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Online Hyphenation of Multimodal Microsolid Phase Extraction Involving Renewable Molecularly Imprinted and Reversed-Phase Sorbents to Liquid Chromatography for Automatic Multiresidue Assays

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Molecular imprinted polymers (MIP) have recently drawn much attention as highly selective solid-phase materials for handling and isolation of organic pollutants in complex matrices. Because of the impaired retention capacity for target species as compared with reversed-phase materials and irreversible sorption of interfering compounds by nonspecific interactions, the implementation of MIP-based solid-phase reactors as permanent components in automatic flow-systems has not received widespread acceptance as of yet. To tackle this limitation, a dynamic microscale solid phase extraction (μSPE) method capitalizing on the principle of programmable flow and bead injection analysis is herein proposed as a front end to liquid chromatography for multiresidue assays. It involves in-line renewable tandem-SPE microcolumns composed of molecularly imprinted polymers and copolymeric *N*-vinylpyrrolidone/divinylbenzene beads integrated within the flow network for multimodal extraction. Chlorotriazine herbicides (namely, atrazine, simazine, propazine) and principal degradation products thereof (namely, deisopropylatrazine and deethylatrazine) were selected as model analytes. The effect of several parameters, including the dimensions and chemical composition of the sorptive microcolumns, the sample loading flow rate, the type and volume of eluent, the interface with liquid chromatography (LC), and the disposable nature of the column on the analytical performance were investigated in detail. The assembled flow setup features appropriate removal of interfering organic species via solvent switch with toluene, the circumvention of analyte band-broadening in LC by in-line merging of the eluate with a water stream, and the transfer of the overall analyte-containing eluate into the LC. For 10-mL sample percolation, limits of detection (S/N = 3) of 0.02−0.04 ng mL^{-1}, limits of quantification (S/N = 10) of 0.07−0.12 ng mL^{-1}, absolute recovery percentages >79%, precision within 1.4−5.5%, and enrichment factors of 46−49 were obtained for the suite of assayed herbicides. The multimodal μSPE method with renewable beads was applied to the multiresidue determination of the target herbicides in crude soil extracts and untreated environmental waters at concentration levels below those endorsed by the current EU Water Framework Directives following appropriate sample preconcentration and/or cleanup.

Multiresidue determination of organic contaminants in environmental matrices does pose several challenges to the analyst as a result of the low concentration levels of target species and the concomitant existence of matrix interferences. Therefore, appropriate sample processing schemes are indispensable for removing interfering components while improving the detection capability of the analyte by application of preconcentration schemes prior to chromatographic separations.[1−3] Different formats of solid-phase extraction (SPE) or capillary sorbent microextraction, also implemented in a mechanized flow-based mode,[4−6] have been proposed and exploited over the past years for effective separation and preconcentration of organic pollutants in real-life samples.[2,3,7] Nonselective reversed-phase sorbent materials with tailored polarity are still the common choice for routine processing of environmental matrices.[1,2] Yet, interfering substances might be retained onto the solid surfaces and eluted concomitantly with the analytes. Although chromatographic methods (e.g., multidimensional chromatography) have excellent peak capacity, the quality of the analytical results and lifetime of chromatographic columns might be severely deteriorated whenever matrix effects are not appropriately overcome.

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(1) Richardson, S. D. *Anal. Chem.* **2009**, *8*, 4645–4677.
(2) Chen, Y.; Guo, Z.-P.; Wang, X.-Y.; Qiu, C.-G. *J. Chromatogr. A* **2008**, *1184*, 191–219.
(3) Hyötyläinen, T. *Anal. Bioanal. Chem.* **2009**, *394*, 743–758.
(4) Hyötyläinen, T. *J. Chromatogr. A* **2007**, *1153*, 14–28.
(5) Miró, M.; Hansen, E. H. *Trends Anal. Chem.* **2006**, *25*, 267–281.
(6) Hansen, E. H.; Miró, M. *Appl. Spectr. Rev.* **2008**, *43*, 335–357.
(7) Hennion, M. C. *J. Chromatogr. A* **1999**, *856*, 3–54.

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Immunoextraction sorbents[8] and man-tailored biomimetic material[9–13] are currently regarded as appealing SPE alternatives for selective isolation and/or preconcentration of target species prior to chromatographic separations. Molecularly imprinted polymers (MIP) are garnering increasing interest as compared to their biological counterparts for emulating molecular recognition as a result of the improved chemical stability and less stringent demands as to the reaction conditions in terms of pH and temperature.[10,12] Notwithstanding of the fact that the covalent or semicovalent approaches for MIP synthesis foster strong interactions of the analyte with functional monomers, the noncovalent approach is by far the most commonly exploited for generation of imprinting binding sites because of its universal applicability, faster sorption kinetics, and operational simplicity.[10,14] Ideally, raw samples and crude extracts could be directly processed on the basis of a selective uptake of analytes via imprinted binding sites. Experimental results, however, revealed that nonspecific interactions of matrix components with the copolymer matrix or residual monomers at the surface might hinder the efficient binding of target species to the specific recognition cavities with the consequent deterioration of the extraction recoveries and sorbent lifetime.[15−17] Matrix effects could be alleviated partially whenever the sample is appropriately buffered prior to MIP percolation[13] and optimized rinsing protocols with a given sequence of solvents are implemented within the analytical procedure.[10,13,17] Loading of the sample onto a nonimprinted polymer and MIP in series has been also proven suitable to minimize the nonspecific sorption of interfering species.[18,19]

