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Development of a New Platform of Highly Inert Fused-Silica Capillary Columns

Gas chromatography technology has consistently improved sensitivity for difficult-to-analyze compounds. Increased sensitivity is especially important in trace analysis, where the response of components depends strongly on the inertness and background of the system. While the sensitivity of GC instruments and detection systems has been improving continuously, column performance has remained relatively constant over the last several years. In order to further increase sensitivity and make use of the instrument improvements, it is necessary to improve the column performance. This can be realized by 1) stationary phase stabilization, which reduces column bleed, and 2) better deactivation techniques, which improve column inertness. New deactivation technology has made it possible to make a more neutral column. As a result, more polar compounds as well as bases/acids can be analyzed with high response using standard-type stationary phases.

Rxi® column technology (**Restek Corp.,** Bellefonte, PA) has been successfully applied with nonpolar phases such as 100% polydimethyl siloxane (PDMS) as well as 5–50% phenyl PDMS phases. A simple test was developed that provides an excellent indication of column quality.

Measurement of trace impurities

When measuring trace-level impurities, the sensitivity of the GC system needs to be optimized. Sensitivity is strongly influenced by the background of the system: A system with high background will always show higher noise levels and reduced sensitivity. To optimize sensitivity, the signal-to-noise ratio (S/N) must be maximized. A lower noise level will increase the S/N, allowing lower concentrations to be detected. There are two ways to maximize the S/N: 1) decrease the noise level (decrease N), and 2) increase the signal (increase S).

Decreasing the noise level

Many parameters need to be optimized to reduce noise levels. The most important are:

- Carrier gas purity and the potential for leaks in lines
- Injection/detection port liners and connections with possible leaks
- Septa, needles, vials, and injection protocols
- Transfer lines and temperatures
- Stationary phase type, film, column length, and i.d.

By optimizing the GC system, not only is an increase in sensitivity obtained, but downtime is also reduced due to less detector contamination. Systems stabilize faster and, when using a mass spectrometric detector, the mass spectra will show better "match" factors because fewer contamination ions are formed.

Increasing the signal

Benefits are also gained from maximizing the signal. Signal can be increased by:

- Concentrating the sample: This will take time and requires more sample purification since the matrix will also concentrate.
- Injecting more volume onto the column: This is growing in popularity because the detection limits can be reduced by a factor of 20–100. There are many developments using large-volume injection techniques. Programmed temperature vaporizer (PTV) injection seems to be quite promising here.
- Optimizing detector settings: Optimizing detector flows, temperatures, and voltages will maximize sensitivity.
- Using inert liners and considering column position: Injection and detection port liners can be highly active. If the column is not positioned correctly in the detection port liner, the liner may adsorb polar/sensitive compounds.
- Increasing gas velocity or temperature program rate: Using faster temperature programs or higher flow rate can elute a component faster, resulting in a smaller peak width. The peak area will be similar, which will result in a higher response.

A factor that is often forgotten is the inertness of the capillary column. The shape of the eluting peak greatly determines the height of the signal and therefore the sensitivity. Rxi column technology was developed to improve the inertness of existing capillary columns.

Reducing noise and increasing signal

Low-bleed stationary phases have been commercially available for some time.

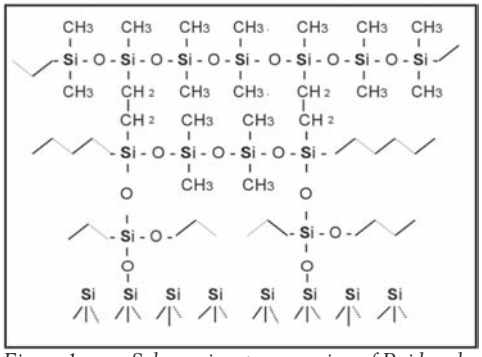

Figure 1 Schematic representation of Rxi bonding process (cross-bonding, surface bonding, deactivation, and shielding for minimization of impact of [re]active silanols).

