Evaluation of Capillary Columns for General Performance Parameters

Application

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Abstract

In recent years, new GC capillary columns have entered the marketplace accompanied by claims of performance equivalent to those from established manufacturers. This application note examines a sampling of these columns, comparing their performance using standard and practical test procedures. Tests were for column bleed, retention index, film thickness, and trace level acid and base performance. Both 5% phenyl- and wax columns were included in this study.

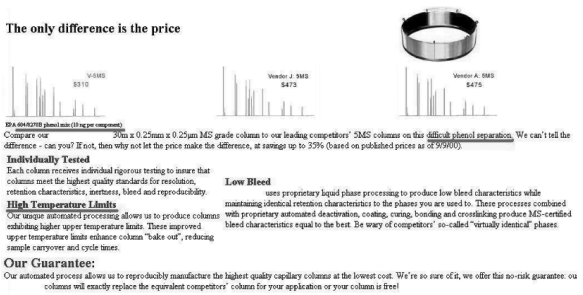


Figure 1. Competitor's column "comparison."

Introduction

The purpose of this application note is to compare, in a rigorous and quantitative way, the performance of Agilent DB- and HP- brand capillary GC columns against a variety of competitive columns claiming performance equivalence. See Figure 1.

Figure 1, derived from a competitor's presentation, purports to make a valid comparison. It concludes that the only difference between the columns presented is their price. Is this a valid conclusion? Closer examination shows that it is not. In this example, the sample is a mixture of phenols, containing 10 ng per component. A lower concentration, yielding an on-column amount between 1 and 5 ng per component, would present a more discriminating challenge. Given the small graphical size of the chromatograms depicted and the fact that no numerical data is presented, it is difficult to discern important differences between the columns that may exist.

Additionally, analysis of phenols tells only part of the story. In the real world, general utility columns such as the 5% phenyl type columns (HP-5 and DB-5 for instance) see a broad range of analytes. While there are two difficult phenols in this mixture (2,4-dinitrophenol and pentachlorophenol), no other class of compounds, such as bases, are examined.

Also, there are claims of low column bleed, but no data is presented to support this claim. The reason you do not see them being compared will be shown.

The brands compared for this study were DB-, HP-, and two column brands new to the marketplace that are being aggressively marketed based on price. The dimensions of all columns tested were 30 m × 0.25 mm id, 0.25 µm film thickness.

General Comparison Methodology

First, all columns were compared using Agilent Technologies quality control (QC) conditions and parameters for the DB-5 column. The parameters tested were column bleed (offset from 135 °C to 325 °C after 2 hours of conditioning at 325 °C), Retention Indices, and film thickness (measured through k).

Then, the effects of thermal-cycling stress on column inertness were monitored with replicate injections of chromatographically active analytes at trace levels (5 ng on-column) in the form of an active acid (2,4-dinitrophenol), an active base (n-octylamine), a very difficult to chromatograph ethanolamine (N,N'-diethylethanolamine), and a chromatographically inert internal standard (naphthalene) at 10 ng on-column. On-column injection was used to eliminate the inlet as a source of activity. A flame ionization detector (FID) was used to ensure long term stability. Test order was randomized and replicate injections (n=6) were performed for inertness testing. On-column probe amounts were the same, verified by naphthalene to within a range of ±3%, and determined to be statistically the same using comparison testing at the 95% confidence level. Flow rate was adjusted in order to elute naphthalene at 9.18 ±.10 min for all columns so that valid peak height comparisons could be made.

Six replicates of the standard were injected at each of the following points:

Initial
After 25 cycles to 350 °C
After 50 cycles to 350 °C
After 25 cycles to 370 °C
After 50 cycles to 370 °C

Finally, after the temperature cycling, the columns were compared again using the DB-5 QC test criteria as described above.

Initial Bleed Out/Condition Times

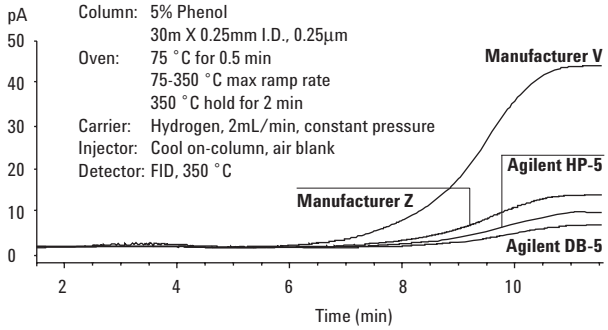


Figure 2. Entire chromatogram of initial bleed profile.

