Analysis of Organic Acids and Alcohols Using the Agilent J&W DB-624UI Ultra Inert GC Column

Application Note

Food Testing & Agriculture

Authors

Pat Sasso and Ken Lynam
Agilent Technologies, Inc.

Abstract

A 26-component mix of aliphatic short chain and aromatic alcohols and carboxylic acids was used to evaluate the recently introduced Agilent J&W DB-624UI column to show acceptable peak shape and resolution. The column was compared to non-Agilent 624 phases. Organic acids had reasonable peak width and peak symmetry for a narrow range of volatilities (C3 through C8) on the DB-624UI column, suggesting it may be possible to analyze these compounds without the need to convert them to methyl esters.

Introduction

Recently, emphasis has been placed on the heart-healthy benefits of long chain omega-3 fatty acids. The accurate quantitation of these acids in various matrixes, ranging from salmon tissue to dietary supplements, typically involves extraction, drying, and derivatization to the methyl esters to permit analysis by gas chromatography. While this technique has gained wide acceptance, the ability to measure organic acids without so much sample preparation has led to the introduction of novel detectors, such as charged aerosol detection coupled to HPLC [1]. The tradeoff is that HPLC columns lack the separation power for closely similar compounds that capillary GC columns can provide. Another problem associated with reversed-phase HPLC is the need to bring samples for injection into an aqueous-friendly environment. GC injection is advantageous because the components of interest possess a nonpolar moiety more easily extracted into GC-friendly nonpolar solvents.

Agilent Technologies

The DB-624UI column acquired its unique phase selectivity over the course of many years, dating back to the original use of the 624 phase for purgeable halogenated hydrocarbons under EPA Method 624 for waste water effluent. By combining sufficient cyanopropyl phenyl with a large percentage of methyl polysiloxane, the column allows GC/MS characterization of previously poorly resolved components with isobaric quantitation ions such as 2-butanone and ethyl acetate [2].

This application note assesses the performance of the DB-624UI GC column against a non-Agilent 624 column for the analysis of organic acids and alcohols, without the need for time-consuming derivatization.

Experimental

An Agilent 6890N GC/FID equipped with an Agilent 7683B Automatic Sampler was used for this series of experiments.

Conditions

Column:	Agilent J&W DB-624UI, 30 m × 0.32 mm, 1.8 µm (p/n 123-1334UI)
Sample:	26-component alcohol and acids mix (C_1 through C_{12}), 100 ng per component on-column
Carrier:	Hydrogen, 38 cm/s, 2.0 mL/min, constant flow mode
Oven:	35 °C (hold 1 min), to 260 °C at 10° C/min (hold 1 min)
Inlet temp:	200 °C
Inlet liner:	Deactivated dual-taper direct connect
Automatic Sampler:	Agilent 7683B, 0.5 µL syringe, 0.01 µL neat, split injection (100:1 ratio)
GC:	Agilent 6890N GC/FID
Detector:	FID at 265 °C

Flow path supplies from Agilent

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:	0.5 µL (p/n G4513-80229)
Septum:	Advanced Green (p/n 5183-4759)
Inlet liner:	Dual-taper direct connect (p/n G1544-80700)
Magnifier:	20× (p/n 430-1020)

Standards preparation

A 26-component checkout mix at equivalent carbon numbers for FID response was prepared from reagents of ACS grade or better, available from Sigma-Aldrich. To provide approximately similar area responses for all components on the FID detector, methanol was added in slight excess to account for evaporation in the automatic sampler vials over time.

Results and Discussion

EPA 624 columns were purchased from another vendor. Agilent and non-Agilent columns had the same dimensions and film thickness, and all columns were conditioned overnight prior to making any injections. Criteria for evaluation included peak symmetry for 3 different organic acids, peak shape for alcohols, and resolution of the critical pair, phenyl ethanol and nonanol. Elution order was verified separately by GC/MS in EI mode on an Agilent 5975D equipped with an EI 350 °C inert ion source.

Figure 1 shows an analysis of the test mixture on the DB-624UI column. The peak symmetry for the 3 organic acids is acceptable.

