Agilent J&W CP-Wax 52 CB Columns with Improved Inertness Consistency from Column to Column

Technical Overview

Introduction

Polyethylene glycol (PEG) also known as Wax-type GC columns have been the industry standard for many years for the analysis of a wide variety of compounds with polar functional groups as well as saturated compounds in flavors and fragrances and industrial chemical quality control. Trace analysis of numerous active analytes of interest still poses big challenges due to the strong adsorption of these compounds onto active sites present on GC columns or elsewhere in the GC flowpath. This may cause significant loss of measurement sensitivity and poor reproducibility. Building on our experience developing enhanced surface deactivation chemistries, Agilent has improved the inertness of the entire GC flowpath, leading to minimal adsorption and breakdown of active analytes [1-4]. This has resulted in a significant improvement in the sensitivity and reproducibility of trace analysis. This technical overview presents improvement of inertness for Agilent J&W CP-Wax 52 CB columns.

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The improved CP-Wax 52 CB columns have been tested through a rigorous procedure using the most active probes that reflect today's demanding applications. The performance of improved CP-Wax 52 CB was evaluated based on a combination of three different perspectives:

- **Inertness:** Performance was evaluated using new, more demanding test mixtures that enable greater scrutiny of column activity. In addition, inertness stability was also investigated using a thermal longevity test at 250 °C as the column's upper temperature limit.
- **Consistency:** A set of 20 improved CP-Wax 52 CB columns was randomly selected from different batches and tested for inertness consistency from column to column.
- **Selectivity:** A combination of retention indices of selected compounds was monitored in QC tests to verify identical selectivity between the standard and the improved CP-Wax 52 CB. Selectivity equivalence to the standard version is important as it facilitates easy upgrading to the improved version, ensuring minimal revalidation or modifications to existing methods. We used compound retention data libraries acquired over years on standard CP-Wax 52 CB columns to ensure validity when switching to the improved version.

A benchmarking study was also performed for a comparison of inertness between an improved CP-Wax 52 CB columns and a variety of non-Agilent wax columns. All experimental conditions (except the columns) were kept constant to provide as fair a comparison as possible among the columns.

With a new treatment technology developed, we obtained strong overall improvement for inertness from column to column. Great thermal stability was demonstrated at the column's upper temperature limit on improved CP-Wax 52 CB columns, allowing the most sensitive and reproducible analytical results for the analysis of more active analytes.

Results and Discussion

Test methods and standards

QC test probes play a key role in the evaluation of column inertness and column-to-column consistency. Highly active analytes have been known to absorb onto active sites of the column. Therefore, the composition and amount on-column of these probes must carefully be selected to allow sufficient detection of important column activity. An easy QC test mixture containing undemanding probes results in poor inertness evaluation because column activity may be insufficiently recognized. Testing wax columns using demanding test probes ensures consistent column inertness performance. This ultimately contributes to improved column-to-column consistency and the reliability of analytical results. A detailed guideline for choosing suitable and effective QC test probes to evaluate column inertness performance can be found in another Agilent technical overview [1].

Following this guideline, we developed two demanding probe test mixtures, Wax Ultra Inert test mixture and a modified Grob test mixture [5] shown in Tables 1 and 2, respectively. These enabled critical assessment of the inertness performance of improved CP-Wax 52 CB columns. These test mixtures contain several challenging test probes such as propionic acid, 2-ethylhexanoic acid, ethyl maltol, ethylene glycol, 2,3-butanediol, and decanal at critical levels. These compounds are representative of several analyte groups seen in flavor and fragrance applications where wax columns have been used over the years. Peak shapes of these active analytes of interest reflect inertness or column activity. Moreover, the influences of other components in the GC flowpath are minimized by using Agilent Ultra Inert liners and Ultra Inert gold seals, which provide more confidence to injection port inertness during the evaluation of the columns.

Table 1. Wax Ultra Inert test mixture in dichloromethane.

Peak no.	Compound	Amount on column (ng)
1	5-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

Analytical conditions for Wax Ultra Inert test mixture analysis	
Injector temperature:	250 °C
Split:	1:75
Injection volume:	1 µL
Carrier gas flow rate:	1.1 mL/min, H_2
Oven temperature:	130 °C isothermal
FID detector temperature:	260 °C

Table 2. Modified Grob test mixture in dichloromethane.