General QC results-Bleed

Some competitors claim that their columns bleed equally or less than DB- and HP- columns. The bleed specifications for DB-5 and DB-5ms are 6 and 4 pA respectively, using an FID as described above. See Figure 3.

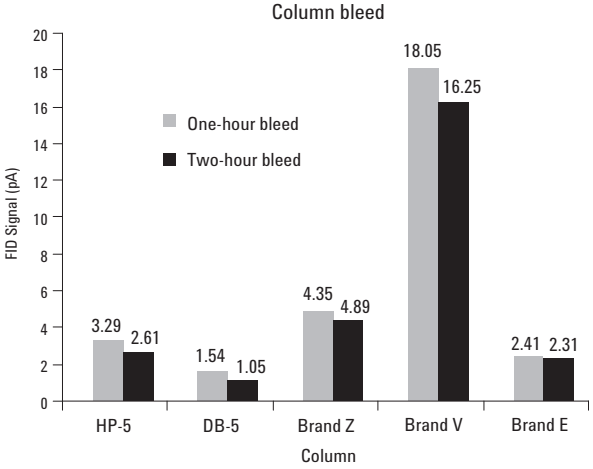


Figure 3. Column bleed at one and two hours of conditioning.

While one competitor's claim is valid, two others did not meet their low-bleed claims. However, the one that did meet the DB-5ms bleed specification, Brand E, had very serious inertness problems. See Figure 5.

Additional QC Results

The columns did perform similarly to DB-5 and HP-5 columns in the parameters of Retention Index and film thickness (as measured by k), all falling within the allowable range for the J&W brand column.

Inertness

Column activity (lack of inertness) is solute adsorption or worse, reaction of the solute by or with the column solute adsorption is chromatographicaly evident as peak shape anomolies (e.g., tailing) or in extreme cases, complete loss of solute response. It is particularly problematic at trace levels, especially on the competitors' columns. See Figure 4. Why is this important? If you are analyzing a broad range of compounds, for which the 5% phenyl columns are especially useful, you may be getting incomplete data. If you are trying to determine trace amounts of impurities, you may miss them because the column retains them or changes them chemically.

NOTE: The diethylethanolamine is likely a reaction/decomposition product of the N,N'-diethylethanolamine according to MS analysis. This was seen on all competitors' columns, and to a small degree on the DB-5, especially after thermal cycling to 370 °C.

The competitors' columns perform well only for acidic compounds, but performed poorly for basic compounds and compounds with active, mixed functional groups.

GC run conditions for Figure 4 were as follows:

Injector: Cool-on-column, 0.5 µL, $MeCl_2$ solvent

Columns: 30 m × 0.25 mm, 0.25 µm film

Temperature program:
40 °C, 0.5 min
40–175 °C at 10 °/min
175–300 °C at 30 °/min
300–325 °C at 20 °/min
325 °C for 2 min

Carrier: Hydrogen, (99.995 plus big universal trap), t_r of Naphthalene = 9.18 min ±0.10 min

