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Approaches for the Simultaneous Extraction of Tetrabromobisphenol A, Tetrachlorobisphenol A, and Related Phenolic Compounds from Sewage Sludge and Sediment Samples Based on Matrix Solid-Phase Dispersion

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A procedure based on matrix solid-phase dispersion (MSPD) for sample preparation in the analysis of some bromophenols and halogenated bisphenols in sediments and sludges has been developed. For the first time ever, MSPD was applied for the extraction of organic contaminants from sediment and sewage sludge samples. The influence of experimental conditions on the yield of the extraction process and on the efficiency of the built-in cleanup step was thoroughly evaluated. Analysis of the extracts was performed by nonaqueous capillary electrophoresis coupled with photodiode array ultraviolet detection, using large-volume sample stacking injection based on the electroosmotic flow pump as an on-column preconcentration technique. The method was applied to the analysis of real sludges from urban sewage treatment plants, as well as river and marine sediment samples.

Brominated flame retardants (BFRs) are ubiquitous substances used in paints, building materials, synthetic textiles, and plastic products, including electronic circuit boards and other electronic equipment, to reduce their flammability and thus to prevent fires.[1,2] Tetrabromobisphenol A (TBBPA) is probably the most widely used flame retardant worldwide.[3,4] Pentabromophenol (PeBP), 2,4,6-tribromophenol (2,4,6-TriBP), and tetrachlorobisphenol A (TCBPA) are also used as halogenated flame retardants,[5] although to a lesser extent than TBBPA. Although some bromophenols are ubiquitous to the marine environment, being readily detected in marine fish, crustaceans, molluscs, polychaetes (which have been shown to contaminate the sediments of their borrows and surroundings to an extent sufficient to influence other biota[6]), and marine algae,[7] 2,6-Dibromophenol (2,6-DiBP) and 2,4,6-TriBP are also generated in the thermal decomposition of TBBPA or from plastics treated with polybrominated epoxy-type flame retardants.[8,9] 2,4,6-TriBP is also the main breakdown product from the degradation of TBBPA when it is exposed to UV light.[10] Biodegradation studies have shown that TBBPA can be partly degraded to lesser brominated analogues under both aerobic and anaerobic conditions in soil and river sediment.[4] Thus, both natural and anthropogenic origins might be attributed to bromophenols in marine environment.

Extensively used under the assumption that BFRs are a safe class of flame retardants, they are widely distributed in the environment. Recently, some concerns have been expressed regarding their persistence and potential bioaccumulation.[5] TBBPA, PeBP, 2,4,6-TriBP, and TCBPA have proved toxicity.[11−15] Some of them (TBBPA, TCBPA) have shown significant thyroid hormonal activities (similar to chlorinated biphenyls), as well as estrogenic activity,[2] and are suspected to be carcinogenic.[12] Although at this moment there is no regulated restriction on the usage of these BFRs, a revision of TBBPA is currently underway in the EU.[16]

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(1) Simonsen, F. A.; Moller, M. L.; Madsen, T.; Stavnsbjerg, M. *Brominated flame retardants: toxicity and ecotoxicity*; Danish Environmental Protection Agency, Project 568, Kobenhavn, Denmark, 2000.
(2) Kitamura, S.; Jinno, N.; Ohta, S.; Kuroki, H.; Fujimoto, N. *Biochem. Biophys. Res. Commun*. **2002**, *293*, 554−559.
(3) WHO (World Health Organization), Environmental Health Criteria 172, Tetrabromobisphenol A and derivatives, World Health Criteria, Geneva, Switzerland, 1995.
(4) Hakk, H.; Letcher, R. J. *Environ. Int.* **2003**, *29*, 801−828.
(5) WHO (World Health Organization), Environmental Health Criteria 192, Flame retardants: A General Introduction, World Health Criteria, Geneva, Switzerland, 1997.
(6) Marinelli, R. L.; Woodin, S. A. *Biochem. System. Ecol.* **2005**, *33*, 559−570.
(7) Joyce Ma, W. C.; Chung, H. Y.; Ang, P. O. *J. Agric. Food Chem.* **2005**, *53*, 2133−2139.
(8) Barontini, F.; Cozzani, V.; Marsanich, K.; Raffa V.; Petarca, L. *J. Anal. Appl. Pyrolysis* **2004**, *72*, 41−53.
(9) Brebu, M.; Bhaskar, T.; Murai, K.; Muto, A.; Sakata Y.; Uddin, Md. A. *Chemosphere* **2004**, *56*, 433−440.
(10) de Wit, C. A. Brominated flame retardants, Swedish Environmental Protection Agency, Report 5065, Stockholm, Sweden, 2000.
(11) Ghost, M.; Meerts, I. A. T. M.; Cook, A.; Bergman, A.; Brouwer A.; Johnson, L. N. *Acta Crytallogr.* **2000**, *56*, 1085−1095.
(12) Meerts, I. A. T. M.; Van Zanden, J. J.; Luijks, E. A. C.; Van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman A.; Brouwer, A. *Toxicol. Sci*. **2000**, *56*, 95−104.
(13) Olsen, C. M.; Meussen-Elholm, E. T. M.; Samuelsen, M.; Holme J. A.; Hongslo, J. K. *Toxicol. Lett.* **2005**, *129*, 55−63.
(14) Olsen, C. M.; Meussen-Elholm, E. T. M.; Samuelsen, M.; Holme J. A.; Hongslo, J. K. *Pharmacol. Toxicol.* **2003**, *92*, 180−188.
(15) Strack, S.; Sander, M.; Detzel, T.; Kuch, B.; Krug, H. F. *Organohalogen Compd.* **2004**, *66*, 3795−3800.