Peak no.	Compound	Amount on column (ng)
1	Decane	2.5
2	Dodecane	2.5
3	Decanal	2.5
4	2,3-Butanediol*	5
5	1-Octanol	2.5
6	C10 FAME	2.5
7	*n*C11-FAME	2.5
8	*n*C12-FAME	2.5
9	2,6-Dimethylaniline	2.5
10	2,6-Dimethylphenol	2.5
11	2-Ethylhexanoic acid	5
12	Ethyl maltol	5

Analytical conditions for modified Grob test mixture analysis	
Injector temperature:	250 °C
Split	1:100
Injection volume:	1 µL
Carrier gas flow rate:	1.35 mL/min, H_2
Oven temperature:	initial temperature 60 °C, ramp 3 °C/min, final temperature 200 °C
FID detector temperature:	260 °C

* 2,3-Butanediol is present in two isomers, RR/SS and *meso* isomers, respectively.

Inertness performance

Figure 1 shows representative FID chromatograms of the modified Grob test mixture on standard and improved CP-Wax 52 CB columns after conditioning for 1 hour at 250 °C. Good peak shapes of 2-ethylhexanoic acid (peak 11) and ethyl maltol (peak 12) were found on both columns. However, column activity of the standard column was revealed by tailing peaks and significant loss of responses of two isomers of 2,3-butanediol (RR/SS isomer for peak 4a and *meso* isomer for peak 4b). Good peak shapes and great improvement in responses for these critical diols were obtained on the improved CP-Wax 52 CB column. These results indicate a significant improvement in inertness performance of improved CP-Wax 52 CB columns. The inertness of decanal (peak 3) is still a constraint of improved CP-Wax 52 CB columns. To obtain an excellent peak shape for aldehydes, Agilent J&W DB-Wax Ultra Inert columns are recommended because of their excellent inertness performance [6].

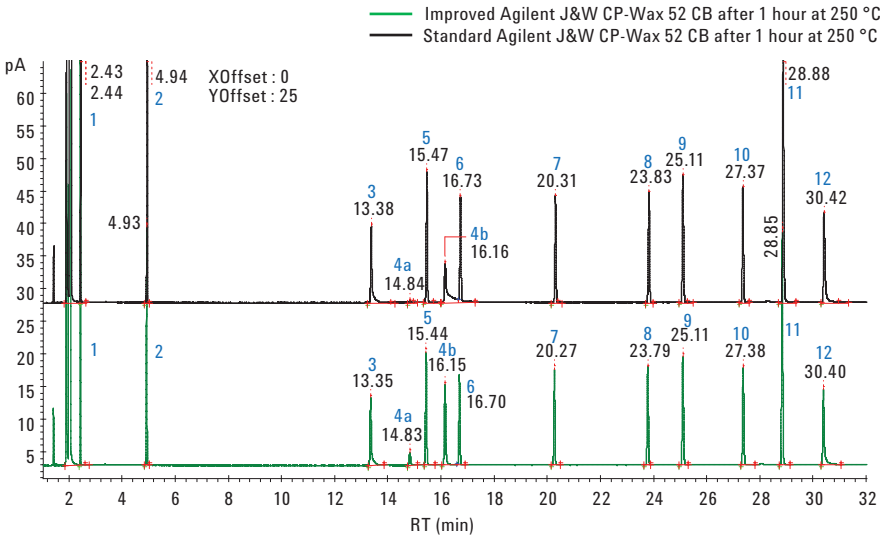

Figure 1. FID chromatograms of the modified Grob test mixture on standard and improved Agilent J&W CP-Wax 52 CB columns after conditioning for 1 hour at 250 °C. See Table 2 for GC conditions and peak identifications.

Figure 2 shows representative FID chromatograms of a complex test mixture containing 50 diols/glycols and alcohols in acetone on both standard and improved CP-Wax 52 CB columns. A critical amount on-column with approximately 3 ng for each component was injected onto the column for efficient inertness evaluation. Significant improvement in peak shape and recovery of critical diols/glycols such as (A) 1,2-propanediol, (B) 1,3-propanediol, (C) neopentyl glycol, and (D) methyl neopentyl glycol were obtained on the improved CP-Wax 52 CB column compared to the standard version. These results show the strong inertness of the improved CP-Wax 52 CB column.

