# Enhanced Performance of the Improved Agilent J&W HP-INNOWax Column

Improved Inertness and Column-to-column Quality

Technical Overview

## Introduction

In current GC and GC/MS applications, the sensitive and reproducible analysis of several highly active analytes at trace levels still poses a major challenge. This is due to the strong adsorption of these analytes onto the active sites present in the flowpath, or their breakdown during the passage from injector to detector. These problems can lead to poor peak shape, poor response, and poor reproducibility. To tackle these challenges, all flowpath components that are directly in contact with analytes of interest need to be highly inert. Agilent has been successful in improving the inertness of the entire GC flowpath [1-4]. This technical overview presents the improved Agilent J&W HP-INNOWax columns (a polyethylene glycol, PEG, stationary phase). These HP-INNOWax columns are regularly used for the analysis of a wide variety of applications.

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The improved HP-INNOWax columns have been tested through a rigorous inertness testing procedure using the most active probes in today's demanding applications. Performance of improved HP-INNOWax was evaluated based on a combination of three different perspectives:

- **Inertness:** Performance was evaluated using more demanding test mixtures, which enabled greater scrutiny of column activity. In addition, inertness stability was also investigated through a thermal longevity test at 260 °C, the column's upper temperature limit.
- **Consistency:** A set of 15 of the improved HP-INNOWax columns were randomly selected from different batches, and tested for column-to-column inertness consistency.
- **Selectivity:** A combination of retention indices of certain compounds were monitored in QC tests to verify that identical selectivity was achieved between the standard and improved HP-INNOWax columns. Maintainiting identical selectivity to the standard version is important for users who have developed and validated methods on the standard HP-INNOWax columns. This identical selectivity makes upgrading to the improved version easy, with minimal method revalidation required.

A benchmarking study was also performed for inertness comparison between improved HP-INNOWax columns and various non-Agilent PEG columns. All experimental conditions (except the columns) were kept constant to provide as fair a comparison as possible among the columns.

Overall, improved HP-INNOWax columns showed strong improvement for inertness consistency from column-to-column for most highly active compounds, with great thermal stability at the column's upper temperature limit of 260 °C. These characteristics allow the most sensitive and reproducible analytical results for strongly critical and active analytes at trace levels.

## Results and Discussion

### Test methods and standards

QC test probes play a key role in the adequate 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 be carefully 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 PEG columns using demanding test probes ensures consistent column inertness performance. This ultimately contributes to improvements in column-to-column consistency, and reliability of analytical results. A detailed guideline for choosing suitable and effective QC test probes to evaluate column inertness performance can be found in the technical overview 5989-8665EN [1].

Following this guideline, new and demanding test mixtures were developed for critical assessment of the inertness performance for improved HP-INNOWax columns. These test mixtures included Wax Ultra Inert and modified Grob test mixtures [5], and are shown in Tables 1 and 2. More challenging test probes were added to allow highly efficient evaluation of inertness of PEG columns, for example decanal, ethylene glycol, propionic acid, dicyclohexylamine, 2-ethylhexanoic acid, ethyl maltol, and 2,3-butanediol at critical concentration levels. Inertness of the PEG columns was evaluated based on peak shapes and peak responses of active analytes of interest. In addition, the influences of other components in the GC flowpath were minimized by using Agilent Ultra Inert liners and Ultra Inert gold seals. These components provide increased confidence in the inertness of the injection port 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 | |
|---|---|
| Injector temperature: | 260 °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 | Dicyclohexylamine | 5.0 |
| 8 | *n*C11-FAME | 2.5 |
| 9 | *n*C12-FAME | 2.5 |
| 10 | 2,6-Dimethylaniline | 2.5 |
| 11 | 2,6-Dimethylphenol | 2.5 |
| 12 | 2-Ethylhexanoic acid | 5 |
| 13 | Ethyl maltol | 5 |

| Analytical conditions for modified Grob test mixture | |
|---|---|
| Injector temperature: | 260 °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 at 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 HP-INNOWax columns after conditioning for 11 hours at 260 °C. Column activity of the standard column was revealed by tailing peaks and loss of responses of active analytes of interest such as two isomers of 2,3-butanediol (RR/SS isomer for peak 4a and *meso* isomer for peak 4b), dicyclohexylamine (peak 7), 2-ethylhexanoic acid (peak 12), and ethyl maltol (peak 13). Good peak shapes and significant improvement in responses for these active probes were obtained for improved HP-INNOWax columns. Inertness of decanal (peak 3) is still a constraint of improved HP-INNOWax columns. To obtain excellent peak shapes for aldehydes, Agilent J&W DB-WAX Ultra Inert columns are recommended because of their excellent inertness performance [6].

