



## Environmental Applications

# Half the Column, Same Chromatogram: Maintain Resolution of BDE 49 and BDE 71 With Proper Method Translation After Trimming an Rtx<sup>®</sup>-1614 Column for Maintenance

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### Abstract

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that are used in many household and office products, including furniture, electronics, and textiles. These lipophilic compounds have been found to be persistent and bioaccumulative in nature. Due to their toxicity and ubiquitous presence, PBDEs are included in many monitoring efforts across a wide array of biological and environmental sample matrices. The analysis of PBDEs is challenging due to structural isomers that need to be chromatographically separated and thermally-labile compounds of interest that may breakdown during gas chromatography. Furthermore, nonvolatile material may still persist even in cleaned final extracts, requiring GC column and inlet maintenance to be performed. PBDEs included in EPA Method 1614 are well resolved on a 15 m x 0.25 mm x 0.10  $\mu\text{m}$  Rtx<sup>®</sup>-1614 GC column that was specifically designed to meet method resolution requirements of less than 40% valley height between BDE 49 and BDE 71. The selectivity of this column, in combination with properly translating the original GC method, allowed the column to be trimmed significantly for maintenance (7.9 m) while maintaining the method resolution criterion. This allows chromatographers to extend GC column lifetime.

### Introduction

Flame retardants have been added to numerous products including polyurethane foams, electronics, plastics, and carpet padding to reduce the risk of burning. Technical mixtures of polybrominated diphenyl ethers (PBDEs) have been extensively used as additive flame retardants since the 1970s [1]. However, the technical mixtures containing penta and octa congeners were voluntarily withdrawn in the United States in 2005 and the last remaining PBDE mixture, decaBDE, is being phased out. Unfortunately, the concentrations of PBDEs in the environment have not been declining and, due to their persistent and bioaccumulative nature, these compounds are still widely monitored. The health concerns of PBDEs are similar to that of polychlorinated biphenyls (PCBs) and at the most recent Stockholm Convention on Persistent Organic Pollutants, two technical mixtures (pentaBDE and octaBDE) were added to the priority pollutant list [2].

Monitoring PBDEs in biota and environmental matrices can be difficult due to the complexity of the sample, structural isomers that must be separated chromatographically, and thermally-labile compounds that can break down during gas chromatography (GC). A 15 m x 0.25 mm x 0.10  $\mu\text{m}$  Rtx<sup>®</sup>-1614 GC column, a 5% diphenyl, 95% dimethyl polysiloxane type stationary phase, was specifically designed by Restek to meet EPA Method 1614 resolution requirements for critical isobaric BDE congeners 49 and 71 [3]. Using a short, thin-film column also allows the elution of decabromodiphenyl ether (BDE 209) without on-column thermal degradation that can compromise its qualitative and quantitative determination [4].

GC column and inlet maintenance is especially important for BDE analysis, because nonvolatile material persists in sample extracts and deposits onto the front of the column and liner. This can cause poor transfer of BDEs to the GC column, which compromises quantification and sensitivity. That same material at the head of the GC column also leads to poor peak shapes and reduced resolution between critical congeners, such as BDEs 49 and 71. Trimming a coil (0.5 m to 0.7 m) off the front of the column and changing

the inlet liner restores performance and significantly increases the column lifetime. While fewer column changes saves time and money, care must be taken to properly translate the method for the shorter column length to ensure that the original separation is maintained. If the GC method is not translated after the column has been trimmed, the column flow would be very fast, which would decrease resolution and detectability. The flow may also exceed the pumping capacity of many mass spectrometers. Method translation software can help users adjust the GC oven program for the shorter column length and maintain the elution profile of the original chromatogram. Using a 15 m x 0.25 mm x 0.10  $\mu\text{m}$  column, we investigated how much of the GC column could be clipped off for maintenance before the Method 1614 resolution requirement for BDE 49 and BDE 71 could no longer be met.

