

Impact of column deactivation on the chromatography of polar compounds at sub-nanogram levels

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Gas chromatography has been consistently improving in order to gain improvements in sensitivity for difficult-to-analyze compounds. Especially in trace analysis the response of components depend strong 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 address the need for improved sensitivity, and make use of the instrument improvements, it is necessary to improve the column performance. This can be realized by stationary phase stabilization, which reduces column bleed and better deactivation techniques, which improves the column inertness. The technology, known as Rxi-technology, allows more symmetrical peak elution, which benefits polar compounds like acids, diols and amines. As an inert surface produces a higher peak response, detection levels will decrease. Also peaks elute at fixed retention times, reducing the chance on misidentification especially if components can be present in different concentration,

The Rxi technology is very promising as it can be applied with near all current stationary phases based on methyl, phenyl, aryl and cyanopropyl substitutions.

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 must be maximized. A lower noise level will increase the signal-to-noise ratio, allowing lower concentrations to be detected.

There are two ways to maximize the signal-to-noise ratio: (A) decreasing the noise level (decrease N), and (B) increasing the signal (increase S).

A: 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 ID.

By optimizing the GC system, not only an increase in sensitivity is obtained, but also downtime is reduced due to less detector contamination. Systems stabilize faster and, when using a mass

spectrometric detector, the mass spectra will show better “match” factors as fewer contamination ions are formed.

B: Increasing the Signal

Besides reducing the noise, we should benefit also from maximizing the signal. Signal can be increased by:

- Concentrating the sample.
- Injecting more volume onto the column.
- Optimizing detector settings.
- Using inert liners & considering column position.
- Increasing gas velocity or temperature program rate.

A factor, which 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. To improve the inertness of existing capillary columns new technology was developed, known as Rxi[®] column technology.

Rxi[®] Column Technology for Reducing Noise and Increasing Signal

Low bleed stationary phases have been commercially available for some time. By using arylene type stabilizing groups, the mobility of siloxanes could be reduced significantly which resulted 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. First by end-capping the reactive silanol groups present in the polymer and on the fused silica surface. Incorporation of systematical cross bonds made it possible to link the siloxane chains up to thicker films, while maintain flexibility without cleavage at higher temperature.

Additionally in the Rxi[®] process, a 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 in 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. 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 show clearly superior characteristics concerning column bleed. Especially when using thicker films, Figure 2b, the stabilization due to the cross-bonds helps in keeping the bleed low. The difference is even more pronounced when inertness is considered.

Rxi: Cross-bonding, Surface-bonding, Deactivation and Shielding for minimization impact of (re)active silanols

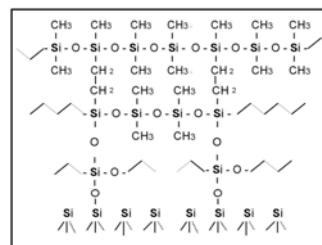
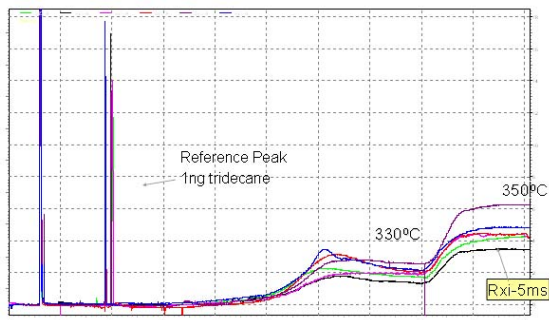


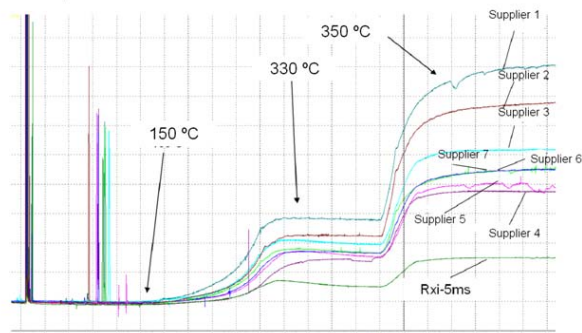
Fig. 1 Schematic representation of Rxi bonding process.