Detector: FID, 325 °C, N_2 makeup, column compensation flow

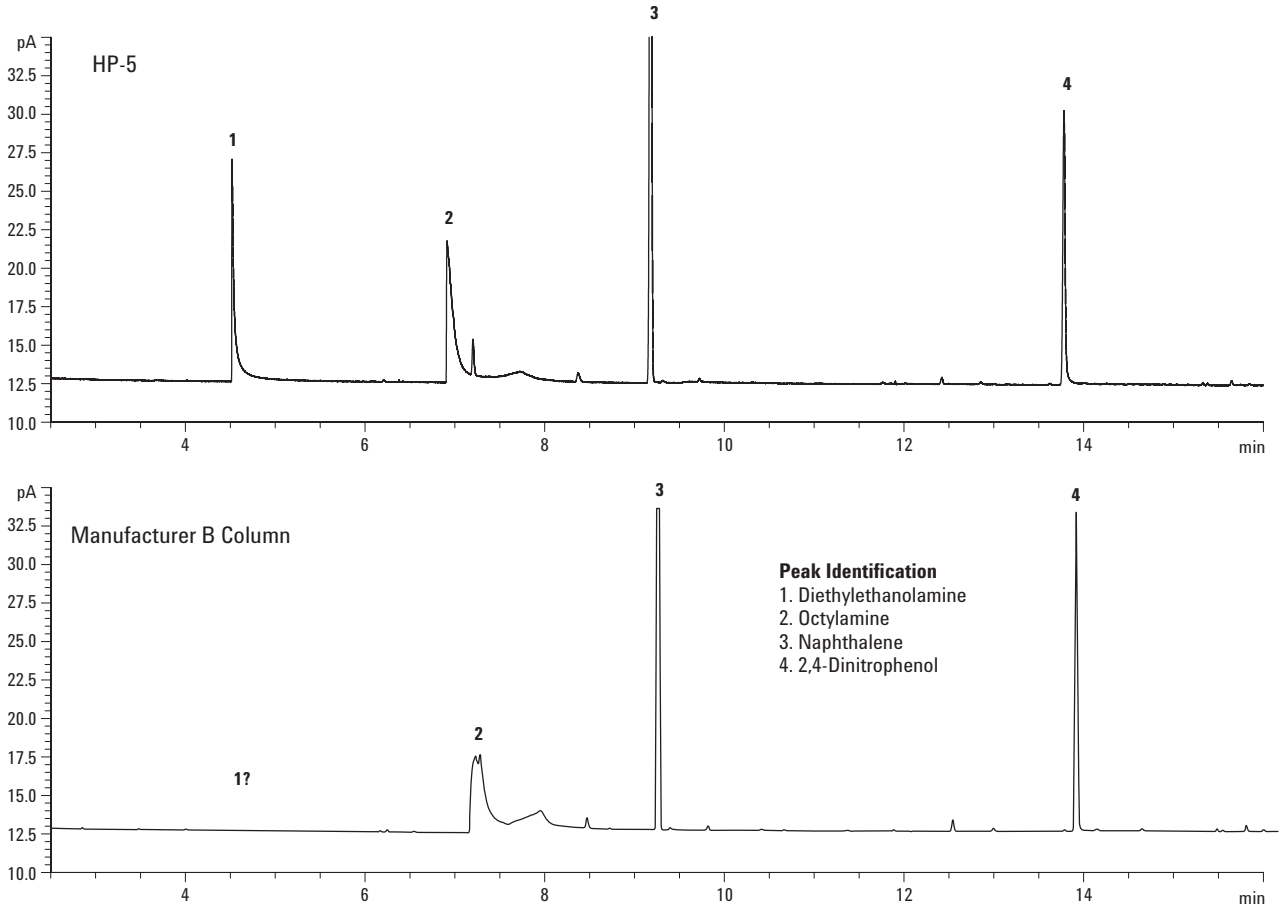


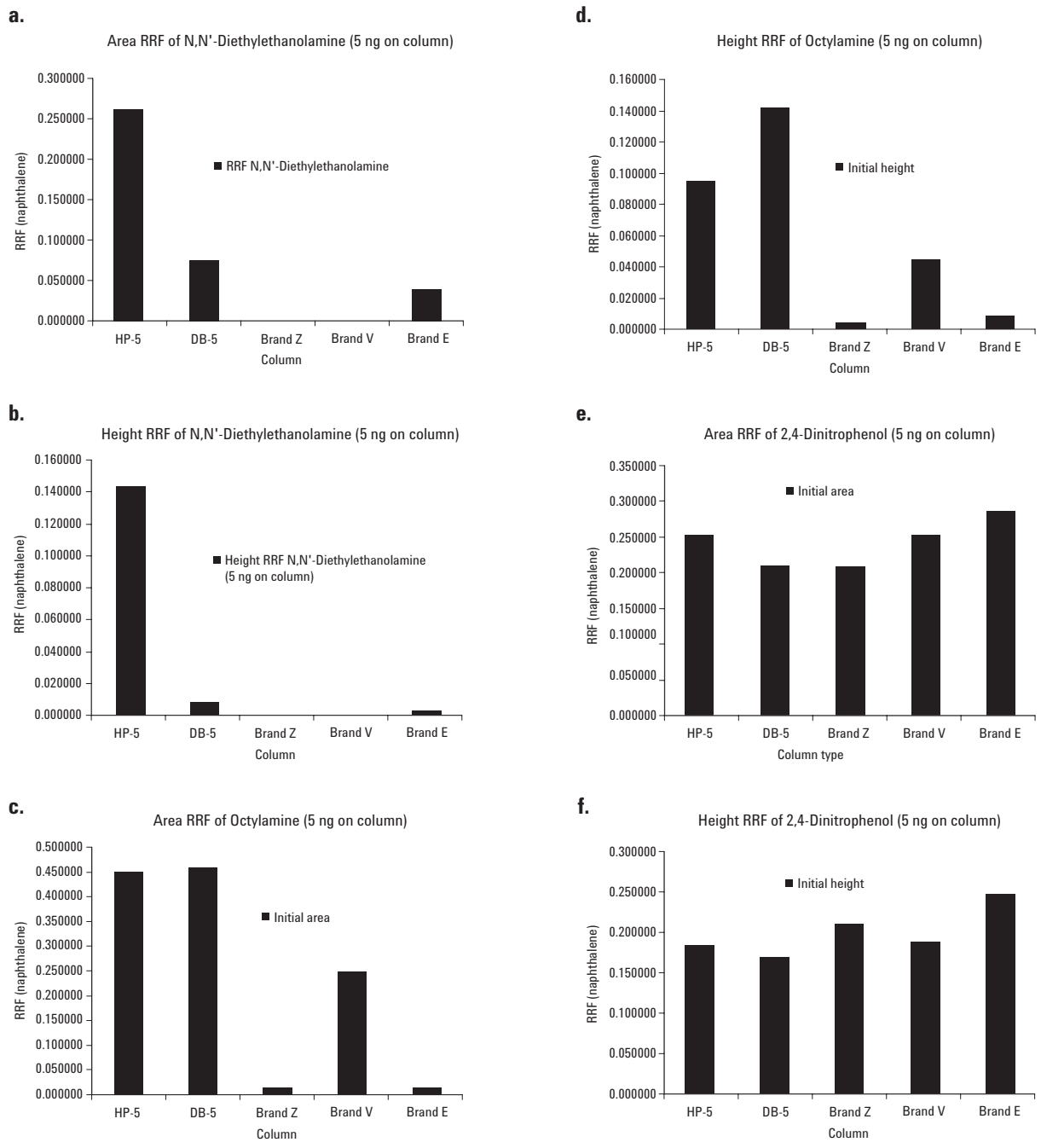
Figure 4. Chromatograms showing typical performance for the trace level compounds on the DB-5 and HP-5 (top) columns, and all of the other competitors' columns (bottom).

Column inertness toward trace active analytes can be measured and compared in two ways:

- **Peak area**—less on one column means permanent loss to the column (irreversible adsorption).
- **Peak height**—less on one column means more activity, but doesn't necessarily mean irreversible loss to the column.

The average relative response factors (ARRF) to naphthalene are used to minimize for injection variation from column to column.

Figure 5 is a collection of charts showing the initial height, area, and ARRFs for all active probes on all columns tested after conditioning but prior to thermal cycling. Note the extremely poor base and active mixed functional group performance on all competitors' columns compared to DB-5 and HP-5 columns, but essentially equivalent acid (2,4-dinitrophenol) performance. Practical interpretation of these results is that you can chromatograph trace acid, base, and mixed functional group compounds on HP-5 and DB-5 columns at trace levels, but reliably, you cannot on the competitors' columns.



Figures 5a-f. Initial AARF for all columns for active probes. n=6 for all samples, n = 1 for each column type.

Effects of Thermal Cycling–Lifetime

Factually and definitively there is not a good way of predicting any given column's lifetime. Relatively speaking, there are many factors that influence useful column lifetime. Most columns fail during use due to contamination from sample matrices, degradation from injection of inorganic acids, bases or salts and oxygen in the carrier gas at high temperatures. The durability of siloxane polymers against abuse is debatibly equal for all columns. Take that away, and the only valid means of comparing column lifetime is by comparing how well columns hold up to thermal stresses in terms of inertness and column bleed under carefully controlled conditions. Bleed is nothing more than degraded stationary phase eluting from the column and creating a signal on the detector. This is more a function of the column manufacturers' proprietary deactivation procedures and the control they have over their manufacturing processes.