The multistage nature of molecularly imprinted-SPE (MISPE) procedures, encompassing an additional solvent switch when processing water samples with noncovalent MIP,[13] made the automation of the overall protocol via flow-based approaches[5,20,21] cumbersome. Further difficulties are associated with the incompatibility of organic solvents inherent to MISPE of water matrices with the components of the flow network and HPLC separation. Moreover, a progressively tighter packing of the polymer is frequently observed whenever the MIP-containing packed reactor is utilized as an integral constituent of the flow network[22] and thus reused for a given number of assay cycles, which gives rise to the deterioration of the retention efficiency of target species.

To tackle the above limitations, we herein propose the exploitation of microscale SPE with renewable surfaces in flow systems, termed bead injection (BI), that automates the packing and retrieval of the sorbent material within the flow conduits in each individual assay.[23,24] To the best of our knowledge, no miniaturized BI−MISPE procedure hyphenated to chromatographic separations for multiresidue analysis has been reported as of yet. As a result of the low binding capacity of MIP by either reversed-phase or normal-phase interactions as compared to conventional nonspecific sorbent materials (e.g., octadecyl-chemically modified silicagel or (polar-enhanced) copolymeric phases) for uptake of target species,[25,26] an automatic tandem-column multimodal-BI approach combining water-compatible MIP and reversed-phase mixed-mode sorption prior to online LC separation has been developed and validated for selective preconcentration and determination of priority environmental pollutants at concentration levels below those endorsed by current EU Water Framework Directives.[27,28] Chlorotriazine herbicides and primary monodealkylated metabolites (deisopropylatrazine and deethylatrazine) of recognized acute and chronic toxicity to biota[29] were selected as model compounds for evaluation of the potential applicability of the automatic bidimensional-μSPE methodology with in-line disposable beads for analysis of untreated complex environmental samples (e.g, ground waters from domestic rural wells and soil extracts).

Sequential injection (SI) analysis based on using programmable, bidirectional, discontinuous flow as precisely computer controlled and coordinated by resorting to syringe pump(s)[5,20,30] has been herein selected as a flow approach for precise metering and automatic handling of given volumes of solutions, organic washing solvents, air, and bead suspensions at the low microliter level, for processing large sample volumes for enrichment and matrix cleanup purposes, and as an appropriate front-end to LC. Further, the versatility of the flow setup devised has been exploited for postcolumn processing of the BI eluate prior to online injection into LC to prevent band broadening of the most polar analytes.

EXPERIMENTAL SECTION

Chemicals and Solutions. Chlorotriazine herbicides, namely, atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine), simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine) and propazine (6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine), and their primary monodealkylated metabolite

