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Focused ultrasound assisted extraction for the determination of PBDEs in vegetables and amended soil

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ABSTRACT

Focused-ultrasound solid–liquid extraction was developed for the extraction of polybrominated diphenyl ethers in vegetables and amended soil. Firstly, solid-phase extraction clean-up using 2 g and 5 g of Florisil and 2-g silica cartridges were evaluated and elution profile was also optimised. Similar recoveries were obtained for most compounds while better recoveries were obtained for 5-g Florisil in the case of the heavier PBDEs. FUSLE extraction time (2 min) guaranteed quantitative extraction of the target analytes in the four studied matrices (69–130%). Method detection limit values were in the range of 1–5 ng g^{-1} for splitless injection in a gas chromatograph coupled to a mass spectrometer and no significant improvement was obtained for large volume injection. Relative standard deviation values were between 1% and 30%. Recoveries obtained using FUSLE were compared with those obtained with microwave assisted extraction and the developed method was also applied to a certify reference material of polybrominated diphenyl ethers and polychlorinared biphenyls in sediment. Similar values were obtained in the case of carrot and pepper matrices (77–130% for FUSLE and 77–112% for MAE). However, MAE provided extraction recoveries higher than 100% for most of the BDE congeners in lettuce and amended soil.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are the most frequently used brominated flame retardants (BFRs). There are three commercial technical mixtures of PBDEs: PentaBDE, OctaBDE and DecaBDE, which are composed of a mixture of congeners and named according to their average bromine content. Congeners 2,4,4′-tribromodiphenyl ether (BDE-28), 2,2,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,3,4,4′-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153), 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154), 2,2′,3,4,4′,5′,6-heptabromodipheny lether (BDE-183) and decabromodiphenyl ether (BDE-209), which are relevant for dietary exposure, are considered as primary interest congeners by the Panel on Contaminants in the Food Chain (CONTAM Panel) due to their occurrence in the composition of the technical BDE mixture, in the environment and in food [1].

PBDEs have been used in a wide array of products, including building materials, electronics, furnishings, motor vehicles, airplanes, plastics, polyurethane foams, textiles and so on. Some of them may be covalently bound into materials during production, but most of them are simply additives. Consequently, they can be released from these products during their production, use, disposal and recycling processes and, as a consequence, PBDEs can leach into the environment and reach animals and humans through water, food chain and dust [2–4]. Although their acute toxicity is low, recently, concerns over the persistence, ability to bioaccumulate and potential for toxicity of the most widely used BFRs have led to increasing regulation and restrictions on their production and use [5–7]. For example, in 2008 the use of DecaBDEs was banned in electrical and electronic applications in the European Union (EU), while Penta- and OctaBDEs have been added to the Persistent Organic Pollutants (POPs) list of the Stockholm Convention (http://chm.pops.int/Programmes/New-POPs/The9newPOPs/tabid/672/%20language/en-US/Default.aspx) [8].

In spite of the processes that influent water is submitted in wastewater treatment plants (WWTPs), potential harmful substances, including PBDEs, are present in both effluent water and sewage sludge, which are a mirror of the chemical and products consumed in modern society [9]. PBDEs are routinely detected in sewage sludge in the low mg kg^{-1} dw range and values have been reported from Sweden [4,10,11], USA [12,13], Germany [14], The Netherlands [15], China [16], Australia [17] and Kuwait [18].

Meanwhile, agricultural application of sewage sludge has become the most widespread method for disposal of sludge since it is the most economical outlet for sludge and offers the opportunity to recycle plant nutrients and organic matter to soil

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for crop production [19]. At present, around 40% of the sewage sludge produced in Europe is used as a fertilizer in agriculture [20]. In general, the EU considers that the re-use of sludge should be encouraged since it represents a long-term solution, provided that the quality of the sludge re-used is compatible with public health and environmental protection requirements [21]. However, concern has increased due to the presence of heavy metals, organic contaminants and pathogenic bacteria in sewage sludge. According to Clarke and Smith [22], PBDEs are included as emerging organic contaminants to be studied in biosolids with agricultural purposes since the contamination of sludge and effluents with PBDEs could have potential implications for disposal and beneficial reuse strategies. One way to study the introduction of organic contaminants to humans via the food chain is to study the uptake of such pollutants by different crop plants. Within this scenario, the measurement of PBDEs in sludge amended soil and crops have gained importance [23,24].

