Improved Inertness and QC Testing Standards for EPA Method 8270D with Zebron™ ZB-SemiVolatiles GC Columns

Kory Kelly and Kristen Parnell
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Increased column activity can lead to poor acid/base sensitivity and analyte misidentification, which are common hindrances to the accuracy of semi-volatile methods. This is most apparent when analyzing compound lists with various types of functional groups and reactivity characteristics, as in EPA Method 8270. This study explores the inertness of leading GC columns used for semi-volatiles methods and presents the advantages afforded by using columns designed and QC tested specifically for EPA 8270D.

Introduction

Environmental chemists have long struggled with the analysis of semi-volatiles by EPA Method 8270. This method contains an exceptionally long compound list, which may number over 200 if adding Appendix IX or client-specified analytes. A varied spectrum of functional groups is present, from reactive acidic phenols to tailing basic amines, as well as neutral compounds like polynuclear aromatic hydrocarbons (PAHs). These analytes are typically extracted from extremely dirty matrices, leading to contaminant build-up and eventually decreased system performance. Active compounds like pentachlorophenol (PCP), 2,4-dinitrophenol, benzidine, and pyridine often exhibit tailing peaks and decreased responses as a result. To remove such contamination and rejuvenate performance, columns require frequent trimming or conditioning, which adjusts analyte retention times and adds to the headache of routine system maintenance.

Through extensive research and testing, Phenomenex has developed a deactivation process, Enviro-Inert™ Technology, designed to combat these issues and provide optimized performance for difficult semi-volatiles analysis. Equipped with this technology, the Zebron ZB-SemiVolatiles GC column features supreme inertness especially for acids, bases, and pesticides while maintaining the same selectivity and retention expected from a 5 % phenyl-arylene phase. To eliminate the guesswork often encountered using typical columns for semi-volatiles methods, this column has been designed to perform under conditions even more demanding than required by the EPA. In addition to the rigorous QC test every Zebron GC column must pass for efficiency, retention, bleed, and activity, each ZB-SemiVolatiles column is QC tested with many stringent criteria specifically implemented to overcome challenges faced in EPA 8270D.

More Sensitive Probes for Column Activity Testing

To ensure that the GC system is performing consistently, EPA 8270D sets system suitability requirements that the instrument must pass using a specific "Tune Mix." This mix contains a mass spectrometer tuning compound (DFTPP) and a collection of active compounds: PCP (an acid), benzidine (a base), and DDT (a reactive pesticide). The system must meet minimum performance requirements for this mix, which are outlined in **Table 1**, before processing analytical samples. Though the Tune Mix is required by the analytical method, GC columns have typically not been QC tested using this mix. Without such screening, analysts must try a column with no guarantee of its performance specifically for their methods.

Table 1. Summary of ZB-SemiVolatiles Performance Requirements

EPA 8270D Method Criteria

Criteria	EPA Requirement	Phenomenex Requirement
Pyridine		
Peak Response	Not Specified	≥ 0.6*
Pentachlorophenol		
Peak Response	Not Specified	≥ 0.3
Peak Skew	≤ 2.0	≤ 2.0
Benzidine		
Peak Skew	≤ 2.0	≤ 2.0
DDT		
Breakdown	< 20 %	< 20 %
Injection	50 ng or less on column	0.2 ng on column

*Requirement of 0.5 for 60 m x 0.25 µm dimensions

Internal testing revealed that benzidine, used to assess a column's basic activity, was not a sufficiently sensitive probe to fully characterize small amounts of such activity. Analysts using general purpose columns may pass benzidine peak skew requirements yet still fail calibration curves for other active compounds. To combat this, analysts often add extra concentration levels to their calibration curves. This approach allows low levels to be ignored because five passing data points may still be attained at high levels. While this achieves the goal of passing a calibration curve, it consequently reveals potential inconsistencies in a lab's low-level detection data and also adds time and complexity to an already challenging method. To overcome these issues, ZB-SemiVolatiles columns are first tested with a QC mix that ensures passing performance for efficiency, bleed, retention, and activity. The columns are then required to pass a second QC test using a composite mix including the EPA 8270D Tune Mix and pyridine, a more active base than benzidine. Passing criteria for this new, more sensitive mix (the ZB-SemiVolatiles QC Test Mix) dictate minimum requirements that are typically unmonitored by GC column manufacturers.

