Development of a magnetic particle immunoassay for polybrominated diphenyl ethers and application to environmental and food matrices

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Abstract

A sensitive magnetic particle enzyme-linked immunoassay (ELISA) was developed to analyze polybrominated diphenyl ether (PBDE) congeners in water, milk, fish, and soil samples. The assay was rapid and can be used to analyze fifty samples in about 1 h after sample cleanup. The assay has a limit of detection (LOD) below 0.1 ppb towards the following brominated diphenyl ether (BDE) congeners: BDE-47, BDE-99, BDE-28, BDE-100, and BDE-153, with the LOD approximately the same as GC–NCI–MS. The congeners most readily recognized in the ELISA were BDE-47 and BDE-99 with the cross-reactivities of BDE-28, BDE-100, and BDE-153 being less than 15% relative to BDE-47. As anticipated, the sensitivities are proportional to the similarities between the hapten structure and the BDE congener structure. Some oxygenated congeners with structural similarity to the hapten showed high to moderate cross-reactivities. Very low cross-reactivity was observed for other PBDEs or chlorinated environmental contaminants. The assay gave good recoveries of PBDEs from spiked water samples and a very small within and between day variance. Comparison with GC–MS demonstrated the ELISA method showed equivalent precision and sensitivity, with better recovery. The lower recovery of the GC–MS method could be caused by the use of an internal standard other than an isotopically substituted material that could not be used because of the fragmentation pattern observed by this method. The cleanup methods prior to ELISA were matrix dependent, no pretreatment was needed for environmental water samples, while fish, milk, and soil samples required various degrees of cleanup. Analysis of this wide variety of environmental samples by both ELISA and GC–MS demonstrated ELISA provides a timely and cost-effective method to screen for PBDEs in a variety of samples.

Keywords:
Analysis
ELISA
Magnetic particle
PBDEs

1. Introduction

Polybrominated diphenyl ether (PBDE) mixtures are manufactured as flame retardant additives for electronic equipment, plastics, and textiles (IPCS, 1994). Three types of commercial PBDE mixtures find wide application (Hardy, 2002), namely “pentaBDEs”, “octaBDEs”, and “decaBDEs”. The penta formulation consists of a mixture of PBDE congeners that includes predominately BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 (deWitt, 2002). The octa formulation consists primarily of BDE-183, while the deca formulation consists primarily of BDE-209. North America accounts for approximately 98% of the global demand for the penta formulation (Boon et al., 2002).

PBDEs are ubiquitous environmental contaminants. Their bioaccumulation has led to the detection of PBDEs in many species of wildlife (Anderson and Blomkvist, 1981), human plasma (Klassen-Wehler et al., 1997) and in human mother’s milk (Meironyte et al., 1999). PBDEs are endocrine disruptors as their structure is similar to thyroid hormones, possibly interfering with thyroid hormone homeostasis (Brouwer et al., 1998). Furthermore, the structural similarity between PBDEs and the polychlorinated biphenyls could result in similar toxicological properties. Some potential problems include the induction of hepatic enzyme activities...
(Chen et al., 2001), increased protein kinase C translocation (Kodavanti and Ward, 2005), and binding to androgen and estrogen receptors (Stoker et al., 2004, 2005). Exposures to PBDEs have been shown to cause developmental and neurological effects (Gill et al., 2004) but many aspects of their toxicity are uncertain at this time. Because of their potential health consequences and their rapidly increasing presence in the environment, usage of PBDEs has been banned by several countries. In 2004, the European Union banned the usage of pentaBDEs and octaBDEs (Cox and Efthymiou, 2003). In 2008, California will ban the usage of pentaBDEs and octaBDEs. Maine was the first state to ban usage of decalBDEs (in 2008) because recent studies indicated decalBDEs can degrade into lower brominated congeners that could potentially have higher toxicities.

The quantification of PBDE samples is usually done by gas chromatography negative chemical ionization–mass spectrometry (GC–NICI–MS) or better by GC/high resolution MS (GC/HRMS). These methods and particularly GC/HRMS are somewhat expensive to operate and limited to a laboratory environment. The analysis of large numbers of samples using these techniques is generally more costly, particularly when the number of assays exceeds 100 samples per week per instrument that can be dedicated to the task. Therefore, a rapid, simple, and cost-effective method for the analysis of PBDEs would be extremely useful for monitoring a large number of environmental and food samples.

