Fast and Accurate Analysis of PBDEs in a Single Run, Including BDE-209

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Polybrominated Diphenyl Ethers (PBDE’s) are aromatic and non-polar compounds that were used as flame retardants. After extensive usage, it was determined that these compounds are toxic and they have been restricted or banned in many areas, including under the Stockholm Convention. PBDE’s result in reproductive and other health effects, are toxic at low levels, and are subject to bioaccumulation. It is therefore important to measure these compounds at very low levels from environmental, food, and biological sources. PBDE’s consist of 209 individual conformations called congeners, which vary in toxicity. It is therefore important to measure and quantitate the most toxic of these individual congeners separately. To achieve the lowest levels of detection and highest degrees of confidence, high resolution gas chromatography with high resolution mass spectrometry (HRGC/HRMS) is used. Even using this advanced instrumentation, accurate separation of all congeners is difficult and requires long run times to provide enough resolution. In addition, not all congeners are stable and may degrade if activity exists in the system. One example is the most substituted congener BDE-209, which often requires a separate analysis using a shorter column to reduce activity and provide sufficient results.

This work utilizes a new method and technology that allows for fast quantitation of toxic congeners with short run times, and includes the quantitation of congener BDE-209 in the same analytical run. This eliminates the need for an extra instrument using an alternative column dimension to quantitate the necessary congeners. Comparison of existing methods and the proposed method are included, highlighting improved sensitivity and shorter run times.
The analysis of PBDE’s is historically problematic for two main reasons: the sheer number of compounds and compound stability. To obtain resolution of the 209 congeners present, labs may use long columns with extended run times. In addition, some congeners are thermally labile, sensitive to column activity, or both. The most notorious of these reactive congeners is the latest eluting congener BDE-209, decabromodiphenyl ether. Complete testing of BDE-209 is especially important because it can break down in the body or environment to even more toxic congeners.

Many labs analyzing PBDE’s in both food and environmental samples run their list of compounds by two separate tests. The first is a detailed method that resolves most congeners. A low polarity column of $60\text{m} \times 0.25\text{mm ID}$ dimensions is traditionally used to achieve resolution. However, this results in nearly hour-long run times. With this extended retention, the important congener BDE-209 has the longest exposure to thermal degradation and column activity as it elutes last. It frequently displays dramatically reduced peak response as a result.

Labs are therefore often forced to analyze this compound with a second test, using a separate instrument and a much shorter column. The column typically has a thinner phase that will result in less retention. This provides a lower elution temperature and helps address the thermal stability of congener BDE-209. However, thinner phase columns may result in greater susceptibility to activity, leading to peak tailing and more difficult quantitation.

This work addresses the contribution of thermal stability to BDE-209 breakdown and also provides a method that results in separation of important congeners as well as BDE-209 in one short run.
FIGURE 1. Demonstration of Thermal Stability of BDE-209

A. 100°C for 1 min to 300°C @ 10°C/min for 30 min

BDE-209
Area = 345
tr = 22 min

B. 100°C for 1 min to 250°C @ 10°C/min for 30 min

Conditions for both columns:

- **Dimension**: 10 meter x 0.18 mm x 0.18 µm
- **Injection**: Split 10:1 @ 250°C, 1 µL
- **Carrier Gas**: Helium @ 3.0 mL/min (constant flow)
- **Oven Program**: As noted
- **Detector**: Electron Capture (ECD) @ 350°C

BDE-209
Area = 2731
tr = 40 min
FIGURE 2.
Comparison of BDE-209 Stability on Columns from Different Manufacturers

Agilent® DB-5ms Ultra Inert
20 m x 0.18 mm ID x 0.18 µm
Mass 959.1679

BDE-209
S/N = 20

Zebron™ ZB-SemiVolatile
20 m x 0.18 mm ID x 0.18 µm
Mass 959.1679

> 40x Greater
Signal-to-Noise
FIGURE 3. PBDE Responses on DB-5ms Ultra Inert and Zebron ZB-SemiVolatiles

Agilent® DB-5ms Ultra Inert

Conditions for both columns:
- **Dimension:** 20 meter x 0.18 mm x 0.18 µm
- **Injection:** Splitless @ 85 °C, 5 µL
- **Carrier Gas:** Helium @ 0.85 mL/min (constant flow)
- **Oven Program:** 70 °C for 1.25 min to 240 °C @ 20 °C/min to 320 °C @ 50 °C/min for 18 min
- **Detector:** High Res Mass Spec (HRMS) @ 325 °C
- **Note:** Used a PTV in Solvent Vent Mode with temperature program to 320 °C in 2 min