Through the use of arylene-type stabilizing groups, the mobility of siloxanes could be reduced significantly, resulting in more stable polymers. As a result, the breakdown reaction that is the basis of stationary phase degradation is less likely to occur.

With Rxi technology, the stabilization has been taken one step further. Endcapping the reactive silanol groups present in the polymer and on the fused-silica surface was the first step. Incorporation of systematic cross-bonds made it possible to link up the siloxane chains to thicker films, maintaining flexibility without cleavage at higher temperature.

Additionally, surface deactivation was developed that allowed surface bonding of all Rxi polymers. The surface bonding makes the Rxi polymers extremely stable for mechanical attack of liquids in, for instance, splitless injections. This typically translates to longer column lifetime. *Figure 1* shows a schematic of the bonding processes used to stabilize Rxi polymers. This deactivation has an important function in shielding any residual activity on the surface, creating a highly inert column. Polymers prepared with dimethyl, diphenyl, and arylene-type stabilization could be made highly inert using Rxi column technology.

Figure 2 shows a comparison of the degradation obtained on several commercially available stabilized or "low-bleed" phases. All columns tested were treated under identical conditions. Although most commercial columns have low-bleed characteristics, the Rxi columns clearly demonstrate very good performance regarding column bleed. This is particularly evident when using thicker films (Figure 2*b*): The stabilization resulting from the cross-bonds helps to keep the bleed low. The difference is even more pronounced when inertness is taken into consideration.

Inertness of the capillary column

To maximize sensitivity, it is also essential to use a highly inert capillary. Ideally, a component elutes as a symmetrical peak. Rxi column technology allows the manufacture of a more inert GC column, which translates to several additional benefits:

- Inert columns will elute components as sharper peaks, providing 1) higher response (lower detection limits with the same hardware), 2) higher peak areas (less adsorption and better sensitivity), and 3) no retention time shifting (reduced chance of misidentification).
- Polar compounds can be measured at increasingly lower levels; this is a challenge for many laboratories.
- Derivatization is often not necessary. Drugs can be analyzed without derivatization, which saves time and improves recovery.
- More analyses without maintenance. Sharper peaks allow sample load to be reduced, which results in wider maintenance intervals and longer column lifetime.

Inertness is critical because peak tailing will increase significantly as activity in the capillary increases (*Figure 3*). This activity is typically based on an adsorption mechanism, and the stronger the adsorption is, the greater the tailing will be.

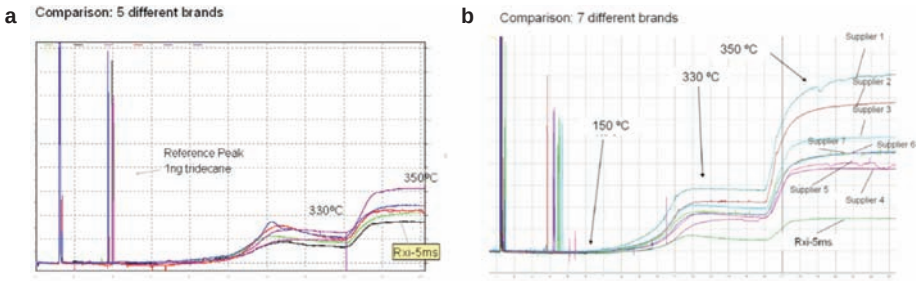

Figure 2 Bleed comparison of different types of 5% diphenyl and aryl phases: a) 0.25-µm film, b) 0.5-µm film.

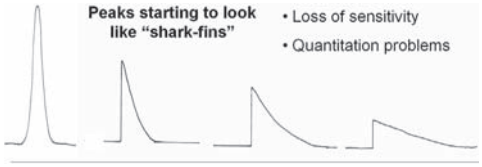

Figure 3 The typical peak shapes seen when adsorption in the column occurs.

Another problem associated with adsorption is that the retention time of the compound depends on the activity of the column. With increased activity, the retention time becomes longer. A practical example is shown in *Figure 4*, where the Rxi column technology is compared with a commercial state-of-the-art capillary column. Using pyridine as a test component immediately reveals how well a capillary is deactivated. The retention, peak shape, and peak height for pyridine eluting from the Rxi-5ms columns are significantly better than when using the standard commercial column.