A way of academically measuring column life is to measure and use the stationary phase bleed rate at a given column temperature, correlate it with the response for a known column bleed component, and make relative comparisons of columns assuming only thermal stress.

Using the 2-hour conditioning bleed values shown in Figure 3, we can compare the relative lifetimes of the columns. See Table 1. This is an academic exercise and not meant to imply lifetime for reasons already stated. The fact that some competitors' columns bleed at rates so much higher suggests that when the variables of sample matrices and oxygen are removed, DB-5 and HP-5 columns have the "longest life," Brand E does not perform too poorly, but Brands Z and V make a poor showing.

Table 1. Summary of Column Bleed Characteristics

	Column				
	HP-5	**DB-5**	**Brand Z**	**Brand V**	**Brand E**
Bleed at 325 °C in pA (2 h value)	2.61	1.05	4.89	16.25	2.31
Total Phase in column (mg)	4.71	4.71	4.71	4.71	4.71
Bleed rate (ng/s)	0.237	0.095	0.445	1.477	0.210
Thermal column "Life" at 325 °C	230	5711	23	36.9	260

Assume value of 11 pAs/nanogram for bleed components

Effects of Thermal Cycling-Inertness

A column rarely gets better, and in particular, more inert with use once it is properly conditioned.

What follows are the ARRF for height and area plotted throughout the thermal cycling test for each one of the probes on each of the columns.

Use the following key when viewing the Figures 6a-c:

1 = Initial
2 = 25 cycles × 350 °C
3 = 50 cycles × 350 °C
4 = 25 cycles × 370 °C
5 = 50 cycles × 370 °C

Unfortunately, two of Brand Z's columns rated to 370 °C broke between the first and second rounds of replicate testing, so only the first round is truly validly comparable. However, the data is still shown.

Unless special high-temperature polyimide tubing is used in capillary columns, a thermal limit of 360 °C for the tubing will give a reasonable lifetime. The fact that two columns broke and also appeared very brittle, and that nearly all tubing used in GC columns comes from one manufacturer, indicates that the robustness of these columns may be compromised by what could be their higher than appropriate thermal limit (370 °C).

A few generalizations can be made about Figure 6. HP-5 and DB-5 are superior columns for analyzing bases and mixed function compounds and appear to offer stable inertness when staying within their normal operating range (up to 350 °C). Brand E appears to improve slightly with continued operation, which suggests that the column may not be fully stabilized by the manufacturer prior to shipping. All columns are fairly close in response for the 2,4-dinitrophenol, with the HP-5 showing a dip after cycling to 370 °C and a continued drop in response, but it was still close to that of DB-5 and other brand columns.

Bleed

- One competitor exhibited much higher bleed.
- Only one competitor met the J&W DB-5ms bleed specification.
- Comparable bleed comes at a price with competitors columns.

