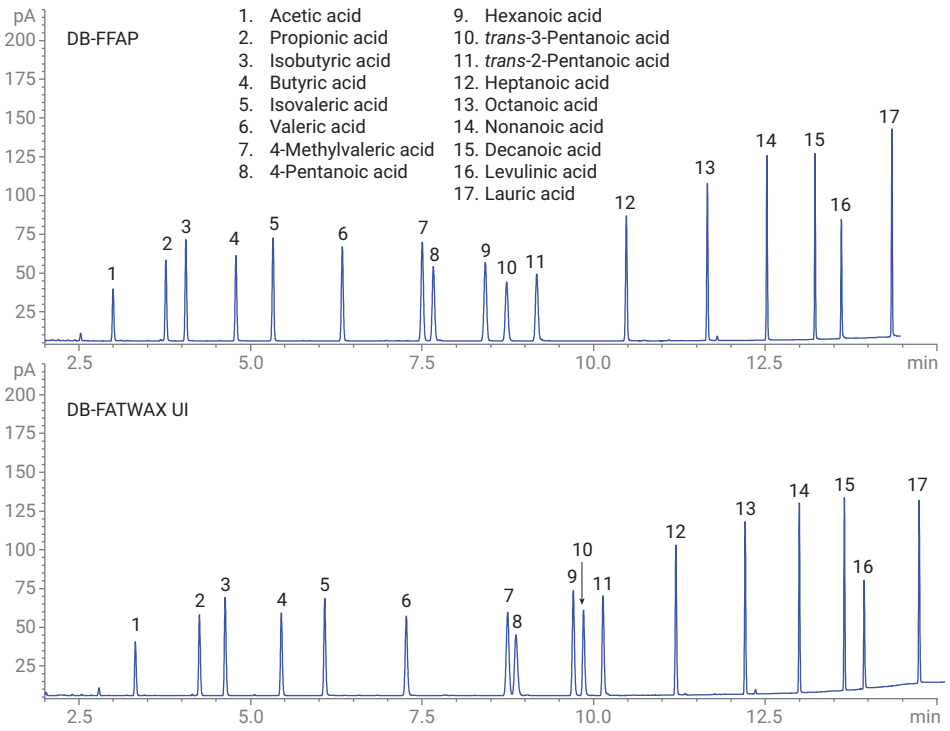

Figure 6. Enlarged section of the GC/FID chromatogram of the organic acids (C2–C18) test mix separated on J&W DB-FFAP and DB-FATWAX UI GC columns (the elution order is the same as Figure 5).

