Competitive Column Inertness Analysis with Active Basic Compounds

Application Note

Environmental

Authors

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Abstract

Agilent J&W DB-5ms Ultra Inert columns were compared to similar versions from three other column manufacturers with respect to their chromatographic behaviors for active analytes. Superior results were observed on the DB-5ms UI columns for basic compounds. Chromatograms depicting peak shape characteristics are presented to show how exceptional inertness leads to a significant reduction in tailing of active basic compounds for more accurate, reliable analytical results. Excellent chromatographic performance was obtained for a diverse group of compounds that included chlorophenols, organophosphorus pesticides, and aromatic positional isomers, demonstrating the applicability of the DB-5ms UI column for a wide range of applications.

Introduction

The chromatographic analysis of very active samples by GC can be a challenging task unless the GC flow path is inert toward these types of compounds. A stable, unreactive GC column has a considerable effect on the inertness of the flow path due to its high surface area, and will not have active sites that interact unfavorably with sample compounds. Both low column bleed and inertness are critical to maximizing sensitivity, yet inertness is central to maintaining optimum column performance for these demanding substances. A high level of inertness is needed to achieve sharp, symmetrical peaks especially at trace levels. Because the GC column is such an integral part of the chromatographic system, it is in an analyst's best interests to have the most inactive column possible to consistently achieve high quality data [1-5].

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The adverse effects of a non-inert column are displayed as differences in peak deformation (tailing and irreversible adsorption) of active compounds. The column can have acidic characteristics that cause unwanted interactions with basic compounds. A group of active basic compounds was analyzed on the Agilent J&W DB-5ms UI column and columns of the same chemistry from three different vendors to measure inertness of the columns toward basic compounds, and assess the acidic nature of the columns. Both qualitative (peak shape) and quantitative (tailing factor, T_f values) data were used to assess the activity of the columns.

Materials and Methods

An Agilent 6890N network GC system equipped with an Agilent 7683B Automatic Liquid Sampler and a flame ionization detector (FID) was used. Sample introduction was done by a single split/splitless injection port. Table 1 lists the columns included in the study.

Table 1. Columns Used in Study

Column 1:	Agilent J&W DB-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 µm (p/n 122-5532UI)
Column 2:	Restek Rxi-5Sil MS, 30 m x 0.25 mm, 0.25 µm
Column 3:	Phenomenex Zebron ZB-5MSi, 30 m x 0.25 mm, 0.25 µm
Column 4:	GL Sciences InertCap 5MS/Sil, 30 m x 0.25 mm, 0.25 µm

Two columns were tested from each vendor to confirm reproducibility of results. All columns were installed in the GC and analyzed in the same manner. The injection of undecane at 0.2% in methanol was done to ensure that a symmetrical peak was obtained, thus verifying proper installation. The composition of the basic test mixture consisted of the six compounds listed in Table 2 and was analyzed using the chromatographic conditions included in Table 3. The basic standard compounds were purchased from Sigma-Aldrich Corp. (≥ 98% purity) and the mixture prepared in methanol (Burdick & Jackson High Purity Solvent). The same standard mixture was injected onto each column at least five times.

Table 2. Basic Test Mixture in Methanol

1. Triethylamine	0.2%
2. Pyridine	0.2%
3. 4-Methylpyridine	0.2%
4. N,N-Dimethylacetamide	0.5%
5. 2,4-Dimethylaniline	0.1%
6. Dicyclohexylamine	0.15%

Table 3. Chromatographic Conditions for Basic Test Mixture

GC:	Agilent 6890N
Sampler:	Agilent 7683B, 10 µL syringe (p/n 5181-3360)
Carrier:	Helium in constant flow mode, 1.5 mL/min
Inlet:	Split/splitless at 250 °C, split 50:1, 0.2 µL injected
Inlet liner:	Single taper, MS certified liner with restriction to hold glass wool (p/n 5188-6576)
Oven:	30 °C (0.5 min) to 60 °C (1 min) at 5 °C/min, then to 150 °C (5 min) at 50 °C/min
Detection:	FID at 250 °C, hydrogen 40 mL/min, air 450 mL/min, nitrogen makeup 45 mL/min

Tables 4 and 5 list the acidic and organophosphorus pesticide compounds tested on the DB-5ms UI column with their respective concentrations. The chlorophenols were purchased from Sigma-Aldrich Corp. (≥ 98% purity) and the organophosphorus pesticides were obtained from ChemService. The positional aromatic compounds included *o*-xylene, *m*-xylene, and *p*-xylene, and were purchased from Sigma-Aldrich Corp. (≥ 98% purity) and prepared in methanol at 0.2%. Table 6 includes the chromatographic conditions used for the acids, pesticides, and aromatic isomers.