Rapid, sensitive, accurate, and cost-effective enzyme immunoassays (EIAs) have provided the analytical chemists an attractive alternate to traditional instrumentation methods. Magnetic particle-based EIAs have previously been described and widely applied to the detection of pesticides and other environmental contaminants (Lawruk et al., 1994; Jourdan et al., 1995; Hottenstein et al., 1996) in various sample matrices, including water, soil, produce, and fish tissue. The uniform dispersion of the particles throughout the reaction mixture allows for rapid reaction kinetics, precise addition of antibody, and excellent analytical sensitivity. In comparison, ELISA in general requires less stringent sample cleanup than instrumental methods. This paper describes the development and assay performance of a magnetic particle-based ELISA directed at PBDEs in water, fish, milk, and soil samples.

2. Materials and methods

2.1. Materials and supplies

Superparamagnetic particles of approximately 1 μm diameter were obtained from Seradyn (Indianapolis, IN). N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma-Aldrich (St. Louis, MO). Rabbit anti-PBDEs serum #122 was produced by immunizing a rabbit with 4-(2,4-dibromo-5-(2,4-dibromophenoxy)phenyl)butyrate-BSA according to Shewater et al. (2005). The PBDE ligand and horse radish peroxidase (HRP) were conjugated via a NHS and EDC reaction to yield PBDE–HRP conjugates (Abraxis, Warminster, PA). TMB peroxidase substrate was purchased from BioFx (Randallstown, MD). Analytical standards (PBDEs, PCBs, PCD and 2,4-D) were obtained either from Chem Service (West Chester, PA) or AccuStandard (New Haven, CT). The purity of these standards were >98%. For the instrumental analysis, the Polybrominated Diphenyl Ether Analytical Standard Solution EO-5278 from Cambridge Isotope Laboratories, Inc. (MA, USA) was used. The components of this solution were BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and decaBDE. The concentration of each compound was of 1 μg/ml except decaBDE which was present at 10 μg/ml. PCB-209 (Cromlab. S.L.) was used as internal standard. The 13C-labeled BDEs-77 and -139 were obtained from Wellington Laboratories (Guelph, ON, Canada). For GC–MS internal standards, the chemical purity was ≥ 98% and the isotopic purity was 99%.

2.2. Activation of magnetic particles with PBDE antibodies

The anti-PBDE antibody coupled magnetic particles were prepared by NHS/EDAC activation, according to the procedure supplied by Seradyn (Indianapolis, IN). The unbound NHS/EDC was removed from the particles by magnetic separation and washing two times with 50 mM of 2-(N-morpholino) ethane sulfonic acid (MES) buffer (pH 6.0). The PBDE antiserum and the activated particles were incubated with agitation overnight at room temperature. The reaction was then quenched with glycine buffer and the covalently coupled anti-PBDEs particles were washed and diluted 1:1000 with a Tris–saline/BSA buffer.

2.3. Sample treatment/cleanup for ELISA

2.3.1. Water

Samples were collected in glass bottles with teflon lined caps and stored refrigerated until used. The samples were analyzed directly without any sample extraction or pre-concentration. Water samples, including a municipal water source, a reservoir, a lake, and a pond were obtained from Warminster, PA, USA. In addition, influents and effluents from waste water treatment plants in the Ebro river basin (Spain) were collected for PBDE analysis. Before analysis, samples were mixed 1:1 with methanol, and the bottle was rinsed with a small amount of methanol to remove any adsorbed PBDEs.

