RESTEK Pure Chromatography

An Evaluation of Deactivations on Gas Chromatographic Stationary Phases for the Analysis of Seized Drugs of Abuse

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Introduction

Gas Chromatography–Mass Spectrometry (GC-MS) is commonly used for screening seized drugs due to its ease of use and high specificity. One key advantage of GC-MS is that many drugs can be analyzed without chemical derivatization, a process that is often time-consuming and complex. However, when drugs of abuse are analyzed in their underivatized form, active functional groups can interact with active sites on the fused-silica column surface. These interactions can result in poor peak shape, potentially leading to inaccurate or unreliable results. Recent advancements in column chemistry, particularly the development of improved deactivation strategies that minimize active silanols on the fused-silica surface, have significantly enhanced the analysis of active compounds without derivatization.

Background

Traditionally, GC column deactivation has focused on either physically shielding silanols or chemically deactivating individual silanol sites. While these approaches generally provide adequate deactivation, physical shielding can contribute to higher column bleed, and individual silanol deactivation often results in incomplete deactivation and increased column-to-column variability. A new deactivation process has recently been developed that integrates both surface coverage and individual silanol deactivation, resulting in a fully integrated deactivation layer that produces more rugged, inert, and consistent columns.

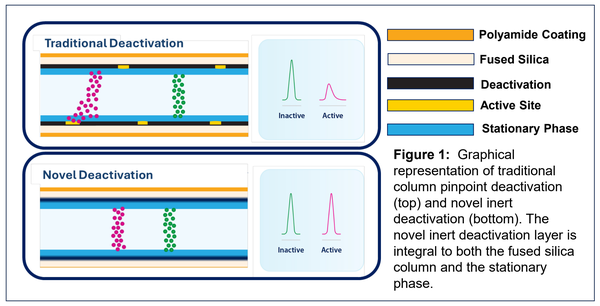

Figure 1: Graphical representation of traditional column pinpoint deactivation (top) and novel inert deactivation (bottom). The novel inert deactivation layer is integral to both the fused silica column and the stationary phase.

Seized Drug Analyte Choice

A comprehensive set of analytes was selected to thoroughly evaluate column performance for acidic, basic, and neutral compounds with a range of functional groups. The analyte list included common seized drugs, as well as representative cutting agents and potential degradation products.

Analyte	Functional Groups	Category	Analyte	Functional Groups	Category
Phenobarbital	barbituric acid	Acid	Norketamine	primary amine	Weak Base
Amobarbital	barbituric acid	Acid	Ketamine	secondary amine	Weak Base
Butabarbital	barbituric acid	Acid	Zolpidem	amide	Weak Base
Butalbital	barbituric acid	Acid	Alprazolam	Diazepine / triazole	Weak Base
Pentobarbital	barbituric acid	Acid	Cotinine	pyrrolidine / pyridine	Weak Base
Secobarbital	barbituric acid	Acid	Diphenhydramine	tertiary amine	Base
Propofol	hydroxyl / phenyl	Weak Acid	Dextromethorphan	tertiary amine	Base
Phenacetin	amide	Neutral	Amitriptyline	tertiary amine	Base
Phencyclidine	piperidine	Weak Base	Cyclobenzaprine	tertiary amine	Base
Codeine	tertiary amine	Weak Base	Sertraline	secondary amine / halogen	Base
Methadone	tertiary amine	Weak Base	Brompheniramine	tertiary amine / bromine	Base
Heroin	tertiary amine	Weak Base	Chlorpheniramine	tertiary amine / halogen	Base
Venlafaxine	tertiary amine / hydroxyl	Weak Base	Tramadol	tertiary amine / hydroxyl	Base
Cocaine	tertiary amine	Weak Base	Imipramine	tertiary amine	Base
Prilocaine	secondary amine / amide	Weak Base	Doxylamine	pyridine / tertiary amine	Base
Tetracaine	secondary/tertiary amine	Weak Base	Benzocaine	primary amine	Base
6-Acetylmorphine	tertiary amine	Weak Base	Procaine	primary/secondary amines	Base

Table 1: Representative analyte mix of common seized drugs and cutting agents, including both acidic and basic compounds. Acid/base classifications are based on gas chromatographic behavior rather than aqueous pKa values.

Model-Assisted GC Method Development

Web-based modeling software was used to facilitate chromatographic method development. This software allows for rapid modeling of compounds of interest and can automatically optimize separations for critical pairs. Alternatively, separations can be modeled using user-defined analytical conditions. One limitation of this software is that analytes must be present in its database. For this study, 18 of the 34 analytes were available in the database, and a model was generated for these compounds.

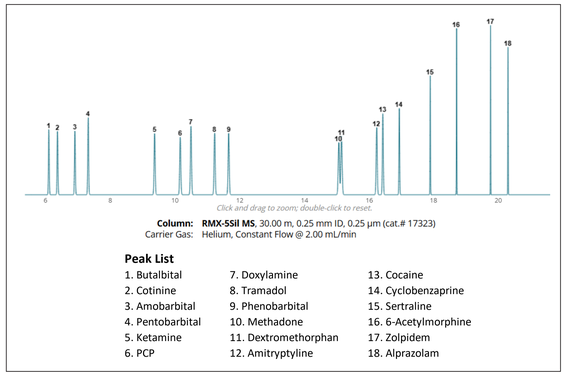

Figure 2: Software-modeled chromatogram with user-customized conditions.