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On the other hand, sewage sludges are disposed and used as an economical organic fertilizer in agriculture. However, it is well known that their field disposal if allowed, or its incineration, may cause the reintroduction and dispersion of toxic compounds in the environment. This can be the case of halogenated phenols and bisphenols, including the formation of polybrominated dibenzodioxins, polybrominated dibenzofurans, or both.[17] Thus, monitoring the levels of these compounds in sewage sludge would be needed to classify and define sludge disposal. TBBPA (log K_{ow} 4.5, solubility in water 0.72 mg/L) and TCBPA and its halogenated analogues are expected to associate with particulates or be adsorbed to organic matter in sludge and sediments.[18] Several environmental studies have shown the presence of significant concentrations of these compounds in sludges from sewage treatment plants in Sweden,[19,20] England,[21] and Canada,[22] as well as in sediments of Japan[23] and England (up to 9.8 μg/g TBBPA in river and estuarine sediments[21]). Additionally, some of these studies have shown the correlation of high TBBPA levels in sludges with electronic[19] or plastic[20] industrial activity or facilities.

Selective extraction of specific compounds from sewage sludge and sediments is recognized as a complicate task because real samples contain a large variety of contaminants differing in polarity and chemical nature, thus requiring highly efficient methods of extraction and sample cleanup. High content of organic matter in sludge makes difficult the attainment of good recoveries and makes necessary exhaustive extraction processes using large amounts of solvents and further multistep cleanup processes of the extract to provide an analyzable solution. Few analytical procedures have been reported for determination of TBBPA, TCBPA, or brominated phenolic compounds in sediment or sewage sludge samples, resorting generally to highly selective detection techniques (LC−MS[18] or GC/MS[19,24,25]). To our knowledge, only liquid−liquid extraction, solid-phase extraction (SPE), centrifugation,[18,24] and pressurized hot water extraction[24] have been successfully applied to the extraction of TBBPA from sediments and sludges. Sample preparation procedures dealing with the simultaneous extraction of TBBPA, TCBPA, and related bromophenols from solid environmental samples have not been reported.

Matrix solid-phase dispersion (MSPD) is a sample preparation method that allows the simultaneous sample homogenization, disruption, extraction of the analyzed compounds, and further cleanup in a single step using solid sorbents without resorting to dedicated or expensive instrumentation, thus providing significant reduction in both sample size and solvent consumption, as well as in analysis time and costs.[26,27] Currently, MSPD has been applied to extracting different types of microcontaminants (drugs, pesticides, PCBs, PAHs) and naturally occurring compounds) from a wide variety of solid or semisolid materials (food, serum, blood, tissues, plants, biota).[27−33] Recently, MSPD has been combined with pressurized solvent extraction, thus automating the final elution stages in MSPD.[28] Li et al.[34,35] have used this technique for the determination of pesticides in soil samples. However, from our knowledge, MSPD has not been reported for the extraction of organic contaminants from sewage sludge and sediment samples.

In this study, a one-step procedure for the simultaneous extraction and cleanup of some brominated phenols and halogenated bisphenols from sewage sludge and river and marine sediment samples using MSPD is described. The influence of important parameters, such as solid sorbent types and eluting solvent, on the yield of MSPD extraction process and on the cleanup efficiency was investigated, to achieve the best performance of the method. Analysis of the extracts was performed by nonaqueous capillary electrophoresis (NACE) coupled with photodiode array ultraviolet detection (DAD). Large-volume sample stacking injection using the electroosmotic flow pump (LVSEP) was applied as an on-column preconcentration technique allowing significant sensitivity enhancements. Finally, the developed method was applied for the determination of TBBPA, TCBPA, and related bromophenols in sewage sludge and river and marine sediment real samples.

EXPERIMENTAL SECTION

Reagents, Standards, and Materials. Methanol and acetonitrile (LiChrosolv gradient grade), ethyl acetate (LiChrosolv), and dichloromethane (Suprasolv) were obtained from Merck (Darmstadt, Germany). Formic acid (95−97%) was purchased from Aldrich (Madrid, Spain), sulfuric acid (96%) was from AnalytiCals Carlo Erba (Milano, Italy), and hydrochloric acid (36%) was supplied by Prolabo (Fontenay-Sous-Bois, France). Ultrapure water was obtained in the laboratory from a Milli-Q system purchased from Millipore (Bedford, MA).