Film = 0.25 μm
Comparison: 5 different brands



A

Film = 0.5 μm
Comparison: 7 different brands



B

Fig. 2 Bleed comparison of different types of 5% diphenyl and arylene phases. A = 0.25 μm film, B = 0.5 μm film.

Rxi[®] Column Technology: 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 in several additional benefits:

- Inert columns will elute components as sharper peaks, providing:
 - Higher response (lower detection limits with same hardware).
 - Higher peak areas (less adsorption, better sensitivity).
 - 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 often is not necessary. Drugs can be analyzed without derivatization which saves time and improves recovery.
- More analyses without maintenance. Sharper peaks allow us to reduce sample load, 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 typically is based on an adsorption mechanism, and the stronger the adsorption is, the greater the tailing will be.

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.

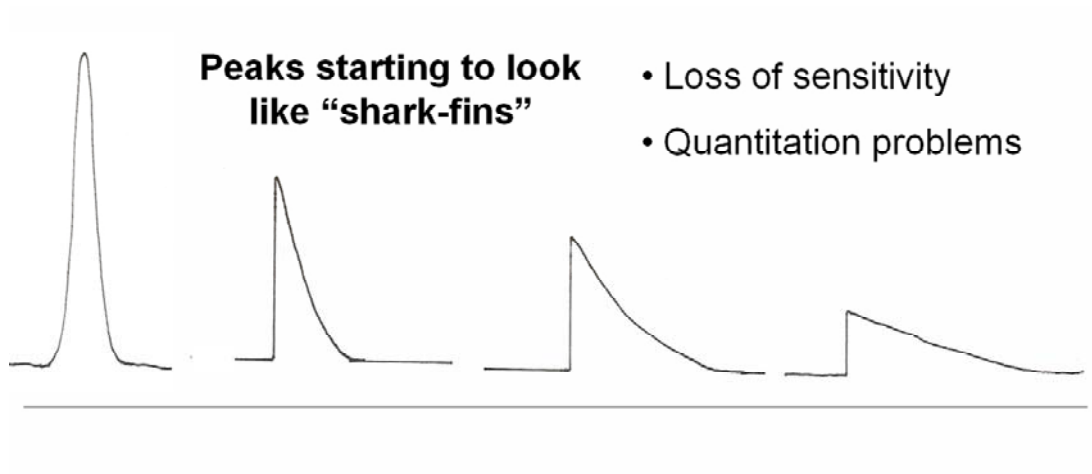


Fig. 3 Typical peak shapes seen when adsorption in the column occurs.

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.

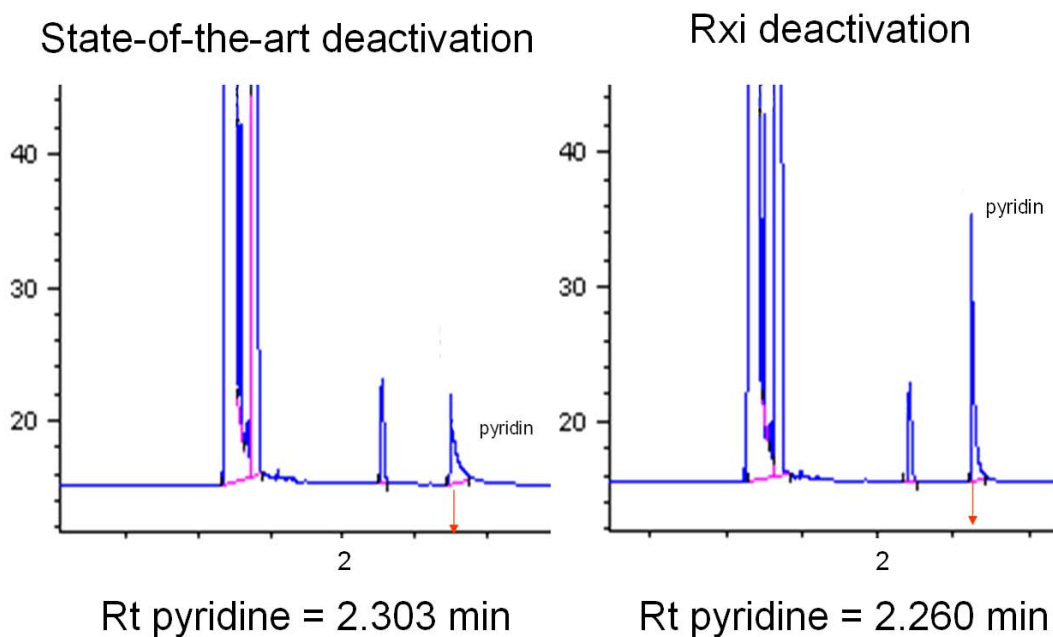


Fig. 4 Impact of column activity on retention of pyridine peak

If activity is present in a liquid phase coated capillary, the activity will act as a second retention mechanism. 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 elute earlier or later.