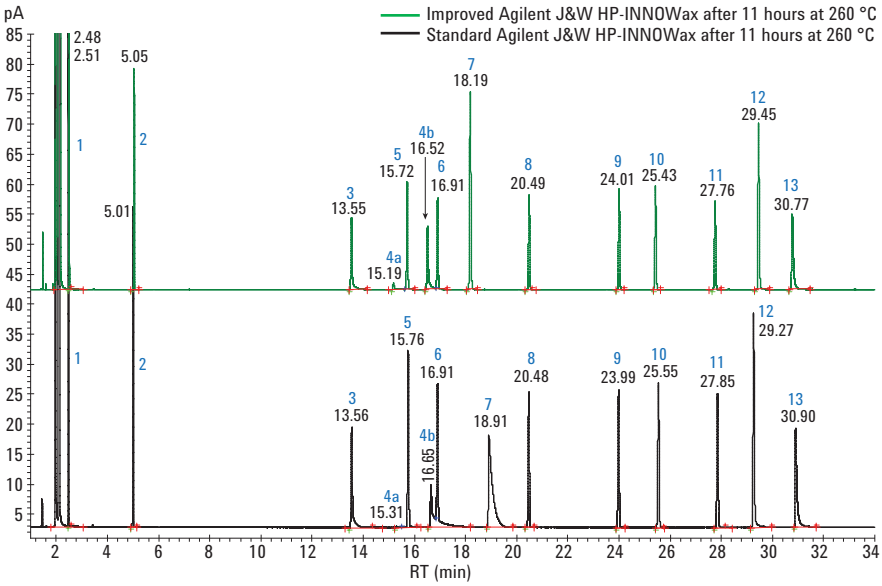
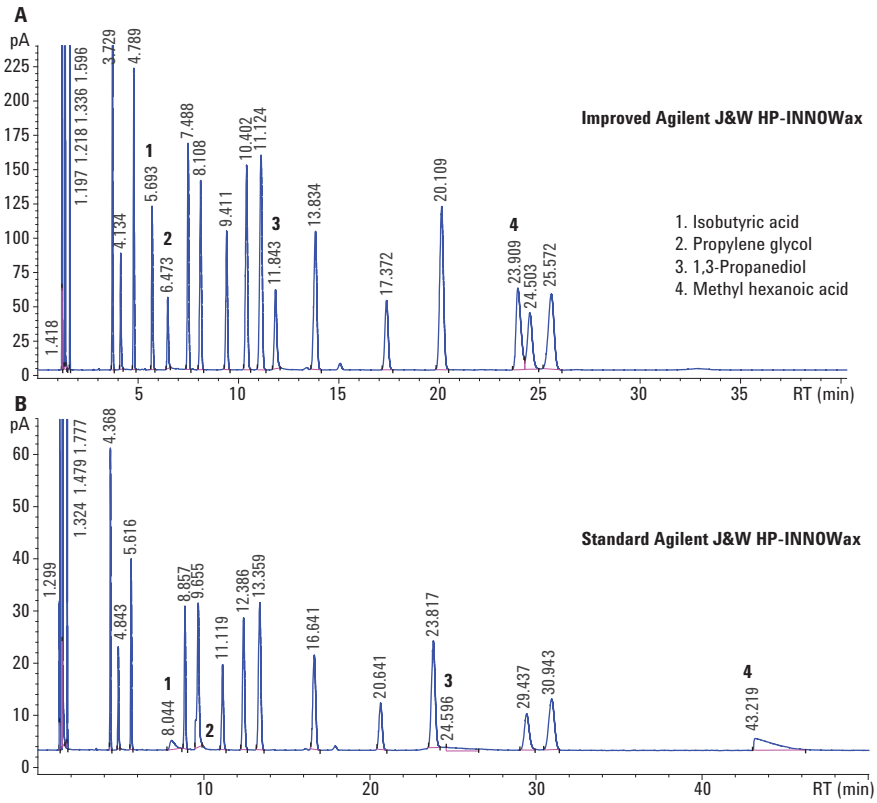



Figure 1. Example FID chromatograms of the modified Grob test mixture on standard and improved Agilent J&W HP-INNOWax columns after conditioning for 11 hours at 260 °C. See Table 2 for GC conditions and peak identifications.

