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How Column Inertness Improves the Chromatography of Basic Compounds

Not all column deactivations are appropriate for analyzing basic compounds. Here we demonstrate the effect of column inertness on peak shape, and discuss its role in improving method accuracy, sensitivity, and development time.

Basic compounds are often problematic to analyze by gas chromatography. Poor peak symmetry, or tailing, is common and can lead to inconsistent integration and calibrations, as well as losses in sensitivity, due to lower signal-to-noise ratios. In both GC and LC alike, peak tailing of basic compounds can be largely attributed to silanol activity, through an adsorption mechanism where the solutes interact with the exposed silica support in LC or the fused silica capillary in GC. In LC, we have the option of modifying our mobile phase with additives, like triethylamine (TEA), that can act to limit the unwanted silanol interaction. In GC, however, we do not have this option and this puts greater importance on choosing an inert column, such as an Rxi® column, which has a balanced deactivation that is effective toward both basic and acidic compounds. In this article we will discuss the relationship between inertness and system activity, illustrate the effect of improper column deactivation with an example for basic compounds, and discuss options for improving peak symmetry.

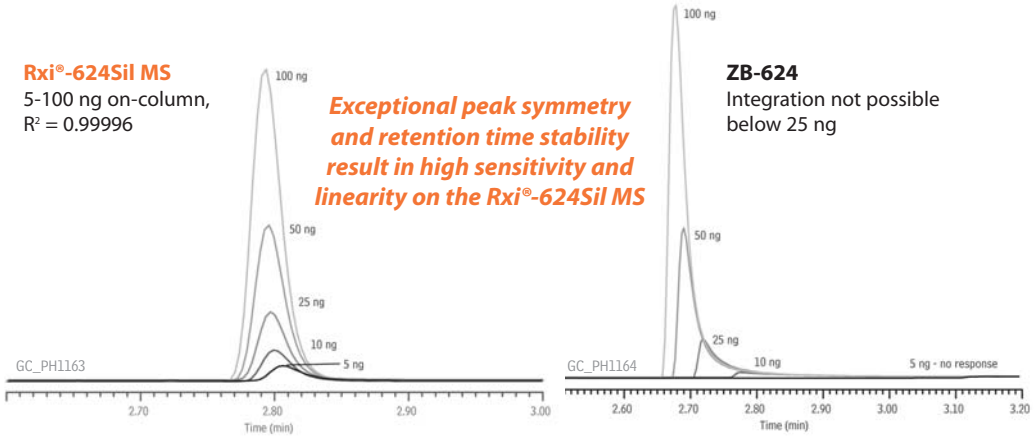
Understanding Inertness in Gas Chromatography

Glass and metal surfaces are chemically reactive and can undergo solute adsorption, which manifests as chromatographic peak tailing. The entire sample pathway, which includes the liner and the chromatographic column, needs to be inert. Deactivation is simply a means by which column manufacturers treat the sample pathway to eliminate unwanted chemical interaction, which may be more practically viewed as imparting inertness. Capillary column deactivation involves treating the fused silica and creating polymers that limit the residual silanol activity that is the basis for the tailing of basic compounds. Not all deactivation processes are the same, however, and most are tailored to specific solute characteristics. Every manufacturer defines a chemical process, or deactivation technology, for deactivating their capillary columns and liners, and this may vary by column line and manufacturer.

Diagnosing System Activity

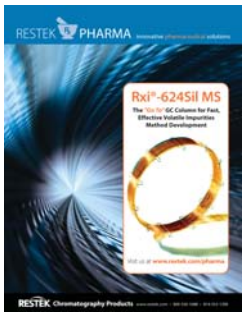
The tailing of basic compounds is certainly familiar to the pharmaceutical researcher as amines are commonly found on active pharmaceutical ingredients and impurities. When troubleshooting our chromatography, improper column deactivation can be easily diagnosed. Peak tailing as a result of solute adsorption is mass dependent. As mass (sample load) increases, tailing becomes less prominent. The ZB-624 column tested in Figure 1 illustrates this point. As the isopropylamine mass on-column drops, tailing

Figure 1 Rxi®-624Sil MS columns are more inert than ZB-624 columns, resulting in more symmetric peaks that give methods greater accuracy, sensitivity, and reproducibility.