Practical this means that when activity is present, high concentrations will elute faster and low concentrations will be more strongly retained and elute later.

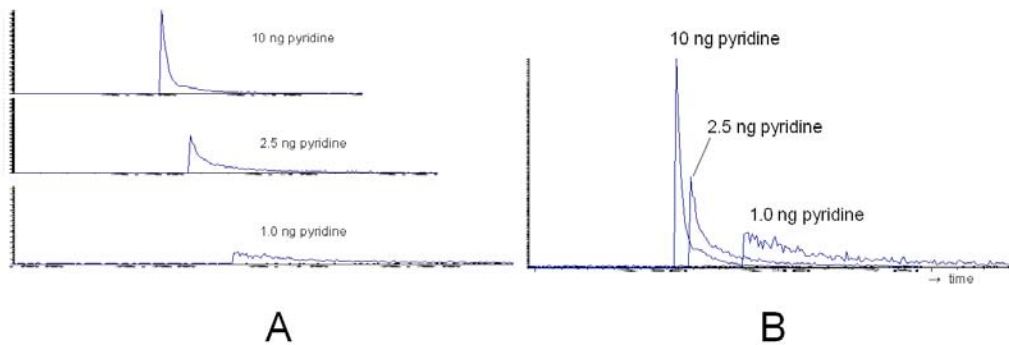


Fig. 5 Impact of the amount of active compound injected on retention time and peak shape. Peak shown: pyridine at 10, 2.5 and 1 nanogram on a conventional state-of-the-art column (30m x 0.25mm df = 0.25 μ m). A : individual runs; B: Overlaid chromatograms

Figure 5 shows the elution of 10, 2.5 and 1 nano gram of 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". Especially when multi-component methods are used, at trace analysis it is very important to set wide windows to look for masses. When using Rxi[®] column technology, the peak shape for pyridine is much better. This translates in similar retention times, even at much lower concentrations, see Figure 6.

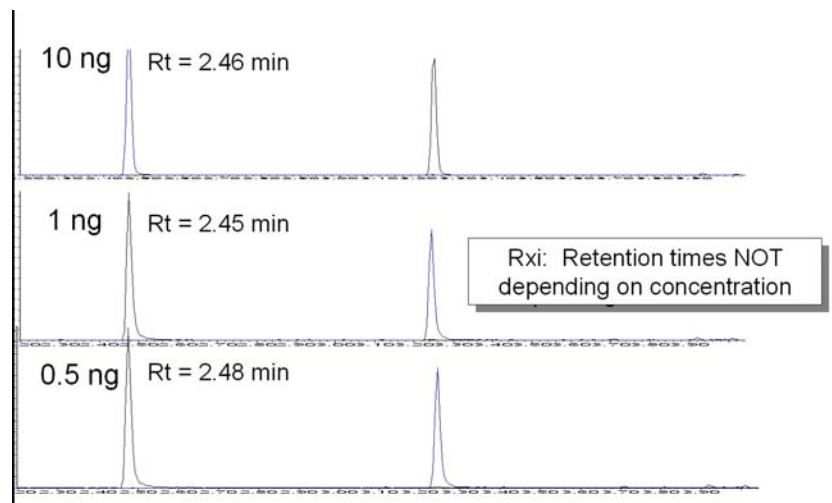


Fig. 6 Peak shape and retention of 10, 1 and 0.5 ng pyridine on similar column dimensions as in Fig. 5, using Rxi[®] column technology

Rxi[®] Column Technology Applied to Rxi[®]-5Sil MS Columns

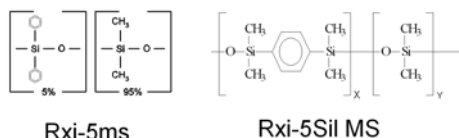


Fig. 7 Structure of Rxi[®]-5ms (5% diphenyl) and Rxi[®]-5-Sil MS phase (arylene stabilized equivalent of 5% diphenyl).