2,4,6-TriBP (99%), PeBP (96%), TBBPA (97%), and TCBPA (98%) were obtained from Aldrich (Steinheim, Germany). 2,6-DiBP (97%) was purchased from Fluka (Buchs, Switzerland). Stock solutions of each phenol derivative were prepared at 2000 μg/mL in methanol. Diluted standard mixtures were prepared in both acetonitrile (used as calibration solutions) and methanol (used

(16) Alaee, M.; Arias, P.; Sjödin, A.; Bergman, A. *Environ. Int.* **2003**, *29*, 683−689.
(17) Wichmann, H.; Dettmer, F. T.; Bahadir, M. *Chemosphere* **2002**, *47*, 349−355.
(18) Chu, S.; Haffner, G. D.; Letcher, R. J. *J. Chromatogr., A* **2005**, *1097*, 25−32.
(19) Öberg, K.; Warman, K.; Öberg, T. *Chemosphere* **2002**, *48*, 805−809.
(20) Sellström, U. Determination of some polybrominated flame-retardants in biota, sediment and sewage sludge. Ph.D. dissertation, Stockholm University, Stockholm, Sweden, 1999.
(21) Morris, S.; Allchin, C. R.; Zegers, B. N.; Haftka, J. J. H.; Boon, J. P.; Belpaire, C.; Leonards, P. E. G.; Van Leeuwen, S. P. J.; De Boer, J. *Environ. Sci. Technol.* **2004**, *38*, 5497−5504.
(22) Lee, H. B.; Peart, T. E. *Water Qual. Res. J. Can.* **2002**, *37*, 681−696.
(23) Watanabe, I.; Kashimoto, T.; Tatsukawa, R. *Bull. Environ. Contam. Toxicol.* **1983**, *31*, 48−52.
(24) Hyötyläinen, T.; Hartonen, K. *Trends Anal. Chem.* **2002**, *21*, 13−29.
(25) Eljarrat, E.; Barceló, D. *Trends Anal. Chem.* **2004**, *23*, 727−736.
(26) Barker, S. A. *LC-GC Int.* **1998**, *11*, 719−724.
(27) Barker, S. A. *J. Chromatogr., A* **2000**, *885*, 115−127.
(28) Kristenson, E. M.; Ramos, L.; Brinkman, U. A. Th. *Trends Anal. Chem.* **2005**, *25*, 96−111.
(29) Barker, S. A. *J. Chromatogr., A* **2000**, *880*, 63−68.
(30) Ramil, M.; Hernánz, D.; Rodríguez, I.; Cela, R. *J. Chromatogr., A* **2004**, *1056*, 187−194.
(31) Carro, A. M.; Lorenzo, R. A.; Fernández, F.; Rodil, R.; Cela, R. *J. Chromatogr., A* **2005**, *1071*, 93−98.
(32) Martínez, A.; Ramil, M.; Montes, R.; Hernánz, D.; Rubí, E.; Rodríguez, I.; Cela, R. *J. Chromatogr., A* **2005**, *1072*, 83−91.
(33) Pensado, L.; Casais, M. C.; Mejuto, M. C.; Cela, R. *J. Chromatogr., A* **2005**, *1077*, 103−109.
(34) Li, Z.-Y.; Zhang, Z.-C.; Zhou, Q.-L.; Gao, R.-Y.; Wang, Q.-S. *J. Chromatogr., A* **2002**, *977*, 17−25.
(35) Li, Z.-Y.; Zhang, Z.-C.; Zhou, Q.-L.; Zhu, C.-S.; Wang, Q.-M.; Gao, R.-Y.; Wang, Q.-S. *J. AOAC Int.* **2003**, *86*, 521−528.

for spiking sludge and sediment samples) to appropriate concentration levels. All solutions were refrigerated at −18 °C and protected against daylight when not in use.

Sodium tetraborate decahydrate and sodium hydroxide were supplied by Merck, and sodium sulfate anhydrous was obtained from Panreac (Barcelona, Spain). Octyldecyl-functionalized silica gel C_{18} (9−12% carbon loading), Florisil (60−100 mesh), and aluminum oxide activated neutral (150 mesh) were purchased from Aldrich, and silica gel 60 (0.040−0.063 mm) was obtained from Merck. Acidic silica gel (40% sulfuric acid) was prepared in the laboratory by mixing neutral silica, previously dried at 120 °C for at least 12 h, with concentrated sulfuric acid. Florisil (when used activated) and acidic silica were heated in an oven at 120 °C for at least 12 h and then allowed to cool at room temperature in a desiccator before use. All these sorbents were stored in sealed bottles until analysis.

Durapore membrane filters (GVHP, 47 mm, 0.22 μm) and Durapore syringe filters (Millex GV, 13 mm, 0.22 μm) were supplied by Millipore. Isolute SPE syringe barrels (15-mL capacity) fitted with a single bottom frit and additional 20-μm polyethylene frits were obtained from International Sorbent Technology (Mid Glamorgan, UK).