Figure 2 shows representative FID chromatograms of a complex test mixture containing several active diols and free fatty acids on both standard and improved HP-INNOWax columns. A critical amount on-column, approximately 20 ng for each component, was injected for efficient inertness evaluation. Significant improvement in peak shape and peak response of critical probes such as isobutyric acid, propylene glycol, 1,2-propanediol, and methylhexanoic acid was obtained on improved HP-INNOWax columns compared to the standard version. These results provide additional evidence for the strong inertness improvement of improved HP-INNOWax columns. Noted in the example is that an active solute's adsorption can, in some columns, be so severe that the solute's retention is shifted beyond a recognized retention index (that is, selectivity shift). Consequences of this shifting could be the misassignment of a peak identification, or the complete loss of the solute to an undetectable response. This problem emphasizes the importance of rigorous testing during column manufacturing such as with the improved HP-INNOWax.




| Parameter | Value |
|---|---|
| GC system: | Agilent 7890B FID equipped |
| Autosampler: | Agilent G4513A, 10 µL syringe (p/n 5181–1267) |
| Columns: | Agilent J&W HP-INNOWax 30 m × 0.53 mm, 1.0 µm (p/n 19095N-123 and 19095N-123i) |
| Inlet: | Inert flowpath Split/splitless weldment (p/n G3970A) |
| Inlet temperature: | 250 °C |
| Injection volume: | 0.2 µL |
| Split ratio: | 1:30 |
| Carrier gas: | Hydrogen, constant pressure mode, 4.6 mL/min |
| Oven temperature: | 135 °C isothermal, 60 min |
| Detector temperature: | 250 °C |
| Detector gases: | Hydrogen (30 mL/min), air (400 mL/min), nitrogen make-up gas (30 mL/min) |
| Flow path supplies: | Ultra Inert low pressure drop liner (p/n 5190–2295)<br>Ultra Inert gold seal (p/n 5190–6144)<br>Graphite ferrules (p/n 5062–3512 10/pk)<br>Long life septa (p/n 5183–4761) |

Figure 2. Example FID chromatograms of a test standard and improved Agilent J&W HP-INNOWax columns.

Longevity testing at 260 °C was also performed for improved HP-INNOWax columns to evaluate inertness stability at the column's upper temperature limit. An extended period of 50 hours conditioning at 260 °C was carried out. QC tests were performed after each 5 hours conditioning at 260 °C. Figure 3 shows example FID chromatograms of the modified Grob test mixture on improved HP-INNOWax columns after conditioning 11 hours and 50 hours at 260 °C. Good peak shapes and responses of 2,3-butanediol (peaks 4a and 4b), dicyclohexylamine (peak 7), 2-ethyhexnoic acid (peak 12), and ethyl maltol (peak 13) in this test mixture indicates a significant enhancement in inertness on the improved HP-INNOWax. This high level of inertness persevered even after 50 hours of thermal exposure at the upper temperature limit of 260 °C. These results show the superior inertness stability of improved HP-INNOWax columns at 260 °C, which contributes to delivering consistent analytical results and enhancing column lifetime.