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, see figure 7. There is a misconception in the industry as 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 however significantly different and can produce complete elution order reversal. Figure 8 shows for instance 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 imagine that especially aromatic compounds will have different interactions with the in-chain arylene groups vs. the side chain diphenyl groups.

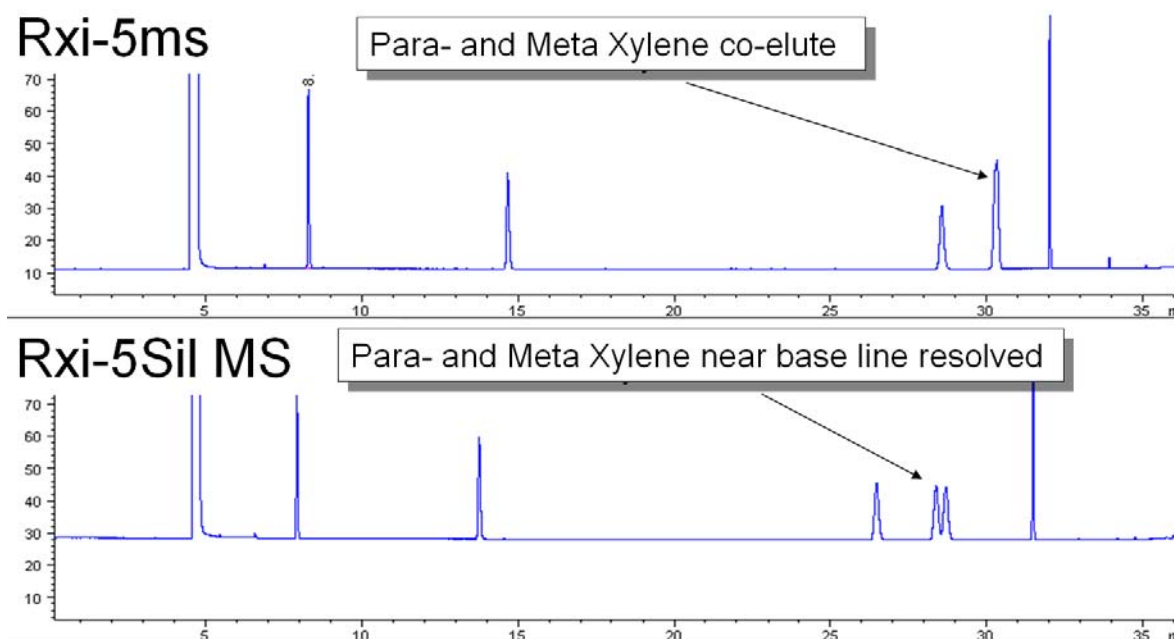


Fig. 8 Separation of xylenes on Rxi[®]-5ms and Rxi[®]-5Sil MS columns, both 60m x 0.25mm df = 0.25 μ m, Temperature = 35°C.

Table 1 Equivalent selectivity of Rxi[®]-5ms / Rxi[®]-5Sil MS columns

	Rxi[®]-5ms	Rxi[®]-5Sil MS
	5% diphenyl- PDMS	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
Mackery nagel	Optima-5	Optima-5ms

Restek makes both phases available and is able to apply the Rxi[®] column technology to both phases. The Rxi[®]-5Sil MS column is a direct substitute for all arylene-stabilized stationary phases (Table1). 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 like 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 excellent symmetry of the acid, base, and diol peaks.