Column	Rxi®-624Sil MS, 30 m, 0.32 mm ID, 1.80 µm (cat.# 13870)	Inj. Temp.:	250 °C
Sample		**Oven**	
Diluent:	DMSO	Oven Temp:	50 °C (hold 1 min.) to 200 °C at 20 °C/min. (hold 5 min.)
Conc.:	100 µg/mL	**Carrier Gas**	He, constant flow
Injection		Linear Velocity:	37 cm/sec.
Inj. Vol.:	1 µL split (split ratio 20:1)	**Detector**	FID @ 250 °C
Liner:	5mm Single Gooseneck with Wool (cat.# 22973-200.1)	**Instrument**	Agilent/HP6890 GC

increases and the retention time shifts to the right. If we consider what is occurring here, this chromatography makes complete sense. As mass on-column increases, the amount of solute that is adsorbed is lower in relation to the total solute mass making tailing less apparent. Also, an adsorbed molecule is retained longer, so as the amount of solute adsorbed relative to the total analyte mass increases, i.e. lower concentrations, the retention time shifts to the right.



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For more information on Rxi®624-Sil MS columns, download PHFL1245 at www.restek.com

Evaluating peak symmetry relative to analyte activity can also be used to detect system activity. Since tailing in this case is based upon adsorption, more reactive analytes will show greater tailing. In this example, we analyzed primary, secondary, and tertiary amines—isopropylamine, diethylamine and triethylamine—with analytical conditions set to eliminate the contributions of injection port activity. The ZB-624 peaks in Figure 2 show exactly what we would expect to see from tailing attributed to solute adsorption. The primary amine is the most reactive and therefore exhibits the greatest degree of peak tailing, while the tertiary amine, the weakest in reactivity, remains symmetric throughout these analyses.