Table 4. Acid Test Mix in Methanol

1. 2,4-Dichlorophenol	0.5%
2. 2,4,6-Trichlorophenol	0.5%
3. Pentachlorophenol	0.5%

Table 5. Organophosphorus Pesticides Test Mix in Methanol

1. Dichlorvos	100 ppm
2. Dimethoate	139 ppm
3. Methyl parathion	109 ppm
4. Malathion	100 ppm
5. Chlorpyrifos	109 ppm
6. Parathion	100 ppm

Table 6. Chromatographic Conditions for Acids, Pesticides, and Aromatic Isomers

Chlorophenols	
Injection:	0.2 µL, split, 100:1
Injection temperature:	250 °C
Carrier:	Helium at 1.5 mL/min in constant flow mode
Oven:	50 °C (1 min), 30 °C to 300 °C (5 min)
Detection:	FID at 250 °C, hydrogen 40 mL/min, air 450 mL/min, nitrogen makeup 45 mL/min
Organophosphorus Pesticides	
Injection:	1 µL, split, 10:1
Injection temperature:	250 °C
Carrier:	Helium in constant pressure mode at 25 psi
Oven:	30 °C (0.2 min), 30 °C/min to 240 °C (4 min)
Detection:	FID at 250 °C, hydrogen 40 mL/min, air 450 mL/min, nitrogen makeup 45 mL/min
Aromatic Isomers (*o*-, *m*- and *p*-Xylene, 0.2% Methanol)	
Injection:	0.2 µL, split, 300:1
Injection temperature:	250 °C
Carrier:	Helium at 1.5 mL/min in constant flow mode
Oven:	30 °C isothermal
Detection:	FID at 250 °C, hydrogen 40 mL/min, air 450 mL/min, nitrogen makeup 45 mL/min

Results and Discussion

A distinct difference in column inertness was observed with the basic compound group, highlighting significant variations in the acidic nature of the columns. Visually, there was noticeable tailing with the first four peaks in the chromatograms for the Restek, Phenomenex and GL Sciences columns, as shown in Figure 1. Excellent peak shapes were obtained for the compounds of interest with the DB-5ms UI column. A measurement of the peak tailing was done using the US Pharmacopeia T_f. This is calculated using the following formula [6].

$$T_f = W_{5.0}/(T_w \times 2)$$

Where: T_w = distance between peak front and retention time of peak (TR) at 5% of peak height; units are the same as used for $W_{5.0}$.