Standardized Performance Criteria for Troublesome Compounds

The EPA method calculates analyte response factors based on peak area in comparison to the internal standard, which does not indicate peak shape performance (a broad peak and a sharp peak could both have the same peak areas). Because poor peak shape negatively impacts signal-to-noise ratios and quantitation, QC testing for ZB-SemiVolatiles measures a more accurate performance indicator – response factors based peak heights as opposed to peak areas. Specific response factors are not defined for the method – the EPA 8270 requirement states that a compound's response must fall within what is typically expected for that analyte. However, to provide analysts with a consistent measure of performance, Phenomenex sets minimum response requirements in the QC test every ZB-SemiVolatiles column must pass (**Table 1**). In this study, minimum response requirements are set at 0.6 for pyridine and 0.3 for PCP. No other column manufacturer has previously set requirements for this level of performance.

Improving Calibration Success at Low-Level Concentrations

ZB-SemiVolatiles columns must demonstrate satisfactory QC performance at levels that typically give analysts the most trouble – the low end of a calibration curve. EPA method 8270D recommends a concentration of "50 ng or less" (1.0 µL splitless injection of a 50 ppm solution). Due to the activity in typical columns, labs often inject the maximum 50 ng to improve their chances of passing calibrations – higher concentrations will saturate any column activity, allowing more data points to respond normally. The QC mix for ZB-SemiVolatiles is injected much lower at 20 ppm to more accurately assess true column activity. Additionally, labs often attempt to improve sensitivity by using splitless or even pulsed splitless (as opposed to split) injections to introduce the most sample onto the column. To ensure their performance at extremely low detection levels, ZB-SemiVolatiles columns are tested using a split injection with a 100:1 split ratio (effectively 0.2 ng on column – 250 times less than the maximum allowed 50 ng).

Experimental

Conditions used for all columns are listed in **Table 2** and reflect the QC conditions used for every ZB-SemiVolatiles column. All comparisons shown were performed within the same time frame by the same analyst on the same instrument. To accurately and consistently measure peak intensities and to account for any minor differences in sample volumes, the internal standard method of comparison was used with the inert compound DFTPP. Peak heights of the analytes of interest were compared to the peak height of the internal standard. This ratio results in a response factor (RF) as similarly calculated in EPA Method 8270D.

Table 2. QC test conditions for ZB-SemiVolatiles

Conditions used for all columns evaluated

Dimensions	30 meter x 0.25 mm x 0.25 µm
Injection	Split 100:1 @ 175 °C, 1 µL
Carrier Gas	Hydrogen @ 40 cm/sec (constant pressure)
Oven Program	40 °C for 2 min to 300 °C @ 15 °C/min for 3.5 min
Detector	FID @ 325 °C
Sample	ZB-SemiVolatiles QC Test Mix Analytes are 20 ppm in dichloromethane 1. Pyridine 2. Pentachlorophenol 3. DFTPP 4. Benzidine 5. DDT 6. DDD
Liner	Single Taper with Wool in Middle
Inlet Seal	Easy Seals™ Inlet Base Seal
Septa	PhenoRed™-400 Injector Septa

Results and Discussion

A chromatogram for the Zebron ZB-SemiVolatiles QC Test Mix is shown in **Figure 1** on a ZB-SemiVolatiles column. The first analyte peak in the chromatogram is pyridine. This active amine tails when a column has activity and will result in a lower peak height and lower RF value. In this chromatogram, the RF for pyridine is 1.06 (ZB-SemiVolatiles minimum requirement is 0.6). The second peak, PCP, would disappear when activity is present. This column still shows an RF of 0.37 (ZB-SemiVolatiles minimum requirement is 0.3). The final peak in the chromatogram is DDT. This is an active pesticide that breaks down into DDD and DDE in the presence of column activity. The ZB-SemiVolatiles column displays low activity as no breakdown products are visible in this chromatogram.

The second column tested was a Restek® Rxi®-5ms column with the chromatogram shown in **Figure 2**. For this column, pyridine shows excessive tailing and has an RF of 0.34. This does not meet the minimum RF requirement of 0.6, and may lead to difficulties in passing calibration curves due to lower sensitivity for bases. While the PCP response factor passes our minimum requirement of 0.3, this Rxi-5ms column would still not pass our QC requirements due to the failing activity toward bases.

The third column tested was the Restek Rxi-5Sil MS as shown in **Figure 3**. This column showed better response for pyridine compared to the Restek Rxi-5ms with a response factor of 0.53. However, this RF is still half that of the ZB-SemiVolatiles column and does not meet minimum requirement of 0.6. Additionally, the increase in pyridine response was achieved by exchanging response of acids for pyridine. The response of PCP has now decreased to 0.26, which leaves phenols subject to greater activity and the possibility of failing calibration curves or decreasing column lifetime. Though an improvement over the Rxi-5ms, this column still does not pass minimum QC requirements.

