Application Note

Food Testing and Agriculture

Reduced carryover and increased reproducibility in the analysis of cold-pressed pink grapefruit essential oil

Using an Agilent J&W DB-HeavyWAX GC column

Author

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Abstract

The analysis of cold-pressed citrus essential oils, such as pink grapefruit, can be challenging due to the presence of higher molecular weight aromatic compounds, which can cause carryover or long run times. The Agilent J&W DB-HeavyWAX column, with a maximum operating temperature of 280 °C isothermal and 290 °C programmed, can operate at temperatures greater than traditional WAX-type columns. The increased temperature range allows faster and more reproducible analysis of compounds in cold-pressed pink grapefruit oil over extended periods of time. The DB-HeavyWAX showed very similar selectivity to the Agilent J&W DB-WAX GC columns.

Introduction

Essential oils are used in many household items, from cleaning products to perfumes, and contain a great deal of aromatic compounds. The two primary extraction methods for these essential oils are distillation and cold-pressing. Steam distillation applies heat to the organic material, while cold-pressing, also referred to as expressed, is a mechanical process where no heat is applied[1]. An advantage of the cold-pressed extraction process is that it contains higher boiling and higher molecular weight compounds that are lost in the steam distillation process. The higher molecular weight compounds produce a fresher and more flavorful aroma, and are widely used to extract citrus oils, such as pink grapefruit essential oil[2].

Analyzing compounds in cold-pressed essential oils is commonly done with a 100 % polyethylene glycol phase column, also known as a WAX column. Due to issues such as decreased thermal stability, traditional WAX columns have a limited maximum operating temperature of 250 °C isothermal and 260 °C programmed. This limit can cause difficulty when analyzing the higher molecular weight compounds found in cold-pressed citrus essential oils. To work around the issue of analyzing all the compounds in cold-pressed citrus essential oils, several options are available:

- Hold the final oven temperature at the maximum for an extended period
- Risk taking the column above the stated Maximum Allowed Operating Temperature (MAOT)
- Select a different column with a phase that allows the use of higher temperatures, but may not have the optimum selectivity

Increasing the final hold time on a GC run may facilitate the elution of some later-eluting compounds, but it will also increase the overall run time. Taking a column above the stated MAOT, to elute all of its volatile compounds, risks damage to the phase. This damage can be seen in shifting retention times, increased column bleed, and sometimes a change in the retention order of certain compounds over extended periods of time[3].

The Agilent J&W DB-HeavyWAX has an extended temperature limit of up to 280/290 °C. This permits the analysis of the higher boiling compounds in cold-pressed citrus oil without risking the integrity of the column phase. This Application Note examined the benefits of the extended temperature limit of the DB-HeavyWAX column. This increased temperature limit can increase the reproducibility and decrease carryover in the analysis of cold-pressed pink grapefruit essential oil. The thermal stability of a traditional WAX column versus the DB-HeavyWAX was examined, with the application of cold-pressed pink grapefruit essential oil. This was done to demonstrate the improved thermal stability of the DB-HeavyWAX.

Experimental

An Agilent 7890 GC/FID equipped with a split/splitless inlet, and an Agilent 7693 sampler with Agilent MassHunter control software was used for GC/FID experiments.

Sample preparation

A sample of cold-pressed pink grapefruit essential oil was purchased from Emily's Oils & Essentials (Fair Oaks, CA), and injected neat.

