Application Note

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# Analysis of Polybrominated Diphenyl Ethers and Novel Brominated Flame Retardants in Soil Using the Agilent 7000 Triple Quadrupole GC/MS


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## Abstract

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardant registered as United Nation's Persistent Organic Pollutants (UN POPs) due to their persistence in the environment, bio-accumulation potential, and toxicity. Replacement novel brominated flame retardants (NBFRs) have exhibited similar health hazards and environmental distribution, becoming recognized as significant contaminants. This Application Note describes the development and validation of a sensitive and reliable method for simultaneous quantitation of eight PBDEs and six NBFRs in environmental soil samples using selective pressurized liquid extraction (S-PLE) and an Agilent 7000C triple quadrupole GC/MS. The method was applied to an environmental soil sample, identifying both PBDEs and NBFRs down to low- and sub-ng/g concentrations.

## Introduction

A range of brominated flame retardants (BFRs) has been incorporated into plastics, electronic equipment, foams, and textiles. The most common of these, PBDEs, have shown a range of adverse effects in humans and animals such as endocrine disruption and developmental neurotoxicity[1,2]. In light of environmental and human health hazards, specific PBDEs have been classified as UN POPs[3], and are subject to legislated bans and voluntary withdrawal by manufacturers in North America, Europe, Australia, and elsewhere. Restriction and regulation of PBDEs, however, has driven a rise in production and use of NBFRs, a subset of which have similar chemical properties to banned PBDEs including toxicity, bioaccumulation potential, and persistence in the environment.

Gas chromatography coupled to mass spectrometry (GC/MS) has been the most commonly used instrumental technique for quantifying BFRs. While selected ion monitoring (SIM) mode using electron capture negative ionization (ECNI) has provided excellent sensitivity for BFR analysis, the complex chromatographic elution profile of combined PBDE and NBFR measurement benefits from the enhanced selectivity of triple quadrupole mass spectrometry in electron ionization mode (GC/(EI)-MS/MS).

Traditional methods of organohalogen separation from solid matrices have typically used Soxhlet extraction, solid phase extraction (SPE), and ultrasonic assisted extraction followed by chromatographic cleanup using a range of adsorbents[4]. These processes have been used successfully for the extraction of various combinations of PBDEs and NBFRs from soil. S-PLE was used in this case for the extraction of PBDEs and other established flame retardants from a variety of matrices.

This study develops a rapid, robust, repeatable, and sensitive method for the simultaneous quantification of PBDEs and NBFRs in environmental soil samples using S-PLE and GC/(EI)-MS/MS.

## Experimental

### Reagents and standards

All native compound standards were purchased from AccuStandard, Inc. (New Haven, CT, USA), and all carbon-labeled surrogate standards were from Wellington Labs (Guelph, ON, Canada). *iso*-Octane, toluene, *n*-hexane, and dichloromethane pesticide grade solvents used in extraction and standard preparation were from Honeywell Burdick and Jackson (Muskegon, MI, USA).

### Sample preparation

Three grams of spiked soil samples and a real environmental soil sample were extracted using pressurized liquid extraction with 50:50 v:v *n*-hexane and dichloromethane at 100 °C and 1,500 psi. Sample cleanup was performed with Florisil, acidified silica, $Na_2SO_4$, and activated copper powder with soil samples dispersed in 2 g of $Na_2SO_4$ and 1 g of Hydromatrix. Extracts were evaporated under a gentle nitrogen stream at room temperature and reconstituted in 100 µL of *iso*-octane:toluene (80:20 v:v) in 250-µL glass inserts. Details of the extraction process are published elsewhere[5].

### GC/MS analysis

A 7000C series gas chromatograph triple quadrupole mass spectrometer was used for GC/MS analysis*. Table 1 provides GC conditions, and Table 2 details ion source and MS conditions.

Target analytes were determined by retention time and two ion transitions using Agilent MassHunter quantitative analysis software. For each compound, one transition was used for quantitation and a second transition was used for qualitative confirmation. Positive identification of analytes in samples was dependent on three criteria:

- The signal-to-noise ratio (S/N) must exceed 3:1.
- The retention time must be within ±5 % of those determined from analytical standards.
- The abundance ratio between quantitative and qualitative ion transitions must be within ±20 % of the ratios measured in standards.