The fourth column tested was an Agilent® HP-5ms Ultra Inert as shown in **Figure 4**. This column had the lowest pyridine response of all columns tested with an RF of 0.28, which fails the requirement of 0.6. Though this column performed poorly for bases, it did have the highest RF for acids with a PCP RF of 0.40. This RF is slightly better than that of ZB-SemiVolatiles, but occurs at the expense of poor response for bases. In addition, this was the only column tested that had significant breakdown of DDT (12.4 %). Though this value is below the 20% required by the method, this was the only column tested that showed such activity.

The final column tested was an Agilent DB-5ms Ultra Inert as shown in **Figure 5**. This column had good response for pyridine with an RF of 0.66 and was the only other column to meet the minimum 0.6 RF requirement for pyridine. As seen with other columns tested, this response comes with a tradeoff. The DB-5ms Ultra Inert had the lowest RF for PCP of any of the columns tested (0.20). This fails the requirement of 0.3.

Of all columns tested, only ZB-SemiVolatiles would have passed the minimum performance requirements for both pyridine and PCP response as summarized in **Table 3**. Though all columns displayed similar peak shapes for benzidine, varying levels of column activity greatly affected the response of more active basic peaks like pyridine. Dramatic differences in performance are apparent when inspecting pyridine peak shape and signal intensity as illustrated in **Figure 6**.