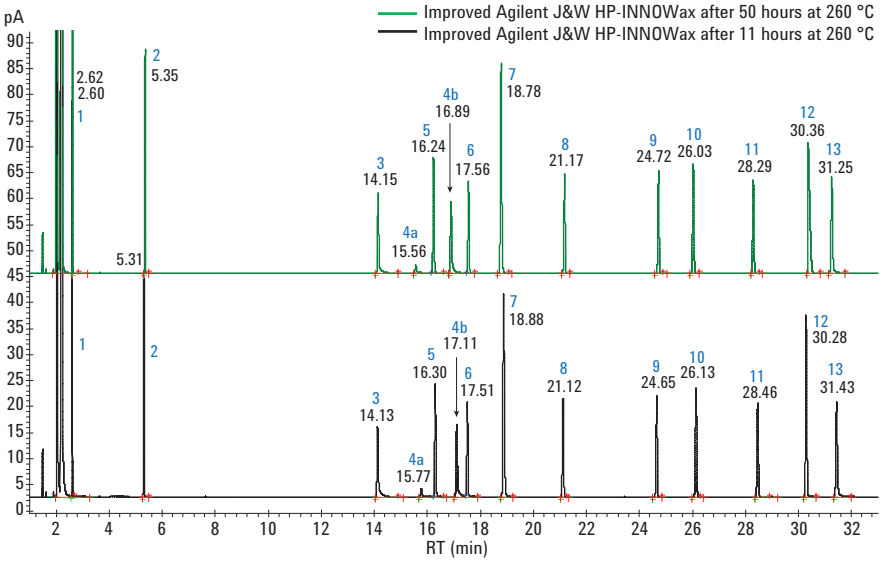



Figure 3. Example FID chromatograms of the modified Grob test mixture on improved Agilent J&W HP-INNOWax columns after conditioning for 11 hours and 50 hours at 260 °C. See Table 2 for GC conditions and peak identifications.

Column-to-column consistency is a key requirement for reproducible qualitative and quantitative analyses. A set of 15 improved HP-INNOWax 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), dicyclohexylamine (peak 7), 2-ethylhexanoic acid (peak 12), and ethyl maltol (peak 13). Figure 4 shows that good column-to-column inertness consistency was achieved on the improved HP-INNOWax column. This consistency is illustrated by the small variation in peak asymmetry at 10% peak height of these active compounds on 15 random improved HP-INNOWax columns.

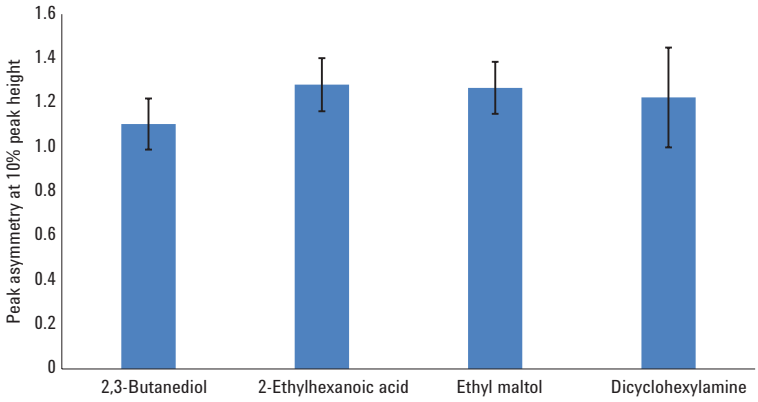
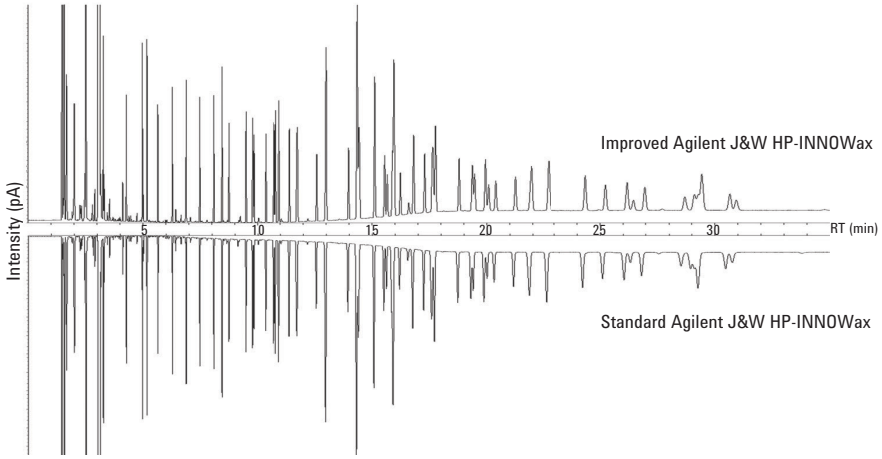



Figure 4. Peak asymmetry at 10% peak height of 2,3-butanediol *meso* isomer, 2-ethylhexanoic acid, ethyl maltol, and dicyclohexylamine on 15 improved Agilent J&W HP-INNOWax columns randomly selected from different batches. QC tests using the modified Grob test mixture were performed after conditioning 1 hour at 260 °C.