2.3.2. Milk

Mother’s milk samples consisted of either 0.5 g of freeze dried milk or 3 ml of liquid milk. De-ionized water was added to each sample (10 ml to dried milk or 5 ml to liquid milk) along with 1 ml of saturated potassium oxalate, and the mixture was vortexed. Ten milliliters of ethanol were added and the mixture vortexed, followed by the addition of 5 ml of ethyl ether and vortexed. After adding 5 ml of n-hexane and vortexing, the vials were inverted 60 times. The organic layer was either allowed to separate or the sample was centrifuged and the organic layer was removed. The hexane extraction was repeated two more times and the organic layers combined. Eight milliliters of DI water were added to the organic extracts and mixed. The vials were inverted 30 times and the organic layer was separated and removed. The organic layer was shaken with 4 g of anhydrous sodium sulfate. After centrifuging the vials at 2000 g, the supernatant was poured into a pre-weighed vial. The organic solvent was removed under a stream of nitrogen and the extracted lipids were weighed. The lipid extract was re-dissolved in 4 ml of hexane along with 1 ml of concentrated sulfuric acid, and the vials were shaken and inverted 30 times. After phase separation, the organic layer was removed. After evaporation of the solvent, the sample was re-dissolved in 2 ml of 50% methanol and analyzed by ELISA.

2.3.3. Fish

Fish samples were obtained from various rivers in Spain. After removing scales, the fish filets were cut into 4–5 cm cubes and homogenized in a blender. After lyophilizing, the fish filets were ground to a fine powder in a mortar and extracted with a Dionex Accelerated Solvent Extractor (ASE) (Dionex, Sunnyvale, CA, USA) using a 50% hexane/dichloromethane solvent with the system pressure at 1500 psi, the temperature at 150 ℃, and a heating time of 5 min. The extraction cycles were performed two additional times and the extract evaporated to 1 ml followed by addition of sulfuric acid (2 ml) and hexane (2 ml). The mixture was vortexed (2 min), centrifuged, and the sulfuric acid layer discarded. The
sulfuric acid wash was repeated five times. The organic layer was evaporated and reconstituted with 50% MeOH.

2.4. Magnetic particle immunoassay for PBDEs

2.4.1. For ELISA analysis, 250 μl of the sample and 500 μl of the anti-PBDE coupled magnetic particles were added to a disposable glass test tube and incubated for 20 min at room temperature. An aliquot of PBDE–HRP solution (250 μl) was added to the tube and the reaction was incubated for an additional 20 min at room temperature. Using a magnetic separation rack and test tube holder (Abraxis, Warminster, PA), a magnetic field was applied to the magnetic solid-phase to facilitate washing and removal of unbound PBDE–HRP as well as to eliminate potential interfering substances. The two-piece design allows sixty tubes to be set up, incubated and magnetically separated without removing the tubes from the tube holder. The enzyme substrate solution (peroxide/TMB) was added (500 μl) and incubated for 20 min. The reaction was stopped with the addition of 2 N H2SO4 (500 μl) and the absorbance at 450 nm recorded (Photometric Analyzer, Abraxis, Warminster, PA, USA). Absorbances were converted to concentration levels using a linear regression line of the log–log standard curve prepared from solutions containing 0, 25, 50, 100, 500 and 1000 parts per trillion (ppt) of BDE-47.

2.4.2. Soil

Soil samples were obtained near a concrete slab at a fire department training station in Fargo, ND where various combustible materials, including some with high levels of PBDEs, were used for burning and extinguishing practice. Top soils were removed adjacent to the slab or 3, 6, 9, or 12 feet away (to produce samples with varying levels of PBDEs) from the slab where burning took place. The soil samples were thoroughly dried, ground, and sieved through a 2 mm screen. One gram of soil and 1.2 g of anhydrous sodium sulfate were thoroughly mixed. The soil sample had 1 steel mixing ball and 2 ml of 20% acetone in hexane added, prior to vortexing and shaking for 10 min. The organic extract was removed and 0.8 ml of sulfuric acid was added to the mixture and vortexed. The organic phase was separated and re-extracted with sulfuric acid until the acid phase was colorless. The organic layer was evaporated and the residue dissolved in 1 ml of DMSO. The sample was filtered through a 2 mm screen. One gram of soil and 1.2 g of anhydrous sodium sulfate were thoroughly mixed. The soil sample had 1 steel mixing ball and 2 ml of 20% acetone in hexane added, prior to vortexing and shaking for 10 min. The organic extract was removed and 0.8 ml of sulfuric acid was added to the mixture and vortexed. The organic phase was separated and re-extracted with sulfuric acid until the acid phase was colorless. The organic phase was evaporated and the residue dissolved in 1 ml of DMSO. The sample was diluted at least 1:50 in 50% MeOH (greater dilution was required for some samples) for ELISA analysis.