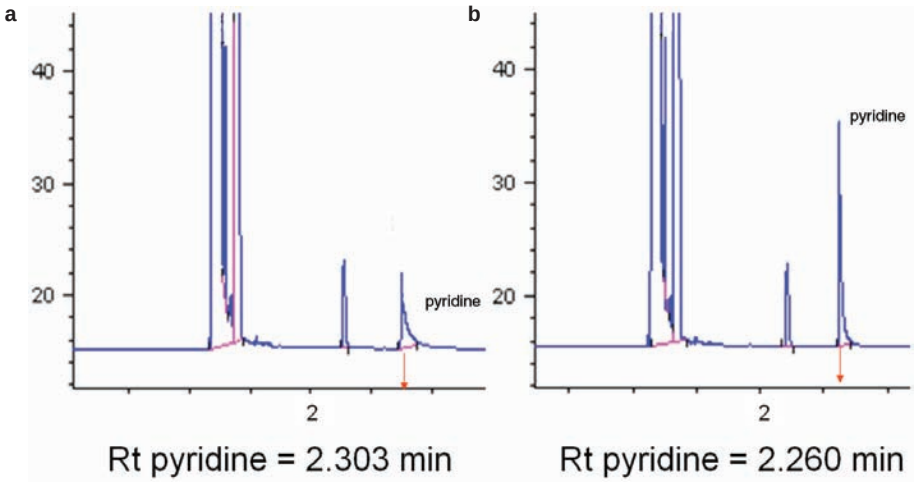

Figure 4 Impact of column activity on retention of pyridine peak. a) State-of-the-art column deactivation, b) Rxi column deactivation.

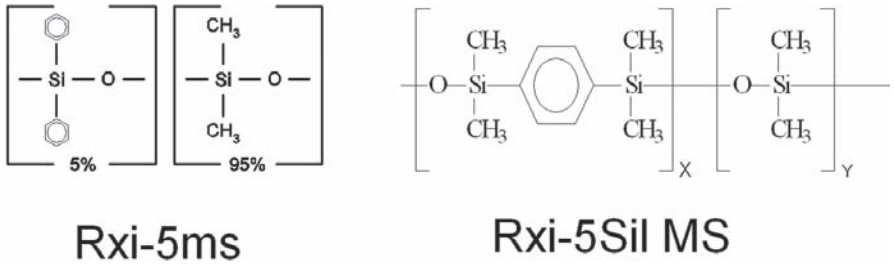

Figure 5 Structure of Rxi-5ms (5% diphenyl) and Rxi-5Sil MS phase (arylene-stabilized equivalent of 5% diphenyl).

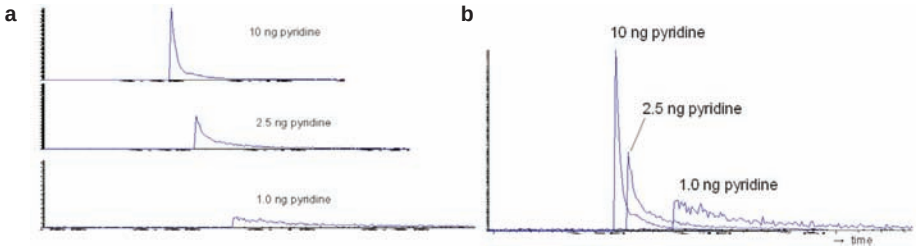

Figure 6 Impact of the amount of active compound injected on retention time and peak shape. Peak shown: pyridine at 10, 2.5, and 1 ng on a conventional state-of-the-art column (30 m × 0.25 mm, df = 0.25 µm). a) Individual runs, b) overlaid chromatograms.

Since quality of deactivation at this level varies widely from column to column, the position of active components will vary. The impact becomes even more relevant when looking at lower levels.