Instrument conditions

GC Conditions	
Column	Agilent J&W DB-HeavyWAX, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132) Agilent J&W DB-WAX, 30 m × 0.25 mm, 0.25 µm (p/n 122-7032) Commercially available WAX column, 30 m × 0.25 mm, 0.25 µm
Carrier	Helium, constant flow, 1 mL/min
Oven	Program 1: 60 °C (2.0 minutes), ramp 5 °C/min to 250 °C (30 minutes) Program 2: 60 °C (2.0 minutes), ramp 5 °C/min to 280 °C (30 minutes)
Inlet	Split mode, 250 °C, split ratio 200:1
Inlet liner	Ultra Inert, low pressure drop (p/n 5190-2295)
GC/FID	Agilent 7890B GC equipped with FID/MSD
Sampler	Agilent 7693 autosampler
FID conditions	
Temperature	280 °C
Hydrogen	30 mL/min
Air	400 mL/min
Col + make up	25 mL/min
Flowpath supplies	
Septum	Bleed and temperature optimized, BTO, 11 mm septa (p/n 5183-4757, 50/pk)
Gold seal	Ultra Inert gold seals (p/n 5190-6145, 10/pk)
Vials	2 mL, screw top, amber, write-on spot, certified (p/n 5182-0716, 100/pk)
Vial inserts	250 µL glass inserts, deactivated (p/n 5181-8872, 100/pk)
Vial caps	9 mm blue screw cap, PTFE/red silicone septa (p/n 5185-5820, 500/pk)
Inlet/FID	85:15 Vespel: graphite ferrules (p/n 5062-3508, 10/pk)

Results and discussion

A sample of cold-pressed pink grapefruit essential oil was injected onto a DB-HeavyWAX column. An initial oven temperature of 60 °C was held for two minutes, then ramped to a final temperature at a rate of 5 °C/min, and held for 30 minutes. Figure 1 demonstrates the effect of achieving a final temperature of 280 °C versus 250 °C on the analysis of pink grapefruit essential oil. By increasing the final temperature, the peak shape is better for the later-eluting compounds, and it is also possible to elute more compounds.

The largest of the two final eluting compounds, Meranzin and Isomeranzin, did not elute with a final temperature of 250 °C and a hold time of 30 minutes. Therefore, these compounds are present in subsequent injections, as seen in Figure 2, as carryover. This carryover causes irreproducible and incorrect results if the column is not operated at a final temperature of 280 °C.