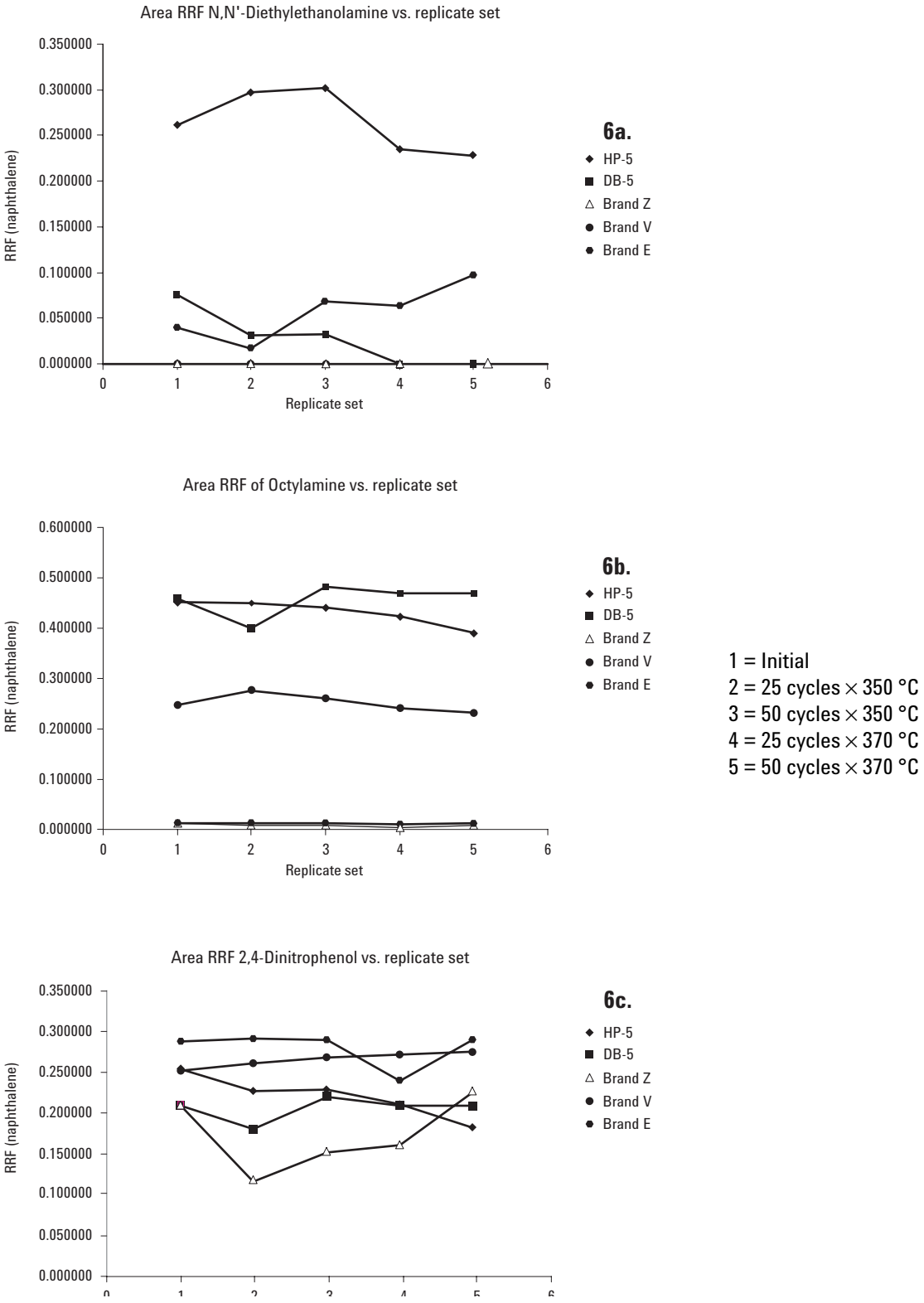


Figure 6. ARRF for all columns as a function of thermal cycling.

Inertness

- The price of lower bleed is a significant response loss for bases and di-functional compounds.

Selectivity

- All appear equivalent within normal variation, before and after thermal cycling (data not shown).

Mechanical Stability

- "Higher temperature limit," competitor's columns seem more prone to breakage.
- Problem may be a side affect of overstressing the tubing's temperature limits to achieve "low bleed" status.

Activity at trace levels

- HP-5 is most universal ("analyte pH neutral") and therefore is most robust for acids, bases, and mixed function compounds. DB-5 is less robust, but it exhibits some low-level universal performance.
- All other columns are good for acids only. Base and mixed function compound performance is very poor.
- Performance for bases diminishes for all columns with extended thermal cycling to 370 °C.

Additional Comparsions

Design of the column comparison methods above is fairly exhaustive work, so the scope of this project was limited to comparing only 5% phenyl capillary columns. However, in early applications, comparison of other competitors' "wax" columns with our DB-Wax column makes it worth noting that the implied "equivalence" in their advertising statements is unsupported.

For such claims to have any use, most analysts would expect to be able to substitute one of these "replacement" columns for a DB- or HP- column and essentially obtain the same results. Resolution, relative elution order, and column selectivity should be nearly equal under the same conditions. However, as the following data show, "replacement" does not mean equivalent, and not recognizing this semantic distinction could jeopardize a lab's time, money, and potentially its reputation.

A comparison of the industry standard, DB-WAX column and another wax column from a manufacturer, who claimed that their columns "replace" DB-WAX columns, was made. A typical application, adulterants and impurities commonly found in distilled alcohol from biological sources, using a sample containing only 18 compounds was chosen. The separation was relatively simple and was readily accomplished on a DB-WAX column. See Table 2.