Peak elution becomes a function of concentration

If activity is present in a liquid phase coated capillary, it will act as a second retention mechanism. *Figure 5* demonstrates that the elution is a function of the activity. Another effect of activity is that the retention becomes a function of concentration. Depending on the number of active sites and their contribution, a component will be "overloaded" and elute earlier or later. In effect, this means that when activity is present, high concentrations will elute faster and low concentrations will be more strongly retained and will elute later.

Figure 6 shows the elution of 10, 2.5, and 1 ng pyridine using a standard capillary. The peak shape of pyridine is already a nice indicator that deactivation is not optimal. By overlaying the chromatograms, it is clear to see what happens with the lower levels of pyridine. This could lead to a false negative, misidentification, or even a false positive. Particularly when multicomponent methods are used, it is very important with trace analysis to set wide windows to look for masses. Using Rxi column technology results in a better peak shape for pyridine. This translates to similar retention times, even at much lower concentrations (*Figure 7*).

New tests for inertness

Historically, column inertness has been tested using test mixtures containing components of different functionality. For column quality, the Grob test mixture has been used for a long time. For differentiation of column inertness, these mixtures are not good enough, since most columns look comparable. A more stringent test is needed to evaluate the quality of the capillary column, and this is achieved by choosing more critical compounds.

A simple, in-house, temperature-programmed test was developed by **Restek Corp.** that uses pyridine and 2,4 dinitrophenol, functioning as markers for the column's base and acid behavior. The pyridine molecule is very sensitive for surface residual acidity and will always show some degree of tailing. On the state-of-the-art type columns, a clear difference can now be measured by analyzing these two test probes when introduced onto the column at nanogram level. A more critical isothermal test was also developed for testing the quality of columns in a production environment. These tests have to be done isothermally to generate reproducible data on all relevant column parameters.

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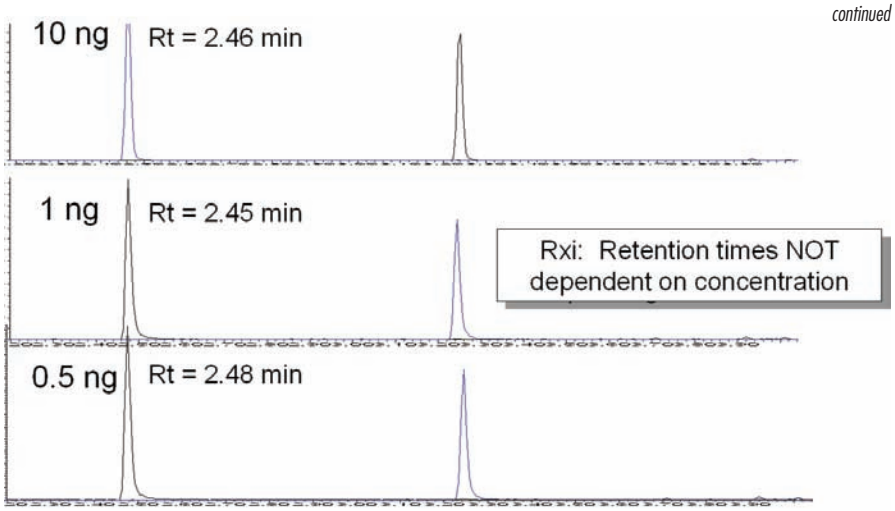

Figure 7 Peak shape and retention of 10, 1, and 0.5 ng pyridine on similar column dimensions as in Figure 8, using Rxi column technology.

Table 1 Typical components used for column quality testing

Previous testing	New testing
Primary alcohol (octanol)	Di-alcohol (2,6-hexane diol)
Aromatic dimethyl aniline	Primary amine (decylamine)
Dimethyl phenol	2,6-Dinitrophenol