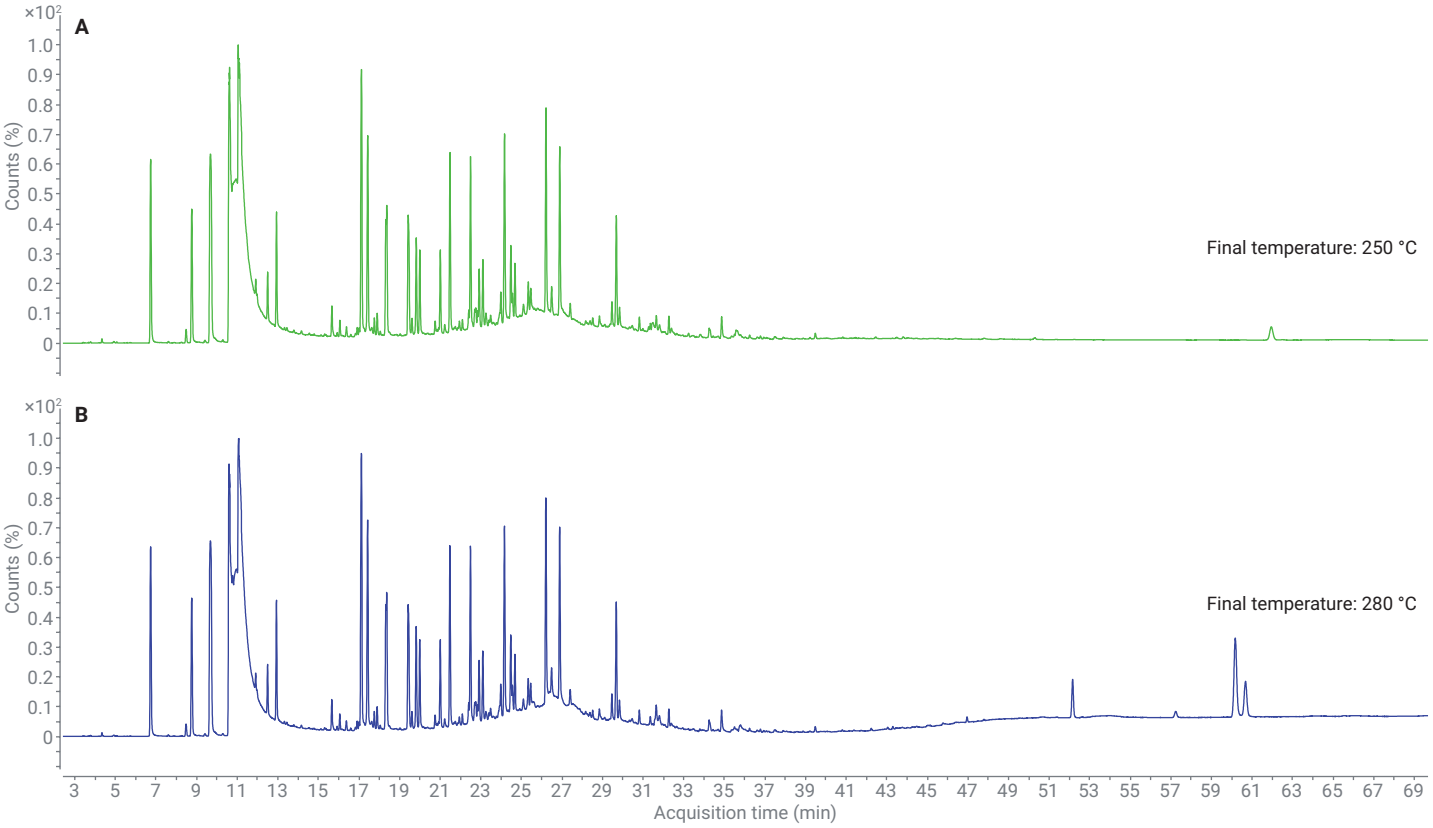

Figure 1. A sample of pink grapefruit essential oil injected on an Agilent J&W DB-HeavyWAX column run to a final oven temperature of 250 and 280 °C.

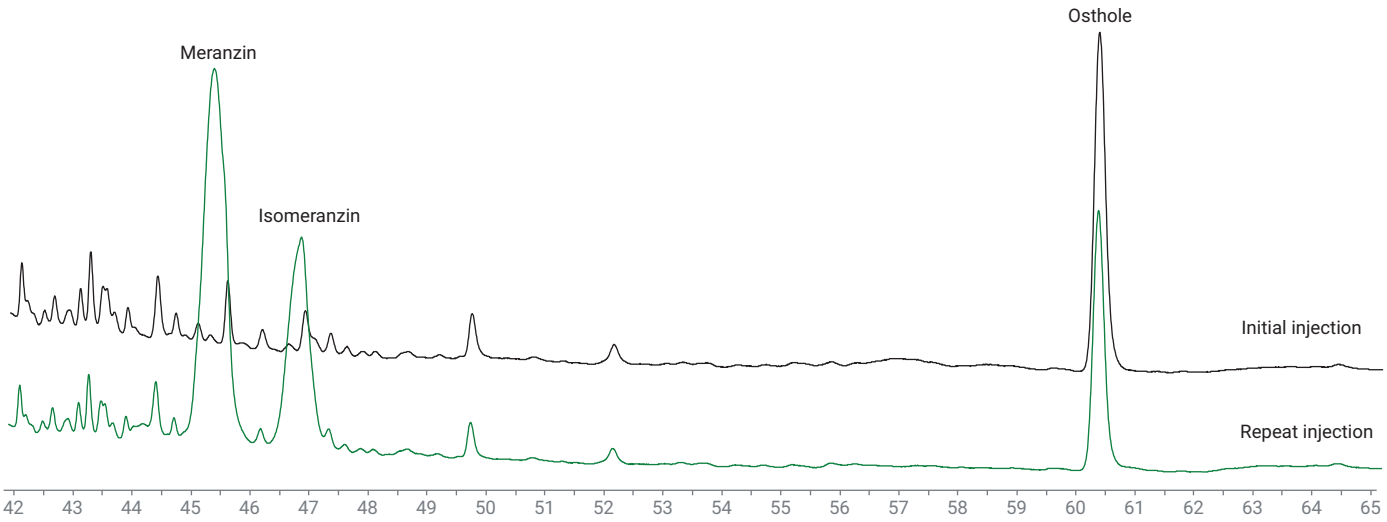

Figure 2. Carryover in repeated injections of pink grapefruit essential oil run to a final oven temperature of 250 °C, held for 30 minutes.