(8) Hennion, M. C.; Pichon, V. *J. Chromatogr. A* **2003**, *1000*, 29–52.
(9) Sellergren, B. *Trends Anal. Chem.* **1999**, *18*, 164–174.
(10) Tamayo, F. G.; Turiel, E.; Martín-Esteban, A. *J. Chromatogr. A* **2007**, *1152*, 32–40.
(11) Caro, E.; Marcé, R. M.; Borrull, F.; Cormack, P. A. G.; Sherrington, D. C. *Trends Anal. Chem.* **2006**, *25*, 143–154.
(12) Alexander, C.; Andersson, H. S.; Andersson, L. I.; Ansell, R. J.; Kirsch, N.; Nicholls, I. A.; O'Mahony, J.; Whitcombe, M. J. *J. Mol. Recogn.* **2006**, *19*, 106–180.
(13) Pichon, V.; Chapuis-Hugon, F. *Anal. Chim. Acta* **2008**, *622*, 48–61.
(14) Pichon, V. *J. Chromatogr. A* **2007**, *1152*, 41–53.
(15) Masqué, N.; Marcé, R. M.; Borrull, F.; Cormack, P. A. G.; Sherrington, D. C. *Anal. Chem.* **2000**, *72*, 4122–4126.
(16) Caro, E.; Marcé, R. M.; Cormack, P. A. G.; Sherrington, D. C.; Borrull, F. *J. Chromatogr. A* **2003**, *995*, 233–238.
(17) Theodoridis, G.; Manesiotis, P. *J. Chromatogr. A* **2002**, *948*, 163–169.
(18) Cacho, C.; Turiel, E.; Martín-Esteban, A.; Pérez-Conde, C.; Cámara, C. *Anal. Bioanal. Chem.* **2003**, *376*, 491–496.
(19) Tamayo, F. G.; Casillas, J. L.; Martín-Esteban, A. *Anal. Bioanal. Chem.* **2005**, *381*, 1234–1240.
(20) Hansen, E. H.; Miró, M. *Trends Anal. Chem.* **2007**, *26*, 18–26.
(21) Miró, M., Hansen, E. H. On-line processing methods in flow analysis. In *Advances in Flow Analysis*; Trojanowicz, M., Ed.; Wiley-VCH:Weinhem, 2008; Chapter 11, pp 291−320.
(22) Dias, A. C. B.; Figueiredo, E. C.; Grassi, V.; Zagatto, E. A. G.; Arruda, M. A. Z. *Talanta* **2008**, *76*, 988–996.
(23) Ruzicka, J.; Scampavia, L. *Anal. Chem.* **1999**, *71*, 257A–263A.
(24) Miró, M.; Kradtap-Hartwell, S.; Jakmunee, J.; Grudpan, K.; Hansen, E. H. *Trend Anal. Chem.* **2008**, *27*, 749–761.
(25) Ferrer, I.; Lanza, F.; Tolokan, A.; Horvath, V.; Sellergren, B.; Horvai, G.; Barceló, D. *Anal. Chem.* **2000**, *72*, 3934–3941.
(26) Bjarnason, B.; Chimuka, L.; Ramström, O. *Anal. Chem.* **1999**, *71*, 2152–2156.
(27) European Commission, Council Directive 98/83/EC of 3 November 1998 relating to the quality of water intended for human consumption. *Off. J. Eur. Commun.* **1998**, *L330*, 32−54.
(28) European Commission, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Off. J. Eur. Commun.* **2000**, *L327*, 1−81.
(29) Ralston-Hooper, K.; Hardy, J.; Hahn, L.; Ochoa-Acuña, H.; Lee, L. S.; Mollenhauer, R.; Sepúlveda, M. S. *Ecotoxicology* **2009**, *18*, 899–905.
(30) Lenehan, C. E.; Barnett, N. W.; Lewis, S. W. *Analyst* **2002**, *127*, 997–1020.

products, namely, deisopropylatrazine (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine; DIA) and deethylatrazine (6-chloro-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine; DEA), were obtained from Sigma-Aldrich (Steinheim, Germany). Prometon (N^2,N^4-diisopropy-6-methoxy-1,3,5-triazine-2,4-diamine) was selected as internal standard for the chromatographic assays. The individual stock solutions of 20 μg mL^{-1} of DEA, atrazine, simazine, and propazine were prepared in methanol by diluting 2 mL of 100 μg mL^{-1} each to a final volume of 10 mL. The stock solutions of 10 000 μg mL^{-1} of DIA and prometon were prepared by dissolving 250 mg of each individual compound in pure methanol. All standards were stored in the darkness at 4 °C and stepwise diluted to the desired concentration for preparation of working solutions. The standard mixtures of triazines for manual injection into LC were prepared in 20% (v/v) acetonitrile/water to prevent band broadening effects. Ultrapure water was obtained from a Milli-Q water generator (Synthesis A10, Milllipore, Billerica, MA). HPLC-grade methanol and acetonitrile were supplied by Merck (Darmstadt, Germany) and toluene CHROMASOLV for HPLC by Sigma-Aldrich. Toluene was further purified by percolation through an Oasis HLB cartridge (200 mg, 6 mL) to remove potential impurities that might be further retained onto the packed sorbent material by nonspecific reversed-phase interactions and interfere with the chromatographic separation.

Sorbent bead materials used for cleaning up of raw samples and preconcentrative extraction of targeted chlorotriazines and metabolites thereof involved the class-specific, terbuthylazine-imprinted polymer from MIP technologies AB (Lund, Sweden) and supplied by Supelco (Bellefonte, PA) as SupelMIP SPE (SPE Triazine 10, Steinheim, Germany), *N*-vinylpyrrolidone−divinylbenzene copolymer (Oasis HLB, 30 μm, Waters, Mildford, MA), and spherically shaped octadecyl chemically modified silica (Upti Clean C18, 50 μm, Interchim, Montluçon, France).