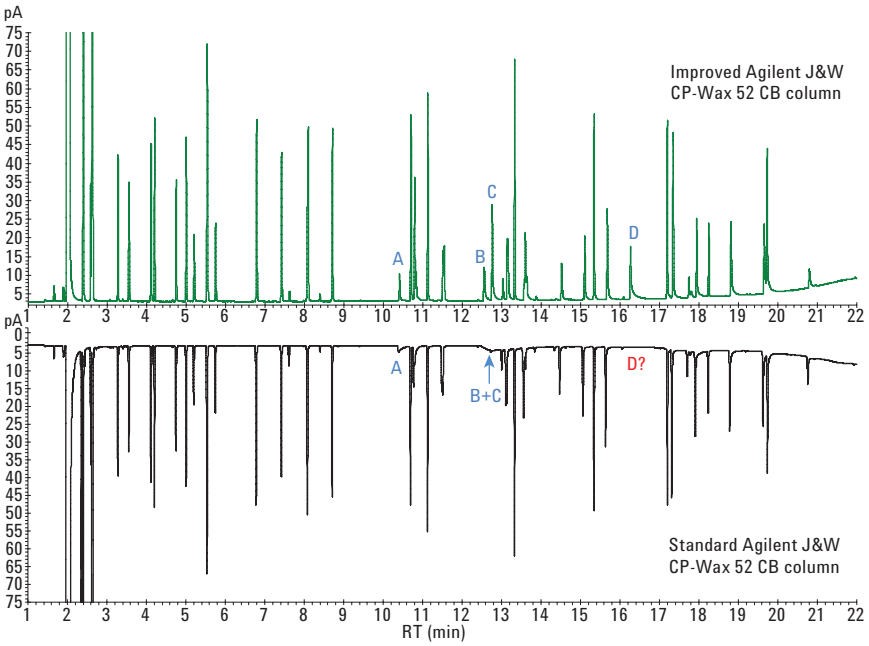

Figure 2. FID chromatograms of a test mixture containing 50 diols/glycols and alcohols on standard and improved Agilent J&W CP-Wax 52 CB columns under conditions listed in table 3.

Table 3. Chromatographic conditions for Figure 2.

Parameter	Value
GC system:	Agilent 7890A FID equipped
Autosampler:	Agilent G4513A, 10 µL syringe (p/n 5181-1267)
Columns:	Agilent J&W CP-Wax 52 CB, 30 m × 0.25 mm, 0.25 µm (p/n CP8713 and CP8713i)
Inlet:	Inert flowpath split/splitless weldment (p/n G3970A)
Inlet temperature:	250° C
Injection volume:	1 µL
Split ratio:	25:1
Carrier gas:	Hydrogen, constant flow mode, 1.35 mL/min
Oven:	40 °C, 0.5 min hold, 10° C/min to 250° C, 10 min hold
Detector temperature:	250 °C
Detector gases:	Hydrogen (30 mL/min), air (400 mL/min), nitrogen make-up gas (30 mL/min)
Flowpath supplies:	Ultra Inert low pressure drop liner (p/n 5190–2295) Ultra Inert gold seal (p/n 5190–6144) Graphite ferrules (p/n 500-2114 10Pk) Long-life septa (p/n 5183–4761)

Longevity testing at 250 °C was also performed for improved CP-Wax 52 CB columns to evaluate inertness stability at the column's upper temperature limit. An extended period of 50 hours conditioning at 250 °C was carried out. QC tests were performed after each 5 hours of conditioning at 250 °C. Figure 3 shows FID chromatograms of the modified Grob test mixture on an improved CP-Wax 52 CB column after conditioning for 1 hour and 50 hours at 250 °C. Good peak shapes and responses of 2,3-butanediol (peaks 4a and 4b), 2-ethyhexnoic acid (peak 11), and ethyl maltol (peak 12) were maintained even after long-term thermal exposure at the upper temperature limit of 250 °C. This demonstrates the superior inertness stability of the improved CP-Wax 52 CB column at 250 °C, which contributes to delivering consistent analytical results, and enhancing column lifetime.

