Application Note

Food

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

Authors

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Abstract

This Application Note highlights the use of Agilent J&W DB-FATWAX Ultra Inert GC columns for the analysis of fatty acid methyl esters (FAMEs) in fish oil and animal fat. The DB-FATWAX UI GC column showed improved resolution for several Omega 3 and Omega 6 FAMEs, including docosahexaenoic acid (DHA), from common interferences.

Introduction

Omega 3 and Omega 6 are important nutritional fatty acids commonly found in food sources such as fish, meat, nuts, and oils. Salmon oil, for example, is an excellent source of polyunsaturated Omega 3 fatty acids (PUFAs), including high content of two important Omega 3s, eicosapentaenoic acid (C20:5n3, EPA), and docosahexaenoic acid (C22:6n3, DHA). Proper identification and quantification of these Omega 3 and Omega 6 fatty acid methyl esters (FAMEs) by gas chromatography is important for food nutritional labeling and quality control tests[1]. For example, the concentration of EPA and DHA is the crucial quality factor for salmon oil capsules.

The Agilent J&W DB-FATWAX Ultra Inert GC column was developed to exceed the requirements for the analysis of Omega 3 and Omega 6 FAMEs, including AOAC Method 991.31 for encapsulated fish oil. This polyethylene glycol (PEG) phase is commonly used to separate FAMEs with minimum overlap in the elution order of different carbon chain lengths. To ensure reproducible column performance, DB-FATWAX UI is designed for the analysis of Omega 3, Omega 6, and other important nutritional labeling FAMEs. It is tested to ensure optimized resolution among key FAMEs and consistent equivalent chain length values from column to column. The DB-FATWAX UI column is also based on Agilent proprietary Ultra Inert technology. This Ultra Inert technology offers high inertness performance to ensure consistently good peak shapes, improved detection limits, and superior long-term thermal stability compared to traditional PEG phases[2,3].

This Application Note investigates the performance of DB-FATWAX UI GC columns for the analysis of Omega 3, Omega 6, and other FAMEs in fish oil and animal fat. Example chromatograms and comparisons with competitor columns are shown to highlight the significant improvement in the separation of key FAME compounds, superior thermal stability, and extended column lifetime of DB-FATWAX UI.

Experimental

Chemicals and Standards

A 37 component FAME standard mixture (p/n CDAA-252795-MIX-1mL) was used for comparison studies. This FAME mix mimics the fatty acid composition of a wide range of food samples, and includes some Omega 3 and Omega 6 FAMEs. The mix was purchased from ANPEL Scientific Instrument Co. Ltd (Shanghai, China). Table 1 lists the concentration of each component in the mixture.

Table 1. 37 component FAME mix.

No.	FAME	Conc. (mg/mL)
1	C4:0	403
2	C6:0	404
3	C8:0	406
4	C10:0	403
5	C11:0	200
6	C12:0	399
7	C13:0	200
8	C14:0	397
9	C14:1	202
10	C15:0	202
11	C15:1	200
12	C16:0	599
13	C16:1	200
14	C17:0	201
15	C17:1	200
16	C18:0	399
17	C18:1 *cis* (n9)	400
18	C18:1 *trans* (n9)	200
19	C18:2 *cis* (n6) (LA)	203
20	C18:2 *trans* (n6)	200
21	C18:3 n6	203
22	C18:3 n3 (ALA)	199
23	C20:0	406
24	C20:3 n6	202
25	C20:2	200
26	C20:3 n6	202
27	C20:3 n3	200
28	C20:4 n6 (ARA)	198
29	C20:5 n3 (EPA)	201
30	C21:0	201
31	C22:0	400
32	C22:1 n9	202
33	C22:2	199
34	C22:6 n3 (DHA)	197
35	C23:0	200
36	C24:0	405
37	C24:1	201

* Names in red are Omega 3 FAMEs; names in blue are Omega 6 FAMEs.