* As of publication, the 7000D MS/MS is the equivalent of the 7000C MS/MS, and gives equivalent or better performance for this analysis.

Table 3 provides optimized acquisition parameters.

Mass-labeled PBDE standards were used as surrogates for internal standard quantitation. Native PBDE congeners were quantified using their corresponding isotopes while NBFRs were assigned the labeled PBDE congener with the closest GC retention time. A 5 ng amount of each surrogate internal standard (100 ng of $^{13}$C-BDE-209) was spiked into each soil sample prior to extraction. Final extracts were spiked with 5 ng of BDE-37 and BDE-77 immediately prior to GC/MS/MS analysis as recovery internal standards to assess surrogate standard recoveries according to procedures described in US EPA method 1614[6]. Five-point calibration curves containing all of the target analytes and each internal standard at its corresponding sample spike concentration were prepared in *iso*-octane/toluene (80:20 v:v) and used for quantitation.

**Table 1.** Agilent 7890 GC conditions.

| Parameter | Value |
|---|---|
| Analytical column | Agilent DB5-ms, 180 µm × 15 m, 0.18 µm (p/n 121-5522UI) |
| Sample injection mode | 2 µL Pulsed splitless using Multimode Inlet (MMI) |
| Injection port liner | Agilent 200 µL dimpled, single-taper liner (p/n 5190-2296) |
| Injection temperature program | 100 °C (0.2 minutes)<br>900 °C/min to 330 ˚C |
| Injection pulsed pressure | 50 psi (1.5 minutes) |
| Purge flow to spit vent | 50 mL/min (2.5 minutes) |
| Carrier gas | Helium |
| Carrier gas flow | 1.8 mL/min (8.25 minutes), 100 mL/min to 4 mL/min |
| Oven temperature program | 80 °C (1 minute), 37 °C/min to 230 °C,<br>30 °C/min to 325 °C (5.3 minutes) |
| Run time | 13.5 minutes |

**Table 2.** EI source parameters and MS conditions.

| Parameter | Value |
|---|---|
| Mass spectrometer | Agilent 7000C with electron impact ionization source operated in multiple reaction monitoring mode (MRM) |
| Ionization mode | Positive |
| Source temperature | 280 °C |
| Quadrupole 1 and 2 temperature | 150 °C |
| Transfer line temperature | 325 °C |

**Table 3.** GC/(EI)-MS/MS acquisition parameters.

| Compound | Acronym | Retention time (min) | Precursor ion (*m/z*) | Product ion (*m/z*) | Dwell (ms) | CE (eV) | EM Gain (V) |
|---|---|---|---|---|---|---|---|
| 2,4,4'-Tribromodiphenyl ether | BDE-28 | 5.79 | 405.8 | 246.0 | 10 | 20 | 10 |
| 2,4,4'-Tribromodiphenyl ether | BDE-28 | 5.79 | 408.0 | 248.1 | 10 | 5 | 10 |
| 2,4,4'-Tribromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-28 (SS) | 5.79 | 417.8 | 258.0 | 10 | 40 | 10 |
| 2,4,4'-Tribromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-28 (SS) | 5.79 | 419.8 | 260.1 | 10 | 60 | 10 |
| Pentabromotoluene | PBT | 5.82 | 485.5 | 247.0 | 10 | 20 | 10 |
| Pentabromotoluene | PBT | 5.82 | 485.5 | 326.0 | 10 | 20 | 10 |
| 3,4,4'-Tribromodiphenyl ether | BDE-37 (RS) | 5.93 | 405.8 | 246.0 | 10 | 20 | 10 |
| 3,4,4'-Tribromodiphenyl ether | BDE-37 (RS) | 5.93 | 408.0 | 248.1 | 10 | 5 | 10 |
| Pentabromoethylbenzene | PBEB | 5.94 | 499.7 | 484.6 | 10 | 20 | 10 |
| Pentabromoethylbenzene | PBEB | 5.94 | 499.7 | 420.5 | 10 | 20 | 10 |
| Hexabromobenzene | HBB | 6.32 | 552.0 | 391.8 | 15 | 25 | 10 |
| Hexabromobenzene | HBB | 6.32 | 549.5 | 389.7 | 10 | 25 | 10 |
| 2,2',4,4'-Tetrabromodiphenyl ether | BDE-47 | 6.43 | 486.0 | 326.0 | 10 | 45 | 10 |
| 2,2',4,4'-Tetrabromodiphenyl ether | BDE-47 | 6.43 | 326.0 | 138.0 | 10 | 20 | 10 |
| 2,2',4,4'-Tetrabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-47 (SS) | 6.43 | 497.7 | 338.0 | 12 | 25 | 10 |
| 2,2',4,4'-Tetrabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-47 (SS) | 6.43 | 495.7 | 336.1 | 12 | 45 | 10 |
| 3,3',4,4'-Tetrabromodiphenyl ether | BDE-77 (RS) | 6.73 | 486.0 | 326.0 | 10 | 45 | 10 |
| 3,3',4,4'-Tetrabromodiphenyl ether | BDE-77 (RS) | 6.73 | 326.0 | 138.0 | 10 | 20 | 10 |
| 2,2',4,4',6-Pentabromodiphenyl ether | BDE-100 | 6.95 | 563.6 | 403.7 | 10 | 35 | 10 |