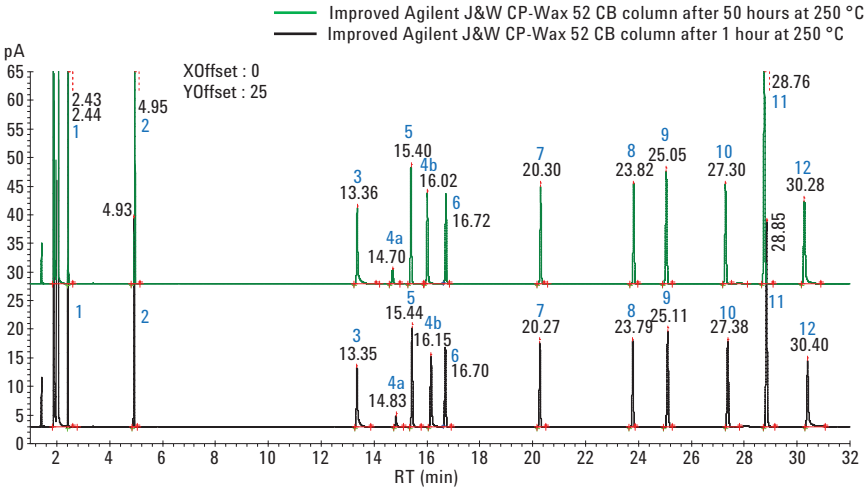

Figure 3. FID chromatograms of the modified Grob test mixture on improved Agilent J&W CP-Wax 52 CB columns after conditioning for 1 hour and 50 hours at 250 °C. See Table 2 for GC conditions and peak identifications.

Consistency from column to column is a key requirement for reproducible qualitative and quantitative analyses. A set of 20 improved CP-Wax 52 CB columns from different batches was randomly selected to evaluate column inertness consistency. Peak asymmetry at 10% peak height was used to evaluate the peak shapes of active compounds such as 2,3-butanediol (*meso* isomer, peak 4b), 2-ethylhexanoic acid (peak 11), and ethyl maltol (peak 12). Figure 4 shows consistent column-to-column inertness illustrated by the small variation in peak asymmetry at 10% peak height of these active compounds on 20 random improved CP-Wax 52 CB columns.

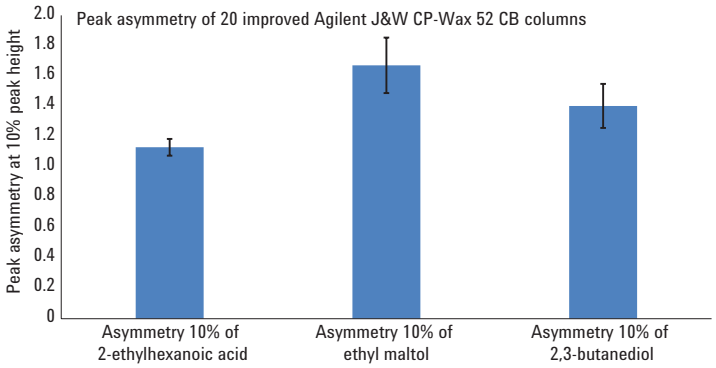

Figure 4. Peak asymmetry at 10% peak height of 2-ethylhexanoic acid, ethyl maltol and 2,3-butanediol *meso* isomer on 20 improved Agilent J&W CP-Wax 52 CB columns randomly selected from different batches. QC tests using the modified Grob test mixture were performed after conditioning for 1 hour at 250 °C.

Identical selectivity

Since standard CP-Wax 52 CB columns have routinely been used for years in many applications, same selectivity between standard and improved versions is an important advantage, if not a requirement, for current users. It ensures a fast and simple column upgrade, with minimal method revalidation, thus avoiding the risks that come with having to recreate or modify existing compound libraries or analytical methods that are based on the standard CP-Wax 52 CB. Column selectivity is often determined using a combination of retention indices of some target compounds in the QC test mixture. Retention indices of improved CP-Wax 52 CB columns were measured and compared to current specifications for standard columns (data not shown). The results indicate no significant difference in retention indices between these two columns, thus verifying the same selectivity between the two columns. The same selectivity between these two columns was also demonstrated in the analysis of fatty acid methyl esters (FAMEs), shown in Figure 5. Here an improved CP-Wax 52 CB column is shown to be identical to the standard CP-Wax 52 CB column, following the parameters established in a retention time locked (RTL) method that has been established for FAMEs and the standard CP-Wax 52 CB column [7].