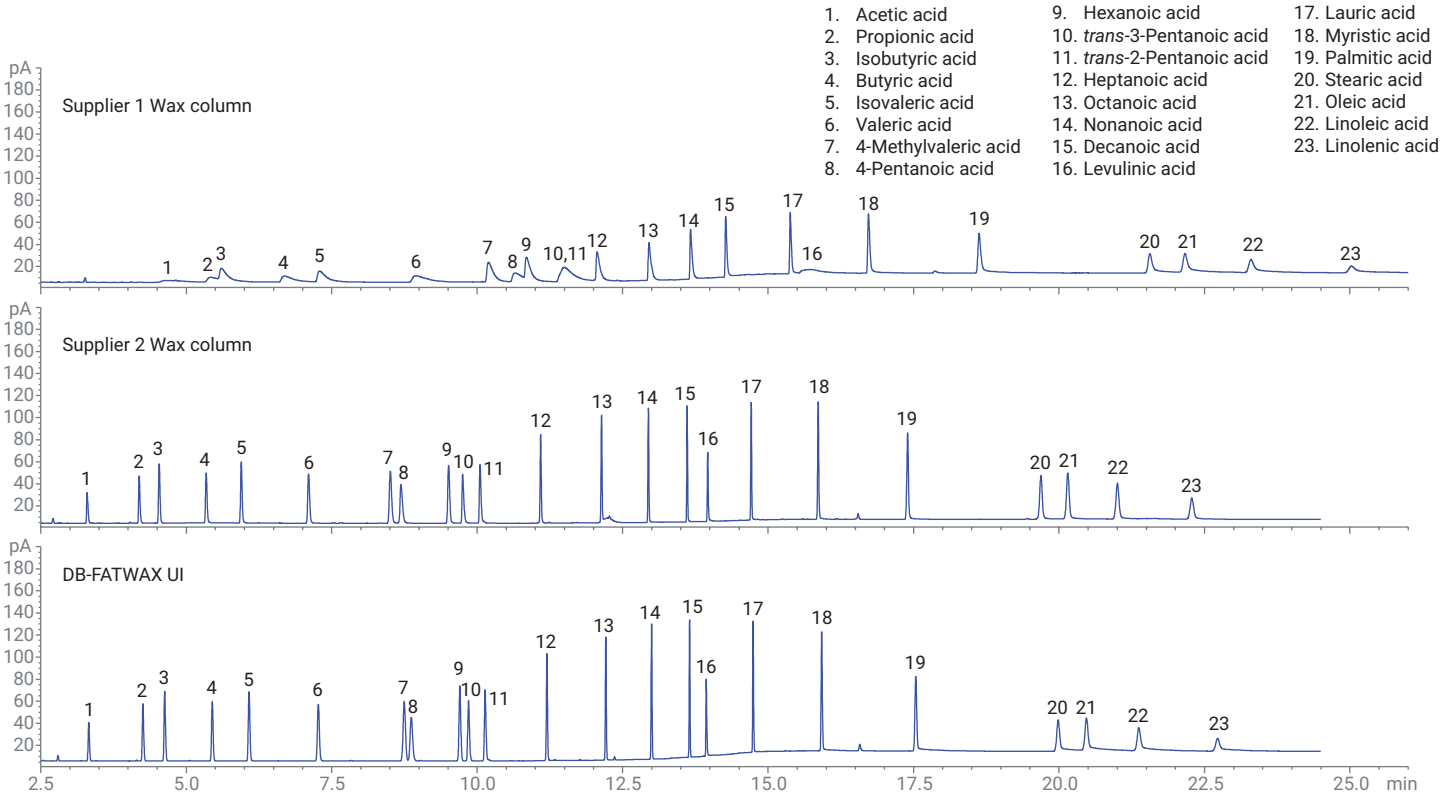

Figure 7. Example GC/FID chromatograms of the organic acids (C2–C18) test mix on a J&W DB-FATWAX UI and other WAX columns from different suppliers (the elution order was the same as Figure 5).

Agilent WAX UI test mixture

DB-FATWAX UI GC columns are part of the J&W Ultra Inert GC column family. To ensure inertness performance for these active polar compounds, every column is tested with the industry's most demanding test probe mixture[6]. The WAX UI test mixture includes propionic acid, ethyl hexanoic acid, and other active compounds. Figures 8 and 9 show the chromatograms of the WAX UI test mixture separated on DB-FATWAX UI, DB-FFAP, and WAX columns from Suppliers 1 and 2. Excellent peak shapes were obtained for the compounds of interest with the DB-FATWAX UI column. DB-FFAP and DB-FATWAX UI share a similar inertness performance for propionic acid and 2-ethylhexanoic acid, but Figure 8 shows that there is noticeable tailing with the peak of ethylene glycol in the chromatograms for the DB-FFAP column. The As. 10 % for ethylene glycol was 0.37 on DB-FFAP, and 0.91 on DB-FATWAX UI. In addition, reduced response is evident in the chromatogram for ethylene glycol on DB-FFAP. The WAX column from Supplier 2 exhibits tailing peaks of decanal, propionic acid, and ethylene glycol, with peak asymmetry values of 1.28, 1.32, and 1.26, respectively. The column activity of the WAX column from Supplier 1 was characterized by tailing peaks and loss of response of critical analytes of interest, such as decanal, propionic acid, ethylene glycol, 2-ethylhexanoic acid, and ethyl maltol (Figure 9).