## Experimental

### Original Instrument Conditions

A 15 m x 0.25 mm x 0.10  $\mu\text{m}$  Rtx<sup>®</sup>-1614 column (cat.# 10296) was used for the column trimming experiments. For all analyses, a 1  $\mu\text{L}$  splitless injection (1 min purge time) of a native PBDE/BFR mix from Wellington Laboratories (Wellington cat.# BFR-PAR) was made into a split/splitless inlet set to 340 °C and fitted with a Restek Premium cyclo double taper inlet liner (cat.# 23310.5 ). The instrument was a 208 V Agilent<sup>®</sup> 7890/5975 GC-MS equipped with an enhanced turbo molecular pump. Electron ionization (-70 eV) and selected ion monitoring (SIM) mode were used for MS.

The initial GC oven and flow conditions were optimized using Pro EZGC<sup>®</sup> methods development software from Restek Corporation (cat.# 21487). This thermodynamic modeling software provided a separation which maximized resolution of target PBDEs with an analysis time of 25 min. The GC oven temperature program started at 75 °C (hold 1 min), then ramped at 18 °C/min to 210 °C, then ramped at 8 °C/min to 310 °C (hold 4 min). Helium was used as the carrier gas with a constant flow of 1.6 mL/min.

### GC Method Translation

The original column length was accurately determined by measuring the retention time of an unretained air peak to determine the holdup time. Using the Agilent<sup>®</sup> GC pressure/flow calculator, the column length parameter was increased until the calculated holdup time matched the experimental holdup time (Table I). The Rtx<sup>®</sup>-1614 GC column that was used for this analysis had an initial length that was calculated to be 16 m. The determined column length was then used for method translation and instrument software inputs.

**Table I:** Column length was increased (or decreased) in the GC pressure/flow calculator until the calculated holdup time matched the measured holdup time.

Measured Holdup Time = 0.420 min	
Column Length (m)	Calculated Holdup Time (min)
15.0	0.381
15.5	0.401
16.0	0.420

After the analysis of the BFR-PAR standard, column maintenance was simulated by immediately trimming approximately two coils (1.6 m) from the front of the column by removing the column from the inlet and pulling two full coils from the column cage and cutting the column where it lined up with the inlet piece again. The new column length was determined again by measuring the holdup time and using the GC pressure/flow calculator to find the column length. The new column length (14.4 m), along with the original GC conditions were input into the Agilent<sup>®</sup> GC method translator software, operated in translate-only mode. The method translator calculated a new oven temperature program so that the PBDEs eluted at approximately the same temperatures as the original program and maintained resolution. The column flow of 1.6 mL/min was held constant. The BFR-PAR standard was reanalyzed to monitor method performance of critical resolution and peak shape. Approximately two coils were trimmed again from the front of the column and the GC method conditions were translated for the shorter column. This process of column trimming, method translation, and analysis of the BFR-PAR standard was repeated until the resolution between BDEs 49 and 71 was compromised or the instrument could no longer maintain the necessary oven programming rate or inlet pressure.