2.5. Sample extraction for the GC–MS analyses

2.5.1. Water Samples

Two hundred milliliters of water were filtered through 1.2 μm pre-weighed glass fiber filters (previously baked at 450 °C for 8 h). After filtration, filters were freeze dried and weighed to determine the amount of particulate matter in the water sample. The filtrate was discarded. Filters were spiked with the standard and extracted with 30 ml of hexane: dichloromethane (1:1, v/v) for 10 min using ultrasonic extraction.

2.5.2. Fish and milk Samples

The extraction protocol for fish and milk samples follows a previous protocol (Lacorte et al., 2006). Briefly, the samples were lyophilized, homogenized, and stored at −20 °C until analyzed. The samples were extracted using a selective pressurized liquid extraction method previously described using a fully automated ASE system with hexane: dichloromethane 1:1 as extraction solvent. The cell was heated to 100 °C until the system pressure reached 1500 psi. The extracts were concentrated, sulfuric acid washed, and re-dissolved with 250 μl of dichloromethane prior to GC analysis.

2.5.3. Soil Samples

One gram of soil was weighed into a sample bottle and 13C-labeled standards for BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209) and 10 ml of toluene:acetone (70:30) was added. After sonicating for 30 min the solvent was filtered through pre-wetted sodium sulfate glass fiber filters and the solvent collected. The process was repeated two additional times with 5 ml of solvent and the combined extracts concentrated to about 0.5 ml. The extract was processed with an automated clean-up procedure using an acid-neutral/basic silica column followed by an alumina column (Power-Prep™, Fluid Management System, Waltham, MA). The extractants were concentrated into dodecane and 13C-labeled injection standards BDE-77 and BDE-139 (final volume 20 μl) prior to GC–HRMS analyses. If any of PBDE congener concentration exceeds the highest calibration point the soil samples were re-extracted using 0.1 g of soil.

2.6. GC–MS analyses for PBDEs

2.6.1. Fish and water samples

GC–NIC–MS of the fish and water samples analysis was performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass spectrometer (Agilent Technologies, Madrid, Spain). The quantitation of mono to hepta-BDEs used PCB-209 as an internal standard because 13C labeled BDEs have the same fragmentation pattern of native PBDEs that are monitored as their Br ion in this analysis. The details of the GC–NIC–MS analysis have been previously published (Eljarrat et al., 2002).

2.6.2. Milk and soil samples

The milk and soil samples were analyzed with GC–HRMS using a VG-AutoSpec-S (Waters Corporation, Milford, MA, USA) mass spectrometer equipped with a Hewlett Packard model 5890 series II gas chromatograph (Agilent, Palo Alto, California, USA). Isotope-dilution methods were used to quantify the analytes and recoveries were corrected by the 13C-labeled standards. The detailed procedure for milk analysis was described by Ikonomou and Rayne (2002). The parameters for soil analysis were adapted from a previous published procedure (Huwe and Larsen, 2005).

3. Results and discussion

3.1. Precision and recovery

Table 1 shows the results from a precision study in which surface and groundwater samples fortified with BDE-47 at three concentration levels (62.5, 125, and 250 ppt) were each measured five times per assay and on five different days for the ELISA technique and similar measurements at 100 ppt for GC–NIC–MS analysis. The within and between day variation was estimated by the method of Bookbinder and Panosian (1986). Coefficients of variation (CV) for both within and between days were below 5% for the ELISA and similar values for the within days for GC–NIC–MS with a slightly higher between day CV (7.6%) for this technique. The ELISA technique gives somewhat superior results for precision and recovery when compared with GC–NIC–MS for a single congener of PBDE.

3.2. Specificity

Table 2 summarizes the cross-reactivity data of the PBDE assay for various PBDE congeners as well as other environmental contaminants such as PCBs, PCP, and 2,4-D. The results indicate that the antibody recognizes BDE-47 and BDE-99 nearly equally. The hapten used in the production of the antibody was designed to detect BDE-47, a major component of the penta mixture and a
Table 1