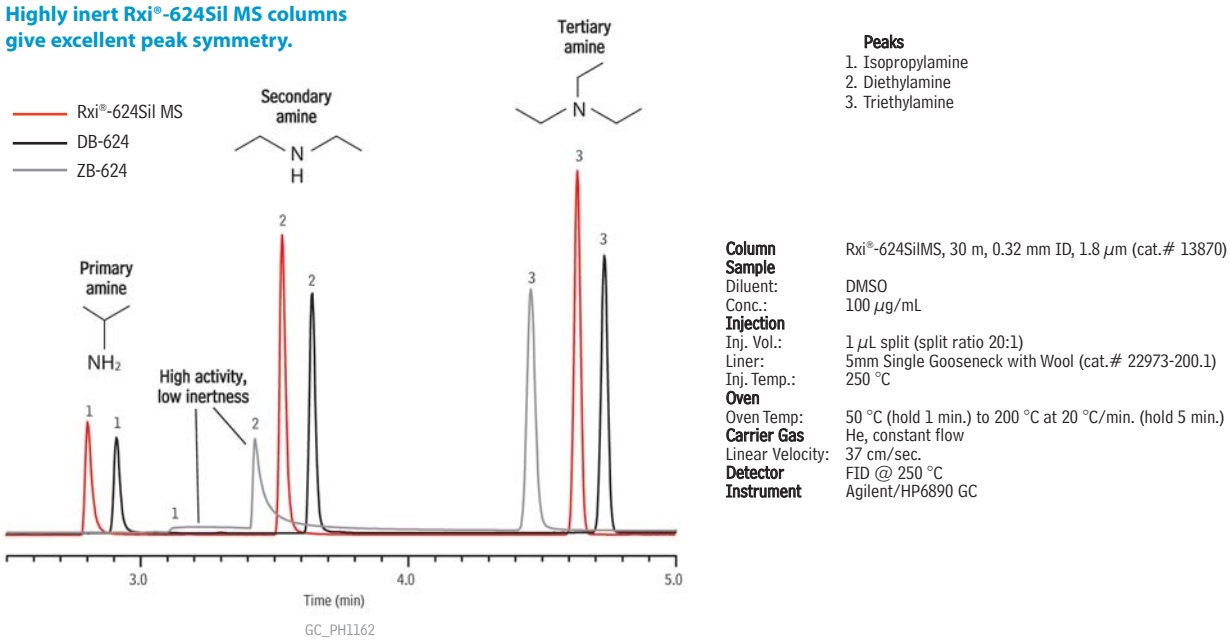
Options for Reducing Peak Tailing

Once we have properly diagnosed system activity, there are certain steps we can take to minimize the deleterious effects on method performance. Since we know that activity is mass dependent, we can simply increase sample load to reduce the effect of improper deactivation. As the mass of solute increases, tailing is less problematic (especially considering that the signal increases while the USP tailing is taken at a consistent 5% peak height). However, increasing sample mass is not always practical or desirable, as it creates a situation where sensitivity and trace analysis is jeopardized. In addition, increased sample loads can contribute to faster column degradation. We can also derivatize compounds into a less reactive state. However, this practice can be lengthy and add uncertainty to methods (derivatization efficiency), so its utility is limited. One simple, easy way to eliminate activity is to consider column inertness. The analytical differences in column inertness can be dramatic. Better sensitivity, linearity, and resolution can all be attributed to column inertness and result in faster, more reliable method development.

Balanced Deactivations Make Column Selection Easy

Often, when choosing an analytical capillary column, we focus entirely on the column type and not the column inertness. Specialty deactivations are often very useful when analyzing compounds with similar activity. For example, when analyzing a group of basic compounds, a deactivation specifically targeting basic analytes could be advantageous. We must consider though, this strategy becomes deleterious when we change analyte polarity. Basic deactivation will cause a high degree of peak tailing for acidic compounds, and vice versa. This brings complexity to analytical development when dealing with a wide vari-

Figure 2 Deactivation processes vary by manufacturer. Rxi® columns provide symmetric peaks for reactive amines, even at trace levels.

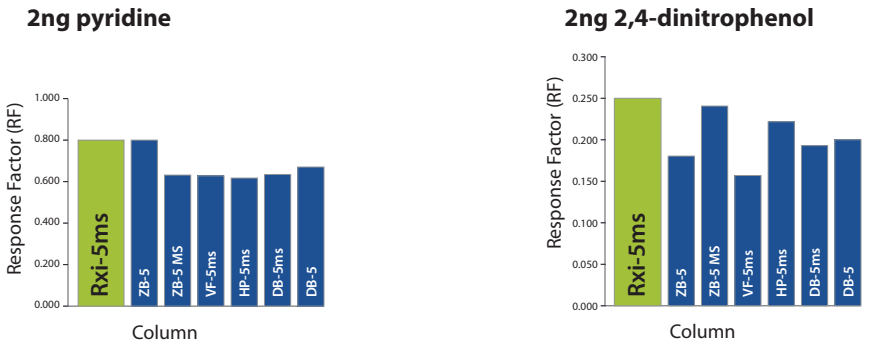


ety of analytes, as is common in pharmaceutical analyses. Rxi® technology was invented to create a practical, comprehensive deactivation that is effective for both acidic and basic compounds (Figure 3). A neutral, or balanced, deactivation as seen in the Rxi® technology, allows for a wider range of compounds—acidic, basic, and polar—to be analyzed with high sensitivity and data quality without the need for changing columns. Rather than spending lab time trying to find the proper column deactivation, researchers can develop methods faster by using columns with a balanced Rxi® deactivation.