## Results and Discussion

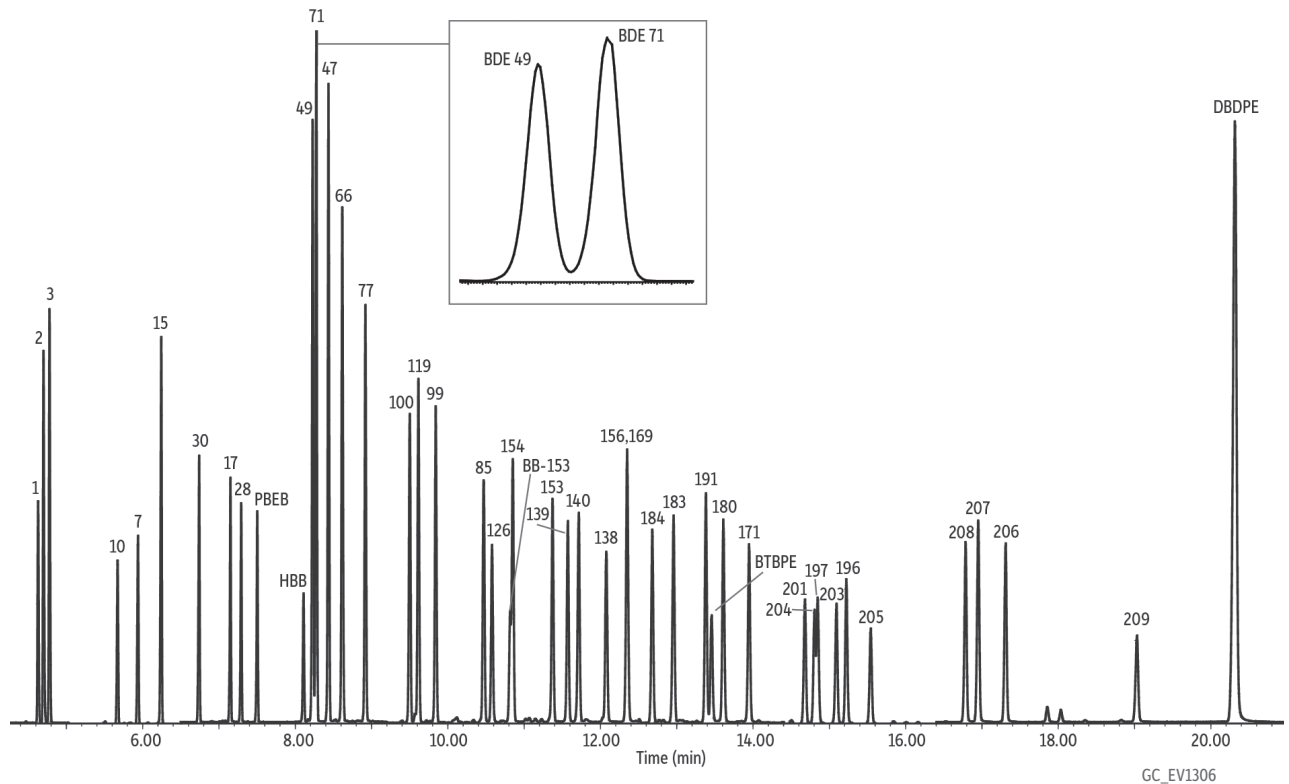
### Optimized Analysis

The Pro EZGC<sup>®</sup> modeling software provided a GC oven temperature program and column flow conditions that maximized the resolution between the tetrabromodiphenyl ethers BDE 49 and BDE 71. These two analytes are isomers that must be chromatographically resolved in order to meet EPA Method 1614 resolution requirements of less than 40% valley height between BDE 49 and BDE 71. The optimized analysis conditions provided by the software yielded baseline resolution of BDE 49 and BDE 71 and a 25 min analysis time to reduce thermal breakdown of decabromodiphenyl ether (BDE 209). Resolution between isomers needs to be optimized, but speed of analysis is also an important consideration for the analysis of PBDEs. Easily debrominating during a fire to suppress the flames is a key characteristic that makes PBDEs ideal flame retardants, but it also makes them difficult to analyze in a hot GC oven. In previous work, we found that a slower analysis or a longer column (30 m) yielded thermal breakdown of BDE 209 [4]. Using the 15 m, 0.25 mm, 0.10  $\mu\text{m}$  Rtx<sup>®</sup>-1614 column and a fast analysis reduces the thermal breakdown of the PBDEs while still providing baseline resolution of the structural isomers. Using an optimized injection port temperature of 340 °C and a cyclo double taper inlet liner was previously found to increase BDE 209 response in comparison to using an inlet temperature of 250 °C or 300 °C. The higher inlet temperature allowed better volatilization and transfer of the higher molecular weight flame retardants onto the column, without substantially increasing the degradation of BDE 209.