Figure 3 illustrates five of the aromatic compounds identified by mass spectrometry in cold-pressed pink grapefruit essential oil that benefit from the increased MAOT of the DB-HeavyWAX column.

Figures 4A and 4B demonstrate the shortcomings of a traditional WAX column with maximum operating temperatures of 250/260 °C, when taken above its MAOT. In Figure 4A, a sample of cold-pressed pink grapefruit essential oil was injected onto a commercially available traditional WAX column, and run to a final temperature of 280 °C. To examine what happens to the resolution and thermal stability of a traditional WAX column taken above the MAOT over an extended period, four well resolved peaks were chosen and identified.

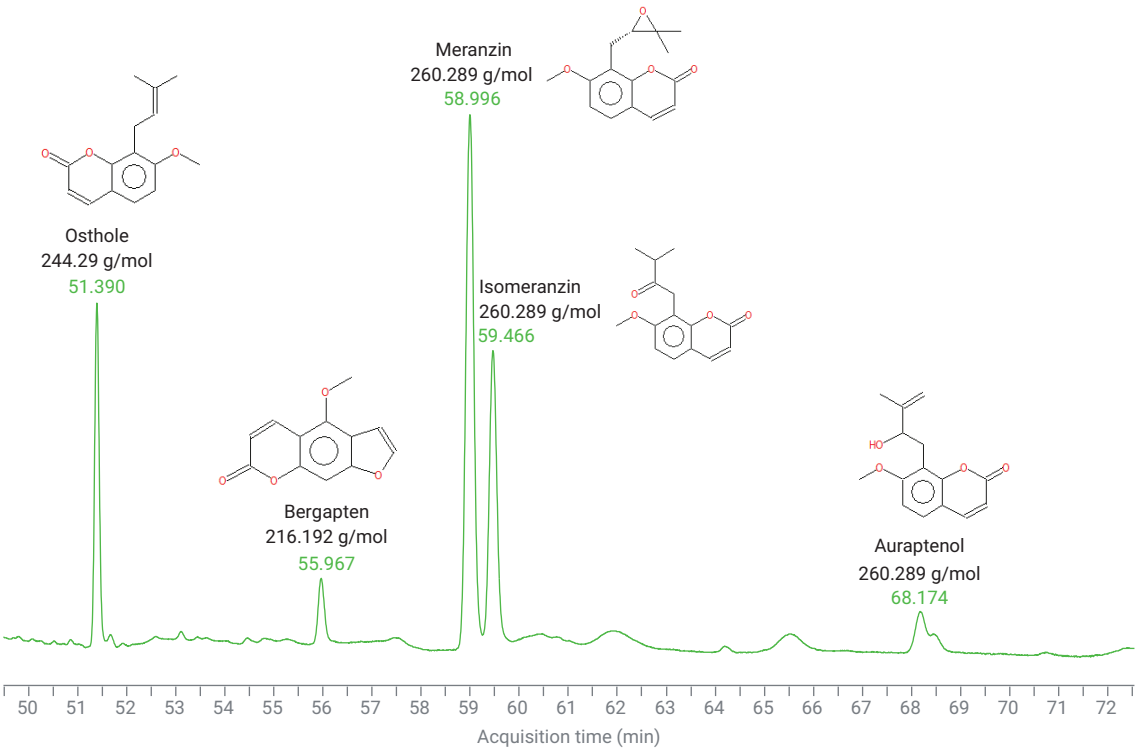

Figure 3. Higher molecular weight compounds in pink grapefruit essential oil (cold-pressed) that elute with a final oven temperature of 280 °C. These compounds were identified using mass spectrometry.