Thus, effective sample pre-treatment, including extraction and clean-up procedures, are compulsory prior to the instrumental analysis with the aim of identification and accurate determination of PBDEs in a variety of solid matrices. Different solid–liquid extraction techniques such as the classical Soxhlet, which requires 4–24 h extraction, has been used for years [25–27]. Several faster extraction techniques have been developed to reduce both the extraction time and the solvent consumption, including microwave assisted extraction (MAE) [28–30] and pressurised liquid extraction (PLE) [31–33]. Recently, focused ultrasound solid–liquid extraction (FUSLE) has gained interest due to its simplicity, low cost and the improved efficiency and reproducibility compared to classical ultrasound baths. FUSLE has been recently applied to extract PBDEs from solid matrices such as dust [34] but no applications to the analysis PBDEs in vegetables and amended soil are found in the literature.

Due to the lack of selectivity of the above mentioned extraction techniques, a clean-up step is also necessary before the analysis step. In the case of PBDEs for almost all the matrices solid phase extraction (SPE) or Gel Permeation Chromatography (GPC) has been mostly used [2,6,29,35–39].

In the present work, FUSLE combined with SPE clean-up was optimised for the determination of PBDEs in vegetables (lettuce, carrot and pepper) and compost-amended soil. The FUSLE extraction was also compared with MAE. This work is included within the CTM2011-24094 Spanish Ministry project where human exposure to different organic contaminants through compost-amended soils is being studied.

2. Experimental section

2.1. Cleaning protocol

All laboratory material was washed with a common detergent, rinsed with abundant Elix water (Millipore, Bedford, MA, USA), sonicated in an acetone bath and maintained there for 24 h. Afterwards the material was rinsed with Milli-Q water (<0.05 μS/cm, Milli-Q model 185, Millipore). All glassware material, except for the volumetric one, was dried in an oven at 120 °C for at least 4 h. In the case of test tubes, the same procedure was employed but later the material was dried in a muffle oven at 400 °C for at least 4 h in order to remove all PBDEs traces and decrease blank signal.

2.2. Reagents and materials

PBDEs (in cyclohexane) at 10 ng μL^{-1} concentration level were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The dilutions at lower concentrations were daily prepared according to the experimentation. All the chemical standards were stored at 4 °C in the dark and the stock solutions at −20 °C. Reference material (PBDEs SQC072 in sediment) was supplied by Sigma-Aldrich (Milwaukee, USA).

Isooctane, *n*-hexane, acetone, dichloromethane (DCM) and toluene (all HPLC grade) were purchased from LabScan (Dublin, Ireland) and copper (powder Cu) from Merck (Darmstadt, Germany). For filtration 0.45 μm polyamide filters (Macherey Nagel, Düren, Germany) were used. LC-Florisil (2 and 5 g) and LC-silica (2 g) cartridges were purchased from Supelco (Walton-on-Thomas, UK) in order to carry out clean-up step.

H_2 gas was used as carrier gas in the detection step and it was obtained by the Hydrogen Generator AD-1020 (CINEL Strumenti Scientifici, Vigonza, Padova, Italy).

2.3. Instrumentation

Samples were frozen and freeze-dried at low temperature (~ −50 °C) using a Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain). For sample extraction, a Sonoplus HD 3100 ultrasonic homogeniser (Bandelin Electronic, Berlin, Germany) equipped with a MS 73 titanium microtip and a Mars X CEM (Matthews, NC, USA) microwave oven were used. All the fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle stream of nitrogen. The SPE clean-up step was performed using Visiprep® SPE manifold which was provided by Supelco (Bellefonte, PA, USA). The extracts were analysed on an Agilent 6890N gas chromatograph (GC) coupled to an Agilent 5975 N mass spectrometer (MS) (Agilent Technologies, Avondale, PA, USA).

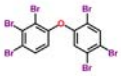
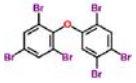
2.4. Spiking of samples

All matrices were freeze-dried (see Section 2.3), homogenised in a mortar and fortified with target analytes at two concentration levels: 6 ng g^{-1} and 58 ng g^{-1}. Hence, a known amount of matrix was weighed, covered with acetone, spiked with PBDEs and stirred during 12 h. After that, acetone was evaporated and the sample was aged for 2 weeks. When pepper matrix was spiked, instead of acetone, *n*-hexane was added since when acetone was used a non homogenous fortified sample was obtained.