Liquid Chromatograph. Liquid chromatographic assays were performed by resorting to HPLC 1100 system from Agilent Technologies (Palo Alto, CA) consisting of a vacuum degasser, a quaternary pump, a thermostat, and a UV/vis diode-array detector. Manual injections were conducted using a Rheodyne high-pressure six-port rotary valve (series 7725i) equipped with a 350 μL stainless steel loop (0.5 mm i.d. × 178.3 cm long). This valve was also exploited as the interface between the flow system and the analytical column. The target chlorocompounds and principal metabolite products thereof were separated and determined on a series of C18 reversed-phase guard column (Kromosil 100 C18, 5 μm, 15 × 4.6 mm, Scharlab, Barcelona, Spain) and analytical packed-bed column of identical chemical composition (Kromosil 100 C18, 3.5 μm, 150 × 4.6 mm, Scharlab) by a linear gradient elution from 20:80 to 70:30 (v/v) acetonitrile/water in 20 min at a flow rate 1.0 mL min^{-1}. Column temperature was controlled at 40 °C throughout the assays. Eluted chlorotriazines were monitored simultaneously at 220 and 225 nm and quantified by internal calibration based on prometon as internal standard using peak area measurement. Running of the LC gradient sequence, recording of chromatogram peaks, and data processing were performed automatically by a PC operated under the Chem Station Rev.10.01 software (Agilent).

Flow Setup. A multisyringe piston pump with programmable speed (MicroBu 2030, Crison Instruments, Alella, Barcelona, Spain) was used as a liquid driver for automation of the μSPE operations. It was equipped with two high-precision bidirectional syringes (Hamilton, Switzerland), labeled as S1 and S2 in Figure 1, with a capacity of 5.0 mL each and connected in block to a 40 000-step motor. S1 and S2 both contained Milli-Q water and were used for fluid delivery and postcolumn remixing of the organic eluate with Milli-Q water prior to LC separation, respectively. A three-way solenoid valve (N-Research, Caldwell, NJ) was placed at the head of each syringe enabling automatic connection with either the liquid reservoirs (OFF) or the flow network (ON). The multisyringe module was coupled to an eight-port multiposition selection valve operating as sample-processing unit (MPV, Valco Instruments, Houston, TX). The selection valve encompasses an ancillary central port and a communication channel (CC) that can be programmed to address each of the peripheral ports. The selection valve was connected via a 3.0 mL holding coil (HC, 1.5 mm i.d PTFE tubing) to S2 for microfluidic handling of the various components of the μSPE procedure (viz., sample, eluent, bead suspensions, and air). Bead containers made of 1 mL pipet tips were mounted vertically on ports 5 and 6 of the MPV using PEEK connectors for MIP and Oasis HLB, respectively. Bead suspensions of 1:4 (w/v) were prepared in pure methanol and 60% (v/v) methanol/water for Oasis HLB and MIP sorbents, respectively. The remaining ports were used for sequential aspiration of sample (port 3), air (port 4), methanol (port 7), and toluene (port 8) or as waste (port 2). The selection valve was connected via a 200 μL transfer line (1.5 mm i.d. PTFE) to a six-port rotary injection valve (IV, Valco) furnished with a cylindrical column (2 mm i.d., internal volume 25 μL) from transparent Kel-F fluoroplastic working as in-line container for capturing of the beads for renewable multimodal μSPE columns. The outlet of the microcolumn was equipped with a 10-μm polyethylene frit (Mo Bi Tec, Göttingen, Germany) for efficient trapping of the sorbent surfaces. The overall IV ports and internal channels were drilled to 1.5 mm i.d. and 2 mm width, respectively, for fostering the reproducible packing and removal of the sorbent column by programmable flow. Otherwise, undue flow impedance was eventually observed. A miniaturized Laboport diaphragm pump (65 W, KNF lab, Freiburg, Germany) was attached to a peripheral port of IV (port 5) for sorbent drying within the μSPE procedure. The IV outlet (port 2) and the delivery line from S1 merged at a Kel-F T-piece, which was connected with the high-pressure IV of LC via a 70 μL transfer PTFE line (0.8 mm i.d.). A schematic illustration of the hyphenated analytical setup for in-line selective preconcentration and chromatographic separation of targeted triazines is depicted in Figure 1. The operational procedures of the automatic μSPE procedure were computer controlled by the software package AutoAnalysis 5.0 (Sciware, Palma de Mallorca, Spain) based on dynamic link libraries (DLLs).

Sample Preparation and Characterization. *Soil Samples and Extraction Procedure.* Two different surface soils from agriculture sites in Mallorca (coded soil 1 and soil 2) were selected to investigate the reliability of the proposed flow assembly. Prior to chemical analysis, soils were oven-dried at 45 °C until constant weight and 2-mm sieved. Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ at a soil to solution ratio of 1:5 (w:v) after 2 h of