| Compound | Acronym | Retention time (min) | Precursor ion (*m/z*) | Product ion (*m/z*) | Dwell (ms) | CE (eV) | EM Gain (V) |
|---|---|---|---|---|---|---|---|
| 2,2',4,4',6-Pentabromodiphenyl ether | BDE-100 | 6.95 | 565.6 | 405.6 | 16 | 35 | 10 |
| 2,2',4,4',6-Pentabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-100 (SS) | 6.95 | 577.7 | 417.8 | 10 | 35 | 10 |
| 2,2',4,4',6-Pentabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-100 (SS) | 6.95 | 417.3 | 309.0 | 10 | 55 | 10 |
| 2-Ethylhexyl-2,3,4,5-tetrabromobenzoate | EH-TBB | 7.13 | 420.5 | 233.0 | 10 | 30 | 10 |
| 2-Ethylhexyl-2,3,4,5-tetrabromobenzoate | EH-TBB | 7.13 | 420.5 | 311.5 | 10 | 30 | 10 |
| 2,2',4,4',5-Pentabromodiphenyl ether | BDE-99 | 7.03 | 563.6 | 403.7 | 10 | 20 | 10 |
| 2,2',4,4',5-Pentabromodiphenyl ether | BDE-99 | 7.13 | 565.6 | 405.6 | 10 | 20 | 10 |
| 2,2',4,4',5-Pentabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-99 (SS) | 7.13 | 577.7 | 417.8 | 10 | 40 | 10 |
| 2,2',4,4',5-Pentabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-99 (SS) | 7.13 | 417.3 | 309.0 | 13 | 55 | 10 |
| 2,2',4,4',5,6'-Hexabromodiphenyl ether | BDE-154 | 7.55 | 643.6 | 483.8 | 10 | 25 | 10 |
| 2,2',4,4',5,6'-Hexabromodiphenyl ether | BDE-154 | 7.55 | 483.7 | 374.9 | 14 | 25 | 10 |
| 2,2',4,4',5,6'-Hexabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-154 (SS) | 7.55 | 655.6 | 495.8 | 10 | 40 | 10 |
| 2,2',4,4',5,6'-Hexabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-154 (SS) | 7.55 | 495.7 | 386.9 | 10 | 45 | 10 |
| 2,2',4,4',5,5'-Hexabromodiphenyl ether | BDE-153 | 7.76 | 643.6 | 483.8 | 14 | 40 | 10 |
| 2,2',4,4',5,5'-Hexabromodiphenyl ether | BDE-153 | 7.76 | 483.7 | 374.9 | 10 | 40 | 10 |
| 2,2',4,4',5,5'-Hexabromodiphenyl[$^{13}C_{12}$] ether | $^{13}$C-BDE-153 (SS) | 7.76 | 655.6 | 495.8 | 10 | 25 | 10 |
| 2,2',4,4',5,5'-Hexabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-153 (SS) | 7.76 | 495.7 | 386.9 | 10 | 25 | 10 |
| 2,2',3,4,4',5',6-Heptabromodiphenyl ether | BDE-183 | 8.28 | 561.7 | 454.9 | 21 | 20 | 10 |
| 2,2',3,4,4',5',6-Heptabromodiphenyl ether | BDE-183 | 8.28 | 721.6 | 561.8 | 10 | 25 | 10 |
| 2,2',3,4,4',5',6-Heptabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-183 (SS) | 8.28 | 733.4 | 573.8 | 10 | 25 | 10 |
| 2,2',3,4,4',5',6-Heptabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-183 (SS) | 8.28 | 733.4 | 575.6 | 10 | 40 | 10 |
| *bis*(2,4,6-Tribromophenoxy)ethane | BTBPE | 8.43 | 356.5 | 118.0 | 16 | 40 | 10 |
| *bis*(2,4,6-Tribromophenoxy)ethane | BTBPE | 8.43 | 356.5 | 90.0 | 16 | 60 | 10 |
| *bis*(2-Ethylhexyl) tetrabromophhtalate | BEH-TEBP | 8.52 | 464.5 | 220.7 | 10 | 30 | 10 |
| *bis*(2-Ethylhexyl) tetrabromophthalate | BEH-TEBP | 8.52 | 464.5 | 380.9 | 10 | 55 | 10 |
| Decabromodiphenyl ether | BDE-209 | 11.62 | 799.4 | 639.5 | 67 | 55 | 100 |
| Decabromodiphenyl ether | BDE-209 | 11.62 | 639.5 | 530.7 | 52 | 55 | 100 |
| Decabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-209 (SS) | 11.62 | 811.8 | 651.4 | 61 | 55 | 100 |
| Decabromodiphenyl [$^{13}C_{12}$] ether | $^{13}$C-BDE-209 (SS) | 11.62 | 651.5 | 543.6 | 57 | 55 | 100 |
| Decabromodiphenylethane | DBDPE | 13.04 | 484.5 | 324.5 | 113 | 25 | 100 |
| Decabromodiphenylethane | DBDPE | 13.04 | 484.5 | 403.5 | 136 | 55 | 100 |