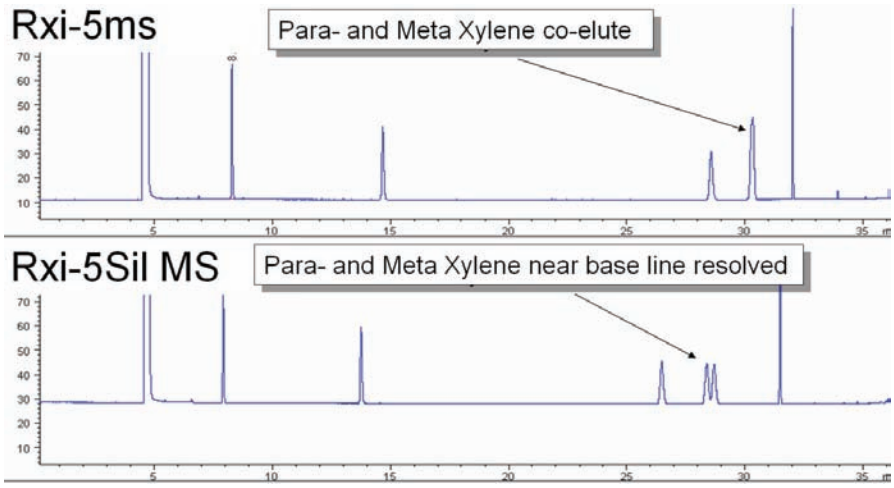

Figure 8 Separation of xylenes on Rxi-5ms and Rxi-5Sil MS columns, both 60 m × 0.25 mm, df = 0.25 µm, temperature = 35 °C.

Table 2 Equivalent selectivity of Rxi-5ms and Rxi-5Sil MS columns*

	Rxi-5ms 5% Diphenyl-PDMS	Rxi-5Sil MS Arylene-stabilized equivalent of 5% diphenyl-PDMS
Restek	Rtx-5	Rtx-5Sil MS
Agilent	HP-5, HP-5ms, DB-5, Ultra-2	DB-5ms
Varian	CP-Sil 8 CB	VF-5ms, CP-Sil 8 CB LB/MS
Alltech	AT-5	AT-5ms
Supelco	Equity-5	MDN-12
SGE	BP-5	BPX-5
Phenomenex	ZB-5	ZB-5ms
Machery-Nagel	Optima-5	Optima-5ms

*__Agilent Technologies__ (Wilmington, DE); __Varian__ (Palo Alto, CA); __Alltech Associates__ (Deerfield, IL); __Supelco Corp.__ (Bellefonte, PA); __SGE__ (Austin, TX); __Phenomenex__ (Torrance, CA); and __Macherey-Nagel__ (Easton, PA).

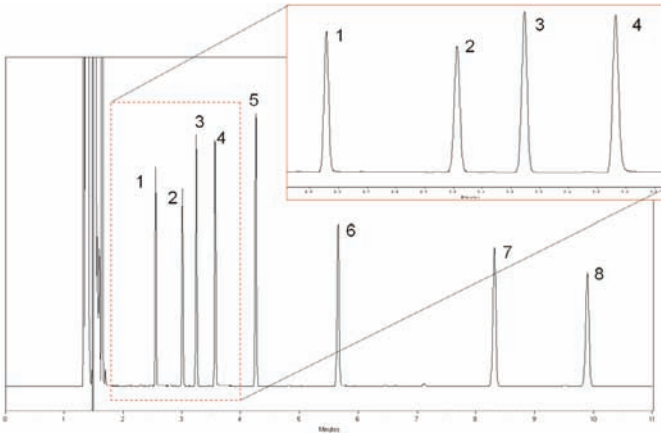

Figure 9 Standard test of Rxi-5Sil MS columns. Column: 30 m × 0.25 mm, df = 0.25 µm. 1) 2,6-hexanediol, 2) 4-chlorophenol, 3) methylnonanoate, 4) 1-decylamine, 5) tridecane, 6) undecanol, 7) acenaphthylene, 8) pentadecane.

The primary change implemented was the type of test probe (see *Table 1*).

The diol functionality reveals the interactions with residual silanols, while the primary amine and nitrophenol are excellent probes for neutrality. Such critical test probes are essential to test and demonstrate the quality of the Rxi line of capillary columns.