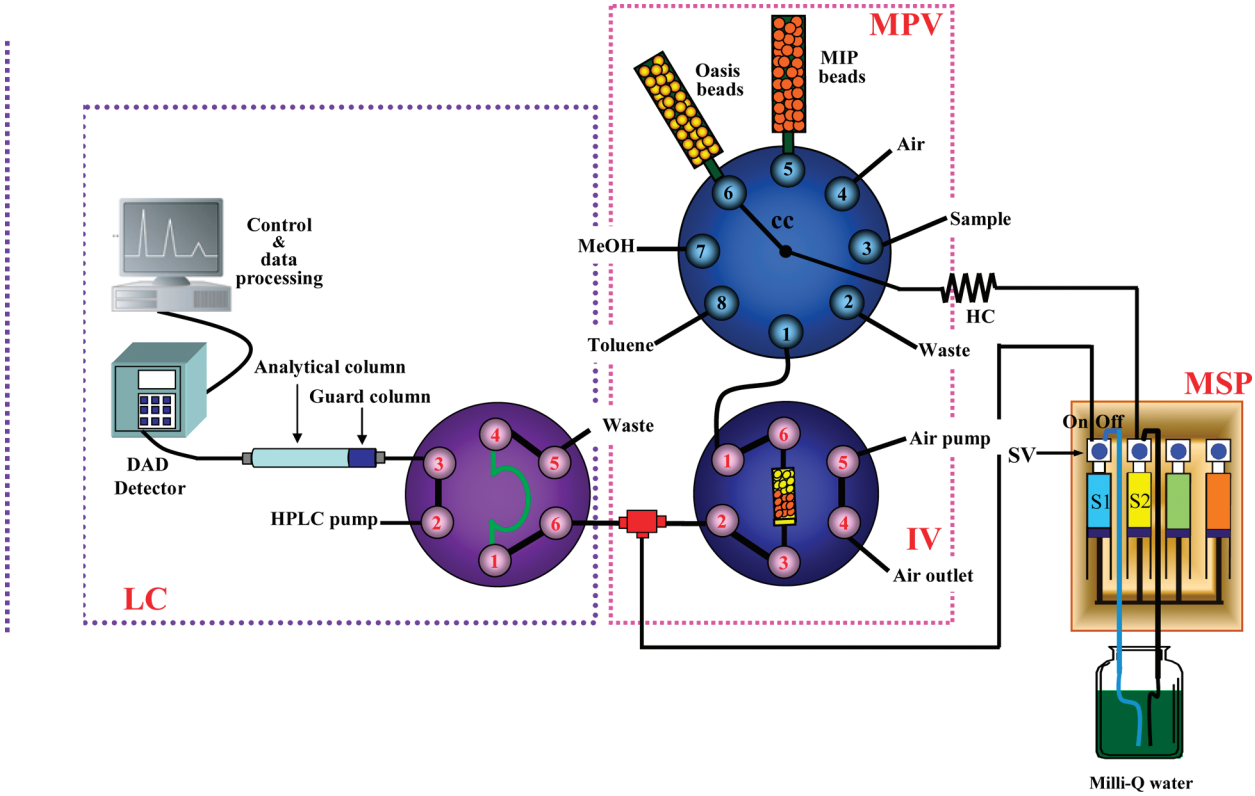

Figure 1. Schematic diagram of the hybrid flow system for automatic multimodal μSPE of trace level concentrations of chlorotriazines utilizing renewable surfaces as a front end to liquid chromatography. MSP, multisyringe pump; S, syringe pump; SV, solenoid valve; IV, injection valve; MPV, multiposition selection valve; HC, holding coil, CC, communication channel; DAD, diode-array detector.

equilibration using a combined pH electrode as endorsed by ISO 10390.[31] pH values of 7.52 ± 0.03 and 7.64 ± 0.07 were obtained for soil 1 and 2, respectively. Total organic carbon contents (TOC) of 8.55% and 8.80% for soil 1 and 2, respectively, were determined by dry combustion at 900 °C following release of carbonates with a few drops of 20% (v/v) HCl solution. Particle size distribution of the fraction <2 mm for determination of soil texture was performed with the Bouyoucos hydrometer method (ASTM type 152H).[32] Soil 1 consisting of 51.1% sand (0.05−2.0 mm), 34.5% silt (2−50 μm), and 14.4% clay (<2 μm) and soil 2 of 53.0% sand, 35.4% silt, and 11.6% clay, respectively, were classified as loam and sandy loam soil, respectively.

Surrogate contaminated soils were prepared by doping 80 g of sample with chlorotriazines and their principal metabolite products at two different concentration levels (20 and 50 ng g^{-1}) using a standard mixture containing 100 ng mL^{-1} each in acetone. Prometon was used as internal standard at the 50 ng g^{-1} level. A metered volume of acetone was added until the solvent completely covered the soil particles, whereupon the samples were gently stirred to ensure homogenization. Doped soils were air-dried at room temperature overnight and extracted thereafter. To this end, 5 g of soil (3 replicates each) was extracted in 40 mL of a dichloromethane/methanol (9:1) mixture as recommended by Chapuis et al.[33] with the aid of ultrasonic energy (150 W, 50 Hz, P-Selecta Asincro, Spain) for 15 min. The extract was filtered through 0.45-μm cellulose acetate membrane and the filtrate was evaporated gently to dryness by a nitrogen stream. The solid residue was dissolved in 150 μL of pure methanol with ultrasonic assistance for 3 min, to which 3 mL of Milli-Q water were added. The methanolic solution was filtered and made up to a final volume of 5 mL with Milli-Q water prior to analysis by the automatic SI−BI−HPLC assembly.