Figure 1. QC Test Mix on ZB-SemiVolatiles

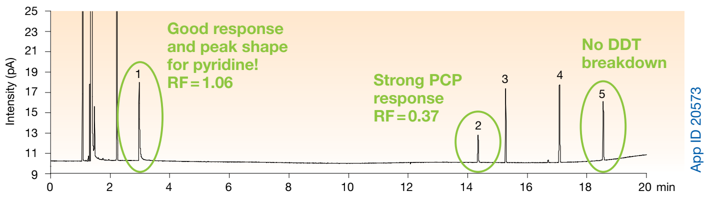

Figure 2. QC Test Mix on Restek® Rxi®-5ms

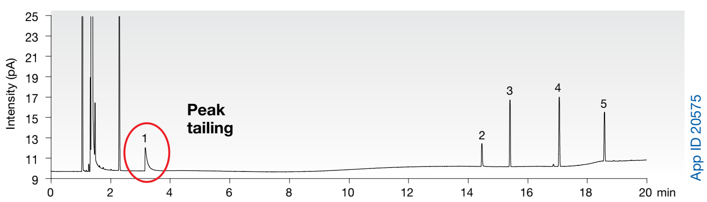

Figure 3. QC Test Mix on Restek Rxi-5Sil MS

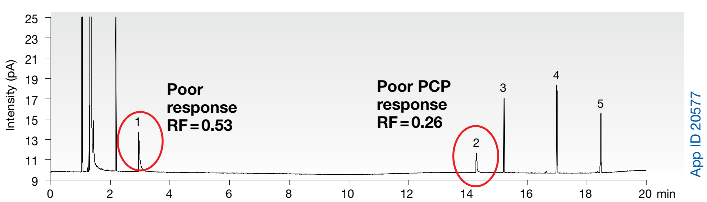

Figure 4. QC Test Mix on Agilent® HP-5ms Ultra Inert

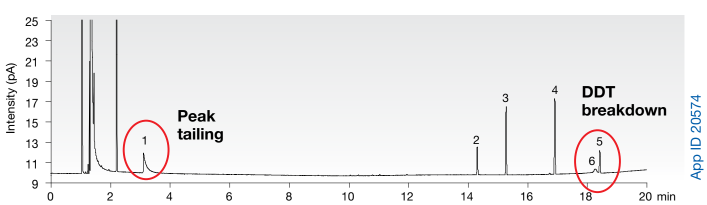

Figure 5. QC Test Mix on Agilent DB-5ms Ultra Inert

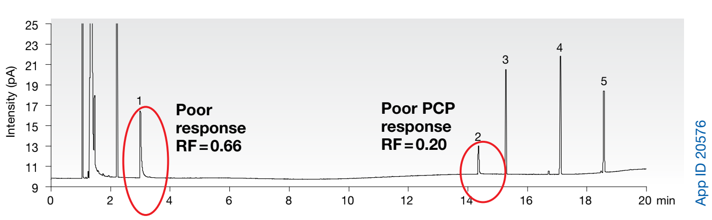

Figure 6. Overlaid pyridine peak for all columns tested

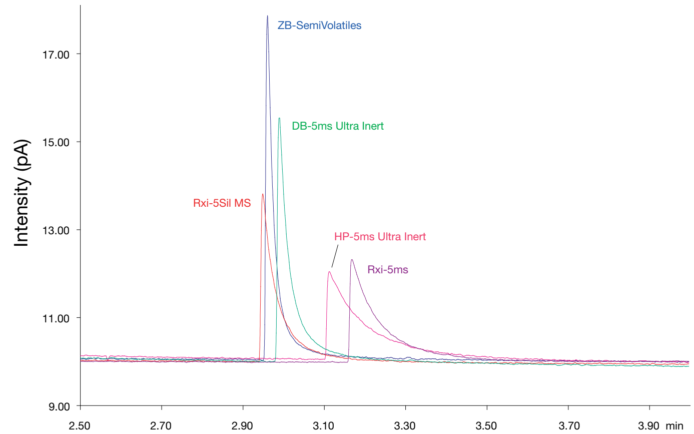

Table 3. Summary of Response Factors (RF) for pyridine and pentachlorophenol (PCP) on each of the columns tested.

Column	Pyridine RF	PCP RF	Result
Zebron™ ZB-SemiVolatiles	1.06	0.37	Pass
Restek Rxi-5ms	0.34	0.34	Fail
Restek Rxi-5Sil MS	0.53	0.26	Fail
Agilent HP-5ms Ultra Inert	0.28	0.40	Fail
Agilent DB-5ms Ultra Inert	0.66	0.20	Fail

RF is calculated by dividing peak height of analyte by peak height of DFTPP as internal standard.

Conclusion

Leading GC columns used for EPA Method 8270D were tested to determine how they would perform to the stringent requirements outlined in the Zebron ZB-SemiVolatiles QC test process. Designed to provide GC analysts with a guarantee of performance specifically for troublesome semi-volatiles methods, this process characterizes both acidic and basic activity and sets previously unmonitored requirements for GC column performance that are even more stringent than required by EPA 8270D. Minimum peak response requirements for pyridine (0.6) and PCP (0.3) serve as a metric for characterizing both basic and acidic column activity, which are both present in methods like EPA 8270. Columns with higher initial peak responses can be expected to maintain performance over time, providing longer lifetimes for the method. Additionally, higher responses allow runs at lower detection levels, improving the sensitivity of the analysis.

Of the columns tested, only ZB-SemiVolatiles had high and balanced responses for both acids and bases. Other columns had good responses for bases but sacrificed the response for acids, or vise versa. The HP-5ms Ultra Inert was also the only column that exhibited any activity for DDT. The improved results on ZB-SemiVolatiles demonstrate the true value of choosing a GC column designed, deactivated, and QC tested to deliver highly inert performance specifically for semi-volatiles methods.

APPLICATIONS

Ordering Infomation

Zebron™ ZB-SemiVolatiles GC Columns

ID (mm)	df (µm)	Temperature Limits (°C)	Part No.
20-Meter			
0.18	0.18	-60 to 325/350	7FD-G027-08
0.18	0.36	-60 to 325/350	7FD-G027-53
30-Meter			
0.25	0.25	-60 to 325/350	7HG-G027-11
0.25	0.50	-60 to 325/350	7HG-G027-17
60-Meter			
0.25	0.25	-60 to 325/350	7KG-G027-11

Easy Seals™ Inlet Base Seals

Part No.	Description	Unit
Standard, single groove for splitless applications, 0.8 mm dia. inlet hole		
AGO-8619	Easy Seals Inlet Base Seal, Gold Plated for Agilent GCs	2/pk
AGO-8620	Easy Seals Inlet Base Seal, Gold Plated for Agilent GCs	10/pk

PhenoRed-400™ Injector Septa

Part No.	Description	Diameter	Unit
PhenoRed-400 GuideRight™ Injector Hole Septa			
AGO-7916	PhenoRed-400, rated to 400 °C	3/8 in (9.5 mm)	50/pk
AGO-7917	PhenoRed-400, rated to 400 °C	7/16 in (9.5 mm)	50/pk

guarantee

If Zebron columns do not provide you with equivalent or better separations as compared to any other GC column of the same phase and comparable dimensions, return the column with comparative data within 45 days for a FULL REFUND.

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions, which may be viewed at http://www.phenomenex.com/TermsAndConditions.

Trademarks
Zebron, Enviro-Inert, Easy Seals, GuideRight and PhenoRed are trademarks of Phenomenex.
Restek and Rxi are registered trademarks of Restek Corporation.
Agilent is a registered trademark of Agilent Technologies, Inc.

Disclaimer
Phenomenex is in no way affiliated with Agilent Technologies or Restek Corporation. Comparative separations may not be representative of all applications.

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries: Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

TN10130113_W

phenomenex ...breaking with tradition℠

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com