SS = Internal surrogate standards, RS = internal recovery standard, CE = collision energy, EM = electron multiplier. The first ion transition was used for quantitation, and the second for confirmation.

## Results and discussion

### Chromatographic performance

Separation of analytes was achieved on the DB5-ms column with a run time of 13.5 minutes. Excellent peak shape was observed at low concentrations in spiked soil extracts to indicate efficient removal of interfering matrices and comprehensive extraction of target compounds. Figure 1 displays the MRM chromatogram from a soil spiked at concentrations approaching respective limits of quantitation (LOQs) for each compound. BEH-TEBP demonstrated instability in acidified sample cleanup steps, and consequently was poorly recovered from spiking tests.

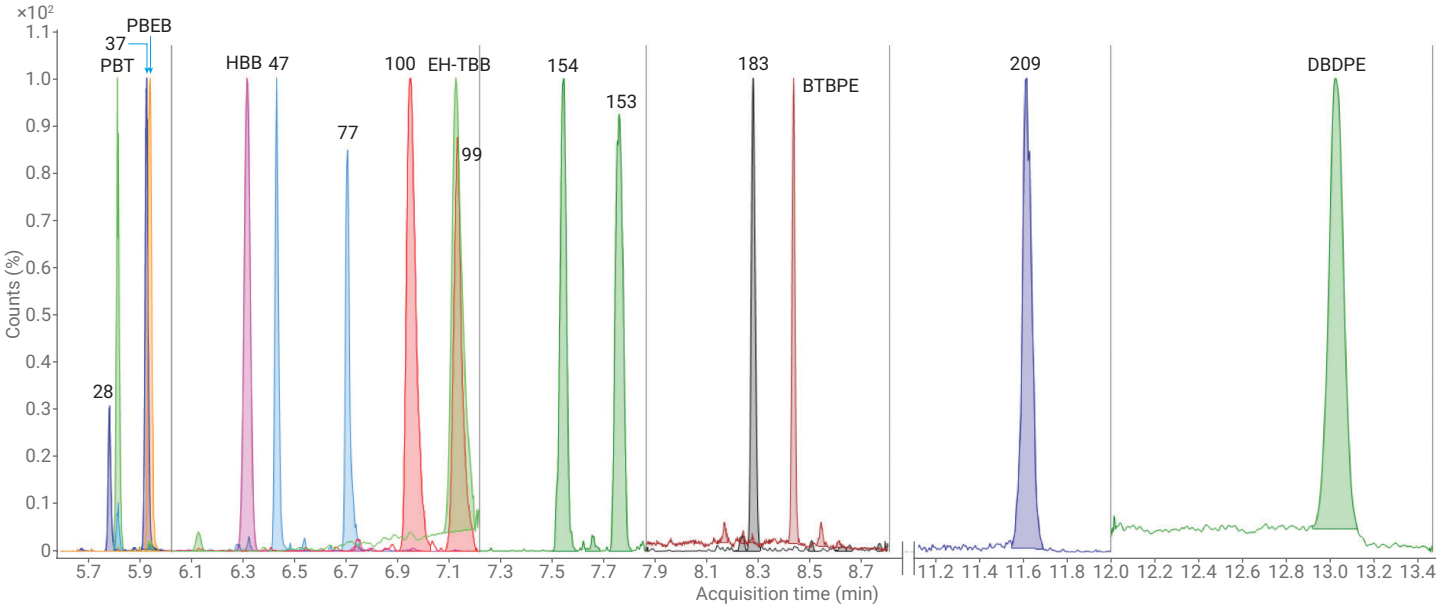


**Figure 1.** Chromatogram of extract from soil spiked at concentrations approaching the LOQ of respective compounds: 0.17 ng/g of BDE-28, -47, -99, -100, -153, -154, -183, PBT, PBEB, and HBB; 13 ng/g EH-TBB; 3.3 ng/g BTBPE; 25 ng/g BDE-209 and 66 ng/g DBDPE. MRM chromatograms have been normalized for each retention window. Numbers above peaks refer to PBDE congeners listed in Table 1.

## Analytical performance

Linear regression was fit to the five-point calibrations with $R^2$ >0.99 for all analytes, while LOQs were below 0.1 ng/g dry weight for most compounds. LOQs were higher for BDE-209 and DBDPE as these compounds have high boiling points and are typically subject to thermal instability during injection and elution from the GC column. Repeated spike and recovery tests using 3 g of clean soil revealed excellent accuracy and precision at two spiking concentrations.