Water Samples. Drinking tap water (Palma de Mallorca), domestic well water (Valldemossa, Mallorca), and potentially contaminated creek water from a rural site (Muro, Mallorca) were analyzed for chlorotriazine content. Water samples were vacuum filtered through 0.45-μm cellulose esters filters (Millipore) previously rinsed with pure methanol and analyzed without pH adjustment.[34] For recovery tests, the overall water samples were spiked at the 0.5 and 2.0 ng mL^{-1} levels with the target herbicides and metabolites thereof using prometon as the internal standard at the 5 ng mL^{-1} level.

Analytical Procedure. The complete operational sequence of the hyphenated analytical setup for automatic preconcentration, separation, and determination of target triazine herbicides in untreated waters and crude soil extracts encompassed the injection of the multimodal beads and packing of the tandem μSPE column, conditioning of the column, sample loading, rinsing or solvent switch, analyte elution, injection of eluate into LC, and

(31) *International Organization for Standardization, ISO 10390: Soil Quality—Determination of pH*; International Organization for Standardization: Geneva, Switzerland, 1994.

(32) Dietrich, H. *SOP METH004.00: Standard operating procedure for determining soil particle size using the hydrometer method*; Environmental Monitoring Branch, California Department of Pesticide Regulation: Sacramento, CA, 2005.

(33) Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, B.; Hennion, M. C. *J. Chromatogr. B* **2004**, *804*, 93–101.

(34) Ferrer, I.; Hennion, M. C.; Barceló, D. *Anal. Chem.* **1997**, *69*, 4508–4514.

discarding beads. The operational details of the overall analytical procedure are detailed in the following.

Sorbent Packing and Equilibration. First, S2 was set to aspirate consecutively 100 μL of air, 100 μL of MeOH, and 30 μL of MIP suspension in 60% (v/v) methanol/water from the external reservoirs into HC, whereupon the MIP beads were delivered by flow reversal to IV, captured in the external microcolumn, and rinsed by methanol and ancillary 600 μL of carrier (Milli-Q water). An identical procedure was programmed for aspiration and trapping of 10 μL of Oasis HLB suspension to generate the multimodal μSPE tandem column. Flow rates for aspiration of bead suspensions and solutions into HC were affixed to 0.3 and 1.5 mL min^{-1}, respectively. Both sorbent materials were packed into the microcolumn container at 1.0 mL min^{-1}. Beads were further conditioned by consecutive delivery of 500 μL of MeOH and 500 μL of carrier.

Sample Loading. S2 was programmed to aspirate 100 μL of air and 2000 μL of water sample consecutively into HC for further perfusion through the renewable tandem column at 1.0 mL min^{-1}. These operational steps were 5-fold repeated in order to handle a total sample volume of 10 mL. The surplus of air in HC was delivered to waste to release the system pressure. Sorbent cleanup was effected by loading 800 μL of carrier solution through the packed microcolumn following sample percolation. For soil extracts, the sample volume perfused was affixed to 1000 μL.

Solvent Switch for Soil Extracts. Further cleanup with toluene was conducted for soil extracts to promote selective interactions of target species with the imprint sites within the MIP. To this end, compressed air was pumped for 10 min through the packed microcolumn once the IV was activated to the inject position in order to strip out the remaining water from the sorbent materials, which would otherwise give rise to partial losses of analytes in the washing step. At the same time, the line communicating this IV with MPV was filled with air. Thereafter, IV was switched to the load position and S2 was programmed to perfuse the analyte-containing tandem column with an air-segmented plug of toluene (800 μL) at 0.5 mL min^{-1}. Prior to elute the sorbed species, the multimodal μSPE column should be again dried with compressed air for 10 min to remove traces of toluene leftover within the column dead volume. The IV loop of LC was simultaneously rinsed by a 1000 μL MeOH plug at 1.5 mL min^{-1}.

Analyte Elution. Retrieval of preconcentrated triazine residues was accomplished by resorting to a 170 μL MeOH segment followed by an air plug (both delivered at 5 μL s^{-1}) to prevent dispersion of the eluate within the carrier solution. The eluate plug was merged downstream with an identical volume of water provided by S1 at 0.5 mL min^{-1} prior to entering into the injection loop of the high pressure IV, whereupon the valve was switched to the injection position, and thus, the LC gradient protocol was initiated. Hence, the LC separation was synchronized with the SI−BI procedure, whereby a given volume of aqueous sample or soil extract was analyzed while the ensuing one was being processed in the hybrid flow system. The automated operational sequence for a single sample lasted 30 min for waters and 40 min for soil extracts, thus matching the time frame of the chromatographic run and re-equilibration of the analytical column to the initial conditions, which amounted to 30 min.

