

GC Analysis of Volatile Free Fatty Acids on the Stabilwax^{fi}-DA Column

Volatile free fatty acids are present in many foods, including eggs (AOAC Official Method 971.11), seafood (AOAC Official Method 973.26), and dairy products (AOCS Official Method Ca 5c-87). Gas chromatographic (GC) analysis is used to quantitate these fatty acids. Unlike fatty acids with longer hydrocarbon chain lengths, these volatile free fatty acids generally do not require methylation to obtain peak symmetry and reproducibility by GC. However, like all free fatty acids, they can be difficult to analyze because of their adsorptive nature. Therefore, a selective stationary phase that is specifically designed to analyze acidic compounds will provide the best results. The Restek Stabilwax^{fi}-DA column fits these criteria and provides good peak shape without methylation.

Preparing Free Fatty Acids

One can analyze existing free fatty acids in a matrix or saponify fats to obtain them. Saponification procedures are outlined in AOAC Methods 971.11D and 938.09D¹. Samples are extracted with solvent and saponified by heating under a reflux with an excess of dilute aqueous ethanolic alkali. After saponification, the sample is neutralized with dilute hydrochloric acid or sulfuric acid. In many cases, an aqueous solution of phosphotungstic acid is added after mixing or shaking. The sample may be centrifuged and/or filtered, and then finally diluted to an aqueous solution.

Injecting Free Fatty Acids

Although free fatty acids can be injected by split or splitless techniques (especially when using smaller bore columns), a direct injection technique is recommended. Direct injection will reduce the risk of losing volatile low molecular weight fatty acids through the split vent, which improves quantitative reproducibility. Because free acids can be adsorbed, the analyst must make every effort to ensure an inert sample pathway by using properly deactivated direct injection liners and inert capillary columns. Regular preventive maintenance of the GC injection port is strongly recommended to prevent surfaces from becoming active over time.

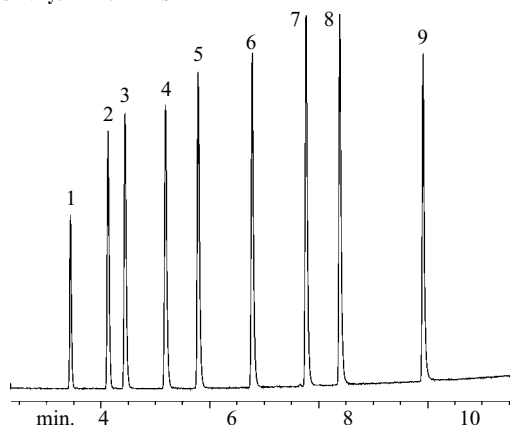
Selecting a Column

Although the official methods listed previously indicate the use of packed column chromatography, capillary chromatography can provide much greater resolving power (i.e., column efficiency). We suggest using a Stabilwax^{fi}-DA capillary column for the analysis of volatile free fatty acids. The Stabilwax^{fi}-DA features a bonded Carbowax^{fi} phase that has been modified specifically for the analysis of acidic compounds. To prove its effectiveness, we analyzed a direct injection of straight chain and branched fatty acids ranging from acetic acid to heptanoic acid. Because the Stabilwax^{fi}-DA column has a strong affinity for free acids, excellent separation was achieved within 10 minutes (**Figure 1**). Also notice how the unique deactivation of the Stabilwax^{fi}-DA column produced sharp symmetrical peaks with minimal tailing.

Figure 1
Stabilwax^{fi}-DA achieved excellent separation within 10 minutes.

15m, 0.53mm ID, 1.0 m Stabilwax^{fi}-DA (cat.# 11052)
0.2 L injection of a 10-20ng/ L free fatty acid standard in water. Direct injection using a Uniliner^{fi} liner.
Oven Temp.: 145 C isothermal
Inj./Det. Temp.: 250 C
Carrier gas: hydrogen
Linear Velocity: 80cm/sec. (flow rate: 10cc/min.)
FID Sensitivity: 4 x 10⁻¹¹ AFS

1. acetic acid
2. propionic acid
3. isobutyric acid
4. n-butyric acid
5. isovaleric acid
6. n-valeric acid
7. isocaproic acid
8. caproic acid
9. heptanoic acid



Summary

An optimized GC system for the analysis of volatile free fatty acids requires direct injection for minimal discrimination and maximum inertness in the injection port, and requires an analytical column like the Stabilwax^{fi}-DA to provide selectivity and good peak shape.

Stabilwax^{fi}-DA Columns

ID	df	15-meter
0.53mm	1.0 m	cat.# 11052
ID	df	30-meter
0.53mm	0.25 m	cat.# 11025

References:

1. AOAC International, *Official Methods of the AOAC*, 15th edition, 1990. Reference not available from Restek.



Order a FREE Foods, Flavors, and Fragrances Catalog! This 52-page document includes important analysis tips and chromatograms for the analysis of fats and oils, carbohydrates, vitamins, amino acids, organic acids, preservatives, flavors and fragrances, essential oils, and chiral compounds. Retention time indices and complete product listings for all of the relevant GC and HPLC products also are included (lit. cat.# 59260). Also, request Applications Note detailing food packaging testing (lit. cat.# 59348).