2.5. Focused ultrasound solid–liquid extraction

PBDEs were extracted from amended soil, carrot, lettuce and pepper using an adaptation of a previously published method [40]. A sample aliquot of 0.5 g was weighed, 10 mL of acetone was added and the vessel was immersed in an ice-water bath (~ 0 °C) for extraction. In the case of amended soil samples, 0.5 g of activated copper, previously treated with HNO_3 30%, rinsed with ultrapure water and DCM, and dried at 50 °C, was added to eliminate sulphur from the soil, which might interfere during the chromatographic analysis [6,27,41]. According to Errekatxo et al. [40] samples were exposed to ultrasonic energy at 20% power and 7 cycles during the optimised extraction time (2 min). Non-fortified extracts were processed in parallel for blank analysis. The supernatant was filtered through a polyamide syringe filter (25 mm, 0.45 μm) and the extract was evaporated to ~1 mL in a Turbovap LV Evaporator using nitrogen blown-down after the addition of isooctane. Isooctane addition was carried out in order to prevent analyte losses and guarantee that the concentrated extract was enriched in a non-polar solvent before SPE clean-up [40,42].

Table 1
Chemical structure, CAS number, log K_{ow} and the ions monitored for each analyte studied. First ion was used as quantifier and the second one as qualifier.

Polibrominated diphenyl ethers (PBDEs)	Structure	CAS number	m/z	Log K_{ow}
2,4,4′-tribromodiphenyl ether (*BDE-28)*		41318-75-6	406/408	5.88
2,2′,4,4′-tetrabromodiphenyl ether *(BDE-47)*		5436-43-1	486/484	6.77
2,3′,4,4′-tetrabromodiphenyl ether *(BDE-66)*		189084-61-5	486/484	6.77
2,2′,3,4,4′-pentabromodiphenyl ether *(BDE-99)*		CASID30342	404/564	ACD/LogP: 8.19
2,2′,4,4′,5-pentabromodiphenyl ether *(BDE-100)*		189084-64-8	404/564	ACD/LogP: 8.03
2,2′,4,4′,6-pentabromodiphenyl ether *(BDE-85)*		182346-21-0	404/564	ACD/LogP: 8.02
2,2′,3,4,4′,5′-hexabromodiphenyl ether *(BDE-138)*		182677-30-1	484/644	ACD/LogP: 7.73
2,2′,4,4′,5,5′-hexabromodiphenyl ether *(BDE-153)*		68631-49-2	484/644	ACD/LogP: 8.98
2,2′,4,4′,5,6′-hexabromodiphenyl ether *(BDE-154)*		207122-15-4	484/644	ACD/LogP: 8.83

2.6. Microwave assisted extraction

The MAE method was based on EPA 3546 method [43]. Briefly, 0.5 g of dried sample was weighed and transferred to the Teflon microwave vessel, 10 mL of acetone was added and the following extraction conditions were studied:

(a) oven set to a power of 1200 W, ramped to 115 °C within 15 min and held for 10 min,
(b) oven set to a power of 1200 W, ramped to 90 °C within 15 min and held for 10 min.

When the irradiation period was completed, samples were removed from the microwave cavity and were allowed to cool down to room temperature before opening. The supernatant was filtered through a polyamide syringe filter (25 mm, 0.45 μm) and

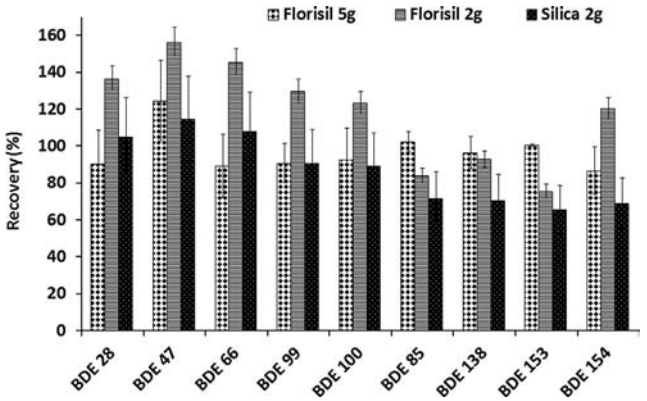


Fig. 1. Recoveries (%) obtained when 2-g and 5-g Florisil cartridges and 2-g silica were used as sorbents in the clean-up step.

the extract was treated as mentioned in Section 2.5. In the case of the amended soil, 0.5 g of activate copper was also weighed in the Teflon vessel.