Bead Discarding. Renewal of the trapped tandem column involved a preliminary step of sorbent moistening with 800 μL of MeOH at 1.5 mL min^{-1}, whereupon the beads were drawn into HC by flow-reversal at 1.0 mL min^{-1} and then dispensed into waste through port 2 by a carrier stream at 3.0 mL min^{-1}. Hence, the hybrid flow system is ready to initiate a new analysis cycle with a fresh portion of beads, thus overcoming the potential irreversible sorption of interfering matrix ingredients and sample cross-contamination as well between consecutive runs.

RESULTS AND DISCUSSION

Selection of Sorbent Material. Preliminary tests in a mesofluidic lab-on-a-valve platform[6,20,35,36] were conducted to ascertain the sorptive preconcentration capabilities of a series of bead materials with different functionalities for uptake of the overall triazine residues along with the more polar dealkylated metabolites in a μSPE format. Three different reversed-phase sorbent materials, namely, water-compatible MIP, copolymeric Oasis HLB, and Upti-Clean C18 silica beads, were investigated in this work as single-use solid extractants in the flow network. The latter two sorptive materials are spherically shaped and uniform in size distribution, thus fostering their reproducible in-valve manipulation and usage in a renewable mode. Despite the lump-type morphology of the commercially available MIP, automatic handling via programmable flow within the flow conduits was proven feasible by appropriate selection of the dispersion medium [in our case 60% (v/v) MeOH/H_2O] to retard bead settlement into the central processing valve unit.

Sorption capabilities of individual packed columns of Oasis HLB (3.0 ± 0.3 mg), Upti Clean C18 (7.0 ± 0.8 mg), and triazine-MIPs (3.2 ± 0.5 mg) were investigated for the enrichment of 1 mL of mix standard of chlorotriazines and chlorinated degradates thereof containing 100 ng mL^{-1} each. Elution was effected with 100 μL of pure MeOH. Oasis HLB was proven to be the most suitable sorbent material in a miniaturized SPE format for the uptake of both parent compounds and monodealkylated metabolites (see Figure 2). This is a consequence of the mixed-mode sorptive behavior of the copolymeric material bearing a balanced ratio of hydrophobic and hydrophilic moieties. On the other hand, the retention efficiency of less hydrophobic DIA and DEA onto the spherical C18 beads dropped by 30−50% as compared with the copolymeric Oasis HLB sorbent (see Figure 2). This also holds true for the MIP material under reversed-phase chemical interactions. This denotes the limited binding capacity of noncovalent MIP for uptake of moderately polar triazines in water matrices via nonspecific sorption onto the residual monomers at the outer surface of the polymer. Despite the miniature dimensions of the renewable in-line MIP reactor herein, this observation is in good agreement with earlier findings in dynamic MISPE using packed-bed permanent columns with >30-fold increased amount of sorbent.[25,37]

(35) Miró, M.; Hansen, E. H. *Anal. Chim. Acta* **2007**, *600*, 46–57.
(36) Quintana, J. B.; Miró, M.; Estela, J. M.; Cerdà, V. *Anal. Chem.* **2006**, *78*, 2832–2840.
(37) Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, S.; Hennion, M. C. *J. Chromatogr. A* **2003**, *999*, 23–33.

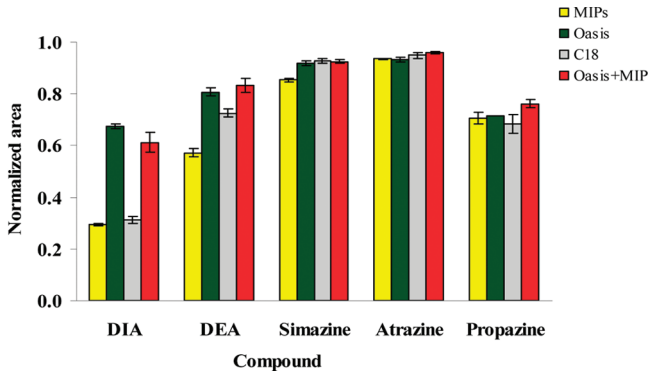

Figure 2. Effect of the chemical nature of the sorbents in a single-μSPE or tandem-μSPE column fashion in the uptake of chlorotriazines and dealkylated metabolites thereof from 1 mL of mixed standard at the 100 ng mL^{-1} level.

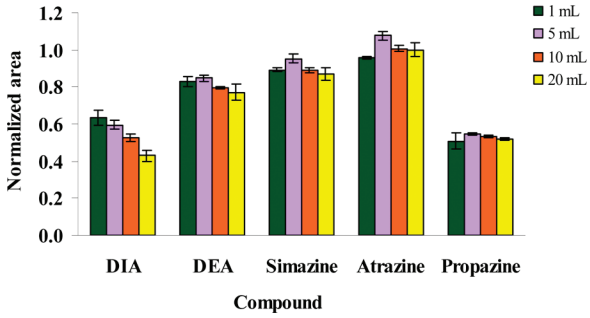

Figure 3. Investigation of breakthrough volumes for the μSPE−BI tandem column in the preconcentration of a given amount of chlorotriazines (100 ng) in 1, 5, 10, and 20 mL of 100, 20, 10, and 5 ng mL^{-1}, respectively. Analytes were eluted by 170 μL of pure methanol at 0.5 mL min^{-1}.