Column technology applied to Rxi-5Sil MS columns

Rxi column technology has been available commercially for approx. three years using the Rxi-5ms and Rxi-1ms phases. The Rxi-5Sil MS column is a nonpolar stationary phase that incorporates arylene groups for extra stabilization. The selectivity of this phase is different from the Rxi-5ms column, as the arylene group will produce different interactions. Both phases have different structures (Figure 5). There is a misconception in the industry because several column manufacturers have claimed that an arylene-stabilized siloxane (type 5ms) is identical to a 5% diphenyl/95% dimethyl-type phase. These phases are significantly different, however, and can produce complete elution order reversal. *Figure 8*, for instance, shows the separation of xylene isomers. On the arylene-stabilized phase, the separation of para- and meta-xylene is near baseline, while on a 5% diphenyl there is no separation at all. One can

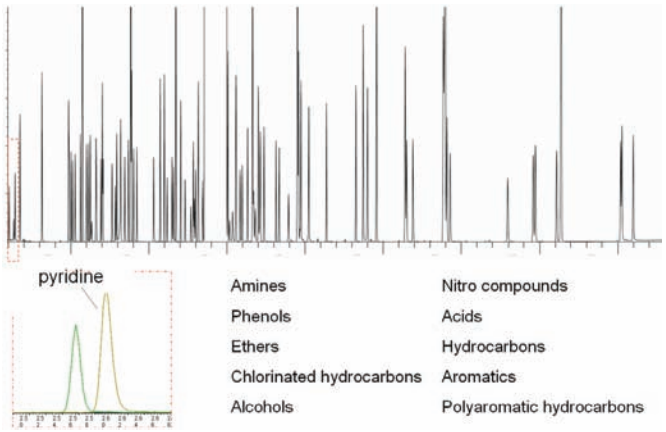

Figure 10 A multicomponent environmental analysis such as U.S. EPA 8270 is usually analyzed using an Rxi-5Sil MS column. All groups of compounds elute as symmetrical peaks. (See Table 3 for conditions.)

Table 3 Conditions for U.S. EPA 8270

Column:	Rxi-5Sil MS column (30 m × 0.25 mm × 0.25 µm with a 5-m Integra-Guard™ (**Restek**), 10 ng of each compound injected on the column
Oven:	40 °C (hold 1 min) → 280 °C at 25 °C/min → 310 °C at 5 °C/min
Carrier gas:	He, 1.2 mL/min
Procedure:	All injections were made in the pulsed-splitless mode with a Siltek® 4-mm-i.d. Drilled Uniliner® (both **Restek**) using an **Agilent** 5975. One-microliter injections were made with an injection port temperature of 250 °C. Carrier flow rate was set at 1.2 mL/min, constant flow. Standards were 10 µg/mL for all compounds except for the internal standards, which were 20 µg/mL.

imagine that aromatic compounds in particular will have different interactions with the in-chain arylene groups versus the side-chain diphenyl groups.

Restek Corp. has applied the Rxi column technology to both phases. The Rxi-5Sil MS column is a direct substitute for all arylene-stabilized stationary phases (*Table 2*). By choosing the correct equivalent, the same separation and elution order will be achieved, but with very low bleed and improved peak symmetry for difficult components such as diols, acids, and primary amines. *Figure 9* shows the elution profile of the developed critical test mixture on the Rxi-5Sil MS column. Note the symmetry of the acid, base, and diol peaks.

Analyzing U.S. EPA 8270 pollutants is a very common application for the Rxi-5Sil MS phase. This is a challenging application because there are many different classes of components to be measured in one method. A 93-component 8270 standards mix was analyzed at levels of 10 µg/mL. All of the analytes performed well at 10-ng load onto the column. Peak shape and separation were excellent for all components, including the challenging pyridine peak (*Figure 10*). Equipment and procedures are described in *Table 3*.

Conclusion

Rxi column technology has been applied successfully to the manufacture of 100% methyl, 5% diphenyl, and arylene-stabilized phases such as the Rxi-5Sil MS. This platform of capillary columns meets the increasingly stringent demands on column quality by combining low bleed with exceptionally high inertness. Trace analysis will become less challenging with these advances because more polar components can be measured without derivatization at increasingly lower levels.

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