Fish oil and animal fat standards were used for this study. These standards, PUFA No. 2 (animal source) and PUFA No. 3 (from Menhaden Oil), are extracted from natural sources, and are used for qualitative identification of FAMEs. These standards were purchased from Minn Bolin Bio-Tech Co. Ltd (Shenzhen, China). The mixtures are available as a 100 mg neat mixture, and were diluted 100 times with acetone for this application.

Instrumentation

The analyses were performed on an Agilent 7890B GC equipped with a flame ionization detector (FID). Sample introduction was done using an Agilent 7683B automatic liquid sampler with a 5 µL syringe (p/n G4513-80213), and a split/splitless injection port. The instrumental configuration and analytical conditions are summarized in Table 2 (37 FAME mix analysis), Table 3 (PUFA No. 2), and Table 4 (PUFA No. 3). Table 5 lists other supplies used in this study.

Table 2. 37 FAME mix analysis method conditions.

Parameter	Value
GC system	Agilent 7890B/FID
Column	Agilent J&W DB-FATWAX Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63008)
Carrier gas	Helium, 40 cm/s, constant flow
Inlet	Split/splitless, 250 °C, split ratio 50:1
Oven	50 °C (2 minutes), 50 °C/min to 174 °C (14 minutes), 2 °C/min to 215 °C (25 minutes)
FID	280 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make-up gas: 25 mL/min
Injection	1 µL

Table 3. PUFA No. 2 mix analysis method conditions.

Parameter	Value
GC system	Agilent 7890B/FID
Column	Agilent J&W DB-FATWAX Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63008)
Carrier gas	Helium, 1.4 mL/min, constant flow
Inlet	Split/splitless, 250 °C, split ratio 100:1
Oven	140 °C, 15 °C/min to 190 °C (11 minutes), 4 °C/min to 220 °C (20 minutes)
FID	280 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make-up gas: 25 mL/min
Injection	1 µL

Table 4. PUFA No. 3 mix analysis method conditions.

Parameter	Value
GC system	Agilent 7890B/FID
Column	Agilent J&W DB-FATWAX Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n G3903-63008)
Carrier gas	Helium, 30 cm/s, constant flow mode.
Inlet	Split/splitless, 250 °C, split ratio 100:1
Oven	180 °C (2 minutes), 2 °C/min to 210 °C (35 minutes)
FID	280 °C, Hydrogen: 40 mL/min Air: 400 mL/min Make-up gas: 25 mL/min
Injection	1 µL

Table 5. Flowpath supplies.

Parameter	Value
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

Figure 1A shows the 37 FAME mix chromatogram generated using the DB-FATWAX UI GC column. As expected, FAMEs are separated primarily by chain length and secondarily by the number of double bonds. The only overlap is C22:6n3 (DHA), which elutes after C24:0. Resolution is optimal for most PUFAs, with good separation for the Omega 3 FAMEs α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexenoic acid (DHA), and the Omega 6 FAMEs linoleic acid (LA) and arachinodic acid (ARA). The separation of LA is of interest, because *cis* and *trans* isomers commonly coelute in traditional WAX-type phases. With DB-FATWAX UI, it is possible to separate the *cis-trans* isomers C18:2 *cis* (LA) and C18:2 *trans* with baseline resolution.

The other pair of *cis-trans* FAMEs, C18:1 *cis* and C18:1 *trans*, were not completely resolved; a resolution of 0.56 was obtained for this pair.

Figure 1B takes a closer look at the baseline resolution achieved for the challenging separation of DHA from saturated C24:0 and the monounsaturated C24:1 using DB-FATWAX UI. This separation is critical for quantification of DHA, as C24:0 and C24:1 are common interferences found in animal fat and fish oil. Similar resolution for these FAMEs was not achieved when using other WAX-type columns designed for FAME analysis.