<table>
<thead>
<tr>
<th>BDE 47 added (ppt)</th>
<th>Mean (ppt)</th>
<th>%R ± SD</th>
<th>% CV (within assay)</th>
<th>% CV (between assay)</th>
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<tr>
<td>ELISA 62.5</td>
<td>56.4</td>
<td>90 ± 3</td>
<td>3.2</td>
<td>4.4</td>
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<tr>
<td>125</td>
<td>131.5</td>
<td>105 ± 5</td>
<td>2.2</td>
<td>4.8</td>
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<td>250</td>
<td>270.6</td>
<td>108 ± 13</td>
<td>2.0</td>
<td>4.0</td>
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<tr>
<td>GC–NCI–MS 100</td>
<td>78</td>
<td>78 ± 9</td>
<td>2.0</td>
<td>7.6</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th></th>
<th>ELISA 50% B/B0 (ppt)</th>
<th>LOD (ppt)</th>
<th>GC–NCI–MS LOD (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-47</td>
<td>0.135</td>
<td>0.017</td>
<td>0.04</td>
</tr>
<tr>
<td>BDE-99</td>
<td>0.15</td>
<td>0.02</td>
<td>0.05</td>
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<tr>
<td>BDE-28</td>
<td>0.9</td>
<td>0.045</td>
<td>0.05</td>
</tr>
<tr>
<td>BDE-100</td>
<td>5.5</td>
<td>0.055</td>
<td>0.04</td>
</tr>
<tr>
<td>BDE-153</td>
<td>10</td>
<td>0.075</td>
<td>0.04</td>
</tr>
<tr>
<td>BDE-154</td>
<td>580</td>
<td>3.5</td>
<td>0.05</td>
</tr>
<tr>
<td>BDE-183</td>
<td>2000</td>
<td>13.5</td>
<td>0.06</td>
</tr>
<tr>
<td>BDE-209</td>
<td>3000</td>
<td>370</td>
<td>ND*</td>
</tr>
<tr>
<td>4′-OH-BDE-49</td>
<td>3.29</td>
<td>0.037</td>
<td>ND</td>
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<td>5-OH-BDE-47</td>
<td>1.53</td>
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<td>6-OH-BDE-47</td>
<td>12.27</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>5-Methoxy-BDE-47</td>
<td>0.08</td>
<td>0.012</td>
<td>ND</td>
</tr>
<tr>
<td>5′-Methoxy-BDE-99</td>
<td>15</td>
<td>0.12</td>
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</tr>
<tr>
<td>Triclosan</td>
<td>11</td>
<td>0.25</td>
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<tr>
<td>Aroclor 1254</td>
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<tr>
<td>PCB-37</td>
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<td>160</td>
<td>ND</td>
</tr>
<tr>
<td>PCB-77</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>ND</td>
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<tr>
<td>PCl</td>
<td>&gt;10,000</td>
<td>3300</td>
<td>ND</td>
</tr>
<tr>
<td>2,4-D</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND: Not determined.

The comparison of ELISA with GC–MS data could be problematic because of the fundamental differences in the analytical methods. The ELISA responds to multiple PBDE congeners with different responses to each congener but all the responses are additive and reported as BDE-47 equivalents because of the use of this congener for the calibration curve. The GC–HRMS or GC–NCI–MS can analyze greater than 35 PBDE congeners ranging from mono to deca bromo substitutions. For the purpose of comparison the amounts of BDE-47, BDE-99, BDE-28, BDE-100 and BDE-153 are corrected for their response in the ELISA by multiplying each concentration by the B/B0 of BDE-47 divided by the B/B0 of the congener and summed. Most of the response, over 95% was due to BDE-47 and BDE-99 since they are present in the largest amounts and show the greatest response to the ELISA. Clearly, the correlation will be successful only to the extent that ELISA is not detecting other components. Significant presence of hydroxy or methoxy PBDEs could have a major effect on correlations as at least one of these gives a pronounced response to the ELISA and they are normally not considered in GC–MS analysis. Twenty-five mothers’ milk samples with the sum BDE-47 and BDE-99 values ranging from 665 to 5881 pg/g lipid were analyzed. Samples, such as milk, containing appreciable amounts of fat give substantial background interference commonly known as a matrix effect. Consequently, even with the lower R² (0.46) and the regression equation observed for the data in Fig. 1 the ELISA still demonstrates satisfactory correspondence to the GC–HRMS technique. Certainly, the high throughput of the ELISA makes it very useful, as a screening assay to select appropriate samples for the more resource intensive congener specific analysis. The somewhat lower response of the ELISA would not pose a problem as the graph shows a screening level could be selected minimizing the false positive and false negative results.

