Agilent J&W Ultra Inert GC Columns: A New Tool to Battle Challenging Active Analytes

Technical Overview

Introduction

QC test probes serve a vital function in ensuring the reproducibility of modern GC columns. These probes ensure that the columns have been properly deactivated, contain the correct amount of stationary phase, and have the same relative retention as the last column purchased. The choice of individual compounds in these test mixes varies widely and can be confusing to the end user, yet these choices can have profound consequences on the performance of a column in the users' applications.

The Test

The ideal column test would run the end-users' demanding compounds under conditions similar to their methods. Unfortunately, it is impractical to test all chromatographically active compounds on every column. It is reasonable to assign, as proxy probes, analytes with active functional groups that are the same as many of the chromatographically active compounds (that is, acids and bases) found in real-world samples. These probes should adequately indicate absolute retention of the column, the relative retention of the column, and the column's behavior toward active analytes.

Absolute retention is measured by the partition ratio, k'. These measurements are commonly made from a normal hydrocarbon or a fatty acid methyl ester in the QC test mix. Holding this measure to a narrow range helps ensure retention time stability from column to column.

Relative retention is generally measured by Kovat's retention indices. These are log functions that describe the placement of a compound between two members of a homologous series. The series most commonly used is that of the normal hydrocarbons. Holding this number for a given compound to a narrow range in conjunction with the k' also ensures retention time stability.

The activity of a column is the measurement of any deleterious effects the column has towards challenging compounds. These interactions may be acidic, basic, or strongly hydrogen bonding in nature. Poor behavior is exhibited by tailing peaks or reduced peak response. Both of these behaviors lead to inaccurate calculation of the peak areas and thus inaccurate quantification of your active compounds of interest.

Probe Selection Is Critical

Most column manufacturers include an organic acid (for example, substituted phenol), a base (for example, organoamine), and an alcohol, along with nonactive probes (for example, alkanes), in their test mixes. Assuming the nonactive probes exhibit good peak shape, any tailing or lost response of the acid indicates that the column is basic in nature. Poor peak behavior of the base indicates that the column is acidic. The alcohol will give an indication if there has been any oxygen damage or if there are any exposed silanols. If the peak shapes for all of these compounds are symmetrical, then the column is considered to be inert towards them.

The choice of test probes can either highlight or mask the deficiencies of a column with respect to activity. By selecting undemanding probes, column activity can go undetected. Figure 1 shows an example, by today's standards, of a series of undemanding probes. The compound list and experimental conditions are provided in Table 1 and Table 2, respectively.