Conclusion

When it comes to peak tailing in GC, we must consider that there are two major areas for solute adsorption, the column and the injection port. Focusing on the column, we can see how choosing a column with an effective deactivation can give us more suitable chromatography—higher sensitivity with decreased sample load, better linearity and accuracy, and increased column lifetimes.

Figure 3 An Rxi® column gives the best overall performance for both basic and acidic compounds.



Comparison of 30m x 0.25mm ID, 0.25µm columns.

Column Cross-Reference Table

POLARITY: nonpolar → polar

Restek	Phase Composition	Agilent	Varian/ Chrompack	SGE	Phenomenex	Machery-Nagel	Supelco
Rxi-1ms	100% dimethyl polysiloxane	HP-1ms UI, DB-1ms UI, HP-1, HP-1ms, DB-1 DB-1ms, Ultra-1	VF-1ms CP-Sil 5 CP Sil 5 CB Low Bleed/MS	BP-1	ZB-1 ZB-1ms	Optima-1 Optima-1ms	SPB-1 Equity-1
Rxi-1HT	100% dimethyl polysiloxane	DB-1HT			ZB-1HT		
Rxi-5ms	5% diphenyl/ 95% dimethyl polysiloxane	HP-5ms UI, HP-5, HP-5ms, DB-5, Ultra-2	CP-Sil 8 CP Sil 8 CB	BP-5	ZB-5	Optima-5	SPB-5 Equity-5
Rxi-5Sil MS	5% phenyl, 95% dimethyl arylene siloxane	DB-5ms UI, DB-5ms	VF-5ms CP-Sil 8 CB Low Bleed/MS	BPX-5	ZB-5MS	Optima-5ms	SLB-5
Rxi-5HT	5% diphenyl/95% dimethyl polysiloxane	DB-5HT	VF-5HT		ZB-5HT		
Rxi-XLB	arylene/methyl modified polysiloxane	DB-XLB	VF-Xms				
Rxi-624Sil MS	6% cyanopropylphenyl, 94% dimethyl arylene siloxane	DB-624, HP-624	VF-624ms	BP-624	ZB-624	Optima-624	
Rxi-35Sil MS	35% phenyl, 65% dimethyl arylene siloxane	DB-35ms	VF-35ms		MR2		
Rxi-17	50% diphenyl/50% dimethyl polysiloxane	HP-17, DB-17, DB-608	CP-Sil 24 CB		ZB-50		
Rxi-17Sil MS	50% phenyl, 50% dimethyl arylene siloxane	DB-17ms	VF-17ms	BPX-50			

GC Columns

Rxi®-624Sil MS Columns (fused silica)

(mid polarity Crossbond® silarylene phase; equivalent to 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.18mm	1.00	-20 to 300/320°C	20-Meter	13865
0.25mm	1.40	-20 to 300/320°C	30-Meter	13868
0.32mm	1.80	-20 to 300/320°C	30-Meter	13870
0.32mm	1.80	-20 to 300/320°C	60-Meter	13872
0.53mm	3.00	-20 to 280/300°C	30-Meter	13871

GC Inlet Liners

Base-Deactivated Inlet Liners

Base Deactivation (BD)

Base deactivation (BD) is ideal for the analysis of basic compounds, such as amines and basic drugs. It prevents analyte adsorption which manifests as either irreproducible results or peak tailing.

For base-deactivated inlet liners, add the corresponding suffix number to the liner catalog number.

qty.	Base-Deactivated Liner		Base-Deactivated Liner w/ Base-Deactivated Wool		Base Deactivated Liner w/CarboFrit	
each	-210.1	addl. cost	-211.1	addl. cost	-229.1	addl. cost
5-pk.	-210.5	addl. cost	-211.5	addl. cost	-229.5	addl. cost
25-pk.	-210.25	addl. cost	-211.25	addl. cost	-229.25	addl. cost

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