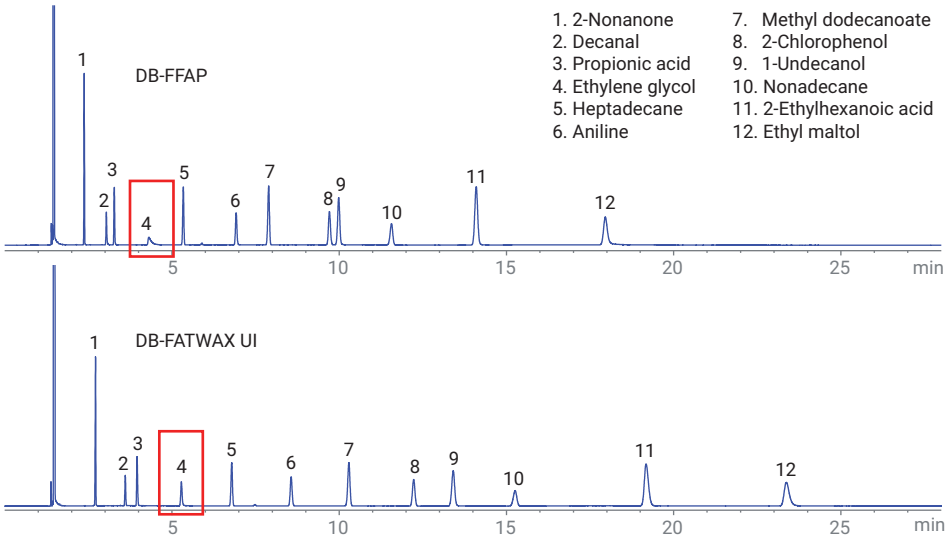

Figure 8. Example GC/FID chromatograms of the WAX UI test mix on J&W DB-FFAP and DB-FATWAX UI GC columns using Method 3 (see Table 4).