PBDEs are lipophilic compounds that will accumulate in the fatty tissue of animals. To determine the amount of PBDEs present in a sample, an extensive extraction and cleanup is required for soil, sediment, and tissue samples [2]. After the extract cleanup there is often still a measurable amount of nonvolatile material that remains in the final sample. The nonvolatile residue collects in the inlet and at the beginning of the GC column and can cause reduced recoveries and peak tailing. Changing the inlet liner and trimming approximately one coil (0.5 m to 0.7 m) off of the front of the column as routine maintenance is often required to restore the desired system performance and continue analyzing samples; however, when this is done the method should be adjusted to accommodate the new shorter column length. Figure 1 shows the chromatographic separation achieved after two coils were trimmed off of the column. Due to the selectivity of the Rtx®-1614 column for the target compounds and the use of properly translated method conditions, the critical separation of BDE 49 and BDE 71 was maintained on the shorter column.

**Figure 1:** Analysis conditions provided by Pro EZGC® software result in baseline resolution of BDE 49 and BDE 71. Resolution is maintained even after trimming 1.6 m from the column.

Peaks	t <sub>R</sub> (min)	Conc. (ng/mL)	Peaks	t <sub>R</sub> (min)	Conc. (ng/mL)	Peaks	t <sub>R</sub> (min)	Conc. (ng/mL)
1. BDE 1	4.63	200	16. BDE 77	8.92	400	32. BDE 191	13.38	800
2. BDE 2	4.70	200	17. BDE 100	9.50	400	33. BTBPE	13.46	400
3. BDE 3	4.78	200	18. BDE 119	9.62	400	34. BDE 180	13.61	800
4. BDE 10	5.67	200	19. BDE 99	9.84	400	35. BDE 171	13.95	800
5. BDE 7	5.94	200	20. BDE 85	10.47	400	36. BDE 201	14.68	800
6. BDE 15	6.24	200	21. BDE 126	10.58	400	37. BDE 204	14.81	800
7. BDE 30	6.74	200	22. BB-153	10.82	400	38. BDE 197	14.85	800
8. BDE 17	7.15	200	23. BDE 154	10.85	400	39. BDE 203	15.09	800
9. BDE 28	7.29	200	24. BDE 153	11.37	400	40. BDE 196	15.22	800
10. Pentabromoethylbenzene (PBEB)	7.50	200	25. BDE 139	11.57	400	41. BDE 205	15.54	800
11. Hexabromobenzene (HBB)	8.11	200	26. BDE 140	11.72	400	42. BDE 208	16.79	2,000
12. BDE 49	8.23	400	27. BDE 138	12.08	400	43. BDE 207	16.95	2,000
13. BDE 71	8.28	400	28. BDE 156	12.35	400	44. BDE 206	17.31	2,000
14. BDE 47	8.44	400	29. BDE 169	12.35	400	45. BDE 209	19.03	2,000
15. BDE 66	8.62	400	30. BDE 184	12.68	800	46. DBDPE	20.32	4,000
			31. BDE 183	12.96	800			



**Column** Rtx®-1614, 14.4 m, 0.25 mm ID, 0.10 µm (cat.# 10296)  
**Sample** Native PBDEs/BFRs (Wellington Laboratories) (cat.# BFR-PAR)  
**Diluent:** Nonane/toluene  
**Injection**  
 Inj. Vol.: 1.0 µL splitless (hold 1.0 min)  
 Liner: 4 mm Restek Premium cyclo double taper (cat.# 23310.5)  
 Inj. Temp.: 340 °C  
**Oven**  
 Oven Temp.: 75 °C (hold 0.9 min) to 210 °C at 21 °C/min to 310 °C at 9 °C/min (hold 3.6 min)  
**Carrier Gas** He, constant flow  
 Flow Rate: 1.6 mL/min

**Detector** MS  
 Mode: SIM  
 Transfer Line Temp.: 330 °C  
 Analyzer Type: Quadrupole  
 Source Temp.: 350 °C  
 Quad Temp.: 200 °C  
**Instrument Notes** Agilent 7890A GC & 5975C MSD  
 Cat.# 10296 is a 15 m column. Two loops were trimmed off the column for a final column length of 14.4 m.