Samples. Spiked and nonspiked sewage sludge and river and marine sediment samples were used in this study. Sludge samples were obtained from an urban sewage treatment plant near Santiago de Compostela (northwest Spain) equipped with primary and secondary treatments, which receives mostly municipal nonindustrial wastewaters. River and marine sediments were collected in different points in northwest Spain. All samples were kept at −18 °C until being lyophilized. After sieving, the fraction with a particle size below 300 μm was taken.

Optimization of sample preparation conditions was performed using a room-temperature air-dried primary sludge material spiked with different concentration levels of analytes.

Sample Preparation. Under finally optimized conditions, accurately weighed 0.2 g of lyophilized sewage sludge sample is first soaked and acidified with 200 μL of 10% aqueous hydrochloric acid and then thoroughly dried with 0.75 g of anhydrous sodium sulfate in a glass mortar. Then 2 g of Florisil is added and the mixture thoroughly blended with the pestle until homogeneous.

The dispersed sample is transferred to the top of a syringe barrel fitted with a single bottom frit containing a layer of 2 g of Florisil (cleanup sorbent in the mixed-mode column, or cocolumn), and the whole solid phase is covered with another 20-μm frit. The column is slightly compressed with a syringe plunger to eliminate voids and channeling.

The analytes are eluted from the cartridge with 2.5 mL of acetonitrile by gravity, allowing the eluate to drip slowly. Extracts are filtered through 0.22-μm Durapore syringe filters and subsequently subjected to CE analysis.

Regarding river and marine sediment samples, the procedure is analogous, except 200 μL of 20% aqueous hydrochloric acid and 300 μL of 36% hydrochloric acid are used respectively to soak and acidify the sample before drying. In both cases, only 1 g of Florisil was used as mixed-mode column.

CE Analysis. Capillary electrophoresis was performed using a HP^{3D} system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column DAD system. Analytes were monitored at 210 and 230 nm.

Uncoated narrow-bore silica capillary (supplied by Composite Metal Services Ltd.) with an effective/total length of 61.5/70 cm and 75-μm i.d. was used. Both the capillary and the samples were thermostated to 25.0 °C.

Unless otherwise stated, standards and sample extracts were injected hydrodynamically by applying a pressure of 50 mbar for 2 s. The applied voltage for separation was −30 kV.

New capillaries were rinsed with 1 M sodium hydroxide for 20 min. Before injections, capillaries were conditioned by washing them with 0.1 M sodium hydroxide for 5 min, Milli-Q water for 5 min, and separation electrolyte for 15 min. After each run, the capillary was flushed with Milli-Q water for 10 min. The inlet and outlet of the capillary were kept overnight in Milli-Q water. The background electrolyte solution was 20 mM sodium tetraborate prepared in methanol. The apparent pH of the solution was 9.4, adjusted by addition of a sodium hydroxide solution in methanol, and measured using a Metrohm 654 pH meter (Herisau, Switzerland) calibrated with aqueous standard buffer solutions. This solution was prepared fresh each 2 days, sonicated for 4 min, and filtered through a 0.22-μm pore size Durapore membrane. Everyday all solutions were filtered through 0.22-μm Durapore syringe filters before use. Data acquisition was carried out by means of HP^{3D} ChemStation Software (Rev. A.06.01[403]) (Hewlett-Packard).

LVSEP. Standard solutions and sample extracts dissolved in acetonitrile were introduced hydrodynamically into the capillary with a pressure of 50 mbar for 30 or 50 s, depending on the experiment. After sample injection, a negative voltage of −30 kV was applied for both sample stacking and subsequent separation. Fresh electrolyte and sample solutions were always used for each injection.

RESULTS AND DISCUSSION

Optimization of MSPD Extraction Conditions. Simplicity is one of the most attractive features in MSPD. Performance of MSPD is mainly affected by column packing and elution procedure, so it is important to select an appropriate sorbent enabling homogenization and disruption of samples, acting at the same time as separation material. Most of the reported MSPD applications have utilized reversed-phase materials, especially C_{18}.[27] Thus, preliminary experiments were performed with the sludge material using C_{18}, and then other sorbent materials were tested. Considering pK_a values of analytes (that range from 4.4 to 8.5 [36]), samples were acidified with formic acid (actual pH of analyzed sludge material was 6.8). Also, a drying step of the acidified sample with sodium sulfate was included to help the sorbent extracting the hydrophobic fraction of samples. In these preliminary experiments, methanol was selected as eluting solvent for compatibility reasons (direct injection of extracts) with the NACE separation and detection system.

In these initial experiments, colorful dark yellow extracts were obtained, even after additional cleanup stages involving 2 g of C_{18} mixed-mode column. NACE injections (not shown) of extracts indicated that analytes were extracted in the first 5-mL eluting

(36) Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (© 1994−2004 ACD/Labs), Chemical Abstracts Service (CAS), American Chemical Society, Washington, DC, 2004.