$W_{5.0}$ = width at 5% of height

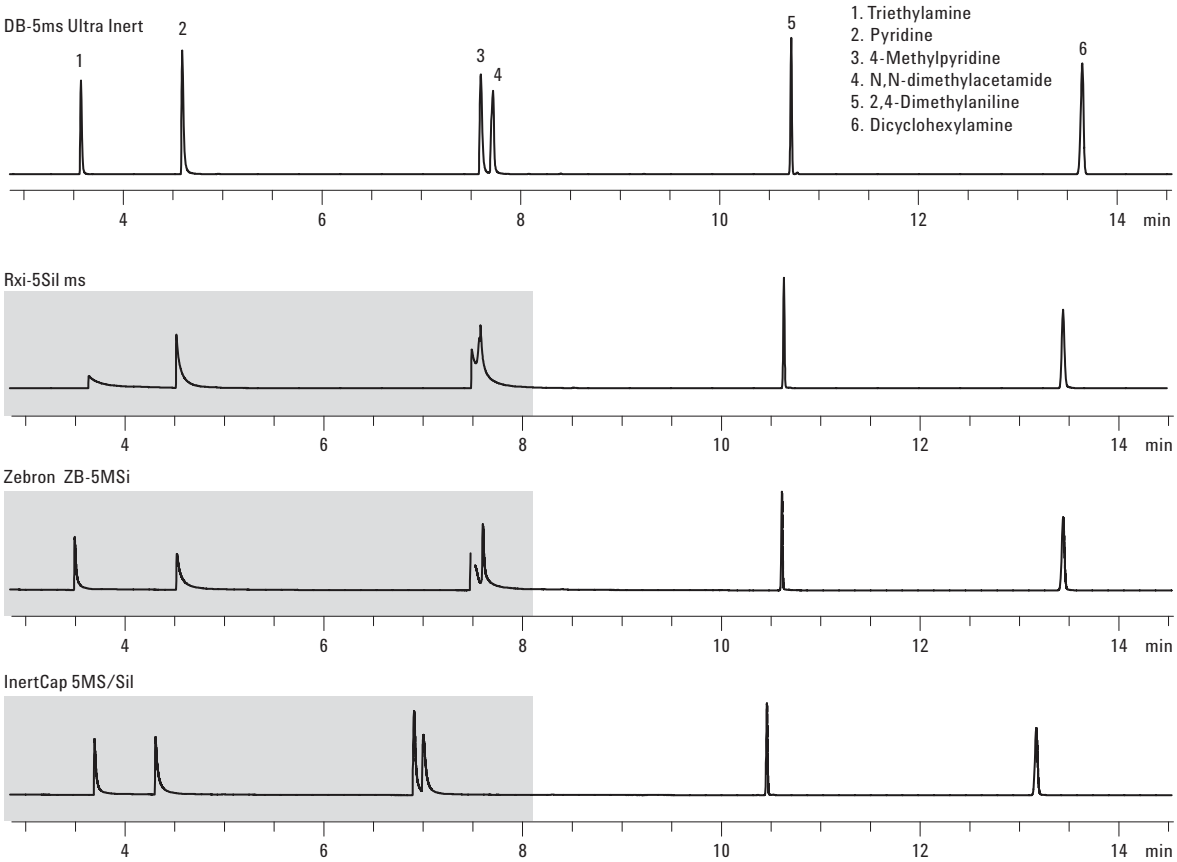

Figure 1. Chromatograms of basic compounds on Agilent and non-Agilent columns, with exceptional peak shapes for these compounds delivered by the Agilent DB-5ms Ultra Inert GC column. Chromatographic conditions are listed in Table 3.

The T_fs are listed in Table 7 for all the basic compounds and the T_f values for the first four peaks are included in the chromatograms in Figure 2. When compared to other columns, the DB-5ms UI column showed a significant reduction in the tailing factors for four of the bases. There was also incomplete resolution of 4-methylpyridine and N,N-dimethylacetamide in the chromatograms for the Restek Rxi-5Sil MS and Zebron ZB-5MSi columns. This observation shows how the lack of column inertness can negatively affect resolution, and consequently selectivity, since resolution is a measure of the degree of separation (selectivity) of two compounds. The selectivity of the non-Agilent columns was not sufficient, with the experimental conditions used, to separate 4-methylpyridine and N,N-dimethylacetamide due to the effects of column activity.

Table 7. Tailing Factors (T_f) for Basic Compounds on -5ms Columns

	Tailing factor (T_f)			
	DB-5ms Ultra Inert	**Zebron ZB-5MSi**	**InertCap 5MS/Sil**	**Restek Rxi-5Sil MS**
Triethylamine	1.5	7.1	7.7	44
Pyridine	2.4	17	5.1	10
4-Methylpyridine	1.7	4.2	4.7	6.1
N,N-dimethylacetamide	0.95	3.9	4.6	1.5
2,4-Dimethylaniline	0.95	1.0	1.0	0.99
Dicyclohexylamine	0.87	0.98	1.1	1.0