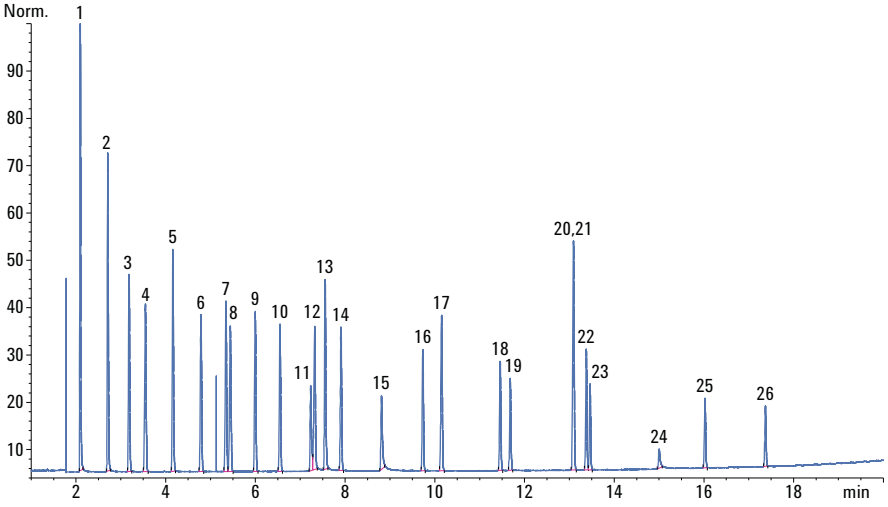

Figure 1. Test mixture showing acceptable peak shape for organic acids on an Agilent J&W DB-624UI GC column.

Table 1 lists the peak symmetry as calculated by the area percent report with performance included. A statistical analysis of 3 replicate injections is provided in Table 2 to demonstrate the reproducible peak symmetry and resolution of the critical pair delivered by the DB-624UI column. It is important to note that under identical temperature programming, 2 non-Agilent columns gave no resolution of the critical pair, leading to the conclusion that not all 624 phases are created equal.

Table 1. Peak width and symmetry for underivatized alcohols and acids following separation on an Agilent J&W DB-624UI GC column.

Component	Peak number	Width	Symmetry
Methanol	1	0.0194	0.6993
Ethanol	2	0.0247	0.7842
Isopropanol	3	0.0293	0.8980
Tert-butanol	4	0.0348	0.9528
1-Propanol	5	0.0299	0.9035
2-Butanol	6	0.0321	0.9521
2-Methyl-1-propanol	7	0.0334	0.9610
2-Methyl-2-butanol	8	0.0367	0.9796
1-Butanol	9	0.0314	0.9466
3.Pentanol	10	0.0323	0.9836
Propanoic acid	11	0.0329	0.5443
3-Methyl-1-butanol	12	0.0354	1.0277
Ethylene glycol	13	0.0309	0.8296
1-Pentanol	14	0.0310	0.9785
Butanoic acid	15	0.0347	0.5953
1-Hexanol	16	0.0312	0.9894
Cyclohexanol	17	0.0342	0.9668
1-Heptanol	18	0.0308	0.9882
1,2-Pentanediol	19	0.0313	0.9390
Benzyl and octanol	20, 21	0.0380	1.0342
Phenyl ethanol	22	0.0321	0.9638
Nonanol	23	0.0305	0.9578
Octanoic acid	24	0.0374	0.6355
Decanol	25	0.0309	0.9398
Undecanol	26	0.0311	0.9527

Table 2. Three replicate injections show reproducible peak symmetry and resolution on an Agilent J&W DB-624UI GC column (serial number USC179032H)

Compound	Avg. symmetry	Std. dev.
Propanoic acid	0.70	0.01
Butanoic acid	1.10	0.03
Octanoic acid	0.87	0.01
	Avg. RS	**Std. dev.**
Phenyl ethanol/nonanol critical pair	3.883	0.021

Figure 2 demonstrates the lack of peak symmetry exhibited by one of the non-Agilent columns. Butanoic acid, also referred to as butyric acid, produced severe tailing on a non-Agilent column, and in replicate injections, it was virtually impossible to integrate properly. This compound can be detected by mammals with good scent detection abilities (such as dogs) at 10 µg/L, whereas humans can detect it in concentrations above 10 mg/L. When butter goes rancid, butyric acid is liberated from the glyceride by hydrolysis, leading to its characteristic unpleasant odor commonly described as acrid.

Figure 3 provides a comparison of the peak symmetry for octanoic acid with the DB-624UI and non-Agilent 624 columns. Symmetry tended to improve as volatility decreased for the DB-624UI column, but the opposite was the case for the non-Agilent column, with octanoic acid eluting so broadly that it appeared to be missing from the sample injections. Octanoic acid, also commonly known as caprylic acid, is present in dairy foods and in palm kernel oil at 6 to 8%, which is the second largest traded edible oil in the world [3].