Column Evaluation and Aging Study

Six 5% phenyl-arylene / 95% dimethylpolysiloxane columns were manufactured. Three columns employed a traditional pinpoint deactivation, while the remaining three were manufactured using the novel deactivation. Column performance for both deactivation types was evaluated by measuring analyte area variability and symmetry for all analytes. Three injections of the test mix were performed on each of the three columns with novel deactivation, followed by three injections on each of the three columns with traditional deactivation. Analytical conditions are shown in Table 2.

Analytical Column:	"5-type" 30m x 0.25mm x 0.25µm
Instrument:	Agilent 7890B
Carrier Gas:	Helium @ 2 mL/min
Inlet:	250°C, 10:1 split
Oven Program:	150°C (hold 1 min)
	4°C/min to 210°C
	30°C/min to 320°C (hold 2 min)
Transfer Line Temp:	280°C
Detector:	5975 MSD, scan 40 - 500 m/z
Source:	230°C
Quad:	150°C
Sample:	**All Analytes @ 25 µg/mL in MeOH/EtAc**

Table 2: Instrumental conditions for all analyses of seized drug analytes mix.

In addition to performance evaluation of unused columns, one column with the novel deactivation was subjected to an accelerated aging study. This study consisted of splitless injections of undiluted black pepper extract combined with extended exposure to the column's maximum temperature of 350 °C. In total, the column was exposed to 60 injections of black pepper extract and 65 hours at 350 °C. Following aging, 1.5 m was trimmed from the head of the column, and six injections of the 25 µg/mL seized-drug analyte mix were performed. Injection reproducibility, FID column bleed during pepper injections, and analyte symmetry were used to evaluate the effects of column aging on performance.

Results and Discussion

A representative chromatogram of the 25 µg/mL analyte mix analyzed on a column with novel deactivation is shown in Figure 3. Analyte resolution closely matched predictions from the web-based modeling software, particularly for the separation of methadone and dextromethorphan.

#	Analyte	#	Analyte	#	Analyte	#	Analyte
1	Propofol	9	Secobarbital	17	Phenobarbital	26	Imipramine
2	Benzocaine	10	Norketamine	18	Chlorpheniramine	27	Tetracaine
3	Butabarbital	11	Prilocaine	19	Procaine	28	Cyclobenzaprine
4	Butalbital	12	Ketamine	20	Venlafaxine	29	Sertraline
5	Phenacetin	13	Diphenhydramine	21	Brompheniramine	30	Codeine
6	Cotinine	14	Phencyclidine	22	Methadone	31	6-Acetylmorphine
7	Amobarbital	15	Doxylamine	23	Dextromethorphan	32	Heroin
8	Pentobarbital	16	Tramadol	24	Amitriptyline	33	Zolpidem
				25	Cocaine	34	Alprazolam

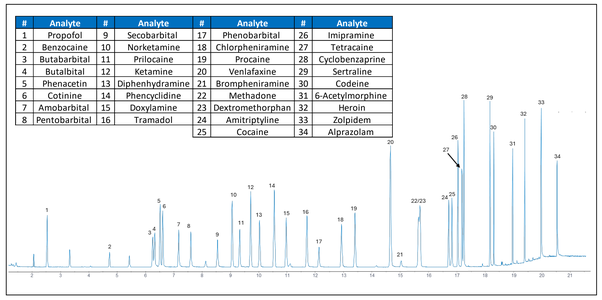

Figure 3: Separation of 34 seized drug analytes on Rmx-5Sil MS.

Figure 4 shows overlaid splitless injections of uncleaned black pepper extract analyzed by GC-FID. The oven program used for this analysis differed from that listed in Table 2; instead, the oven was ramped from 40 °C to 350 °C over 15 minutes and held at 350 °C for an additional 15 minutes. Figure 5 shows the pepper extract used for column aging, the used inlet liner, and the aged column compared with a non-aged column.

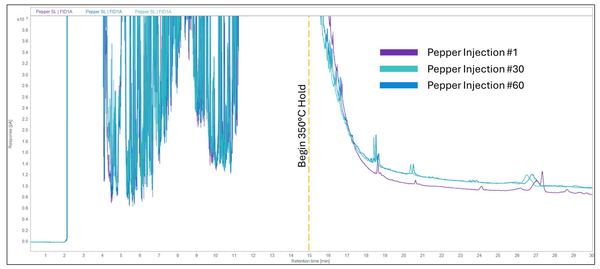

Figure 4: Overlay of FID trace for black pepper extract injections 1, 30, and 60 on column with novel Rmx deactivation.

Over the course of 60 injections of a dirty matrix at the column's maximum temperature, the novel deactivation produced minimal column bleed, which did not increase significantly over the duration of the experiment. Column bleed was evaluated during pepper extract analysis rather than following a bakeout. Slight discoloration of the polyimide coating on the aged column reflects exposure to high oven temperatures, while the extensive non-volatile contamination observed on the inlet liner highlights the harshness of the aging conditions.