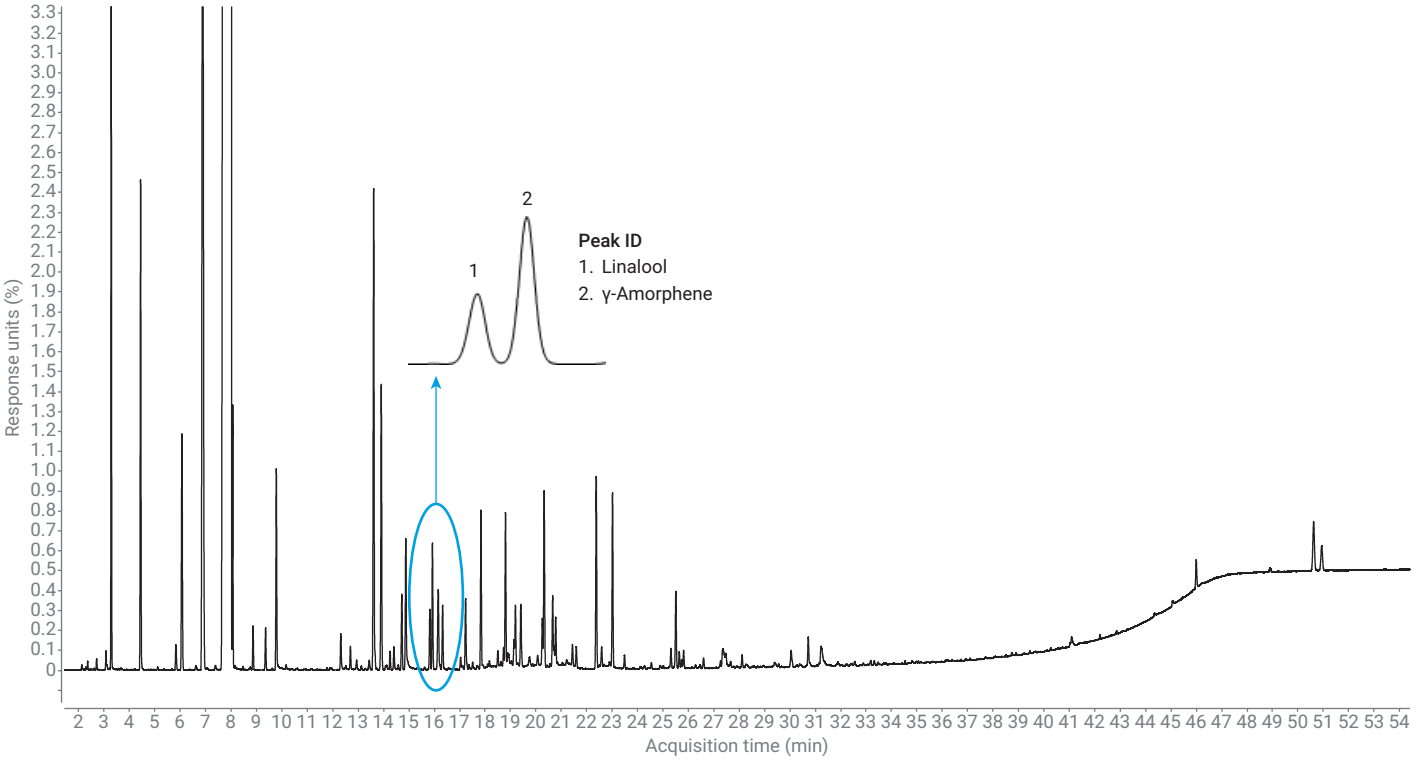

Figure 4a. A sample of cold-pressed pink grapefruit essential oil injected onto a commercially available WAX column, run to a final oven temperature of 280 °C