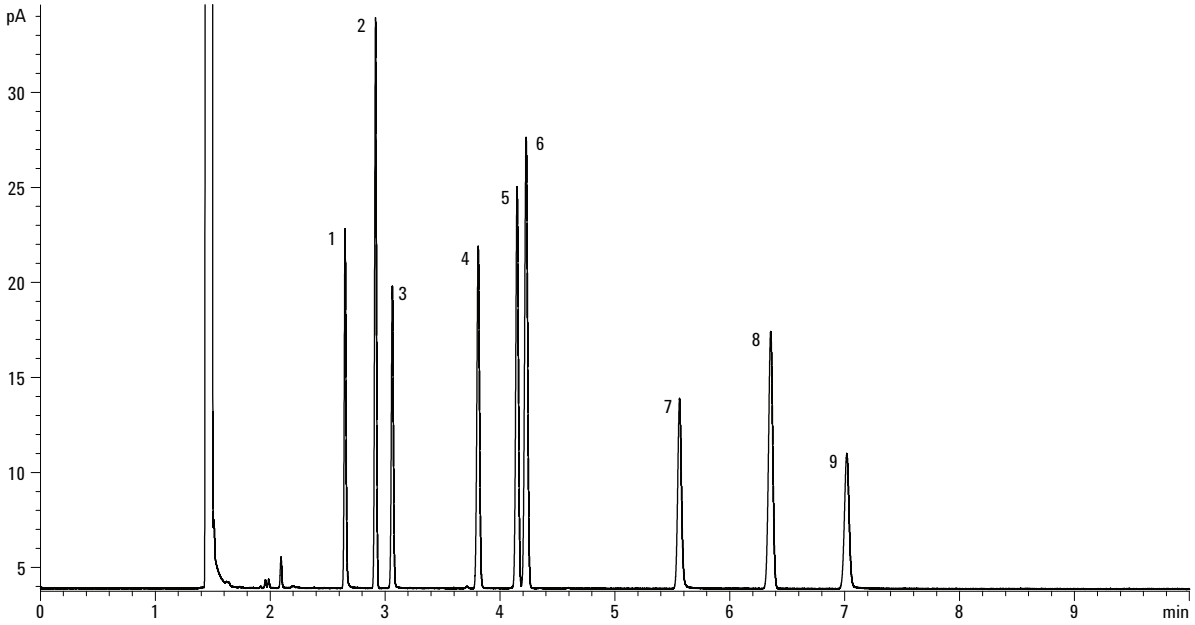

Figure 1. Example chromatogram of today's less demanding test mix. Conditions are shown in Table 2.

Table 1. Less Demanding Test Mix Components

1. 1-Octanol
2. n-Undecane
3. 2,6-Dimethylphenol
4. 2,6-Dimethylaniline
5. n-Dodecane
6. Naphthalene
7. 1-Decanol
8. n-Tridecane
9. Methyldecanoate

Table 2. Chromatographic Conditions for Figure 1

GC:	Agilent 6890N
Sampler:	Agilent 7683B, 5-µL syringe (Agilent p/n 5181-1273), 1.5-µL split injection, 4 ng each component on column
Carrier:	Hydrogen constant pressure 38 cm/s
Inlet:	Split/splitless; 250 °C, 1.4 mL/min column flow, split flow 75 mL/min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column:	30 m x 0.25 mm × 0.25 µm 5%-Phenyl column
Oven:	120 °C isothermal
Detection:	FID at 325 °C, 450 mL/min air, 40 mL/min hydrogen, 45 mL/min nitrogen makeup

Ideally, probes should be molecules with low molecular weights, low boiling points, and no steric shielding of the active group. When summed together, these characteristics allow the probative portion of the test molecule to penetrate and fully interact with the column's stationary phase and surface. In the above example, both the acidic and basic portion of the molecules are shielded by the methyl groups of a 2,6-dimethyl substituted phenyl ring, making these molecules far less probative. Likewise, the alcohols are long-chained alcohols. As the chain length increases, the molecules become more hydrocarbon like and thus less probative.

In addition, this test must be run at an elevated temperature, 120 °C, in order to elute the analytes from the column in a reasonable time. At elevated temperatures the interactive forces of the analytes are diminished, thus weakening their usefulness as test probes. Higher temperatures give probes in the mobile phase more kinetic energy, allowing the molecules to sweep past active sites on the column and effectively mask solute/column interactions.

A QC Test for Today's Demanding Applications

Taking all of the above under consideration and working through nearly 60 test probe candidates, the following QC test mixture (Table 3) was selected to evaluate our new Agilent J&W GC column family of DB-5ms and HP-5ms Ultra Inert columns:

Table 3. Über One Mix

Probe	(ng on column)	Column functional test
1. 1-Propionic acid	1.0	Basicity
2 1-Octene	0.5	Polarity
3. n-Octane	0.5	Hydrocarbon marker
4. 4-Picoline	1.0	Acidity
5. n-Nonane	1.0	Hydrocarbon marker
6. Trimethyl phosphate	1.0	Acidity
7. 1,2-Pentanediol	1.0	Silanol
8. n-Propylbenzene	1.0	Hydrocarbon marker
9. 1-Heptanol	1.0	Silanol
10. 3-Octanone	1.0	Polarity
11. n-Decane	1.0	Hydrocarbon marker

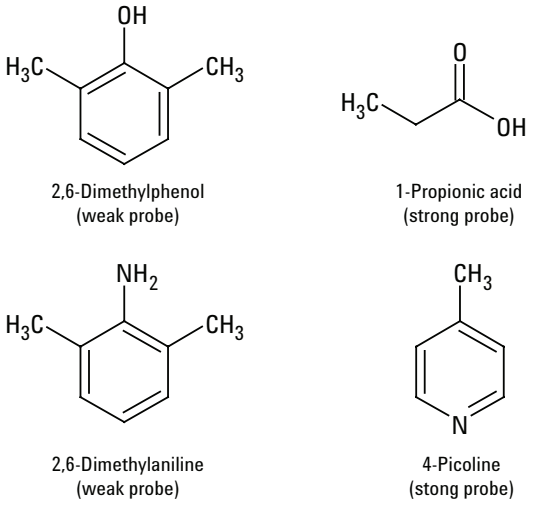

Figure 2. Weak probes vs. strong probes

The probes in the Über One mix were chosen to be highly probative of the stationary phase and surface. The active end of each compound is available to interact with any active sites on the column. In the examples in Figure 2, active portions of the weak test probes are shielded by the two methyl groups on the phenyl rings.