### GC Method Translation

The GC method translation software allows the user to input the original GC oven program conditions and scale the new program appropriately to the new GC column length so that the elution temperatures, and thus the retention order, of peaks remain the same. There are two possible ways to translate the method after column trimming. The first maintains the original retention times and oven program by reducing the column outlet flow. The benefit to this approach is that it is easier to maintain selected ion monitoring windows because the analytes elute at approximately the same retention times as when the column was longer. However, the utility of this approach is limited by the operating limits of the system. For example, starting with a 16 m column and an outlet pressure of 0 psi, due to the mass spectrometer, the minimum column length is 12.5 m (approx. 6 coils). As the column flow is reduced, the inlet pressure decreases. If another coil (0.7 m) is trimmed off, the column length would be approximately 11.8 m and the calculated inlet pressure would be an inoperable -0.4 psi.

The second way to translate the GC method after column trimming is to maintain the original column outlet flow while increasing the oven temperature program rate (Table II). Using this approach is beneficial because the column outlet flow into the mass spectrometer is constant. Decreasing or increasing the outlet flow into the MS will affect the vacuum pressure and change analyte response. In addition, as the column gets shorter the analysis becomes faster, which increases sample throughput. Maintaining the outlet flow and reducing the inlet pressure allows for a minimum column length of 7.9 m (~ 12 coils), which allows 6 more coils to be removed for maintenance compared to the method where retention times are held constant. At 7.9 m the inlet pressure was 0.85 psi. If another coil is trimmed off of the column, the length becomes approximately 7.2 m and when a vacuum-outlet detector (e.g., MS) is used the inlet pressure would be negative. In this case then, the inlet pressure was the limiting factor in determining whether to install a new column, not the failure to meet key congener separations, which were more than adequate (Figure 2).

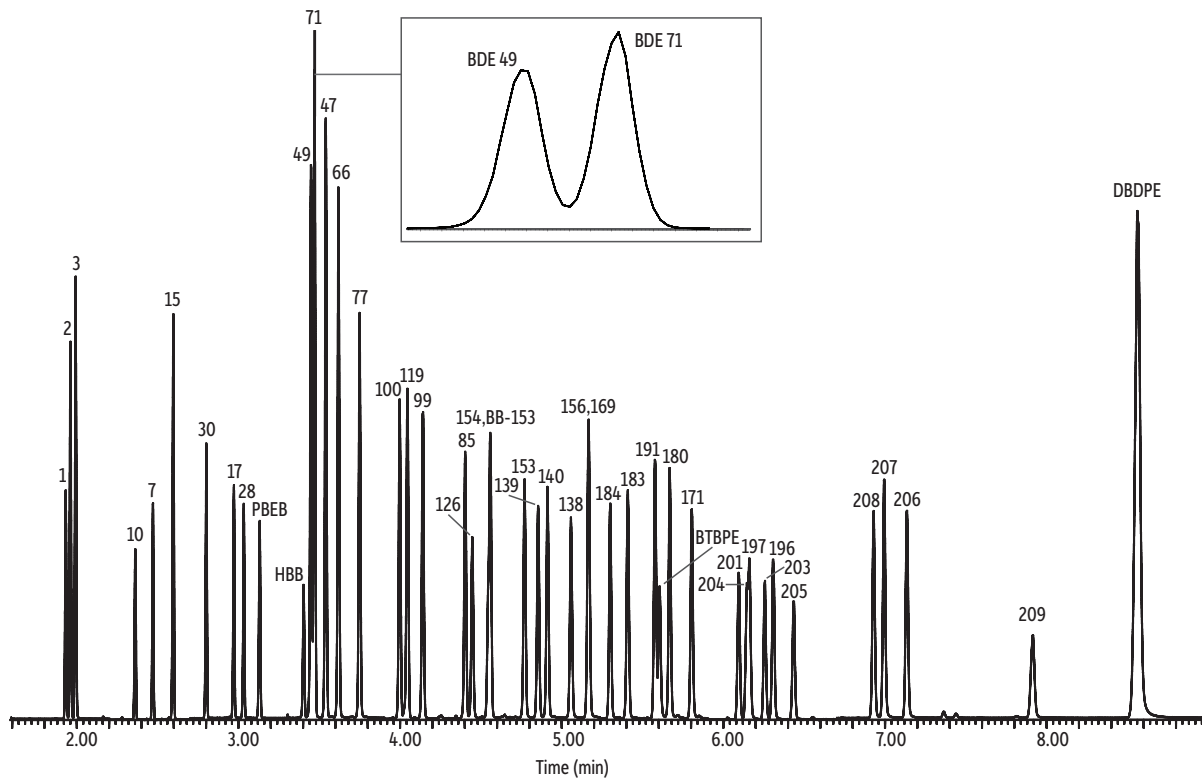
When adapting the method to the shorter column length, the ion monitoring windows must also be adjusted to accommodate the faster analysis time. The new windows can be determined using the speed gain factor calculated in the method translation software. For example, at 10.4 m the speed gain factor is 1.6 compared to the 14.4 m method. The retention times are all approximately shifted by a factor of 1.6; therefore, taking the previous method ion monitoring windows and dividing by the speed gain factor will give the expected new elution time (Table III). When column maintenance is later repeated, the new ion monitoring windows can again be accurately calculated from the actual measured retention times in the previous method (instead of from the measured retention times in the original method) using the speed gain factor that was calculated by the method translation software.