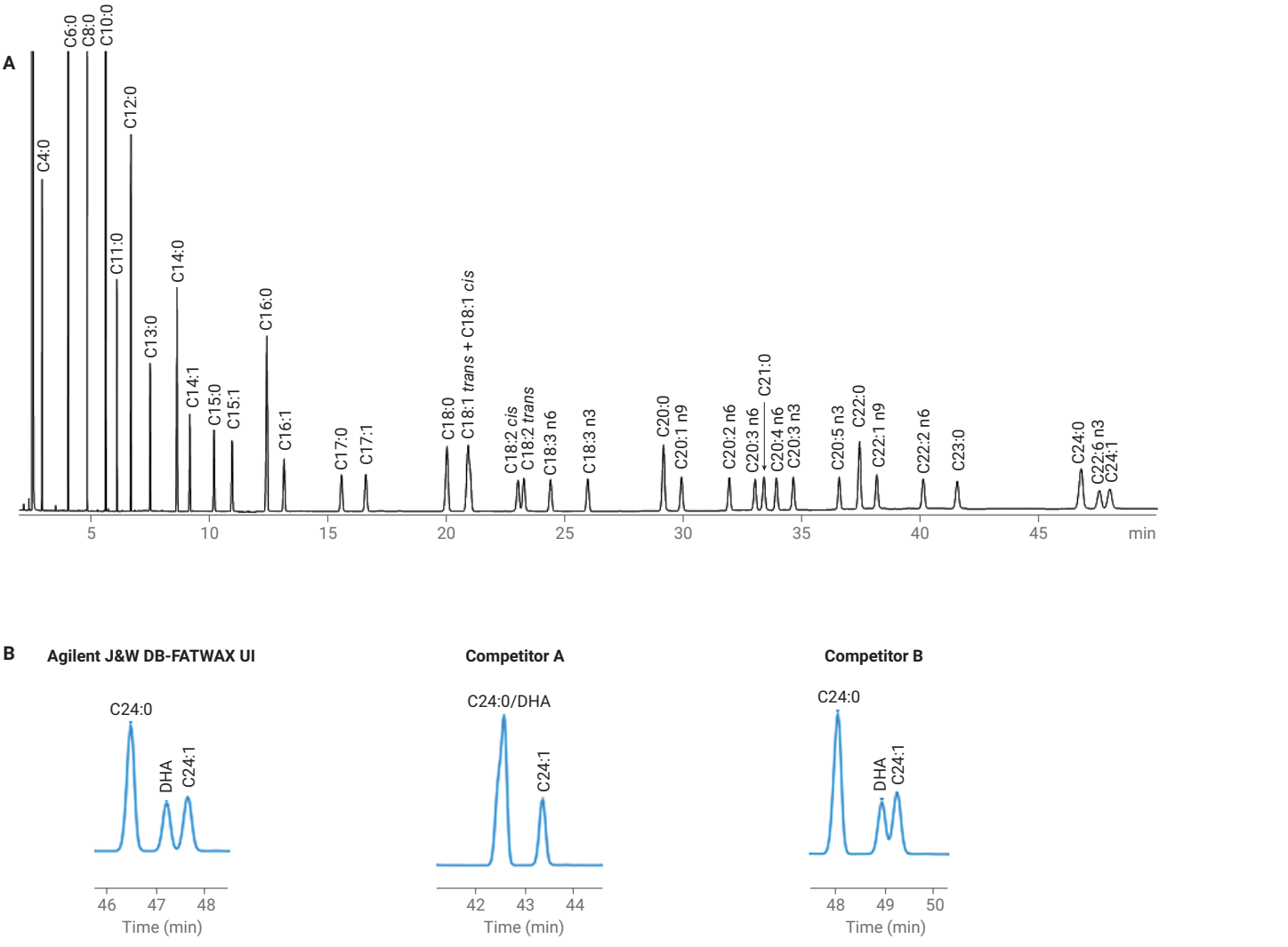

Figure 1. A) GC/FID chromatogram of 37 component FAMEs standard mixture on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column using the method in Table 2. B) Comparison of the separation of DHA C24:01 and C24:1 between the Agilent J&W DB-FATWAX UI and two other columns using a similar method to Figure 1A.