Zebron ZB-SemiVolatiles

Note: Used a PTV in Solvent Vent Mode with temperature program to 320 °C in 2 min
FIGURE 4.
Separation of PBDE Congeners on a Zebron ZB-SemiVolatiles (20 m x 0.18 mm x 0.18 µm)

- **Tribromophenyl ethers**
  - 13C-BDE-28 + BDE-28

- **Hexabromophenyl ethers**
  - 13C-BDE-154 + BDE-154
  - 13C-BDE-153 + BDE-153
  - BDE-138

- **Tetrabromophenyl ethers**
  - 13C-BDE-47 + BDE-47
  - 13C-BDE-77 + BDE-77
  - BDE-66

- **Heptabromophenyl ethers**
  - 13C-BDE-183 + BDE-183
  - BDE-190

- **Pentabromophenyl ethers**
  - 13C-BDE-100 + BDE-100
  - 13C-BDE-99 + BDE-99
  - BDE-119
  - BDE-85

- **Decabromophenyl ether**
  - 13C-BDE-209 + BDE-209
Breakdown of BDE-209 is often attributed solely to the column, but this is not always the case. This is shown in Figure 1, where PBDEs are injected under identical conditions using a 10 m x 0.18 mm ID x 0.18 µm column, with the exception of the final temperature. The final temperature is elevated to 300 °C in run A but is limited to 250 °C in run B. Though BDE-209 elutes in under 22 minutes in run A, the peak is small (with an area of only 345) and is preceded by a hump that is the degradation product. Run B has a much longer retention time for BDE-209, allowing the analyte to react with column activity for an extended period. Degradation actually decreases, resulting in a much stronger peak (with area 2731, 8X larger), though it is more broad because of later isothermal elution. This result contradicts the theory that BDE-209 degrades due to column activity alone and demonstrates that degradation is also the result of thermal stability.

Column activity does play a significant role in PBDE stability, however. In Figure 2, BDE-209 is analyzed using the same temperature program on two different columns of the same dimensions, but from different manufacturers. The top chromatogram is an ion chromatogram for BDE-209 on a new Agilent® DB-5ms Ultra Inert. The signal-to-noise (S/N) for this peak is 20. The bottom chromatogram is the same sample on the same dimension of a Phenomenex Zebron™ ZB-SemiVolatiles column. This chromatogram results in a S/N value of 815, over 40x that of the DB-5ms Ultra Inert!

This difference in intensity does not result from injection volume or split ratio differences. A comparison of absolute intensities for other PBDEs (Figure 3) shows that the intensity of all other peaks on the DB-5ms Ultra Inert are actually slightly more intense than on the ZB-SemiVolatiles column. Therefore, the difference in peak intensities of BDE-209 is due to the inertness of the ZB-SemiVolatiles column. This inertness allows analysts to run a single test to achieve both separation of the more toxic congeners and quantitation of BDE-209 in one run. Labs can now reallocate free HRMS systems to other projects, as well as reduce the quantity of columns that need to be purchased.

Detailed separations of other key PBDE's are provided in Figure 4, including tribromo-, tetrabromo-, pentabromo-, hexabromo-, heptabromo-, and decabromo- biphenyl ether congeners.
The lack of stability of BDE-209 has forced some labs to add a second PBDE test to analyze this compound separately. This extra testing requires additional instrumentation, extra columns, and decreases the overall productivity of the laboratory.

The breakdown of BDE-209 can be attributed to a combination of both temperature stability and column activity. If a lab is able to reduce either cause, the overall response of BDE-209 can be improved.

With a narrow bore 20m x 0.18mm ID x 0.18µm film Zebron ZB-SemiVolatiles GC column, labs are now able to successfully analyze a range of PBDEs from BDE-28 to BDE-209 in a single run. Use of ZB-SemiVolatiles roughly halves the time required for analysis as there is no longer a need for a second injection with a shorter column. ZB-SemiVolatiles displays superior inertness to active compounds and provides improved response when compared to other ‘Ultra Inert’ columns. Using the same system, sample, column dimensions, and identical conditions, signal-to-noise of BDE-209 increased over 40x when using ZB-SemiVolatiles.

Zebron ZB-SemiVolatiles represents a major improvement in the GC analysis of highly brominated flame retardants that can save labs both time and money, resulting in decreased maintenance, increased revenues, and improved productivity.
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