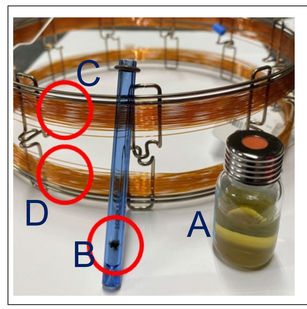

Figure 5A: A: Black pepper extract used for column aging, 2g pepper extracted using EN QuEChERS salts, no further cleanup. **5B:** Liner removed from instrument after 60 splitless injections of pepper extract. **5C:** Column used for aging study. **5D:** Non-aged column

Results Cont.

Compound	Novel Deact. Area %RSDs	Traditional Deact Area %RSD	Aged Novel Deact. Area % RSD
Propofol	8.26%	11.72%	2.94%
Benzocaine	7.26%	8.55%	2.72%
Butabarbital	24.24%	11.77%	3.54%
Butalbital	22.60%	11.11%	2.71%
Phenacetin	7.28%	14.20%	3.19%
Cotinine	7.37%	13.21%	2.62%
Amobarbital	19.32%	9.27%	3.44%
Pentobarbital	17.91%	11.86%	3.52%
Secobarbital	23.16%	12.77%	3.47%
Norketamine	8.09%	12.99%	3.06%
Prilocaine	9.35%	13.24%	3.55%
Ketamine	9.02%	13.91%	2.57%
Diphenhydramine	8.70%	13.50%	2.60%
Phencyclidine	7.65%	13.02%	2.55%
Doxylamine	8.01%	14.45%	3.31%
Tramadol	9.73%	17.64%	4.48%
Phenobarbital	47.49%	27.99%	3.09%
Chlorpheniramine	8.35%	14.88%	3.31%
Procaine	8.05%	15.00%	2.98%
Venlafaxine	8.51%	17.24%	4.79%
Brompheniramine	4.51%	16.51%	2.89%
Methadone	9.02%	15.49%	2.58%
Dextromathorphan	8.21%	15.24%	2.25%
Amitriptyline	8.09%	15.57%	2.41%
Cocaine	9.13%	14.93%	2.88%
Imipramine	8.41%	15.87%	2.82%
Tetracaine	8.36%	16.28%	2.61%
Cyclobenzaprine	7.95%	14.94%	2.38%
Sertraline	7.10%	15.83%	2.07%
Codeine	6.22%	14.06%	2.75%
6-Acetylmorphine	14.58%	8.47%	3.92%
Heroin	15.85%	10.61%	2.03%
Zolpidem	17.08%	16.95%	2.96%
Alprazolam	9.61%	16.85%	2.71%

Table 3: Average area %RSDs for 34 analytes across multiple columns with different types of deactivation, as well as triplicate injections on an aged column with novel deactivation.

Table 3 shows percent relative standard deviation (%RSD) for replicate injections of the analyte mix on new columns and on the column that underwent the aging study. Data for non-aged columns were collected across three different new columns, spanning multiple days and multiple MS venting and tuning cycles. No internal standard was included and attempts to normalize the data using a well-behaved analyte (e.g., phencyclidine) did not improve variability.

Although the four acidic compounds exhibited higher variability on the column with novel deactivation, overall performance was improved compared to columns deactivated using the traditional process.

Figure 6 shows the results of symmetry evaluation for all analytes on a traditionally-deactivated column, a column with novel deactivation, and on the aged column with novel deactivation. The novel deactivation resulted in improved peak shapes for 25 of the 34 analytes. The greatest improvement in peak shape was seen for basic analytes, which is consistent with improved silanol deactivation. After aging, although overall peak symmetry degraded versus a new column with novel deactivation, symmetry on the column with novel deactivation outperformed the column with traditional deactivation for 26 of the 34 analytes, showing the ruggedness of the novel deactivation even when subjected to high temperatures and harsh matrices.

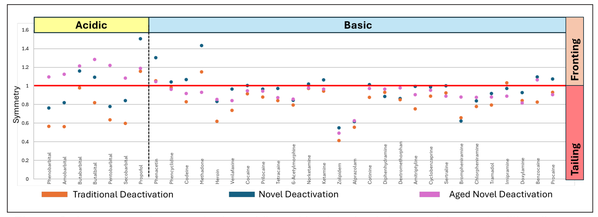

Figure 6: Average peak symmetry for acidic and basic seized-drug analytes on traditionally deactivated, novel deactivated, and aged novel deactivated columns. A symmetry value of 1.0 (red line) represents ideal peak shape, with values >1 indicating fronting and values <1 indicating tailing.

Conclusions

The novel RMX column deactivation evaluated in this study produced a more inert and rugged stationary phase compared to traditional deactivation approaches. Improved peak shape for underivatized seized drugs, reduced column bleed, and sustained performance following exposure to high temperatures and dirty matrices demonstrate the suitability of this deactivation for seized drug analysis. Collectively, these results suggest that the novel deactivation can enhance analytical reliability while extending column lifetime in demanding forensic applications.