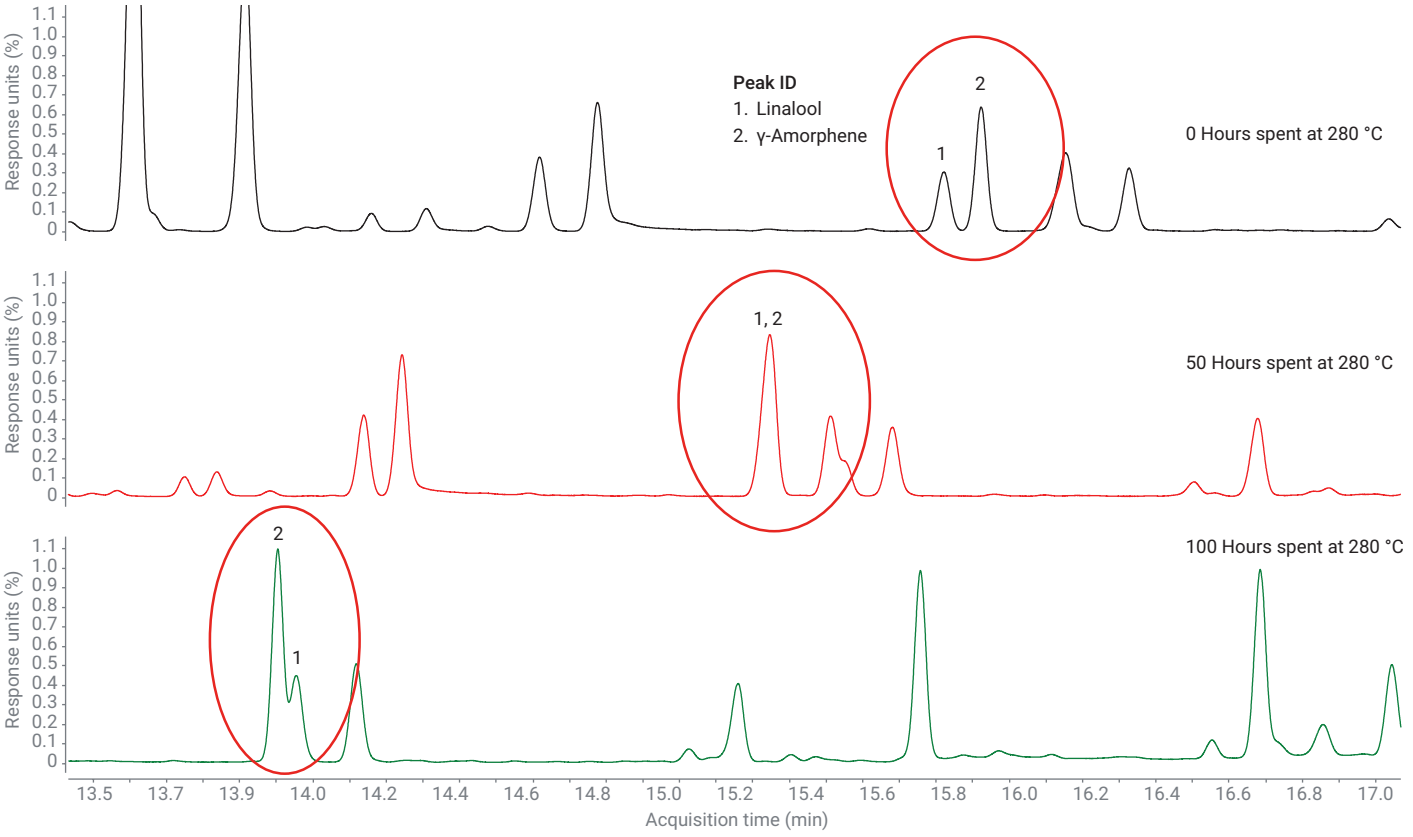

Figure 4b. A sample of pink grapefruit essential oil (cold-pressed) run on a commercially available WAX column, operated for 100 hours at 280 °C.