## Analysis of real soil samples

A soil sample collected from an electronic waste recycling facility was analyzed using the optimized S-PLE and GC/MS methods, and was found to contain both PBDEs and NBFRs (Figure 2). Individual PBDEs were measured at concentrations ranging 0.11 ng/g dry weight (dw) (BDE-28) to 1,900 ng/g dw (BDE-209), while HBB, BTBPE, and DBDPE were present at 0.56, 20, and 380 ng/g dw, respectively. BEH-TEBP was also identified in the analysis, but was not quantified due to low extraction efficiency. The MRM chromatogram shown in Figure 2 depicts good peak shapes with high S/N. These results demonstrate the extraction efficiency of the S-PLE method and excellent sensitivity of the 7000C GC/MS.

**Table 4.** Analytical performance and recovery from repeated soil spikes.

| | Low spike (n = 5) | | High spike (n = 3) | | | |
|---|---|---|---|---|---|---|
| Compound | Recovery (%) | RSD | Recovery (%) | RSD | Linearity ($R^2$) | LOQ (ng/g soil) |
| BDE-28 | 98 | 2 | 98 | 2 | 0.9999 | 0.03 |
| BDE-47 | 98 | 2 | 99 | 3 | 0.9999 | 0.04 |
| BDE-99 | 100 | 5 | 101 | <1 | 0.9999 | 0.04 |
| BDE-100 | 97 | 1 | 100 | 1 | 0.9999 | 0.06 |
| BDE-153 | 98 | 1 | 96 | 7 | 0.9998 | 0.03 |
| BDE-154 | 93 | 2 | 92 | 2 | 0.9999 | 0.03 |
| BDE-183 | 68 | 3 | 104 | 47 | 0.9999 | 0.05 |
| BDE-209 | 101 | 19 | 91 | 1 | 0.9980 | 16 |
| PBT | 93 | 2 | 86 | 2 | 0.9998 | 0.03 |
| PBEB | 92 | 2 | 88 | 2 | 0.9999 | 0.03 |
| HBB | 95 | 5 | 106 | 3 | 0.9976 | 0.03 |
| EH-TBB | 88 | 7 | 91 | 16 | 0.9934 | 1.8 |
| BTBPE | 110 | 9 | 108 | 2 | 0.9959 | 0.49 |
| BEH-TEBP | N/A | N/A | N/A | N/A | 0.9908 | N/A |
| DBDPE | N/A | N/A | 103 | 2 | 0.9915 | 45 |