## Identical selectivity

The standard HP-INNOWax columns have been routinely used for years in many applications, therefore same selectivity between standard and improved versions is an important advantage for current users. It ensures an easy, fast, and simple column upgrade, with minimal method revalidation. This avoids the risks that come with having to recreate or modify existing compound libraries or analytical methods that are based on the standard HP-INNOWax. Column selectivity is often determined using a combination of retention indices of some target compounds in QC test mixture. Retention indices of improved HP-INNOWax columns were measured, and compared to current specifications for standard columns (data not shown). The ranges of retention indices of improved HP-INNOWax columns fall into normal distribution of current specifications for retention indices of standard HP-INNOWax columns. This indicates that there is identical selectivity between standard and improved HP-INNOWax columns. In addition, the same selectivity between these two columns was also demonstrated in the analysis of fatty acid methyl esters (FAMEs), as shown in Figure 5. Figure 5 shows that an improved HP-INNOWax column is identical to the standard HP-INNOWax column, following the parameters established in a retention time locked method that has been established for FAMEs and the standard HP-INNOWax column [7].




| Parameter | Value |
|---|---|
| GC system: | Agilent 7890B FID equipped |
| Autosampler: | Agilent G4513A, 10 µL syringe (p/n 5181–1267) |
| Columns: | Agilent HP-INNOWax 30 m × 0.25 mm, 0.25 µm (p/n 19091N-133 and 19091N-133i) |
| Inlet: | Inert flowpath split/splitless weldment (p/n G3970A) |
| Inlet temperature: | 250 °C |
| Injection volume: | 1 µL |
| Split ratio: | 1:25 |
| Carrier gas: | Hydrogen. Methyl stearate is retention time locked to 14.00 min, constant pressure mode (average linear velocity is approximately 35.6 cm/s at 50 °C) |
| Oven temperature: | 50 °C, 1 min hold, 25 °C/min to 200 °C, 3 °C/min to 230 °C, 18 min hold |
| Detector temperature: | 280 °C |
| Detector gases: | Hydrogen (40 mL/min), air (450 mL/min), nitrogen make-up gas (30 mL/min) |
| Flowpath supplies: | Ultra Inert low pressure drop liner (p/n 5190–2295)<br>Ultra Inert gold seal (p/n 5190–6144)<br>Self-tightening column nut (p/n 5190–6194)<br>Graphite/Vespel ferrules (p/n 5181–3323 10/pk)<br>Long life septa (p/n 5183–4761) |

Figure 5. FID chromatograms of extended FAMEs Mixture 72 compounds retention time locked on improved and standard Agilent J&W HP-INNOWax columns.

## Benchmarking research

A benchmarking study was performed to compare the inertness performance and thermal stability between improved HP-INNOWax and selected PEG columns from three different vendors: X, Y, and Z. Two different types of PEG column 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 the columns were conditioned for 1 hour at 250 °C using the Wax Ultra Inert test mixture. A longevity test at 250 °C and 260 °C for 50 hours was also carried out for PEG columns from other vendors and the improved HP-INNOWax. This longevity test examined 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 or 260 °C.

Figure 6 shows FID example chromatograms of PEG columns from different vendors compared to improved HP-INNOWax columns after 1 hour conditioning using the Wax Ultra Inert test mixture. The improved HP-INNOWax columns showed good peak shapes for most critical components in the test mixture, such as propionic acid (peak 3), ethylene glycol (peak 4), 2-ethylhexanoic acid (peak 11), and ethyl maltol (peak 12). The inertness was maintained after 50 hours of conditioning at 260 °C. Tailing peak shapes and reduced responses for propionic acid (peak 3), 2-ethylhexanoic acid (peak 11), and ethyl maltol (peak 12) were observed for PEG columns from vendors Y and Z. The inertness of these columns rapidly deteriorated during the longevity test at 250 °C, shown in Figure 7, after 50 hours conditioning at 250 °C. Figure 6 shows that the PEG columns from vendor X showed reasonable inertness for active compounds after 1 hour conditioning at 250 °C, but the inertness significantly dropped during the longevity test at 250 °C, as shown in Figure 7.