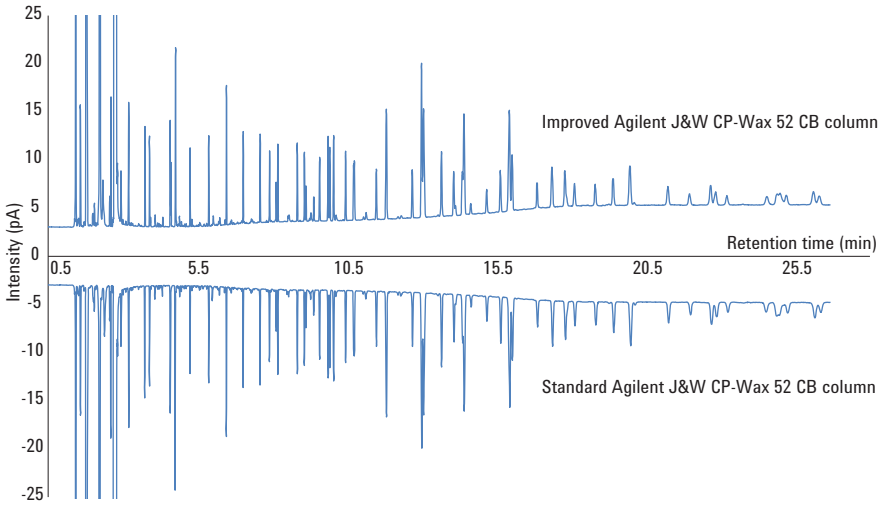

Figure 5. FID chromatograms of extended FAMEs mixture 72 compounds. The retention time was locked using methyl stearate on improved and standard Agilent J&W CP-Wax 52 CB columns.

Table 4. Chromatographic conditions for Figure 5.

Test conditions	
GC system:	Agilent 7890A FID equipped
Autosampler:	Agilent G4513A, 10 µL syringe (p/n 5181-1267)
Columns:	Agilent J&W CP-Wax 52 CB, 30 m × 0.25 mm, 0.25 µm (p/n CP8713 and CP8713i)
Inlet:	Inert flowpath split/splitless weldment (p/n G3970A)
Inlet temperature:	250° C
Injection volume:	1 µL
Split ratio:	25:1
Carrier gas:	Hydrogen
Constant pressure mode:	Approximately 53 kPa
Oven temperature:	50° C, 1 min hold, 25° C/min to 200° C, 3° C /min to 230° C, 18 min hold
Detector temperature:	250° C
Detector gases:	Hydrogen (30 mL/min) Air (400 mL/min) Nitrogen make-up gas (25 mL/min)
Flowpath supplies:	Ultra Inert low pressure drop liner (p/n 5190–2295) Ultra Inert gold seal (p/n 5190–6144) Graphite ferrules (p/n 500-2114 10Pk) Long-life septa (p/n 5183–4761)

Benchmarking research

A benchmarking study was performed to compare the inertness performance and thermal stability between an improved CP-Wax 52 CB column and selected wax columns from vendors X, Y, and Z. Two different types of wax columns were tested from vendor Z. All other analytical conditions except the testing columns were kept constant to provide a fair comparison. Inertness testing was performed after columns were conditioned for 1 hour at 250 °C using the Wax Ultra Inert test mixture. A longevity test at 250 °C for 50 hours was also carried out, testing for column inertness versus modest thermal stress at each column's upper temperature limit. QC tests were performed after each 5 hours of conditioning at 250 °C.