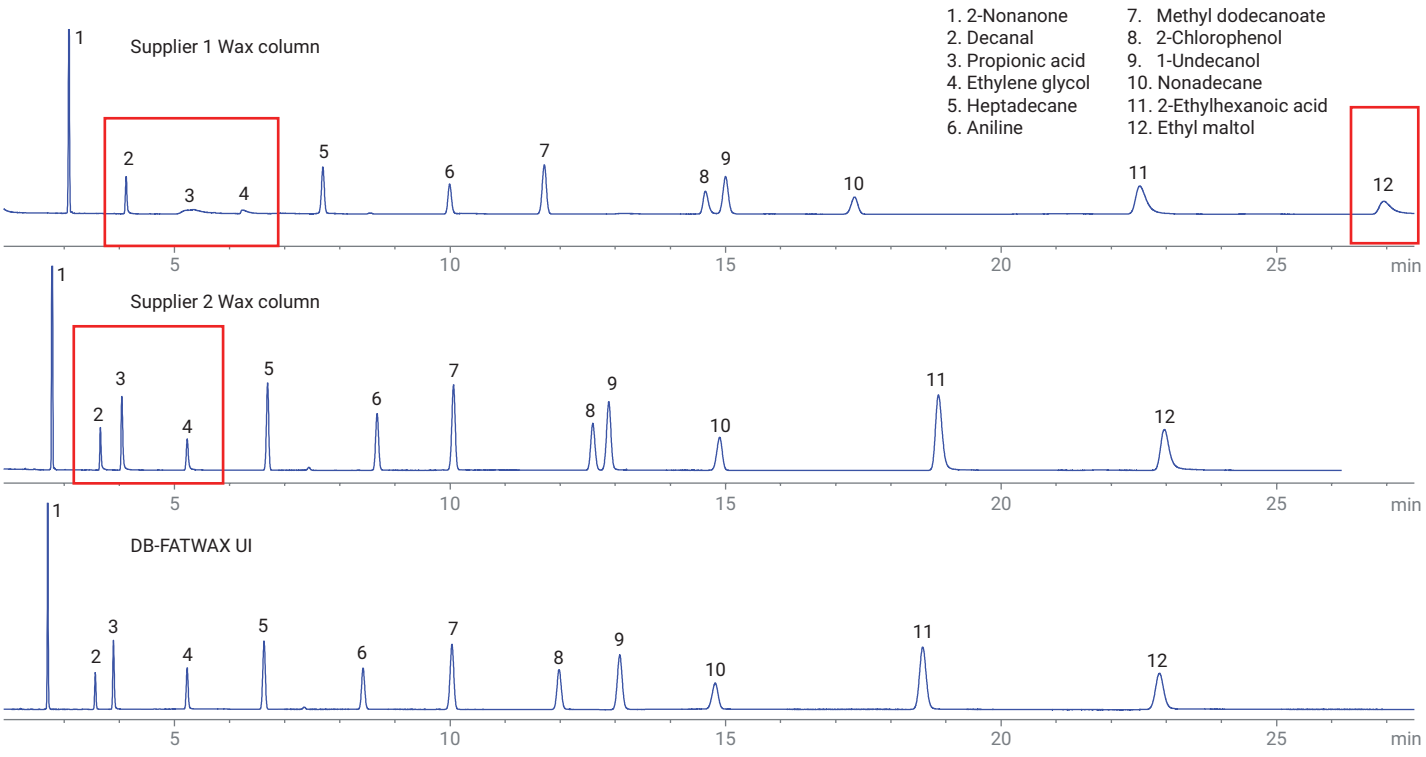

Figure 9. Example GC/FID chromatograms of the WAX UI test mix on a J&W DB-FATWAX UI and WAX GC columns from other suppliers using Method 3 (see Table 4).

Conclusions

A J&W DB-FATWAX Ultra Inert GC column was evaluated by analyzing organic acids and WAX UI test mixtures using GC/FID. High inertness and the improved thermal stability of the DB-FATWAX UI provided better peak shapes and more consistent analytical results than the other suppliers' WAX columns evaluated in this report. This was especially true in the presence of strong short-chain volatile organic acids and aqueous samples. Volatile organic acids and free fatty acids are well resolved, with sharp and symmetrical peaks on both DB-FATWAX UI and DB-FFAP GC columns. The better column for C18 fatty acids analysis is the DB-FFAP column due to reduced column bleed at high temperatures (250 °C) and shorter analysis times. The best column for complex samples including alcohols, diols, glycols, and organic acids is the DB-FATWAX Ultra Inert because it produces sharper peaks and higher responses especially for these most active compounds, enhancing the sensitivity and reproducibility for these challenging analytes. The excellent phase stability of DB-FATWAX UI for aqueous injections was demonstrated by the reproducible analysis of C2–C7 free fatty acids in water.

References

1. Merrick-Gass, M.T. Gas Chromatography in G-Bacteria Identification. *American Clinical Products Review*. Dec. **1986**.
2. Henderson, M.H.; Steedman, T.A. Analysis of C2–C6 monocarboxylic acids in aqueous solution using gas chromatography. *Journal of chromatography A*, 244(1982), 337–346.
3. Vickers, A.K. High efficiency FAMEs analyses using capillary GC. *Agilent Technologies Article Reprint*, publication number 5989-6588EN, **2007**.
4. Van Der Meer, I.; Zou, Y.; Serrano, G. Analysis of Omega 3 and Omega 6 FAMEs in Fish Oil and Animal Fat Using an Agilent J&W DB-FATWAX Ultra Inert GC Column. *Agilent Technologies Application Note*, publication number 5991-8744EN, **2017**.
5. Zou, Y.; Wu, H. Improving the Analysis of 37 Fatty Acid Methyl Esters. *Agilent Technologies Application Note*, publication number 5991-8706EN, **2017**.
6. Dang, N.-A; Vickers, A.K. A New PEG GC Column with Improved Inertness Reliability and Column Lifetime. *Agilent Technologies Competitive Comparison*, publication number 5991-6683EN, 2016.
7. Alur, M.D.; *et al.* Biochemical methods for determination of spoilage of foods of animal origin: a critical evaluation. *Journal of Food Science and Technology (India)* **1995**, *32*, 181–188.

www.agilent.com/chem

Printed in the USA, April 5, 2018
5991-9223EN

Agilent
Trusted Answers