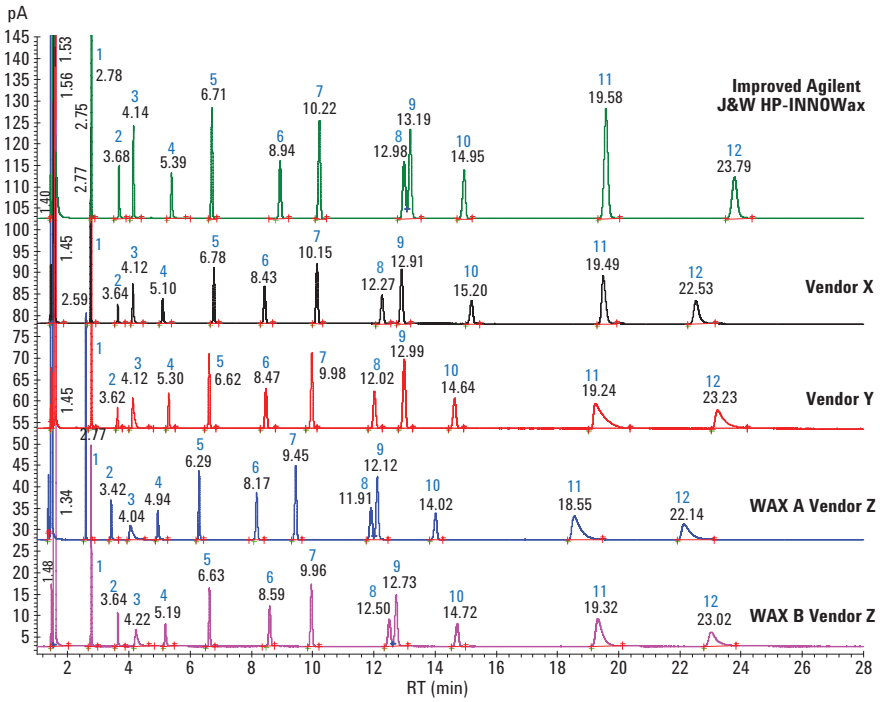



Figure 6. FID chromatograms of the Wax Ultra Inert test mixture on improved Agilent J&W HP-INNOWax columns after conditioning for 1 hour at 260 °C, and a wide variety of PEG columns from different vendors after conditioning for 1 hour at 250 °C. See Table 1 for GC conditions and peak identifications.