**Table II:** As column length decreases with maintenance trimming (reduced by 0.5 m to 0.7 m for each coil removed), properly translated method conditions maintain the column flow rate and increase the oven temperature ramp rate so that analytes still elute at approximately the same temperature as with the original method.

	Original Column			~2 Coils Removed			~8 Coils Removed			~12 Coils Removed		
Column Length (m)	16			14.4			10.4			7.9		
Internal Diameter (mm)	0.25			0.25			0.25			0.25		
Film Thickness (µm)	0.10			0.10			0.10			0.10		
Carrier Gas	He			He			He			He		
Inlet Pressure (psi)	7.4			6.3			3.1			0.85		
Flow Rate (mL/min)	1.6			1.6			1.6			1.6		
Average Velocity (cm/sec)	63			67			79			90		
Oven Temperature Program	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)	Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)
	—	75	1.0	—	75	0.9	—	75	0.5	—	75	0.3
	18	210	0.0	21	210	0.0	34	210	0.0	52	210	0.0
	8.0	310	3.6	9.0	310	3.6	15.0	310	1.9	23.0	310	1.4

**Figure 2:** The minimum column length of 7.9 m achieved before the inlet pressure was no longer positive maintained adequate resolution between BDE 49 and BDE 71. Approximately twelve coils were trimmed from the column, and the analysis time was reduced by 16 min from the original 25 min method.

Peaks	Conc. (ng/mL)	Peaks	Conc. (ng/mL)	Peaks	Conc. (ng/mL)	Peaks	Conc. (ng/mL)
1. BDE 1	200	12. BDE 49	400	24. BDE 153	400	36. BDE 201	800
2. BDE 2	200	13. BDE 71	400	25. BDE 139	400	37. BDE 204	800
3. BDE 3	200	14. BDE 47	400	26. BDE 140	400	38. BDE 197	800
4. BDE 10	200	15. BDE 66	400	27. BDE 138	400	39. BDE 203	800
5. BDE 7	200	16. BDE 77	400	28. BDE 156	400	40. BDE 196	800
6. BDE 15	200	17. BDE 100	400	29. BDE 169	400	41. BDE 205	800
7. BDE 30	200	18. BDE 119	400	30. BDE 184	800	42. BDE 208	2,000
8. BDE 17	200	19. BDE 99	400	31. BDE 183	800	43. BDE 207	2,000
9. BDE 28	200	20. BDE 85	400	32. BDE 191	800	44. BDE 206	2,000
10. Pentabromoethylbenzene (PBEB)	200	21. BDE 126	400	33. BTBPE	400	45. BDE 209	2,000
11. Hexabromobenzene (HBB)	200	22. BB-153	400	34. BDE 180	800	46. DBDPE	4,000
		23. BDE 154	400	35. BDE 171	800		