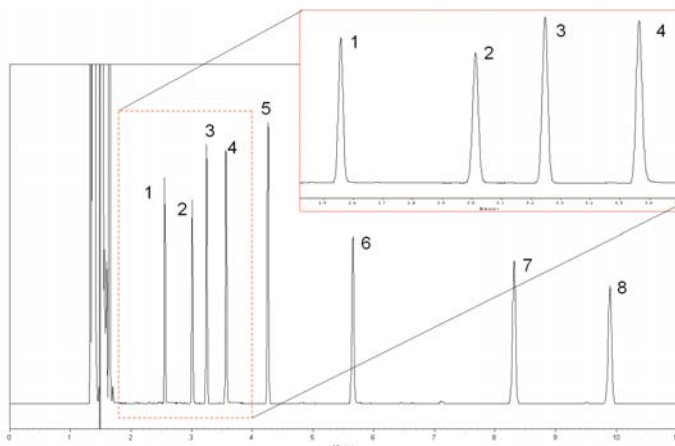


Fig. 9 Standard test of Rxi[®]-5Sil MS columns; column: 30m x 0.25mm df = 0.25 um; 1=2,6-hexanediol; 2=4-chlorophenol; 3=methylnonaanoate; 4=1-decylamine; 5=tridecane; 6=undecanol; 7=acenaphthylene; 8=pentadecane.

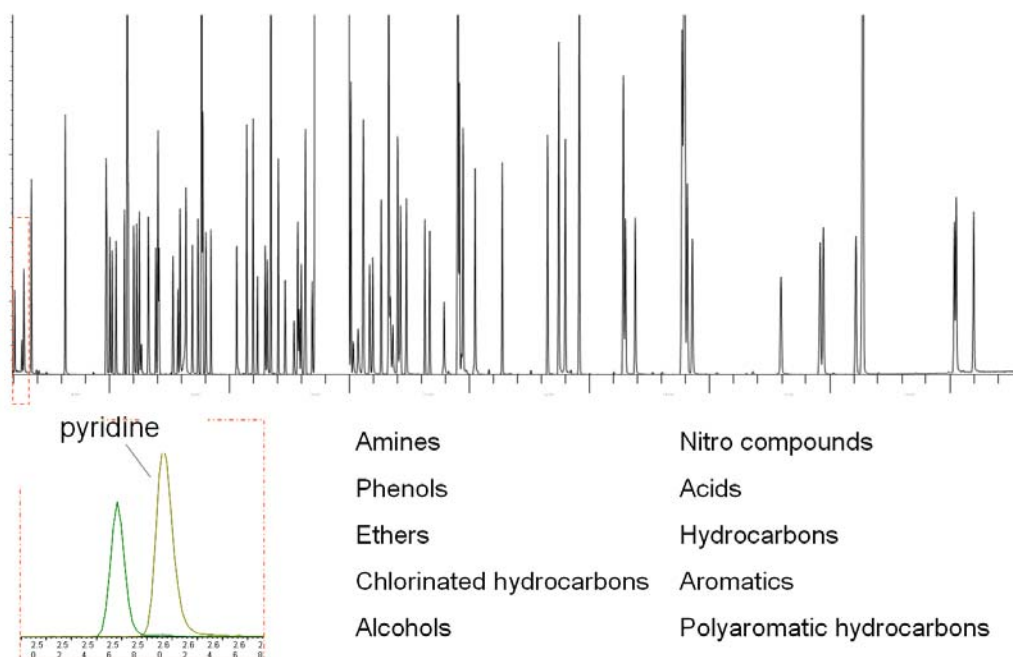


Fig. 10 EPA 8270 is usually run on an Rxi®-5Sil MS column, 30m x 0.25mm x 0.25µm with a 5m Integra-Guard™; 10ng of each compound on the column; Oven:40°C (hold 1min.) ? 280°C @ 25°C/min. ? 310°C @ 5°C/min. ; Carrier gas: He, 1.2mL/min., constant flow; Procedure: All injections were made in the pulsed-splitless mode with a Siltek® 4mm ID Drilled Uniliner® using an Agilent 5975. constant flow.

Another example of the unique inertness is shown in figure 10: Analyzing EPA 8270 pollutants is a very common application for the Rxi®-5Sil MS phase. All analytes elute as sharp peaks.

Conclusion

Rxi® column technology has successfully resulted in improved inertness of a wide series of stationary phases, allowing more polar, basic and acidic components to be analyzed at lower levels.

Because the peak shapes are improved, retention time shifting due to column activity and amount of component injected, will be minimized which will make trace analysis methods more reliable.

The Rxi® column technology is also successfully applied to Rxi-5Sil MS phase, which is now also made available to replace commercial arylene stabilized stationary phases, and obtain similar order of peak elution, but with the unique combination of low bleed AND high inertness.