When the same column as exhibited in Figure 1 was evaluated with this new Über One mix (Figure 3), very poor performance was observed for the aromatic amine of 4-picoline and for the trimethylphosphate, Peaks 4 and 6, respectively. There was also increased tailing of the 1,2-pentanediol, Peak 9, indicating a poor deactivation or possible oxygen damage to the stationary phase. Chromatographic conditions are shown in Table 4.

Contrary to what the first QC test indicated, this column will not perform well with demanding analytes and fails the new column inertness QC testing.

A properly deactivated column will deliver the peak shapes found in Figure 4. You can be more confident that this column will meet your chromatographic needs when analyzing challenging analytes. The symmetrical peak shapes, along with the increased peak heights, allow for accurate integration and detection of trace analytes. Chromatographic conditions are shown in Table 4.

Testing of each column with the new Über One test mix will ensure consistent column inertness. With an established inertness baseline for each column, more demanding analytes can be done routinely.

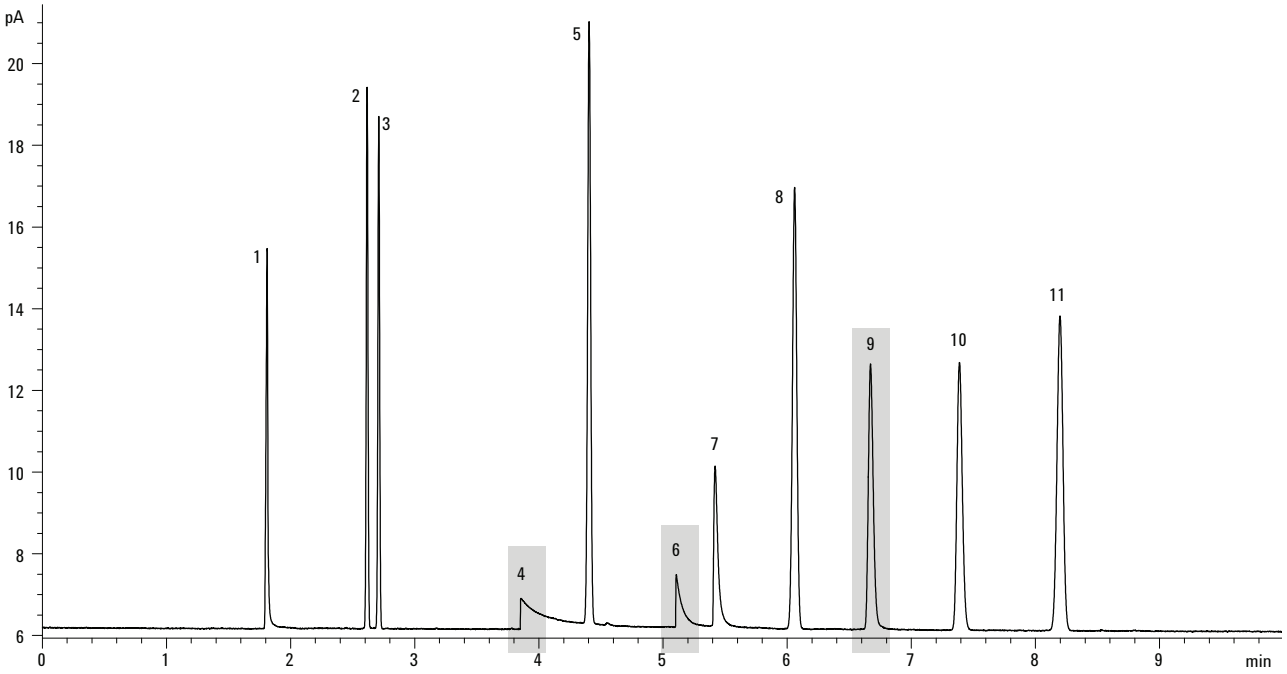

Figure 3. Example chromatogram of Über One Mix on same column as in Figure 1. Chromatographic conditions are shown in Table 4.