GC\_EV1307

**Column** Rtx®-1614, 7.9 m, 0.25 mm ID, 0.10 µm (cat.# 10296)  
**Sample** Native PBDEs/BFRs (Wellington Laboratories) (cat.# BFR-PAR)  
**Diluent:** Nonane/toluene  
**Injection**  
**Inj. Vol.:** 1.0 µL splitless (hold 1.0 min)  
**Liner:** 4 mm Restek Premium cyclo double taper (cat.# 23310.5)  
**Inj. Temp.:** 340 °C  
**Oven**  
**Oven Temp.:** 75 °C (hold 0.3 min) to 210 °C at 52 °C/min to 310 °C at 23 °C/min (hold 1.4 min)  
**Carrier Gas** He, constant flow  
**Flow Rate:** 1.6 mL/min

**Detector** MS  
**Mode:** SIM  
**Transfer Line**  
**Temp.:** 330 °C  
**Analyzer Type:** Quadrupole  
**Source Temp.:** 350 °C  
**Quad Temp.:** 200 °C  
**Instrument Notes** Agilent 7890A GC & 5975C MSD  
 Cat.# 10296 is a 15 m column. Twelve loops were trimmed off the column for a final column length of 7.9 m.

**Table III:** Retention times of new translated method can be predicted using the speed gain factor calculated by the method translation software.

	14.4 m Column		10.4 m Column		Difference (min) = Calculated t <sub>R</sub> - Actual t <sub>R</sub>
	Actual t <sub>R</sub> (min)	Calculated t <sub>R</sub> (min) = 14.4 m t <sub>R</sub> / 1.6	Actual t <sub>R</sub> (min)		
BDE 1	4.63	2.89	2.91		-0.01
BDE 2	4.70	2.94	2.95		-0.01
BDE 3	4.78	2.99	3.00		-0.01
BDE 10	5.67	3.55	3.56		-0.01
BDE 7	5.94	3.71	3.72		-0.01
BDE 15	6.24	3.90	3.92		-0.01
BDE 30	6.74	4.21	4.23		-0.01
BDE 17	7.15	4.47	4.48		-0.01
BDE 28	7.29	4.56	4.57		-0.01
PBEB	7.50	4.69	4.71		-0.02
HBBZ	8.11	5.07	5.11		-0.04
BDE 49	8.23	5.14	5.18		-0.04
BDE 71	8.28	5.17	5.21		-0.04
BDE 47	8.44	5.27	5.31		-0.04
BDE 66	8.62	5.39	5.43		-0.04
BDE 77	8.92	5.57	5.62		-0.04
BDE 100	9.50	5.94	5.98		-0.05
BDE 119	9.62	6.01	6.06		-0.05
BDE 99	9.84	6.15	6.20		-0.05
BDE 85	10.47	6.54	6.59		-0.05
BDE 126	10.58	6.61	6.66		-0.05
BB-153	10.82	6.76	6.80		-0.04
BDE 154	10.85	6.78	6.83		-0.04
BDE 153	11.37	7.11	7.15		-0.04
BDE 139	11.57	7.23	7.27		-0.04
BDE 140	11.72	7.32	7.36		-0.03
BDE 138	12.08	7.55	7.58		-0.03
BDE 156/169	12.35	7.72	7.75		-0.03
BDE 184	12.68	7.93	7.95		-0.02
BDE 183	12.96	8.10	8.12		-0.02
BDE 191	13.38	8.36	8.37		-0.01
BTBPE	13.46	8.41	8.42		0.00
BDE 180	13.61	8.51	8.51		0.00
BDE 171	13.95	8.72	8.72		0.00
BDE 201	14.68	9.18	9.16		0.01
BDE 204	14.81	9.25	9.24		0.02
BDE 197	14.85	9.28	9.27		0.01
BDE 203	15.09	9.43	9.41		0.02
BDE 196	15.22	9.51	9.49		0.03
BDE 205	15.54	9.71	9.68		0.03
BDE 208	16.79	10.49	10.44		0.05
BDE 207	16.95	10.60	10.54		0.06
BDE 206	17.31	10.82	10.75		0.07
BDE 209	19.03	11.90	11.86		0.04
DBDPE	20.32	12.70	12.76		-0.06