Table 1. Recoveries of Analytes from a Primary Sewage Sludge Sample Processed in Different Conditions[a]

MSPD conditions		recovery (%)					
mixed-mode column	solvent	2,4,6-TriBP	PeBP	2,6-DiBP	TBBPA	TCBPA	comments
1 g C_{18} + 2 g Alu	MeOH	74.7[b]	83.6[b]	nd	nd	nd	clean extracts, large solvent volume
1 g C_{18} + 0.5 g Alu	MeOH	84.5	82.8	57.8	55.9	58.1	many interferences
1 g C_{18} + 2 g Sil	MeOH	63.4	73.7	50.4	54.5	52.4	low recoveries
1 g C_{18} + 2 g Flo	MeOH	91.3	86.9	87.5	80.4	80.7	best sorbent option
1 g C_{18} + 3 g Flo	MeOH	70.4	73.7	65.0	68.9	71.0	decrease in recoveries
1 g C_{18} + 2 g Flo	DCM[c]	9.6	nd	26.6	4.4	nd	clean extracts, loses in evaporation
1 g C_{18} + 2 g Flo	AcOEt	43.7	16.9	19.0	46.1	40.1	clean extracts, loses in evaporation
1 g C_{18} + 2 g Flo	DCM−MeOH (70:30)	78.8[b]	87.3[b]	72.3[b]	74.2[b]	74.2	dirty extracts, large volume
1 g C_{18} + 2 g Flo	DCM−MeOH (1:1)	53.9[b]	50.7	42.6	47.1[b]	39.7	dirty extracts, large volume
1 g C_{18} + 2 g Flo	AcN[d]	79.8	47.0	84.7	62.9	62.8	clean extracts, best solvent option

[a] Solvent volume for elution 5 mL except in marked cases. Alu, neutral alumina; Sil, neutral silica; Flo, Florisil; MeOH, methanol; DCM, dichlorometane; AcOEt, ethyl acetate; AcN, acetonitrile; nd, nondetected. [b] Two succesive 5-mL fractions were collected and measured for analytes. [c] One fraction of 10 mL. [d] Fractions of 5 + 2 mL were collected.

fraction although very poor recoveries appeared. Moreover, a lot of peaks, some of them interfering with the analytes, appeared in the electropherograms. Consequently, other sorbents (including activated and nonactivated Florisil, neutral aluminum oxide, and acidic and neutral silica) were assayed as a mixed-mode column. Also, some other eluents (dichloromethane, ethyl acetate, acetonitrile, dichloromethane−methanol mixtures) were tested to screen the possibilities of developing a practical sample preparation procedure. The most significant results in these series of preliminary experiments have been summarized in Table 1.

When 2 g of aluminum oxide was used as a mixed-mode column, clean, colorless extracts were produced although two solvent fractions were needed to elute 2,4,6-TriBP, PeBP, and 2,6-DiBP. Only traces of TBBPA and TCBPA were eluted under those conditions. Reducing four times the amount of alumina allows eluting all the analytes from the cartridge, but the number of interferences also grows.

Better results in terms of recoveries were obtained with nonactivated Florisil. Neutral silica provided results similar to those with Florisil regarding the interference level although lower recoveries. On the other hand, higher amounts of Florisil do not improve recoveries as can be seen in Table 1.

Regarding solvents, dichloromethane produced clean, colorless extracts although with very low recoveries. Mixtures of dichloromethane with methanol allow better recoveries although yet unsatisfactory, and large volumes of solvent are needed to extract the analytes from the cartridge.

Acetonitrile provided the better performance in effectively removing the analytes from the cartridge using low solvent volumes and keeping the interferences under reasonable levels. Yellowish extracts fully compatible with the NACE procedure are produced. It should be noticed that some of the above comments regarding extracting solvents and the eventual need of solvent exchange before injecting the extracts are a consequence of the separation−detection NACE technique applied in this study. Other determination approaches or the use of more selective detection techniques (e.g., MS) probably would alleviate some these aspects. However, the use of NACE has allowed not only enlarging the analytical possibilities of this technique but also clearly showing the ability of the proposed sample preparation procedure to produce clean, easily analyzable extracts

After these preliminary experiments, it was clear that MSPD might be applied for the extraction of TBBPA, TCBPA, and the remaining related phenols from sludge although the experimental conditions need a systematic optimization procedure. Also, Florisil appeared as the most promising sorbent with these aims. Thus, it was decided to thoroughly evaluate the process using a mixed-level experimental design in 24 runs. Some experimental factors (sample amount 0.2 g; anhydrous sodium sulfate 0.75 g; 2 g of Florisil as dispersant material; and 2.5 mL of acetonitrile as eluent) were fixed on the basis of the described preliminary observations. Factors included into the experimental design were the predisruption additive and the column building layers structure. Both experimental design generation and data treatment were performed using the NemrodW V-2000D package.[37] Table 2 summarizes the assignment of factor levels in the experiment and the standardized main effects for the analytes. Significant factors (95% confidence level) are in boldface type in Table 2. The higher the absolute value of an effect, the larger the influence of the corresponding variable in the extraction yields. A positive sign indicates an improvement in the extraction efficiency and oppositely for negative signs.