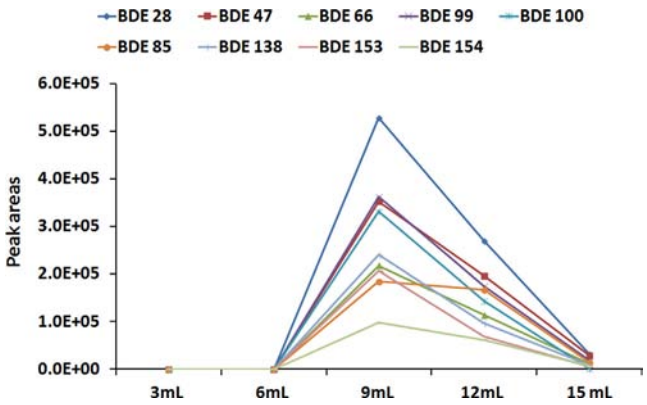


Fig. 2. Solvent elution profile for 5-g Florisil cartridges using 3–15 mL of *n*-hexane: toluene (80:20, v/v) as elution solvent mixture.

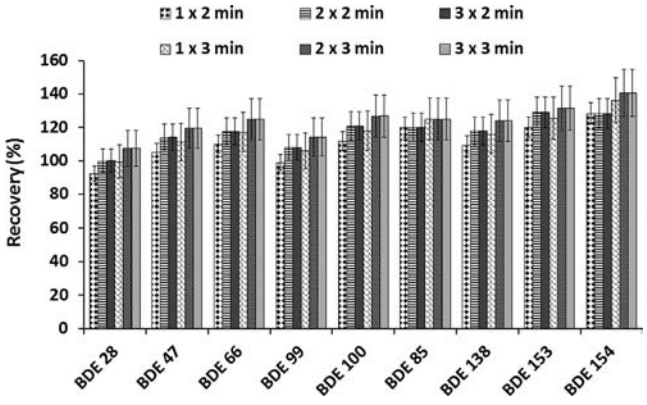


Fig. 3. Recoveries ($n=3$) (%) obtained for spiked pepper samples at different extraction times.

2.7. Clean-up of the extracts

Different strategies were studied for the clean-up of the extracts:

(a) 200 μL of the concentrated extract was loaded onto a 2-g Florisil cartridge, previously conditioned using 5 mL of *n*-hexane, and the target analytes were eluted with 18 mL *n*-hexane: toluene (80:20, v/v).
(b) 200 μL of the concentrated extract was loaded onto a 2-g silica cartridge, previously conditioned using 5 mL of *n*-hexane, and the target analytes were eluted with 18 mL of a (80:20, v/v) *n*-hexane:toluene mixture.
(c) 200 μL of the concentrated extract was loaded onto a 5-g Florisil cartridge, previously conditioned using 15 mL of *n*-hexane, and the target analytes were eluted with 25 mL of a (80:20, v/v) *n*-hexane:toluene mixture.
(d) 200 μL of the concentrated extract was loaded onto a 10-g Florisil cartridge, previously conditioned using 20 mL of *n*-hexane, and the target analytes were eluted with 40 mL of a (80:20, v/v) *n*-hexane:toluene mixture.

In all the cases the eluate was evaporated to dryness and then reconstituted in 120 μL of *n*-hexane before gas chromatography-mass spectrometry (GC–MS) analysis.

2.8. Analysis of the extracts

In the case of splitless mode 2-μL extract was injected at 300 °C.

In the case of LVI-PTV, 20 μL of the extract was injected at 3.5 μL s^{-1} in a cooled PTV (60 °C) at 7.7 psi vent pressure using 100 mL syringe placed in a MPS2 autosampler. *n*-Hexane was purged out with a vent flow of 75 mL min^{-1} for 3 min (vent time), then splitless mode was programmed for 1.5 min, while the temperature increased at 12 °C min^{-1} to 300 °C where it was held for 1 min.