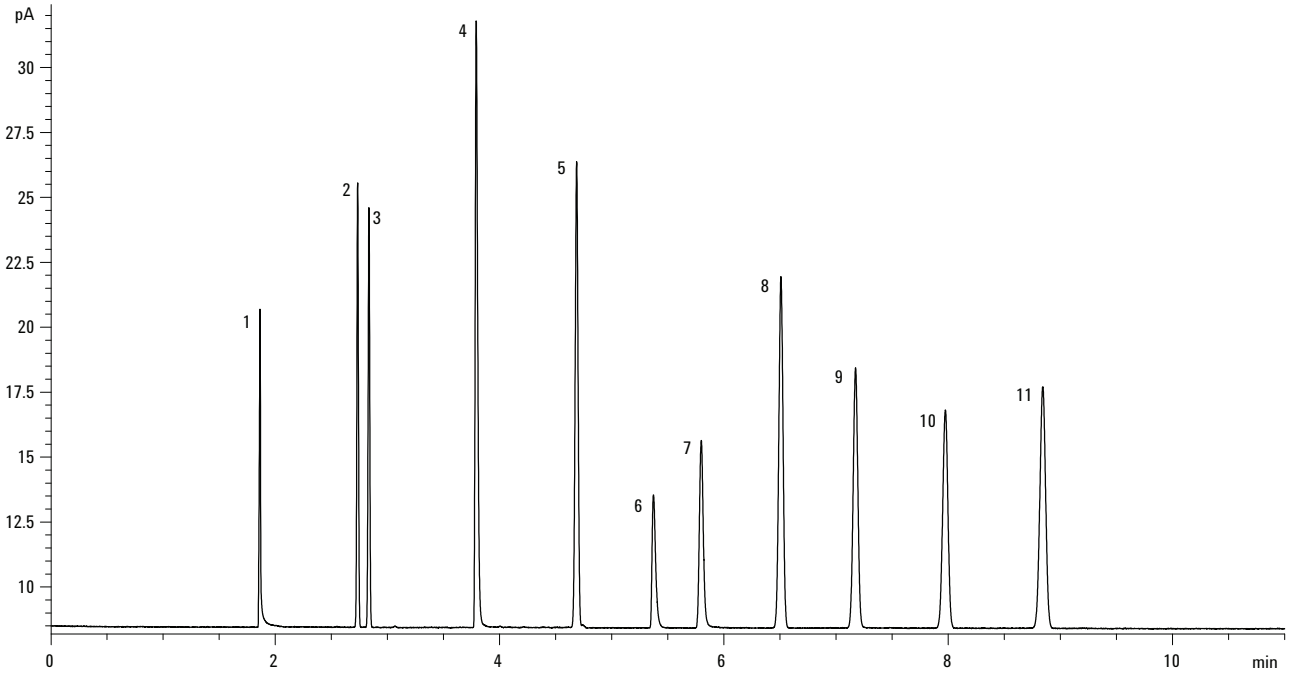

Figure 4. Example chromatogram of Über One Mix on an Agilent J&W DB-5ms Ultra Inert column. Chromatographic conditions are shown in Table 4.

Table 4. Chromatographic Conditions for Figures 3 and 4

GC :	Agilent 6890N
Sampler :	Agilent 7683B, 0.5-µL syringe (Agilent p/n 5188-5246), 0.02-µL split injection
Carrier:	Hydrogen constant pressure 38 cm/s
Inlet:	Split/splitless; 250 °C, 1.4 mL/min column flow, split flow 900 mL/min, gas saver flow 75 mL/min on at 2.0 min
Inlet liner:	Deactivated single taper w/glass wool (Agilent p/n 5183-4647)
Column 1: (for Figure 3)	30 m × 0.25 mm × 0.25 µm 5%-Phenyl column
Column 2: (for Figure 4)	Agilent J&W DB-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)
Oven:	65 °C isothermal
Detection:	FID at 325 °C, 450 mL/min air, 40 mL/min hydrogen, 45 mL/min nitrogen makeup

Conclusions

Today's demanding analysis of trace, active analytes demands the most inert columns available. Unfortunately, not all QC test mixes are capable of differentiating which columns will meet these requirements. With the new Über One Mix, carefully selected chemical species known to adsorb onto active surfaces provide the means for an in-depth evaluation of column inertness.

Each Agilent J&W Ultra Inert column is individually tested against very demanding test probes. This testing goes a long way to ensuring a successful analysis for challenging analytes, providing analysts the most consistent column inertness performance, and hence the utmost confidence in their analytical results.

For More Information

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