Surprisingly (see Table 2), the type of additive was the important factor in all cases and not the kind of sorbent used as mixed-mode column for cleanup, although this second factor appeared important for PeBP extraction (the higher the ratio Florisil/C_{18}, the higher the recovery for PeBP). The use of formic or hydrochloric acid in the first stage of the procedure clearly improves the extraction efficiency. Regression analysis of the experimental results indicated that the maximum recoveries were attained using HCl 5% as additive and Florisil as mixed-mode column. Under these conditions, recoveries around 95% were obtained for all analytes except for PeBP (77%). Using more concentrated hydrochloric acid solutions allows increasing the extraction efficiency (87%) for PeBP that exhibits the lower pK_a value among the considered analytes.

LVSEP. Sensitivity Improvement. As mentioned previously, NACE-DAD in this study allowed us to evaluate the ability of MSPD to produce clean extracts, not needing additional time-consuming cleanup stages or resorting to highly selective analyti-

(37) NemrodW, Mathieu, D.; Nony J.; Phan-Tan-Luu, R.; LPRAI, Marseille, France, 2000.

Table 2. Experimental Domain for the Selected Factors in the Mixed Design and Effects Significance for the Considered Analytes

factor	level	key	2,4,6-TriBP	PeBP	2,6-DiBP	TBBPA	TCBPA
mixed-mode column	2 g C_{18}	A1	−0.050	**−0.193**	−0.262	0.117	−0.134
	1.34 g C_{18} + 0.66 g Florisil	A2	−0.007	−0.071	0.102	−0.143	0.157
	0.66 g C_{18} + 1.34 g Florisil	A3	−0.027	0.083	0.082	−0.046	−0.089
	2 g Florisil	A4	0.085	**0.181**	0.078	0.072	0.066
additive	without additive	B1	**−0.986**	**−0.562**	**−0.367**	**−1.234**	**−1.702**
	200 μL Milli-Q water	B2	**−0.306**	−0.139	−0.184	**−1.254**	**−1.455**
	200 μL formic acid 2%	B3	0.061	0.036	0.048	−0.419	−0.350
	200 μL formic acid 5%	B4	**0.396**	**0.201**	0.218	**0.921**	**1.198**
	200 μL HCl 2%	B5	**0.389**	0.106	0.146	**0.599**	**0.990**
	200 μL HCl 5%	B6	**0.446**	**0.358**	0.138	**1.384**	**1.320**

[a] Coefficients in boldface type correspond to significant effects (95% confidence level).

cal techniques for the analysis of the extracts. However, the NACE-DAD technique may only be applied for the analysis of environmental samples provided low detection limits are attained. In a previous paper,[38] we have proven that detection limits achieved with the NACE-DAD method, using methanol as running buffer solvent could be improved significantly by concentrating methanolic sample extracts directly in the capillary using LVSEP. In this case, acetonitrile has been used as eluting solvent so the LVSEP procedure needs adjustments to be applied.

Figure 1 shows the separation produced and the enhancement obtained using LVSEP-NACE-DAD with 50 s of hydrodynamic injection time. Graphs in Figure 1 compares the electropherograms for the spiked and nonspiked sludge sample, including also the electric current registry during the process. As can be seen (Figure 1, E trace), the sludge sample contains quantifiable amounts of 2,4,6-TriBP and, probably, traces of TBBPA and TCBPA although under the quantification limits. Enhancement of analytical signals is clearly visible, and calculated recoveries in spiked samples for LVSEP-NACE-DAD agreed with those calculated for NACE-DAD procedure.

Another obvious option to produce higher signals is processing higher sample amounts. Figure 2 shows the LVSEP-NACE-DAD separation obtained by processing 0.4 g of sample. In that case, 30 s of hydrodynamic injection was used to avoid peak splitting by overloading. Of course, in that case, the concentration of hydrochloric acid must be doubled as well.

Performance of the Method. The analytical performances of both the NACE-DAD (using 2 s of injection time) and LVSEP-NACE-DAD (50 s of injection time) procedures were compared using standard mixtures of the analytes. Results have been summarized in Table 3. Calibration curves were built by triplicate injections at four concentration levels in the range shown in the third column of Table 3. Precision was examined by performing six replicate injections of a 500 (in NACE-DAD) and 50 ng/mL (for LVSEP-NACE-DAD) standard mixtures in the same day or along different days). Instrumental detection and quantification limits were calculated at signal-to-noise ratios of 3 and 10, respectively, following IUPAC recommendations.[39] As can be seen, LVSEP improved 14−21-fold LODs and LOQs depending of the analytes.