Figures 4A and 4B demonstrate that the two peaks, linalool and γ-amorphene, confirmed with a mass spectrometer, are fully resolved upon their initial injection of pink grapefruit essential oil, and zero operating hours at 280 °C. After 50 hours of operation at 280 °C, the retention times of the peaks have not only shifted earlier, but linalool and γ-amorphene coelute. Operation of this column at 280 °C for 100 hours demonstrates greater retention time shifting, coelution, and a change in position of the linalool and γ-amorphene peaks.

The shift in retention time, seen in Figure 4B, indicates that the phase in the commercially available WAX column has been significantly degraded after repeated use at 280 °C. This loss of phase has caused the four compounds to elute at different parts of the temperature programs, and has changed their elution order.

Figure 5 demonstrates the improved thermal stability of the DB-HeavyWAX column when operating at high temperatures for extended periods of time. Even after 100 hours of use at 280 °C, the elution order on the DB-HeavyWAX is still the same, which is not the case with the competitor WAX column. The stability of the elution order over long periods of time at high temperatures demonstrates the improved thermal stability of the DB-HeavyWAX.

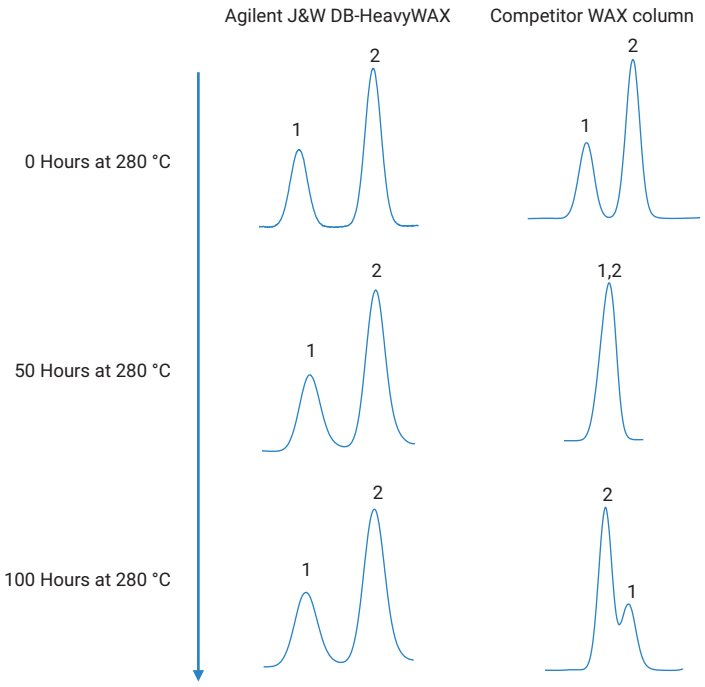

Figure 5. Comparison of stationary phase thermal stability over 100 hours operating at 280 °C for the Agilent J&W DB-HeavyWAX and a traditional commercially available WAX column.

Figure 6 shows the same sample of cold-pressed pink grapefruit essential oil run on a DB-WAX and a DB-HeavyWAX column to a final oven temperature of 250 °C. This temperature was held until the final peaks eluted after 60 minutes. Both the DB-WAX and the DB-HeavyWAX show similar selectivity for the analysis of cold-pressed pink grapefruit essential oil.

Conclusion

The Agilent DB-HeavyWAX column provides an increased maximum temperature range. This range allows for more reproducible analysis of higher boiling compounds in cold-pressed citrus essential oils, without fear of carryover from sample to sample. Traditional WAX columns have a lower upper temperature limit, and cannot maintain retention time stability at high temperatures, such as 280 °C, for extended periods of time. The increased temperature limit of the DB-HeavyWAX provides a more thermally stable column, even after 100 hours operating at 280 °C. The DB-HeavyWAX has a similar selectivity to an Agilent DB-WAX, allowing for easy method translation when replacing a DB-WAX column with a DB-HeavyWAX. The DB-HeavyWAX has the added benefit of the extended temperature limit, up to 280 °C isothermal and 290 °C programmed.