Figure 6 shows FID example chromatograms of several wax columns from three different vendors compared to an improved CP-Wax 52 CB column after 1 hour of conditioning at 250 °C using the Wax Ultra Inert test mixture. Improved CP-Wax 52 CB showed good peak shapes for critical components in this test mix, such as propionic acid (peak 3), ethylene glycol (peak 4), 2-ethylhexanoic acid (peak 11), and ethyl maltol (peak 12). The good inertness performance on improved CP-Wax 52 CB was also maintained after 50 hours of conditioning at 250 °C, shown in Figure 7. However, tailing peak shapes for propionic acid (peak 3), 2-ethylhexanoic acid (peak 11), and ethyl maltol (peak 12) were observed for wax columns from vendors Y and Z (Figure 6). Moreover, the inertness of these columns rapidly deteriorated during the longevity test, as shown in Figure 7, after 50 hours of conditioning at 250 °C. Wax columns from vendor X showed reasonable inertness for active compounds after 1 hour of conditioning at 250 °C. However, the inertness significantly drops during the longevity test at 250 °C, as shown in Figure 7.

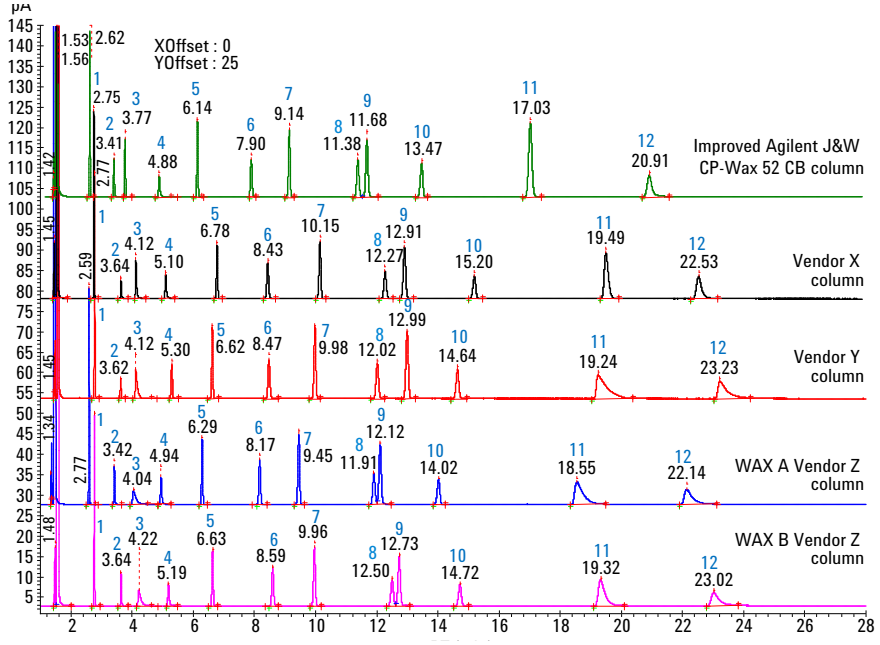

Figure 6. FID chromatograms of the Wax Ultra Inert test mixture on an improved Agilent J&W CP-Wax 52 CB column, and a wide variety of wax columns from different vendors after conditioning for 1 hour at 250 °C. See Table 1 for GC conditions and peak identifications.