### Fast Analysis

When properly translating the GC method, the column length of 7.9 m did not prevent the EPA Method 1614 resolution requirement for BDE 49 and BDE 71 from being met. The new method keeps the column flow the same but the oven programming rates are faster to accommodate the higher linear velocity through the column. Analysis times are decreased by a factor of 2.8 from the 16 m method to the 7.9 m method and peak widths are reduced when trimming the column and translating the method (Table IV). Narrower peak widths can increase the signal-to-noise ratio and detectability of the analytes if the data acquisition rate is adjusted to properly define the peak for quantitation.

**Table IV:** Full width half heights (FWHH) of the BDEs are reduced when the column length is reduced.

	Column Length		
	14.4 m	10.4 m	7.9 m
	FWHH (s)	FWHH (s)	FWHH (s)
BDE 49	1.1	0.84	0.66
BDE 71	1.1	0.84	0.60
BDE 209	2.2	1.6	1.3
DBDPE	2.9	2.2	1.8

The Agilent® 7890/5975 GC-MSD that we used for this work was a 208 V instrument with a fast ramping oven that allowed for an oven ramp rate of 52 °C/min to 210 °C and 23 °C/min to 310 °C for the shortest column length (7.9 m). Having the multi-ramp program with a slower ramp at higher temperatures also allowed for the faster programming of shorter columns. A single, fast oven programming rate may require additional systems so that the instrument can accurately provide a faster ramp rate at higher temperatures.

### Conclusions

Trimming a coil (~0.5 m to 0.7 m) off of a GC column as part of routine maintenance extends column lifetime; however, in order to maintain resolution of the target analytes following maintenance, the method must be properly translated for the shorter column length. By translating the GC oven program method for the new column length, an Rtx®-1614 column (0.25 mm x 0.10 µm) can be trimmed down to 7.9 m for maintenance and still exceed the EPA Method 1614 resolution requirement for BDEs 49 and 71 and produce a chromatogram very similar to the original 16 m column. Based on a “1 coil trim”, approximately 12 maintenance trims could be performed for the analysis of BDEs before the GC oven ramping speed and inlet pressure limitations did not allow further use of the column.

### References

- [1] Polybrominated Diphenyl Ethers (PBDEs), Toxipedia., <http://toxipedia.org/pages/viewpage.action?pageId=296> (accessed May 2013).
- [2] Stockholm Convention on Persistent Organic Pollutants, C.N.524.2009.Treaties-4, May 2009.
- [3] U.S. Environmental Protection Agency, Method 1614, *Brominated Diphenyl Ethers in Water, Soil, Sediment and Tissue by HRGC/HRMS, 2007.*
- [4] J. Thomas, G. Stidsen, J. Cochran, *3-Fold Faster Polybrominated Diphenyl Ether (PBDE) Short Column Method, Restek Corporation.* [http://www.restek.com/Technical-Resources/Technical-Library/Environmental/env\\_A035](http://www.restek.com/Technical-Resources/Technical-Library/Environmental/env_A035) (accessed May 2013).