In the next study, the thermal stability and inertness of DB-FATWAX UI was tested and compared to two other WAX-type columns for FAME analysis. Figure 2 shows the separation of DHA from C24:0 and C24:1 after conditioning the three columns at the maximum allowable operating temperature (MAOT) for 20 and 40 hours, respectively. As the graph shows, there is minimum change in retention time and selectivity when operating DB-FATWAX UI at its maximum operating temperature. That is not the case with the two other columns. For competitor A, the pair C24:0 and DHA, which were coeluting before conditioning the column, is now resolved, but with a clear change in selectivity, with DHA eluting before the unsaturated C24:0. The opposite happens with the competitor B column, although DHA was partially resolved from C24:1; after conditioning the column at its MAOT, DHA now completely overlaps with C24:0. This change in selectivity is well documented for traditional PEG-type phases when operating at their MAOT. Other than a loss of phase due to column bleeding for long periods of time, there are extra processes taking place, which change the PEG phase irreversibly. In the DB-FATWAX Ultra Inert, column bleeding can explain the slight shift in retention time, with no change in selectivity; but no other irreversible interactions are taking place that affect the phase structure.

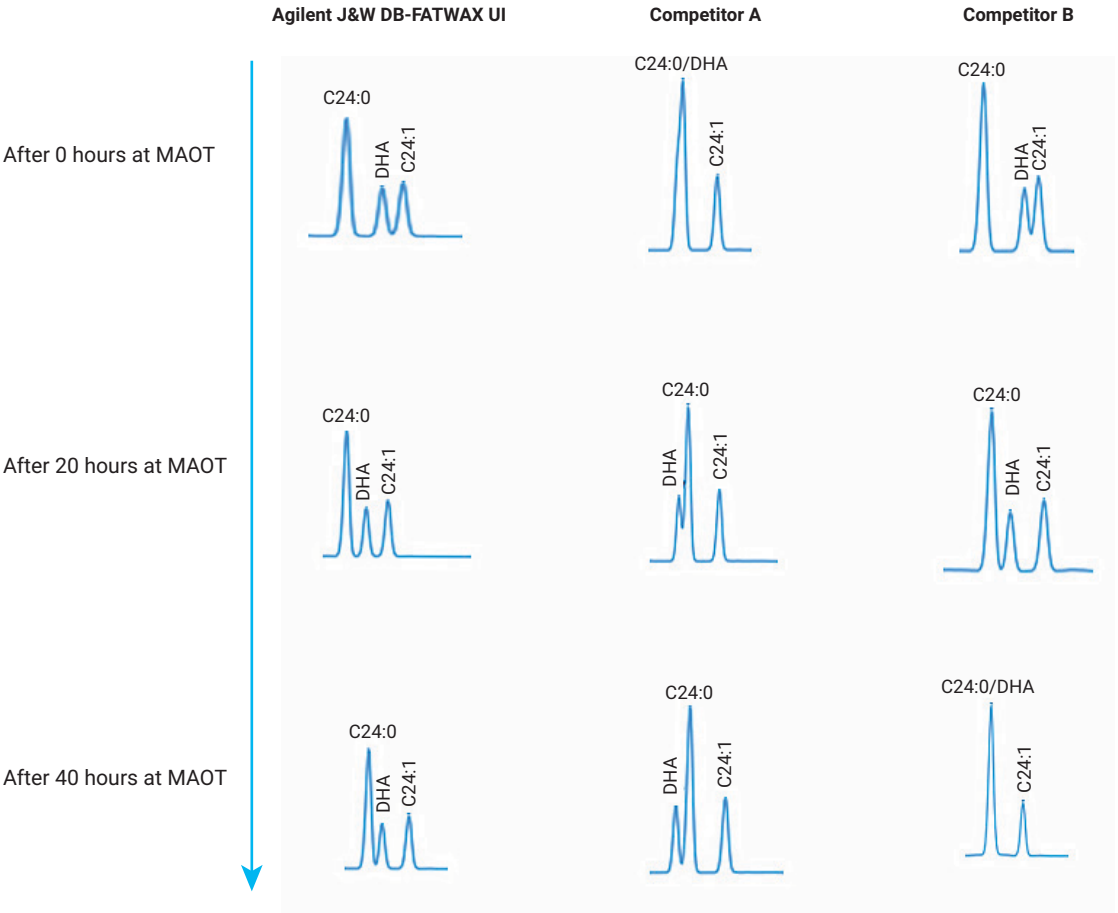

Figure 2. Comparison study of thermal stability and inertness after conditioning the columns for 20 and 40 hours at their MAOT, for the Agilent J&W DB-FATWAX Ultra Inert and two other WAX-type columns.

Figures 3 and 4 show the use of the DB-FATWAX Ultra Inert column for the analysis of meat fat and fish oil, respectively. In both chromatograms, we can see a clean baseline with little interference from contaminants, and an optimal resolution for several key Omega 3 and Omega 6 FAMEs. As it is typical from animal fat, in Figure 3 we can see the higher concentration of Omega 6 such as LA and ARA, relative to the Omega 3 FAMEs such as ALA, EPA, DPA, and DHA. In contrast to animal fat, marine fish oil contains large amounts of Omega 3 fatty acids. Figure 4 shows how DB-FATWAX UI provides good resolution for various Omega-3 FAMEs in an extract from Menhaden oil. Similar to other marine fish oils, Menhaden oil contains large amounts of EPA and DHA, and small amounts of Omega 6s such as LA and ARA. In both chromatograms, we can see that the DB-FATWAX UI column provides good separation and resolution for these various key Omega 3 and Omega 6 FAMEs. It is also possible to easily measure the differences and relative ratios among these components.