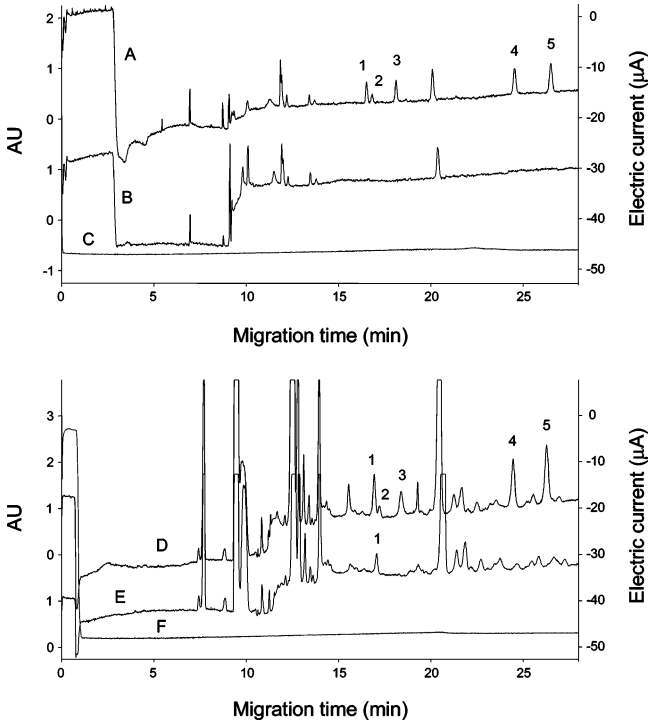

Figure 1. Electropherograms obtained for MSPD extracts of 0.2 g of primary sludge sample (A, D) spiked at a concentration of (A) 5 and (D) 0.5 μg/g for all compounds and (B, E) nonspiked. (C) Electric current during the NACE process. (F) Electric current during the LVSEP-NACE process. Hydrodynamic injection: 50 mbar during 2 s (A−C) and (D−F) 50 s. Peak assignation: (1) 2,4,6-TriBP, (2) PeBP, (3) 2,6-DiBP, (4) TBBPA, and (5) TCBPA.

At the time of conducting these experiments, to the best of our knowledge, no certified reference material for bromophenols and halogenated bisphenols in sludge or sediment was available, so a true validation could not be carried out. Recoveries of the MSPD extraction method were evaluated using six independent 0.2-g portions of primary sludge sample, freshly spiked at two concentration levels (5 μg/g and 500 ng/g), for all studied compounds. Nonspiked samples (blanks, $n = 3$) were also processed and taken into account for calculations. Only 2,4,6-TriBP was quantified in primary sewage sludge at a concentration of 252 ± 10 ng/g. Table 4 summarized the average recoveries obtained for each compound, ranging from 75.7 to 105.4%, with relative standard deviations below 7.7%. As shown in the table,

(38) Blanco, E.; Casais, M. C.; Mejuto, M. C.; Cela, R. *J. Chromatogr., A* **2005**, *1071*, 205−211.
(39) Currie, L. A. *Pure Appl. Chem.* **1995**, *67*, 1699−1723.

Table 3. Linearity, Precision, Detection, and Quantification Limits of NACE Methods

method	compound	calib range (ng/mL)	corr coeff (r)	repeatability[a] (RSD %)	reproducibility[a] (RSD %)	LOD (ng/mL)[b]	LOQ (ng/mL)[b]
NACE $t_{inj} = 2$ s[c]	2,4,6-TriBP	400−10000	0.9999	5.2	5.6	80.6	268.5
	PeBP		0.9999	4.7	4.6	76.0	253.3
	2,6-DiBP		0.9978	4.1	4.2	77.7	259.1
	TBBPA		0.9980	3.3	3.9	75.4	251.4
	TCBPA		0.9979	5.5	4.8	51.0	170.0
LVSEP-NACE $t_{inj} = 50$ s[c]	2,4,6-TriBP	25−600	0.9998	3.1	2.9	3.9	12.9
	PeBP		0.9999	3.5	3.2	5.3	17.7
	2,6-DiBP		0.9997	5.0	4.7	4.8	16.1
	TBBPA		0.9988	2.9	2.5	4.3	14.3
	TCBPA		0.9981	3.3	3.6	2.7	9.1

[a] $n = 6$ replicate injections. [b] Limit of detection (LOD), S/N = 3; limit of quantification (LOQ), S/N=10; detection at 210 nm, except of PeBP at 230 nm. [c] t_{inj} = injection time; other CE conditions as described in CE Analysis.