In both cases analytes were introduced into a HP-5 capillary column (30 m × 0.25 mm, 0.25 μm). PBDEs were separated using the following oven temperature programme: 60 °C (hold 1 min),

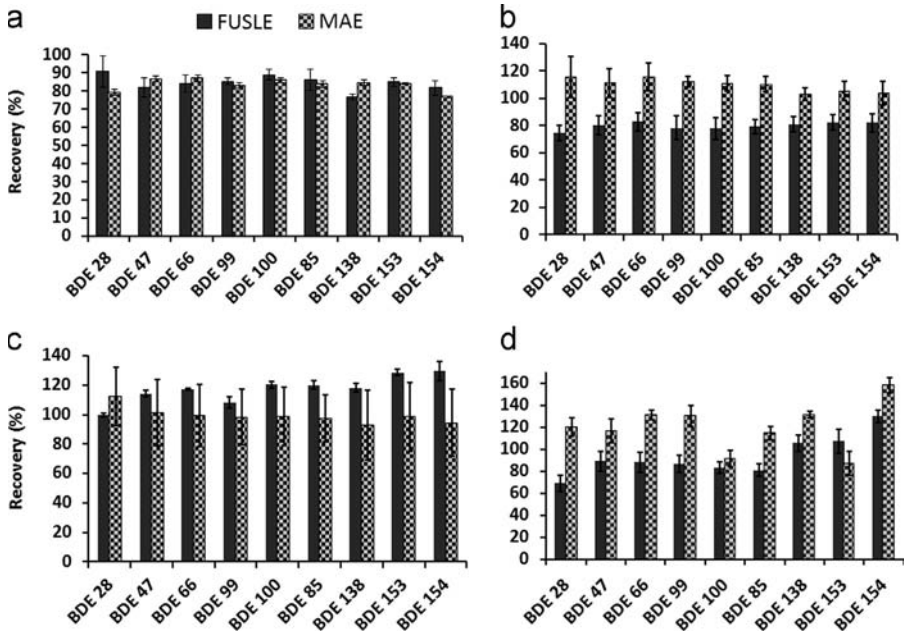


Fig. 4. Recoveries ($n=3$) obtained for FUSLE and MAE in the case of (a) carrot, (b) lettuce, (c) pepper and (d) amended soil using 5-g Florisil clean-up.

temperature increase at 7.0 °C min^{-1} up to 300 °C, where it was finally held for 15 min (carrier gas H_2 at 1.3 mL min^{-1} flow-rate).

The MS was operated in the electron impact ionisation mode (EI) and the energy of the electrons was kept at 70 eV. The interface temperature was set at 310 °C and the ionisation source and the quadrupole temperatures at 230 °C and 150 °C, respectively. Measurements were performed in the selected-ion-monitoring (SIM) mode and the ions monitored for each analyte are listed in Table 1. The first ion was used as quantifier and the second one as qualifier.

3. Results and discussion

3.1. Optimisation of the clean-up step

In order to optimise the clean-up step for PBDEs, non-fortified carrot was extracted using FUSLE (2 min at 20% power and 7 cycles) and the extract was spiked at 240 ng mL $^{-1}$ concentration level. Both Florisil and silica cartridges were tested due to their wide applicability to lipid removal from the samples [5,6,35]. In the case of Florisil, 2-g and 5-g cartridges were evaluated, while only 2-g cartridges were used in the case of silica. The study was repeated in triplicate for each of the cartridges used and the results can be observed in Fig. 1. In the case of 2-g Florisil cartridges, recoveries exceeding 100% were obtained for most of the PBDEs studied, especially for the lighter congeners, with recovery values up to 156%. The results obtained for 2-g Florisil cartridges clearly indicate the presence of co-eluting interferences. In the case of 5-g Florisil and 2-g silica cartridges, similar results (according to ANOVA test) were obtained for BDE-28, BDE-47, BDE-66, BDE-99, BDE-100 and BDE-154 ($F_{experimental}$=1.13–6.44 < $F_{critical}$=10.13), while better recoveries were obtained for 5-g Florisil in the case of the heavier PBDEs (BDE-85, BDE-138 and BDE-153) ($F_{experimental}$=10.93–13.13 > $F_{critical}$=10.13). Finally 5-g Florisil cartridges were chosen as optimised sorbent and used in further experiments.

Further experiments were performed in order to fix the elution volume when 5-g Florisil cartridges were used (previous experiments had been performed with 25 mL of a (80:20, v/v) *n*-hexane: toluene mixture). Aliquots were separately collected every 3 mL. According to the results in Fig. 2, 15 mL was sufficient for quantitative recovery (99–106%) of target analytes. Therefore 15 mL of the elution solvent were used in further experiments.