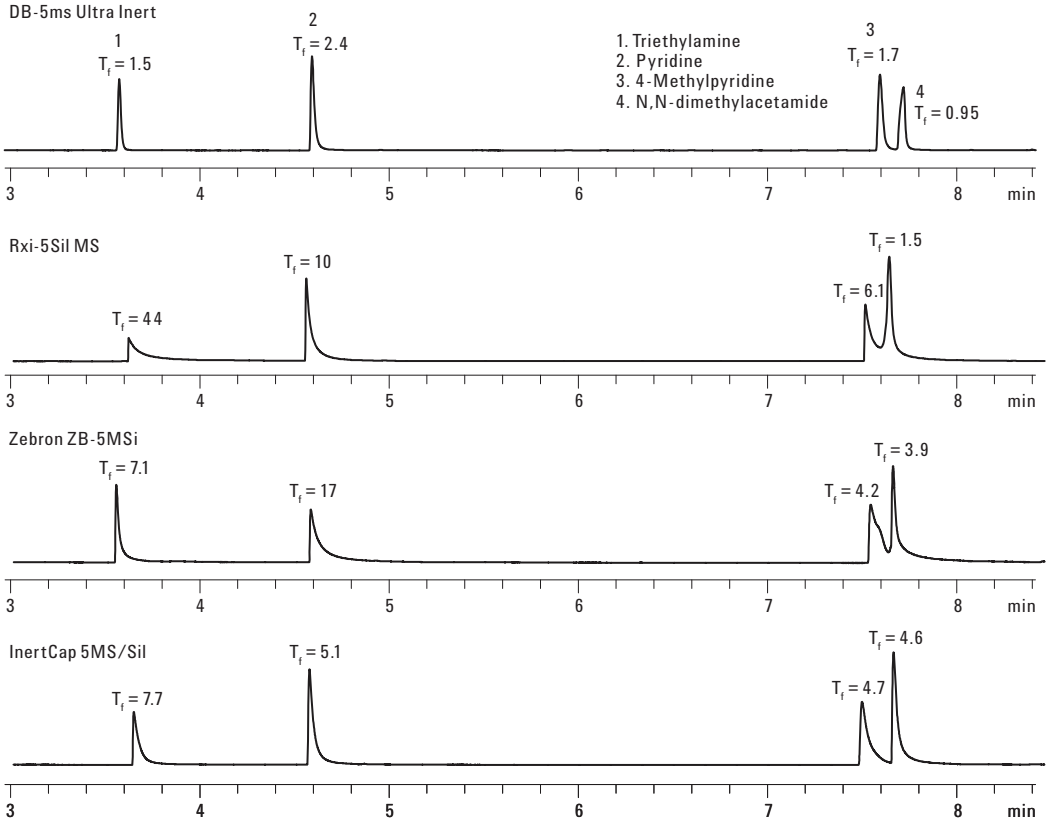

Figure 2. T_f values for four basic compounds on Agilent and non-Agilent columns, with extraordinary inertness of the Agilent J&W DB-5ms Ultra Inert column revealed by significantly lower T_f values. Chromatographic conditions are listed in Table 3.

A diverse group of compounds that included acids (chlorophenols), organophosphorus pesticides, and positional aromatic isomers was analyzed on the DB-5ms UI column. Excellent chromatographic results were obtained for these compounds, in addition to the bases. The separation of the aromatic isomers demonstrated that the column retained unique selectivity to separate isomers that was not compromised as a result of the enhanced inertness. The chromatograms in Figure 3 depict the very favorable chromatographic results attained for this wide-ranging group of compounds and illustrate the versatility of the DB-5ms UI column for a variety of applications.