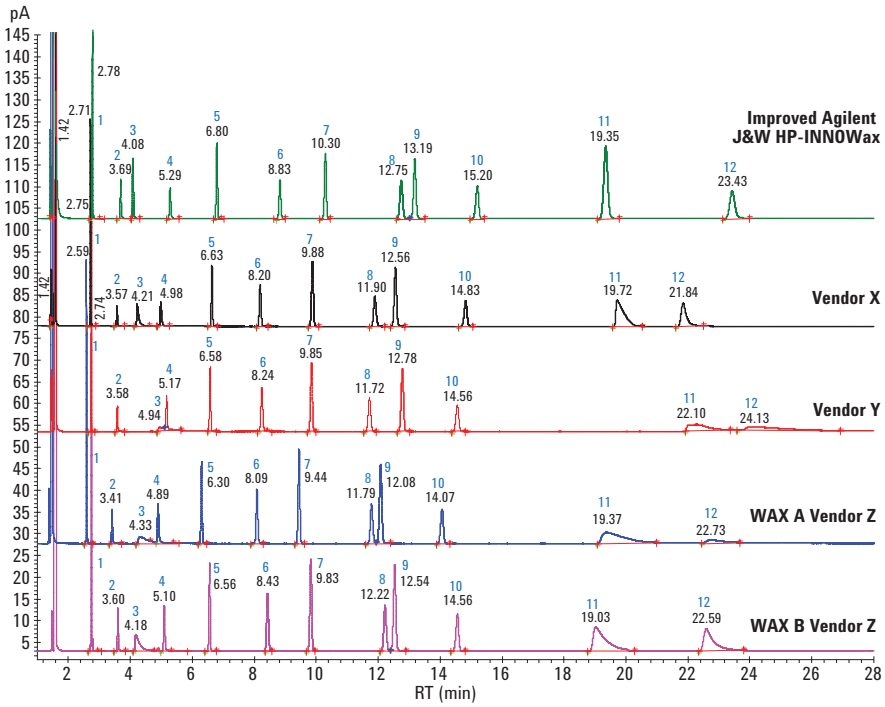



Figure 7. FID chromatograms of the Wax Ultra Inert test mixture on improved Agilent J&W HP-INNOWax columns after conditioning for 50 hours at 260 °C, and a wide variety of PEG columns from different vendors after conditioning for 50 hours at 250 °C. See Table 1 for GC conditions and peak identifications.

In summary, the use of new demanding QC test probes allowed column activity to be detected, and provided better classification of inertness performance of PEG columns in this benchmark study. Compared to standard HP-INNOWax and other PEG columns, the improved HP-INNOWax columns show, overall, far better inertness performance. This improved inertness strongly contributes to improved sensitivity and reproducibility for analyses of a wide variety of active analytes of interest at critically low levels. In addition, the high thermal longevity of the improved HP-INNOWax column at its upper temperature limit of 260 °C provides better column durability and more consistent analytical results over its lifetime.

## Conclusions

In comparison to other PEG columns evaluated in this benchmark study, the improved Agilent J&W HP-INNOWax column shows strong overall improvement and superior performance of inertness, thermal stability, and consistency in column-to-column inertness. In addition, identical selectivity, as the standard HP-INNOWax demonstrated, facilitates a simplified upgrade to this improved version with minimal method revalidation. For current applications, there is no requirement to recreate or modify existing compound libraries or methods based on the standard HP-INNOWax column.

Table 3. Ordering guide for the improved Agilent J&W HP-INNOWax columns. Various inner diameters, lengths, and film thickness are available.

| ID (mm) | Length (m) | Film (µm) | Temp limits (°C) | 7 in Cage | 5 in Cage | Agilent 7890/6890 LTM II module |
|---|---|---|---|---|---|---|
| 0.18 | 20 | 0.18 | 40 to 260/270 | 19091N-577i | 19091N-577iE | |
| 0.20 | 25 | 0.20 | 40 to 260/270 | 19091N-102i | | |
| | 50 | 0.20 | 40 to 260/270 | 19091N-105i | | |
| | | 0.40 | 40 to 260/270 | 19091N-205i | | |
| 0.25 | 15 | 0.25 | 40 to 260/270 | 19091N-131i | | |
| | | 0.50 | 40 to 260/270 | 19091N-231i | | |
| | 30 | 0.15 | 40 to 260/270 | 19091N-033i | | |
| | | 0.25 | 40 to 260/270 | 19091N-133i | 19091N-133iE | 19091N-133iLTM |
| | | 0.50 | 40 to 260/270 | 19091N-233i | 19091N-233iE | |
| | 60 | 0.25 | 40 to 260/270 | 19091N-136i | 19091N-136iE | |
| | | 0.50 | 40 to 260/270 | 19091N-236i | | |
| 0.32 | 15 | 0.25 | 40 to 260/270 | 19091N-111i | | |
| | 30 | 0.15 | 40 to 260/270 | 19091N-013i | | |
| | | 0.25 | 40 to 260/270 | 19091N-113i | 19091N-113iE | |
| | | 0.50 | 40 to 260/270 | 19091N-213i | 19091N-213iE | |
| | 60 | 0.25 | 40 to 260/270 | 19091N-116i | | |
| | | 0.50 | 40 to 260/270 | 19091N-216i | 19091N-216iE | |
| 0.53 | 15 | 1.00 | 40 to 240/250 | 19095N-121i | | |
| | 30 | 1.00 | 40 to 240/250 | 19095N-123i | 19095N-123iE | |
| | 60 | 1.00 | 40 to 240/250 | 19095N-126i | | |

## References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