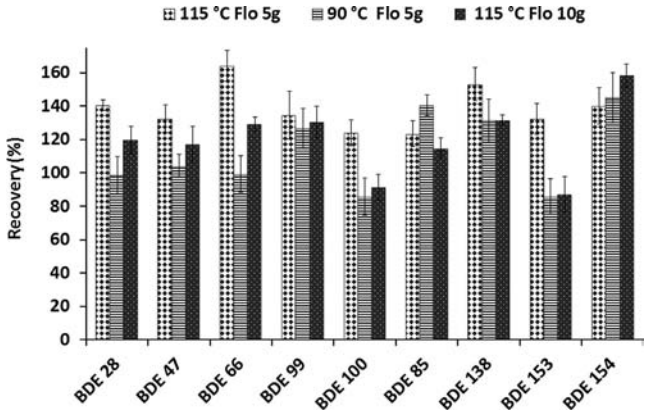


Fig. 5. Recoveries (n=3) (%) obtained for different MAE extraction conditions (90–115 °C extraction temperature) and 5-g and 10-g Florisil clean-up cartridges for amended soil matrix.

3.2. FUSLE vs MAE

FUSLE and MAE were applied and compared in the analysis of PBDEs in vegetables (lettuce, carrot and pepper) and amended soil. In a first attempt, FUSLE extraction conditions were previously optimised in published work for the determination of different organic contaminants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PEs) and alkylphenols (APs) [40]. MAE method was based on EPA 3546 [43].

In the case of FUSLE extraction, although in the Errekatxo et al. method [40] 2 min FUSLE extractions were satisfactorily optimised and performed, 2 × 2 min, 2 × 3 min, 1 × 3 min, 2 × 3 min and 3 × 3 min extraction periods were also evaluated here in order to see whether extraction recoveries could be improved in order to obtain exhaustive extraction. The recoveries obtained are included in Fig. 3. According to the ANOVA of the results, no significant differences were observed ($F_{experimental}$=1.00–3.03 < $F_{critical}$=4.07) and, therefore, one single 2-min extraction was finally chosen as

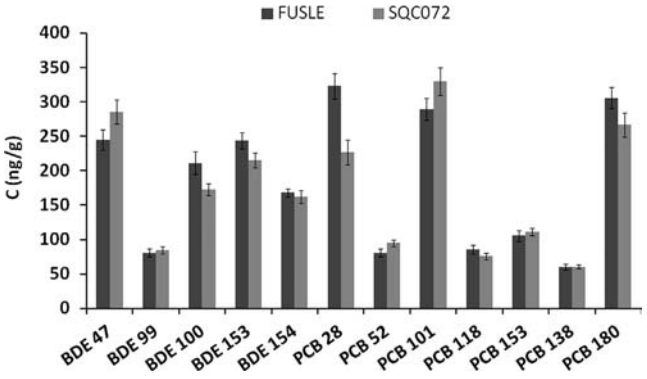


Fig. 6. Comparison of the results obtained using the present methodology (n=5) and the certified values of the reference material SQC072 for PBDEs and PCBs.

Table 2
Average (n=3) recovery (%) and RSD (%) at high (58 ng g^{-1}) and low concentration(6 ng g^{-1}) and MDL values obtained for PBDEs in spiked matrices (carrot, pepper, lettuce and amended soil).

Analyte	r^2 (MDLs-1 ng µL^{-1})	RSD (%) n=3 in two ranges 6/58 ng g^{-1}				Recovery (%) in two ranges 6/58 ng g^{-1}				MDLs (ng g^{-1}) at 6 ng g^{-1}			
		Pepper	Carrot	Lettuce	Amended soil	Pepper	Carrot	Lettuce	Amended soil	Pepper	Carrot	Lettuce	Amended soil
BDE-28	0.996	13/1	18/3	21/7	15/11	105/100	102/90	103/75	73/69	3	2	1	1
BDE-47	0.996	13/2	20/3	10/9	16/10	101/114	108/125	107/80	93/89	2	3	1	2
BDE-66	0.994	13/1	23/14	10/8	14/11	98/117	122/89	105/83	91/88	1	2	1	2
BDE-99	0.996	23/3	20/2	13/11	30/9	117/108	99/91	113/78	94/87	3	3	3	2
BDE-100	0.989	13/2	19/9	12/10	23/7	106/120	117/93	111/78	88/83	2	3	1	3
BDE-85	0.997	23/2	17/11	14/7	24/7	122/120	97/102	110/79	107/81	3	2	2	2
BDE-138	0.996	16/2	23/5	11/7	24/7	116/108	108/96	124/71	120/106	2	2	1	5
BDE-153	0.993	15/2	3-Dec	11/7	22/10	117/128	101/101	120/82	106/107	3	2	1	1
BDE-154	0.994	10/5	15/4	18/8	14/4	115/130	100/87	118/82	130/108	1	2	1	3

Table 3
MDLs (ng g^{-1}) and LODs (ng g^{-1}) found in the literature for PBDEs in different solid samples.