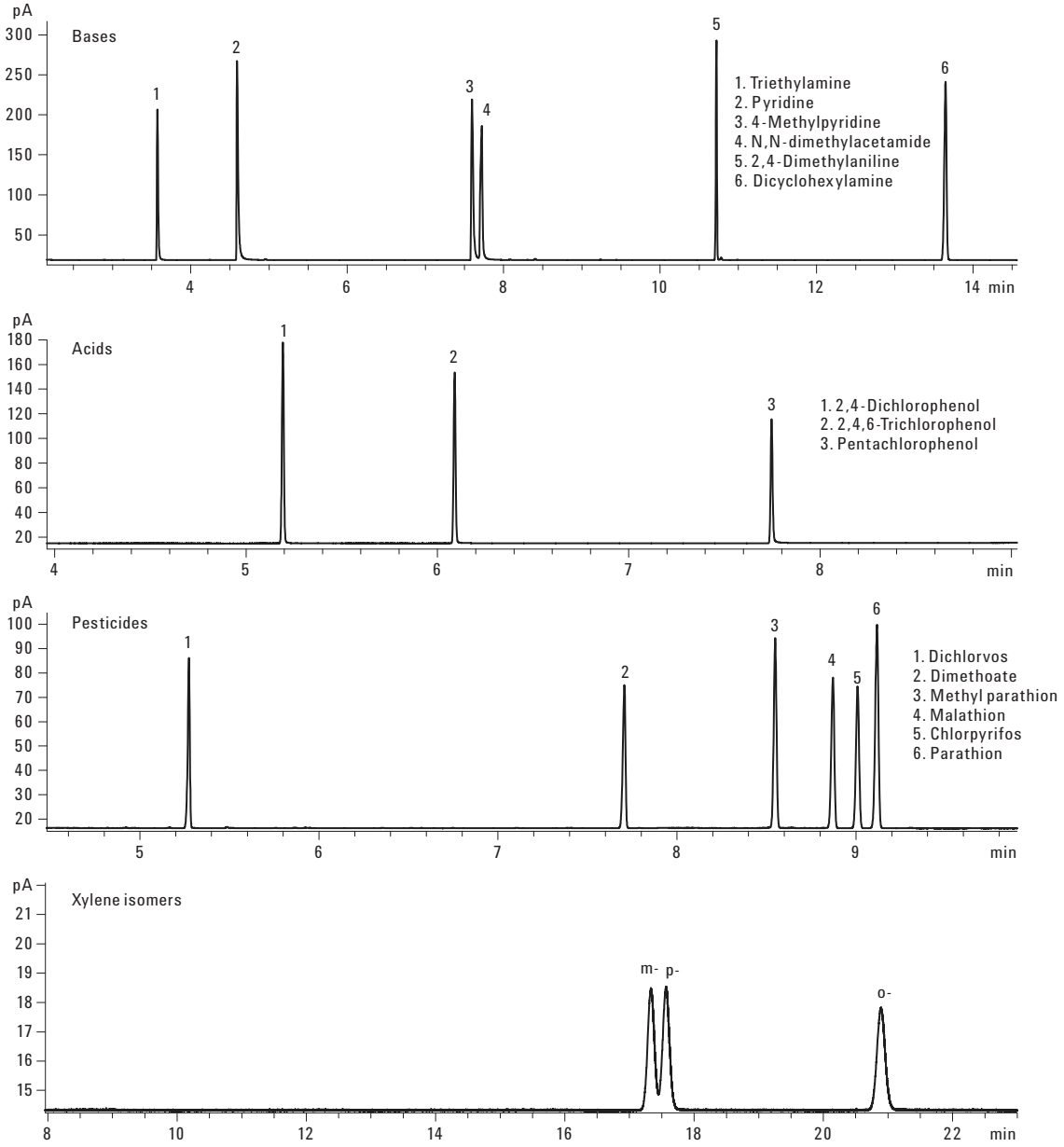

Figure 3. Chromatograms for bases, chlorophenols, organophosphorus pesticides, and xylene isomers on an Agilent J&W DB-5ms Ultra Inert column, with excellent chromatographic performance across a diverse group of compounds. Chromatographic conditions are included in Tables 3 and 6.

Conclusions

Agilent J&W DB-5ms Ultra Inert GC columns were superior to non-Agilent columns in the same category for the analysis of basic compounds. The high level of inertness resulted in a better peak shape for active compounds and was visually apparent and verified by tailing factor (T_f) calculations. A high level of sensitivity that translates to lower detection limits was achieved because of the excellent peak shape obtained with the highly inert DB-5ms UI column. Excellent chromatographic performance for a varied group of compounds, including chlorophenols, aromatic isomers, and pesticides, was clearly demonstrated.

References

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