Table 2. Comparison of Retention Time and Retention Index (RI) for Test Compounds on DB-WAX and "Replacement" Columns

	Compound	DB-WAX time (min)	"Replacement" time (min)	DB-WAX RI	"Replacement" RI	RI difference
1	Methane	1.184	1.195			
2	Pentane	1.230	1.236	500.0000	500.0000	n/a
3	Acetaldehyde	1.430	1.436	704.1436	715.2015	–11.0579
4	Methyl formate	1.587	1.890	764.2420	775.2326	–10.9906
5	Propionaldehyde	1.700	1.694	794.3359	803.6293	–9.2934
6	Acetone	1.818	1.806	819.4102	828.2317	_8.8215
7	Methyl acetate	1.889	1.872	832.3342	840.6943	–8.3601
8	Butyraldehyde	2.213	2.181	878.3749	886.3760	–8.0012
9	Ethyl acetate	2.317	2.271	890.0975	896.9889	–6.8913
10	Acetal	2.366	2.271	895.2625	896.9889	–1.7364
11	Nonane	2.413	2.298	900.0000	900.0000	n/a
12	Methanol	2.446	2.392			
13	Methyl propionate	2.505	2.450			
14	Isopropanol	2.852	2.744			
15	Ethanol	3.021	2.902			
16	1-Propanol	5.150	4.879			
17	Isobutanol	7.653	7.117			
18	1-Butanol	10.642	9.917			
19	Active amyl alcohol	16.421	15.303			
20	Isoamyl alcohol	16.442	15.303			

The comparison was done under identical conditions of sample, temperature, column dimensions, and carrier gas linear velocity. The separations were all performed isothermally, thus minimizing any minor differences in column dimensions that may affect this comparison. In addition to these components, we incorporated a few hydrocarbons to check retention indices (RI), a measure of selectivity. If these measurements are the same (within normal variation, generally ±2 RI units for this phase type), then the replacement column really could be considered an equivalent, and the manufacturer's claim could be considered practical. However, as the data in Table 2 show, the selectivity of these columns is very different.

The chromatograms in Figures 7 and 8 show that these same columns are far from direct replacements.

If you replaced a DB-Wax with one of these other wax columns, you would:

- Lose resolution of ethyl acetate and acetal
- Lose all resolution existed between active amyl and isoamyl alcohol

Further, you can optimize separation between ethanol and isopropanol without compromising other separations simply by increasing the linear velocity on a DB-WAX column.