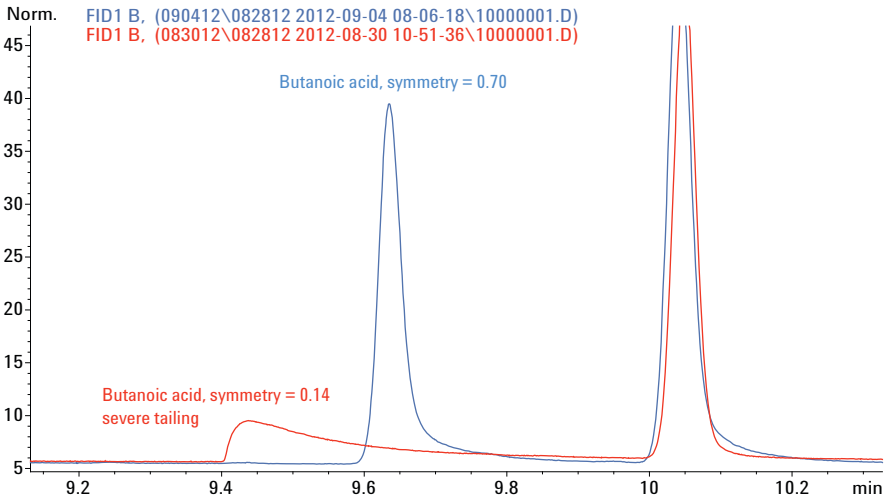

Figure 2. Peak shape for butanoic (butyric) acid on an Agilent J&W DB-624UI GC column (blue) and a non-Agilent 624 column (red).

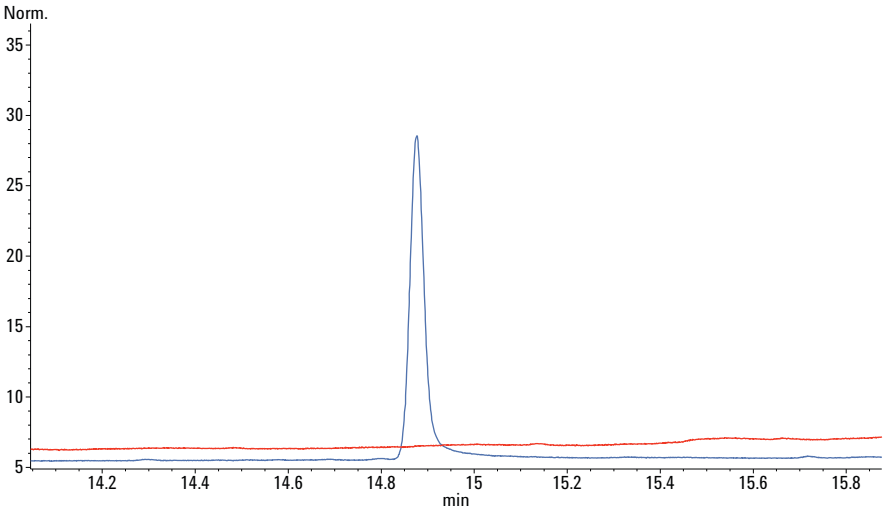

Figure 3. Peak shape for octanoic (caprylic) acid demonstrating complete loss of symmetry on a non-Agilent 624 column (red).

Conclusions

The DB-624 Ultra Inert GC column provided better performance than non-Agilent 624 columns. When considering the potential for time-saving without derivatization, it becomes apparent that this stationary phase can be suitable for the analysis of numerous organic acids. Given that only the Agilent column gave suitable peak symmetry and resolution, the option of analytical work-around procedures, such as foregoing ester derivatization, makes column selection very important to the success of the assay.

References

1. I. Acworth, M. Plante, B. Bailey, C. Crafts. "Quantitation of Underivatized Omega-3 and Omega-6 Fatty Acids in Foods by HPLC and Charged Aerosol Detection". Thermo Fisher Scientific, Inc. Publication Number LPN 2931-02 01/12 (2011).

2. EPA. EPA Method 624 - Purgeables. U.S. Environmental Protection Agency. http://water.epa.gov/scitech/methods/cwa/organics/upload/2007_07_10_methods_method_organics_624.pdf

3. C. Coslett. Facts on Palm Kernel Oil. http://www.ehow.com/facts_5783441_palm-kernel-oil.html

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
November 8, 2012
5991-1441EN

Agilent Technologies