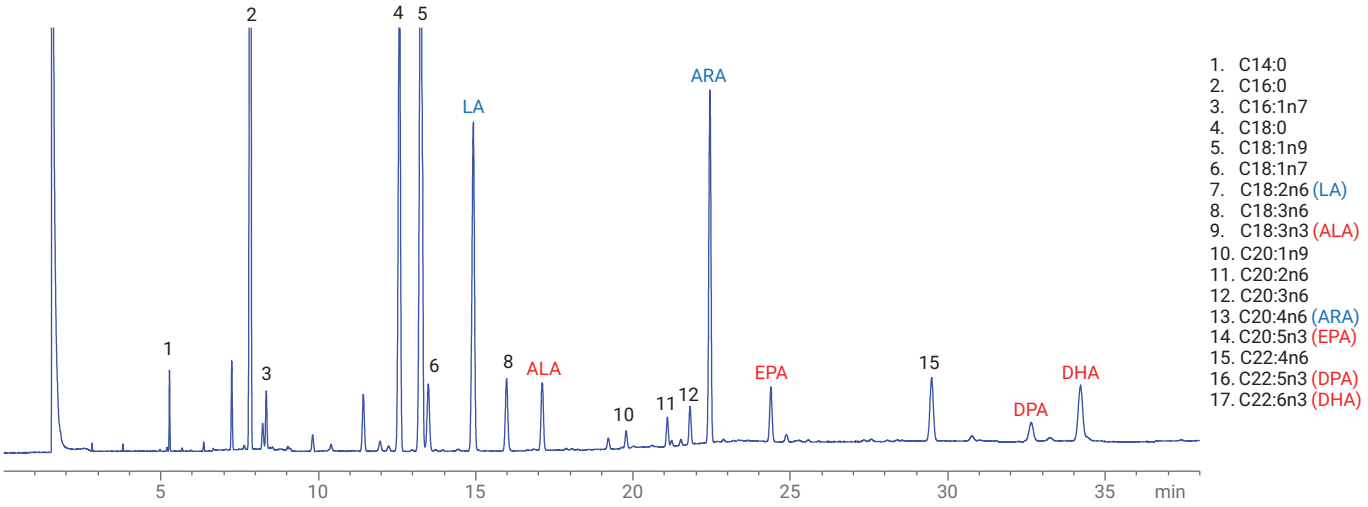

Figure 3. GC/FID chromatogram of PUFA No. 2 mix (animal source) on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column.

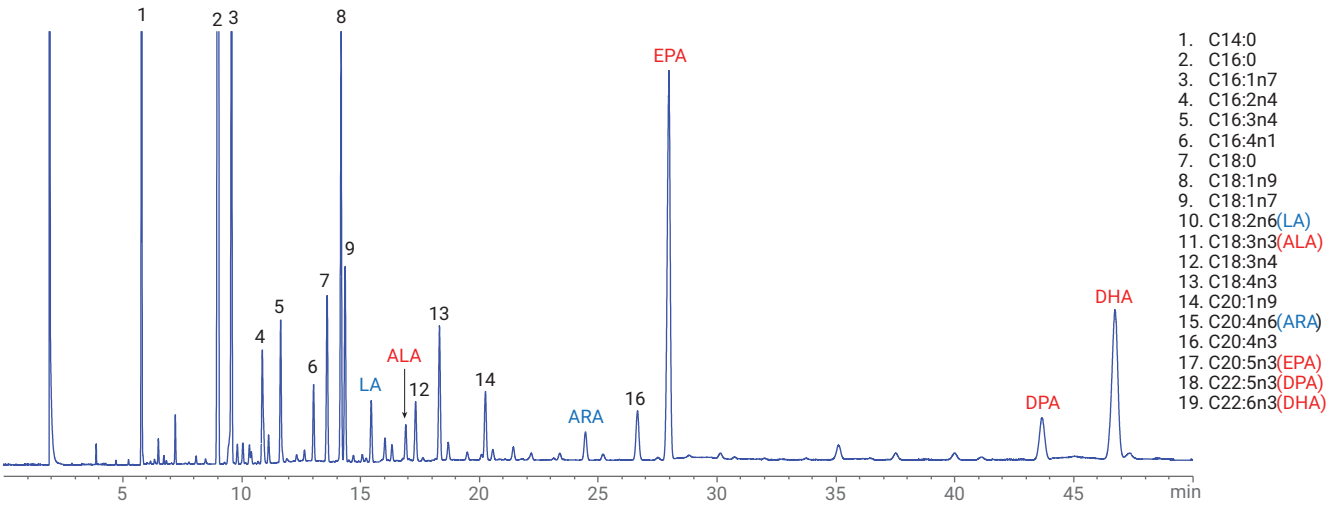

Figure 4. GC/FID chromatogram of PUFA No. 3 mix (from Menhaden oil) on a 30 m × 0.25 mm id, 0.25 µm Agilent J&W DB-FATWAX Ultra Inert GC column.

Conclusions

This Application Note highlights the benefits of the Agilent J&W DB-FATWAX Ultra Inert GC column for the analysis of FAMEs, in particular Omega 3, Omega 6, and other FAMEs commonly found in fish oil and animal fat. The DB-FATWAX Ultra Inert column provides excellent resolution and unique selectivity for critical FAMEs, including the challenging separation of DHA from C24:0 and C24:1. In addition, the superior inertness and enhanced thermal stability of this phase makes it possible to use the column for long periods of time without changes in retention time and selectivity. These are important factors for proper identification of the analytes by retention time, and for consistent, reproducible analysis over the column lifetime.

References

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Printed in the USA, November 27, 2017
5991-8744EN

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