Sample	Extraction	Analysis	MDL	References
Soil and vegetables	FUSLE	GC–MS	1–3	In this work
Sediment	SPLE	GC–NCI–MS	1–46 × 10^{-3}[a]	[6]
Sediment	Soxhlet	GC–NCI–MS	3–50 × 10^{-3}[a]	[6]
Sediment	USAL-DSPE-DLLME	GC–MS/MS	0.02–0.08[a]	[34]
Soil	ASE	GC–NCI–MS	1–2[b]	[48]
Biosolid	ASE	GC–NCI–MS	2–10[b]	[48]
Corn	ASE	GC–NCI–MS	1–5[b]	[48]
Soil	Soxhlet	GC–Ion Trap	1 × 10^{-3}[a]	[46]
Vegetables	Soxhlet	GC–Ion Trap	1 × 10^{-3}[a]	[46]
Cow milk	Soxhlet	GC–Ion Trap	0.8 × 10^{-3}[a]	[46]
Foodstuff	Soxhlet	GC–MS	5–40 × 10^{-3}[a]	[47]
Foodstuff	MSPD	GC-LRMS/MS	0.01–0.55 × 10^{-3}[c]	[48]
Soil	Soxhlet, ASE and MAE	HRGC/HRMS	1.52–24.8 × 10^{-3}[c]	[32]
Sewage sludge	MAE	GC–NCI–MS	1.8–6.1	[30]
Soil	PLE	GC–TOF–MS	0.1–1	[49]
Dust	FUSLE	GC–MS/MS	0.05–0.8[a]	[36]

ASE: accelerated solvent extraction, GC–MS/MS: gas chromatography–tandem mass spectrometry, GC–NCI–MS: gas chromatography negative chemical ionisation mass spectrometry, GC–TOF–MS: gas chromatography time-of-flight mass spectrometry, HRGC/HRMS: high-resolution gas chromatography coupled with high-resolution mass spectrometry, MAE: microwave assisted extraction, MDL: method detection limit, MSPD: matrix solid-phase dispersion, PLE: pressurised liquid extraction, SPLE: selective pressurised liquid extraction, USAL-DSPE-DLLME: ultrasound-assisted leaching-dispersive solid-phase extraction followed by dispersive liquid–liquid microextraction.

[a] LODs werecalculated based on three times based the signal-to-noise ratio.
[b] Method quantitation limit.
[c] LODs, the calculation mode was not reported.

optimum. This FUSLE method was applied to the four matrices of interest and taking into account the results included in Fig. 4a, b, c and d for carrot, lettuce, pepper and amended soil, respectively, satisfactory results (~100%) were obtained in most of the cases except for BDE-28 in amended soil, which showed an extraction yield of 69%.

Fortified samples were analysed also by means of MAE based on EPA 3546 method. Firstly, extraction conditions described in Section 2.5 (protocol (a)) and clean-up with 5-g Florisil cartridges were tested. According to the ANOVA of the results included in Fig. 4, comparable recovery values were obtained by means of FUSLE and MAE in the case of carrot and pepper ($F_{experimental}$=1.15–8.03 < $F_{critical}$=10.12, $F_{experimental}$=1.14–6.55 < $F_{critical}$=10.12 for carrot and pepper, respectively), although repeatability was significantly lower for MAE when applied to pepper matrix ($F_{experimental}$=25–1181 > $F_{critical}$=9) based on an *F*-test of the results. In the case of lettuce and amended soil matrices, recoveries obtained for MAE exceeded 100% for most of the congeners studied, indicating the lower selectivity obtained with MAE extractions. It should be underlined that the clean-up step was optimised using FUSLE extracts and not MAE extracts, which were more colourful than the former, indicating the extraction of more interferences. Thus, in order to improve the results for MAE, two other new set of experiments were performed in the case of amended soil:

(a) Extraction at 115 °C and clean-up with 10-g Florisil.
(b) Extraction at 90 °C and clean-up with 5-g Florisil.