Table 4. MSPD Extraction Recoveries from Spiked Sewage Sludge and River and Marine Samples, Obtained under Optimized Conditions[a]

	primary sludge			secondary sludge		river sediment		marine sediment	
compound	recovery[b] (RSD %)	recovery[c] (RSD %)	LOQ[c] (ng/g)	recovery[c] (RSD %)	LOQ[c] (ng/g)	recovery[d] (RSD %)	LOQ[d] (ng/g)	recovery[d] (RSD %)	LOQ[d] (ng/g)
2,4,6-TriBP	99.6 (6.9)	98.2 (3.8)	129	106.4 (4.2)	162	78.1 (7.1)	232	92.2 (8.5)	232
PeBP	87.2 (4.8)	75.7 (3.0)	176	87.8 (2.5)	221	77.1 (7.0)	336	81.3 (8.0)	336
2,6-DiBP	91.7 (5.6)	97.1 (5.4)	161	105.0 (6.4)	202	92.2 (6.2)	182	92.6 (4.7)	182
TBBPA	101.3 (5.6)	102.3 (5.5)	143	98.0 (6.3)	179	83.6 (10.9)	285	90.5 (4.1)	285
TCBPA	105.4 (7.0)	100.3 (7.7)	91	102.3 (5.1)	114	79.4 (7.9)	236	96.8 (6.5)	236

[a] Limits of quantification of the compounds studied for each matrix using the proposed MSPD-NACE-DAD and MSPD-LVSEP-NACE-DAD methods. Relative standard deviation (RSD) $n = 6$ replicates; limit of quantification (LOQ), S/N=10, expressed in dry weight; detection at 210 nm, except of PeBP at 230 nm. [b] Spiked samples analyzed by MSPD-NACE-DAD, injection time 2 s; level of addition 5 μg/g. [c] Spiked samples analyzed by MSPD-LVSEP-NACE-DAD, injection time 50 s; level of addition 0.5 μg/g. [d] Spiked samples analyzed by MSPD-LVSEP-NACE-DAD, injection time 30 s; level of addition 0.5 μg/g.

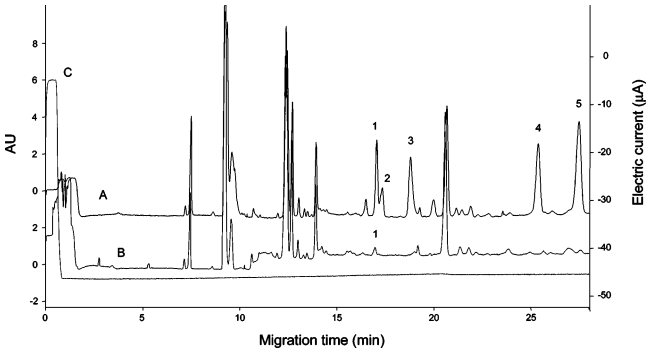

Figure 2. Electropherograms obtained for MSPD extracts of 0.4 g of primary sludge sample (A) spiked at a concentration of 5 μg/g for all compounds, and (B) nonspiked. (C) Electric current during the LVSEP-NACE process. Hydrodynamic injection: 50 mbar during 30 s. Peak assignation as in Figure 1.

recoveries achieved with MSPD-LVSEP-NACE-DAD analysis were in good agreement with those obtained for MSPD-NACE-DAD, thus showing that under these experimental conditions there were no matrix effects influencing the sample stacking process.

Application to Spiked Real Samples. To verify the real applicability of the proposed procedure, several samples of secondary biological sludge and river and marine sediments were analyzed.

For secondary sludges, a slightly decrease in extraction recoveries was observed, although this effect can be avoided by increasing the eluting solvent volume to 2.5 mL. For sediment samples, especially those containing higher amounts of calcium carbonate, higher concentrations of hydrochloric acid need to be used. Best results in terms of extraction recoveries were obtained using 200 μL of 20% HCl for river sediment samples and 300 μL of 36% HCl for marine sediments. On the contrary, Florisil amount used as mixed-mode column can be reduced to 1 g while granting clean extracts. A 2.5-mL aliquot of acetonitrile allows quantitative elution of analytes from the cartridges. Because of the higher ionic strength of these types of sample extracts, LVSEP injection times have to be reduced to 30 s at the logical expense of LODs. In these conditions, RSDs ranging from 0.9 to 6% were obtained. Results for these sample types are also summarized in Table 4.

CONCLUSIONS

For the first time ever, MSPD was applied for the extraction of brominated organic contaminants (TBBPA, TCBPA, and related bromophenols) from sediment and sludge samples. MSPD was demonstrated as a suitable preparation technique for the isolation of the compounds under study from sewage sludges and river and marine sediments, obtaining good recoveries and reproducibility. This opens new perspectives in sample preparation for important environmental matrixes of recognized analytical difficulty. Although NACE-DAD methodology was applied here, allowing us to show the cleanup potential of the MSPD technique, other more selective analytical techniques might be tested and

eventually being advantageous in the analysis of MSPD extracts. Employing acetonitrile as eluting solvent, MSPD extracts can be directly injected into the NACE system without any dilution or solvent exchange. It allows good compatibility between extraction and electrophoretic processes, allowing also LVSEP to improve quantification limits.

The whole method is fast as compared with the usual procedures of sludge sample extraction, with very low cost and minimum sample handling, and suitable for the routine determination of TBBPA, TCBPA, and related bromophenols in sewage sludge and river and marine sediment samples. Although not explored here, the combination of the developed MSPD procedure with pressurized solvent extraction could enhance and automate sample processing of this type of material.

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