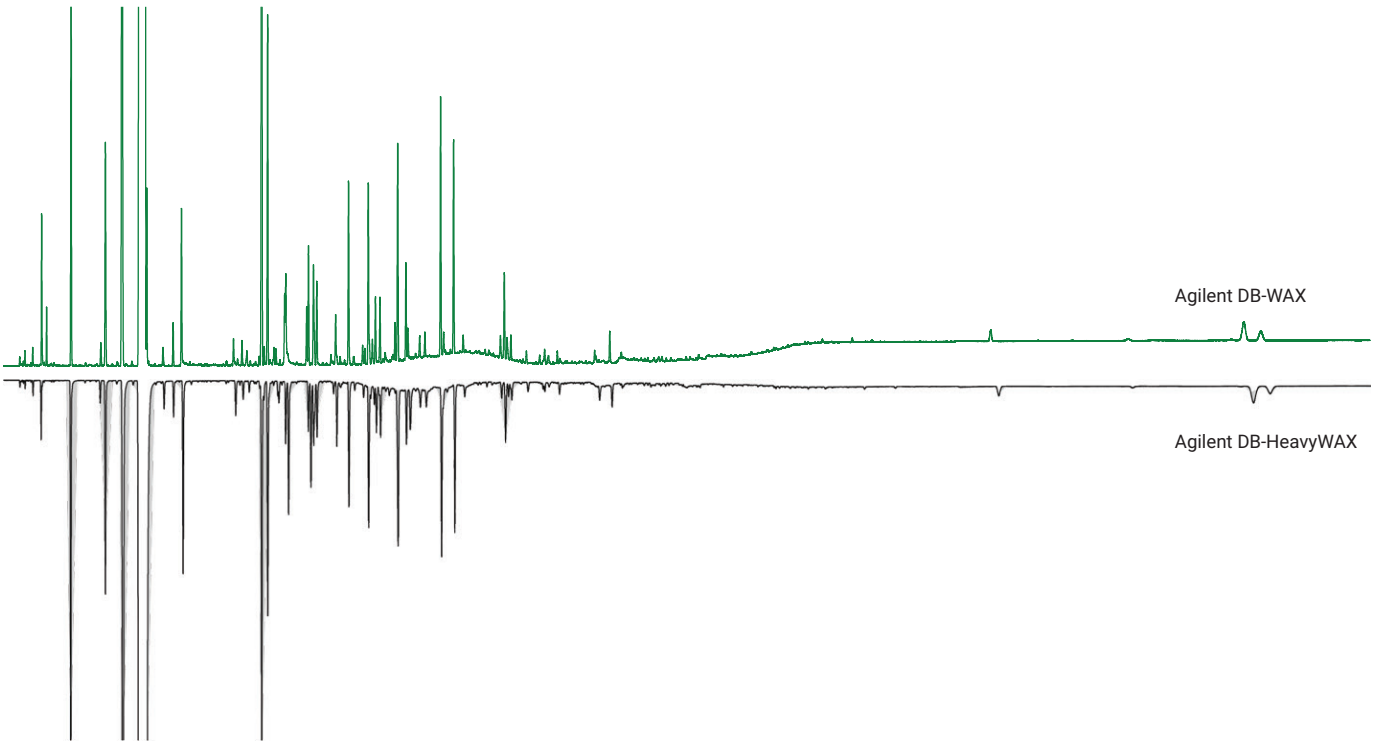

Figure 6. Agilent J&W DB-WAX has a similar selectivity to Agilent J&W DB-HeavyWAX, as demonstrated in the analysis of pink grapefruit essential oil.

References

1. https://naha.org/explore-aromatherapy/about-aromatherapy/how-are-essential-oils-extracted, Accessed 8 January **2018**.
2. Lin, J.; Rouseff, R. L. Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time–intensity GC–olfactometry and GC–MS. *Flavor and Fragrance Journal* **2001**, *16*, 457–463.
3. Abercrombie, V.; Provoost, L. Increased Thermal Stability and Maximum Temperature of the Agilent J&W DB-HeavyWAX Column. *Agilent Technologies Application Note*, publication number 5991-9035EN, **2018**.

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