The best results (see Fig. 5) were obtained when milder MAE conditions followed by 5-g Florisil clean-up were applied and the use of 10-g Florisil cartridges did not imply any improvement of the results. However, even under the mildest conditions tested, extraction yields exceeded 100% for most of the congeners and further MAE optimisation should be performed for application to amended soil and lettuce.

3.3. Validation of the method

In the absence of a certified reference material (CRM) for PBDEs in vegetables or soil, two approaches were followed for method validation. On the one hand, fortified samples of the four matrices studied were analysed under optimal conditions. On the other hand, the developed method was applied to CRM SQC072, certified sediment for both PBDEs and PCBs.

In terms of obtained recovery from fortified samples, FUSLE combined with 5-g Florisil clean-up provided acceptable results (see Table 2) for the four matrices studied at two spiking levels (6 and 58 ng g^{-1}). Recovery ranges of 97–122%, 103–124%, 98–122% and 73–120% were at the lowest concentration level (6 ng g^{-1}) and 87–125%, 71–83%, 100–130% and 69–130% at the highest level (58 ng g^{-1}) in the case of carrot, lettuce, pepper and amended soil, respectively.

The average values (n=5) obtained for CRM SQC072 under optimised conditions are compared to the certified values in Fig. 6. Although not included in the present work, the results for PCBs were also included. In terms of recovery, the recovery values were within the 86–120% for PBDE and in the 85–115% for PCBs, except for CB-28 which showed recoveries up to 142%. It could be concluded that results obtained under optimal conditions are in good agreement with the certified values, both for the target PBDEs and for PCBs.

Method detection limits (MDLs) and relative standard deviations (RSDs) were also determined for the four matrices tested. Instrumental calibration curves were performed from the MDLs up to 1 ng μL^{-1} and squared correlation coefficients (r^2) values higher than 0.993 were obtained (see Table 2) for all the congeners. MDLs were calculated according to US Environmental Protection Agency Method (http://www.epa.gov/waterscience/methods/det/rad.pdf) and matrices (n=7) were spiked at 6 ng g^{-1}. The results for splitless injection are included in Table 2 and were in the 1–5 ng g^{-1} range. In order to improve the MDLs, LVI (20 μL) in a PTV system was also assayed. Although the signal for LVI-PTV injection of the standards increased ten time compared to splitless injection, no improvement was observed for real samples (see Table 2). The MDL values obtained in the present work were compared with other values found in the literature (see Table 3). In this sense, MDL values are in the same order of magnitude as those obtained by Shin et al., Hale et al. and Park et al. [28,44,45]. Better limits of detection (LODs) defined as three times the signal-to-noise ratio (S/N) were obtained in other works [6,30,32,46–49]. For instance,

in the case of FUSLE applied to dust samples [34] but it should be highlighted that tandem mass spectrometry (MS/MS) was used. Besides, and as recommended by the US Environmental Protection Agency (EPA), we think that MDLs calculated using real samples give a more realistic value of the detection limit. Tandem mass spectrometry [32–34,48], ion trap [46] or negative chemical ionisation-mass spectrometry (NCI–MS) [6,49] provided, in general, the best values. In terms of precision, RSD values were in the 1–30% range for the fortified samples and in the 3–12% for CRM SQC072. The latter were in good agreement with the RSD of the certified values which were in the 5–8% range for both PBDEs and PCBs.

The present method was applied to the determination of PBDEs in carrots, lettuces and pepper from local markets and concentrations were always lower than the MDL values.

4. Concluding remarks

2-min FUSLE extraction combined with 5-g Florisil clean-up was optimised for the determination of PBDEs in vegetables. FUSLE has turned out to be an alternative to more expensive extraction techniques such as MAE or PLE providing good MDLs (1–5 ng g^{-1}), precision (1–24%) and recoveries (71–130% for vegetables and 69–130% amended soil).

MAE and FUSLE were compared as alternative extraction techniques and although, similar recoveries were obtained in the case of carrot (77–91% for FUSLE and 77–87% for MAE) and pepper (100–130% for FUSLE and 93–109% for MAE) matrices, recoveries higher than 100% were attained for lettuce and amended soil in the case of MAE.

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