Product Listing

Stabilwax^{fi}-DA (Fused Silica)

(Crossbond^{fi} Carbowax^{fi} for acidic compounds) Stable to 250 C
 Ideal for the analysis of free acids (no need for derivatization).
 High thermal stability (250 C) and long column lifetime.
 Crossbond^{fi} technology results in reduced bleed, increased column lifetime, and solvent rinsability.
 100% bonded polyethylene glycol stationary phase.
 Similar polarity to DB-FFAP, OV-351, NUKOL, and HP-FFAP columns.

ID	df (m)	Temp. Limits	15-Meter	30-Meter	60-Meter
0.25mm	0.10	40 to 250/260 C	11005	11008	11011
	0.25	40 to 250/260 C	11020	11023	11026
	0.50	40 to 250/260 C	11035	11038	11041
0.32mm	0.10	40 to 250/260 C	11006	11009	11012
	0.25	40 to 250/260 C	11021	11024	11027
	0.50	40 to 250/260 C	11036	11039	11042
0.53mm	1.00	40 to 240/250 C	11051	11054	11057
	0.10	40 to 250/260 C	11007	11010	11013
	0.25	40 to 250/260 C	11022	11025	11028
	0.50	40 to 250/260 C	11037	11040	11043
	1.00	40 to 240/250 C	11052	11055	11058
	1.50	40 to 230/240 C	11062	11065	11068

Uniliner^{fi} Liners



Uniliners ^{fi}	each	5-pk.
Agilent Open-top Uniliner ^{fi} w/ Wool	20843	20844
Varian Open-top Uniliner ^{fi} w/ Wool	20845	20846
Shimadzu 94mm Uniliner ^{fi} w/ Wool	21713	21719
CE/Thermoquest ^{fi} Open-top Uniliner ^{fi} w/Wool	20841	20842
PE Auto Sys Open-top Uniliner ^{fi} w/ Wool	20837	20838
PE Auto Sys Cyclo-Uniliner ^{fi}	20839	20840

Trademarks: Restek: Stabilwax, Uniliner, Thermolite, and Crossbond. DB (J&W Scientific), OV (Ohio Valley Specialty Chemical Co.), NUKOL (Supelco), Agilent.

Thermolite^{fi} Septa Green

Lowest bleed on FIDs, ECDs, and MSDs.
 Each batch is tested to ensure lowest bleed.
 Excellent puncturability.
 Preconditioned/ready to use.
 Does not adhere to hot metal surfaces.
 Usable to 340 C inlet temperatures.
 Packed in non-contaminating glass jars.



Thermolite ^{fi} Septum Diameters	25-pk.	50-pk.	100-pk.
9.5mm (3/8")	20359	20360	20361
10mm	20378	20379	20380
11mm (7/16")	20363	20364	20365
Shimadzu Plug	20372	20373	20374

Encapsulated Ferrules

Will not deform or stick in fittings.
 Allows reuse of the ferrule.
 Less torque needed to seal ferrule.
 Unique blend of graphite provides less fragmentation and outgassing.



Encapsulated Ferrule ID	Fits Column ID	10-pk.
0.4mm	0.25mm	21036
0.5mm	0.32mm	21037
0.8mm	0.53mm	21038

Literature Request List

Lit. Cat. #	Title
59128	Application Note: Determination of Omega 3 and Omega 6 Fatty Acids
59136	Application Note: Analysis of Saw Palmetto using Rtx-Stabilwax
59177	Application Note: Ultra Aqueous C18 HPLC Column
59181	Application Note: Analysis of Vitamins Using Ultra C18 HPLC Columns
59186	Application Note: HPLC Analysis of Vanillin and Ethyl Vanillin in Vanilla Flavors
59241	HPLC Minicatalog
59546	Application Note: The Analysis of Chiral Flavor Compounds in Apple Juices using the Rt-bDEXsm & Rt-bDEXse Columns
59553	Application Note: Grape Flavor Analysis using Rt-bDEXsa Column
59579	Application Note: Analysis of Flavor Volatiles in Alcoholic Beverages
59580A	Application Note: Fast, Selective Triglyceride Analysis
59581	Application Note: Analysis of Cholesterol
59582	Application Note: Detection of Synthetic and Natural Antioxidants in Foods
59583	Application Note: Analyzing Free Fatty Acids
59584	Application Note: Analyzing Fatty Acid Methyl Esters (FAMES)
59627B	Genuine Restek Replacement Parts Catalog for Agilent GCS
59889	Guide to the Analysis of Chiral Columns by GC
59890	Selection Guide for Polar Wax GC Column Phases
59199	Applications Note: Analyzing Heat Level of Peppers and Hot Sauces Using an Ultra C18 HPLC Column

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