Eighteen fish samples obtained from various regions of Spain having a sum of BDE-47 and BDE-99 ranging from 55 to 4101 pg/g dry weight (dw) were analyzed (Fig. 2). For the same reasons described above, the direct comparison of GC–NCI–MS with ELISA for fish samples presents some problems. The R² (0.84) for fish was...
much better than that for milk, and the regression coefficient (slope) was similar (0.45). The graph (Fig. 2) clearly demonstrates the usefulness of ELISA as a high throughput screening technique easily identifying significant PBDE contamination.

Polyurethane foam in furniture contains a substantial amount of pentaBDEs as flame retardants (Prevedouros et al., 2004). Hence, a facility where furniture was burned for training firefighters was chosen as the soil sampling site. Samples were obtained in different directions and different distances from the burning slab. The correlation between the amounts determined by the ELISA and by GC–HRMS showed excellent correlation (0.81) and the regression coefficient was close to one (1.12) (Fig. 3). The better statistics can probably be attributed to a much smaller matrix effect for the ELISA since unlike fish and milk samples very little fat would be expected in these samples. Although in general the PBDE concentration decreased with distance from the burning site, exceptions indicated some randomization occurred (data not shown). The ratios of the congeners were relatively constant and different from control sites remote from the burn site indicating the PBDEs at the burn were derived from a common source, most probably the articles burned at the site.

In conclusion, this work describes the performance characteristics of a magnetic particle-based ELISA for the detection of PBDEs in fish, milk, soil, and water samples. The assay is fast, exhibits high sensitivity, excellent precision, and good recovery, thus providing consistent and cost-effective monitoring of environmental and food samples. Using this ELISA, fifty water or processed samples can be analyzed in about 1 h. The method was shown to be applicable to samples of milk, soil, and fish with some cleanup. Sample preparation required for complex matrices added considerable time and expense to the procedure, as it does in other analytical methods requiring extensive cleanup. The correlation studies comparing GC–MS with ELISA demonstrated the ELISA to be a useful screening assay for these complex matrices complementing the more expensive GC–MS or GC–HRMS analyses which provide detailed congener information.

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3.4. Comparison of GC–MS with ELISA for water samples

None of the groundwater samples in Warminster, PA had significant levels of PBDEs. The results for PBDE levels collected from the influents and effluents of a waste water treatment plant in the Ebro river basin (Spain) are shown in Fig. 4. All the influents had trace amounts of PBDEs (64–107 ppt) while the effluents were below the level of detection (17 ppt). This data is compatible with concept of the water treatment process removing PBDE contaminants and the ability of the magnetic particle assays to quickly screen for PBDEs in environmental samples. This was confirmed by GC–NCI–MS analysis of the samples that showed no detectable PBDE’s in the ELISA procedure. Of those showing significant levels by ELISA one was a false positive showing no PBDE’s in the GC–MS analysis. The reason for this is unknown. The sample size was insufficient for a correlation study.

In conclusion, this work describes the performance characteristics of a magnetic particle-based ELISA for the detection of PBDEs in fish, milk, soil, and water samples. Using this ELISA, fifty water or processed samples can be analyzed in about 1 h. The method was shown to be applicable to samples of milk, soil, and fish with some cleanup. Sample preparation required for complex matrices added considerable time and expense to the procedure, as it does in other analytical methods requiring extensive cleanup. The correlation studies comparing GC–MS or GC–HRMS with ELISA demonstrated the ELISA to be a useful screening assay for these complex matrices complementing the more expensive GC–MS or GC–HRMS analyses which provide detailed congener information.

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Fig. 2. PBDE concentrations (pg/g dw) in fish samples measured by ELISA BDE-47 equivalents and GC–NCI–MS (BDE-47 + BDE-99 + BDE-28 + BDE-100 + BDE-153) corrected for the ELISA response based on the congeners cross-reactivity. The regression equation was BDE-47 equivalents (ELISA) = 31.8 + 0.45 ((BDE-47 + BDE-99 + BDE-28 + BDE-100 + BDE-153) GC–NCI–MS corrected for ELISA with cross-reactivity data) R² = 0.84.
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