Low- and high-spiked soils (3 g) received: 5 and 20 ng of BDE-28, -47, -99, -100, -153, -154, -183, PBT, PBEB, and HBB; 10 and 40 ng of EH-TBB and BTBPE; 25 and 100 ng of BEH-TEBP; 50 and 200 ng of BDE-209 and DBDPE, respectively.

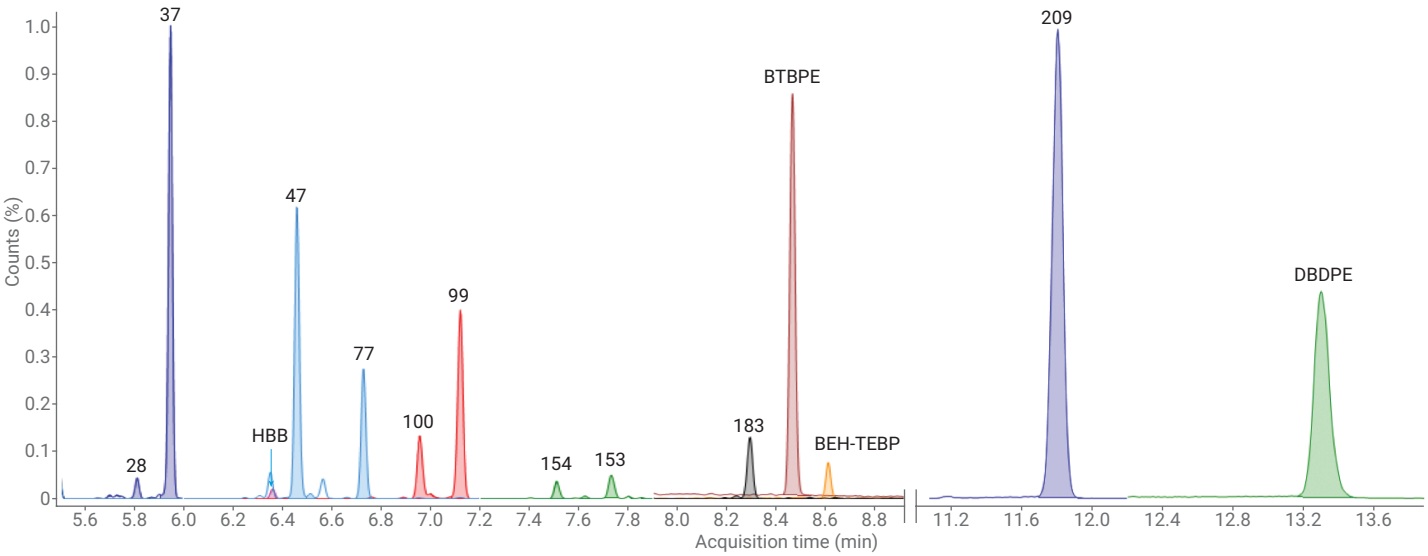


**Figure 2.** MRM chromatogram of PBDEs and NBFRs detected in a real environmental soil sample.

## Conclusion

This Application Note presents the simultaneous analysis of eight PBDEs and six NBFRs using the 7000C Series triple quadrupole GC/MS.

The 7000C triple quadrupole GC/MS was demonstrated to provide reliable and robust quantification of PBDEs and NBFRs in soil.

Good peak shapes were achieved for all analytes at low and sub-ng/g concentrations to provide excellent sensitivity due to high S/N ratios. The S-PLE protocol delivered good recoveries for all analytes with typically low RSDs across repeated analyses.

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Printed in the USA, August 7, 2018
5994-0195EN

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