SPE Method Development Recommendations for Extraction of Brominated Flame Retardants from Plasma

This represents recommendations for SPE method development. The proposed steps are based on experience with similar analytes and matrices, but have not been verified in Biotage laboratories. Please refer to section below for the analyte and matrix considerations that were made in developing this method.
As for all method development, this procedure should first be developed using pure solvent spiked with analyte. Only after the chemistry is established should spiked matrix samples be tested.
Non-aqueous samples: Spike a solvent similar to sample matrix.
Aqueous samples: Spike reagent water or 10 to 20 mM buffer. An appropriate buffer is usually the same as that used in the equilibration step.

This suggested method describes the extraction of BFRs from plasma, using a non-polar retention mechanism.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE ENV+ 200 mg/6 mL (part number 915-0020-C)

There may be more than one phase that could be effective in the extraction of this compound. The method development should include testing phases in parallel in order to optimize the procedure.

Pre-treatment:	1. Add formic acid/2-propanol (4:1, v/v, 5 Ml) to plasma sample. 2. Sonicate for 5 min. Allow to stand for 1 hour. 3. Dilute with water/2-propanol (19:1, v/v, 5 Ml). 4. Sonicate for 5 min.
Solvation:	Condition the column with methanol (5 mL).
Equilibration:	Equilibrate with acidified water (5 mL).
Sample application:	Apply sample under gravity. Check for analyte breakthrough.
Interference elution:	1. Decompose lipids on the column by applying conc. sulfuric acid (6 mL) under gravity. 2. Rinse with water (12 mL). 3. Rinse with acetate buffer (0.1M, 6 mL). 4. Rinse with water/methanol (9:1, v/v, 6 mL). 5. Rinse with water/methanol (3:2, v/v, 6 mL). 6. Thoroughly dry the column under nitrogen (0.5 bar, ~20 min.).
Analyte elution:	To elute analytes, apply first volume of elution solvent to extraction cartridge. Soak for two minutes. Add second volume of elution solvent to extraction cartridge and collect. Elute analytes with DCM/methanol (7:3, v/v). Concentrate under a stream of nitrogen to approx. 50 uL Derivatise (if required) with diazomethane and store in amber glass vials at -18 Celsius until required.

Structure	Various, not shown.

Structural considerations	The analytes are relatively non-polar.
Matrix considerations	The matrix is aqueous with various potential interference compounds.
Analytical method	N/A.
Reagents	1. Methanol, Analytical grade 2. Water, PR grade 3. Formic acid, Analytical grade 4. Sulfuric acid, Analytical grade 5. DCM, Analytical grade
General comments	Reference: Thomsen, -C et al J. Environmental Monitoring, 2001, 3; 366-370

ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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