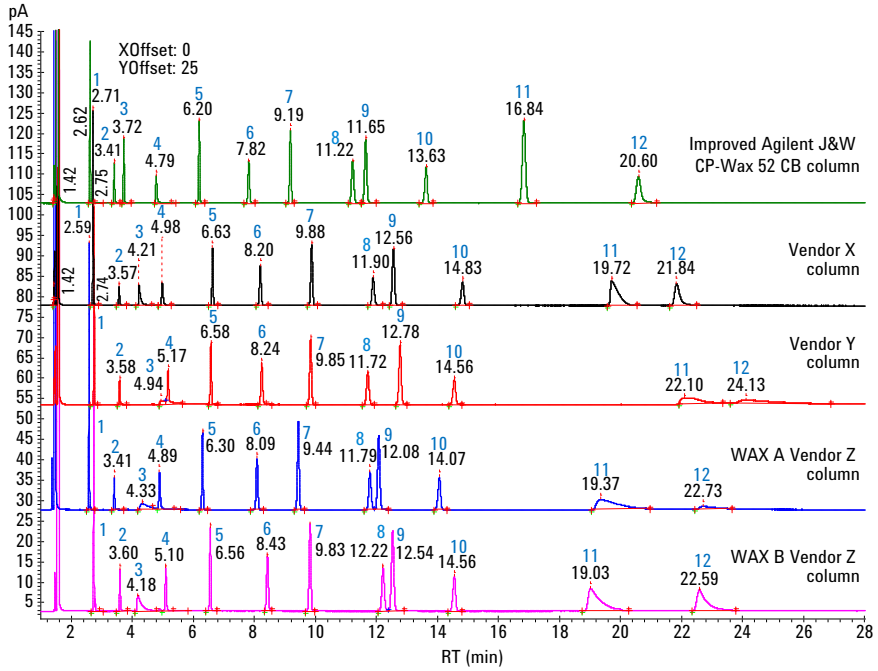

Figure 7. FID chromatograms of the Wax Ultra Inert test mixture on an improved Agilent J&W CP-Wax 52 CB and a wide variety of wax columns from different vendors after conditioning for 50 hours at 250 °C. See Table 1 for GC conditions and peak identifications.

Conclusions

In summary, by using new demanding QC test probes, column activity can be detected, and allow for better classification of inertness performance among several different wax columns in this benchmark study. Compared to standard CP-Wax 52 CB columns and wax columns from other vendors, improved CP-Wax 52 CB columns show significantly better overall inertness when testing a wide variety of active analytes of interest, as well as consistent thermal stability at the column's upper temperature limit. These characteristics strongly contribute to improved sensitivity and reproducibility of trace analyses and column lifetime.

The improved CP-Wax 52 CB shows better overall performance for inertness, thermal stability, and consistent column-to-column inertness. In addition, it shows the same selectivity as the standard Agilent J&W CP-Wax 52 CB column, making it simple to switch to this improved version with minimal method revalidation.

Ordering Information

ID (mm)	Length (m)	Film (µm)	Temp limits (°C)	7 in Cage	5 in Cage
0.10	10	0.10	20 to 250/265	CP7334i	
		0.20	20 to 250/265	CP7335i	
	20	0.20	20 to 250/265	CP7345i	
0.15	15	0.12	20 to 250/265	CP7791i	
	25	0.25	20 to 250/265	CP7792i	
0.20	30	0.20	20 to 250/265	CP7775i	
	50	0.20	20 to 250/265	CP7785i	
0.25	10	0.20	20 to 250/265	CP7703i	
	15	0.25	20 to 250/265	CP8513i	
	25	0.20	20 to 250/265	CP7713i	CP7713ii5
		1.20	20 to 250/265	CP7673i	CP7673ii5
	30	0.15	20 to 250/265	CP8745i	
		0.25	20 to 250/265	CP8713i	CP8713ii5
		0.50	20 to 250/265	CP8746i	
	50	0.20	20 to 250/265	CP7723i	CP7723ii5
	60	0.25	20 to 250/265	CP8723i	
		0.50	20 to 250/265	CP8748i	
0.32	15	0.25	20 to 250/265	CP8543i	
		0.50	20 to 250/265	CP8553i	
	25	0.20	20 to 250/265	CP7743i	
		0.40	20 to 250/265	CP7879i	
		1.20	20 to 250/265	CP7763i	
	30	0.15	20 to 250/265	CP8757i	
		0.25	20 to 250/265	CP8843i	
		0.50	20 to 250/265	CP8763i	
	50	0.20	20 to 250/265	CP7753i	
		0.40	20 to 250/265	CP7889i	
		1.20	20 to 250/265	CP7773i	CP7773ii5
	60	0.25	20 to 250/265	CP8853i	
		0.50	20 to 250/265	CP8773i	
		1.20	20 to 250/265	CP8073i	CP8073ii5
0.53	10	1.00	20 to 250/265	CP7628i	
		2.00	20 to 250/265	CP7648i	
	15	1.00	20 to 250/265	CP8718i	
	30	1.00	20 to 250/265	CP8738i	
	25	1.00	20 to 250/265	CP7638i	
		2.00	20 to 250/265	CP7658i	CP7658ii5
	50	1.00	20 to 250/265	CP7698i	
		2.00	20 to 250/265	CP7668i	
	60	1.00	20 to 250/265	CP8798i	
	100	2.00	20 to 250/265	CP7678i	

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