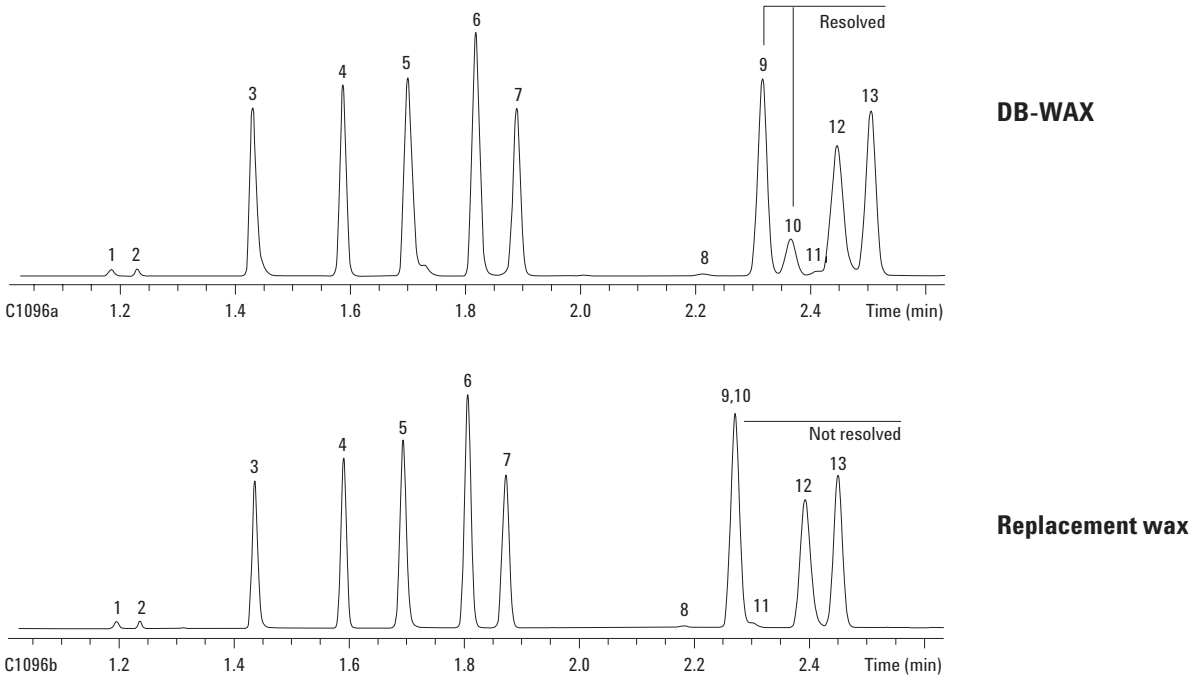


Figure 7. Comparison of DB-WAX and a "Replacement" column Chromatograms; Earliest eluants.

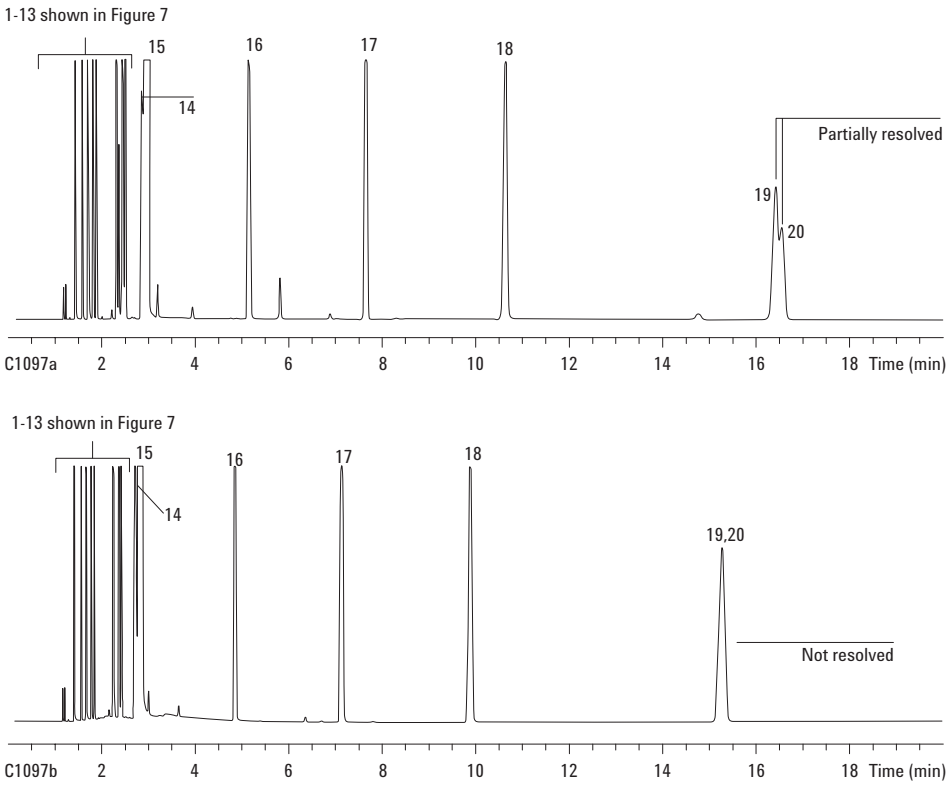


Figure 8. Comparison of DB-WAX and a "Replacement" column Chromatograms; Later eluants.

Ethyl acetate and acetal and active amyl and isoamyl alcohol could not be resolved on the replacement column under the range of conditions used for this comparison. Often, with these "replacement" columns, the manufacturer claims that the "only difference is the price."

For even simple applications like that shown in Figure 7, when you factor in the time it takes to re-verify elution order and the potential risk that either the column will not work for your application or an elution order change might be missed, minor "savings" in the price of a column are erased and may rapidly become major liabilities.

Most analysts would agree that consistent column performance from one column to the next is the most prized column performance characteristic.

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