To tackle the aforementioned drawbacks that impede the processing of large volumes of untreated water samples onto MIP reactors, a flow-through multimodal renewable-bead sorptive procedure encompassing a prior uptake and enrichment of species onto *N*-vinylpyrrolidone−divinylbenzene copolymer (Oasis HLB) followed by further cleanup on MIP is herein proposed for preconcentration and determination of triazine residues at environmentally relevant levels. An in-loop microcolumn (in lieu of LOV) arrangement (see Figure 1) was devised to admit larger amounts of sorbents (2.7 ± 0.3 mg of Oasis HLB plus 7.0 ± 1.0 mg of MIP) and foster the efficient drying of beads prior to elution. The idea behind this configuration was to ensure the selective and repeatable sorptive preconcentation of target species when handling complex matrices. The improved recoveries for the less hydrophobic dealkylated metabolites compared to MIP alone (see Figure 2) demonstrated the suitability of this bidimensional μSPE procedure for processing samples containing chlorotriazines with a broad spectrum of polarities.

Investigation of the maximum enrichment factors attainable with the tandem column arrangement was performed in terms of breakthrough volumes. To this end, a given amount of target chlorotriazines (100 ng each) in a mixed standard was loaded onto the column in increasing sample volumes, as shown in Figure 3. No breakthrough was observed for any of the target species up to 20 mL, excepting for DIA, for which significant pre-elution was observed at volumes >10 mL (as compared to 1 mL). In fact, absolute recoveries of merely 51% for DIA have been earlier reported in online MISPE procedures when loading water volumes as low as 1.5 mL.[38] For appropriate preconcentration of the most polar metabolite residue and parent compounds as well, sample percolation volumes were affixed to ≤10 mL.

The sample loading and elution flow rates for the μSPE procedure were affixed to 0.5 and 0.3 mL min^{-1}, respectively, to ensure quantitative uptake of the overall target species including the more polar chlorinated degradates onto the miniaturized sorptive columns and efficient retrieval in a minimum volume of eluent for subsequent online injection into LC without pressure drop within the analytical sequence.

Online Coupling of Bead-Injection Tandem Column to LC. A survey of the literature revealed that online hyphenation of class-specific MIP sorbents with chromatographic separations is not a common practice.[10] This is most likely a consequence of the band-broadening of moderately polar species whenever the optimum hydroalcoholic MIP eluent with high methanol or ethanol content is delivered to the LC.[38,39] In our case, DIA and DEA could not be accurately quantified whenever volumes >50 μL in pure methanol were directly injected into LC because of undue peak dispersion and eventual coelution in the void volume.

To overcome the lack of compatibility of the eluent medium with the isocratic or gradient LC separation, a plethora of authors exploited online column-switching methods[7,40] involving the elution of the sorbed species with the mobile phase itself. This approach might, however, be inappropriate for attaining high enrichment factors, because of the incomplete elution of hydrophobic species or excessive spreading of the elution band. In-line heart-cut elution schemes[41] encompassing the injection of a small segment of solvent with increased elution strength into LC do not render improved sensitivity because of the partial loss of the preconcentration capabilities gained during analyte sorption.

In this work, a SI-based programmable elution mode was selected aimed at the introduction of the overall solvent volume of optimum elution strength into the LC. One of the cornerstones of flow-based systems is the controllable sample/reagent dispersion, which was initially exploited for in-tube dilution of the eluate band into the carrier stream toward the LC and within the injection loop itself. By this means, no appreciable band broadening for either triazine metabolite was observed for 150 μL of 90% (v/v) MeOH/H_2O or 120 μL of 100% MeOH utilizing the SI manifold described previously. Quantitative elution of the most hydrophobic parent compounds was, however, not accomplished. In fact, the higher the volume of pure methanolic eluent percolated through the tandem column, the better were the absolute recoveries of the target species up to 170 μL eluent, which was chosen for the remainder of the work.

To ensure quantitative stripping out of triazines from the multimodal-μSPE column and efficient LC band-focusing, the SI manifold was hyphenated with a multisyringe flow setup for appropriate in-line dilution. To this end, the 170 μL methanolic plug was merged downstream with a water-stream provided concurrently by one of the liquid drivers of the multisyringe device

(38) Koeber, R.; Fleischer, C.; Lanza, F.; Boos, K.-L.; Sellergren, B.; Barceló, D. *Anal. Chem.* **2001**, *73*, 2437–2444.
(39) Zamora, O.; Paniagua, E. E.; Cacho, C.; Vera-Avila, L. E.; Pérez-Conde, C. *Anal. Bioanal. Chem.* **2009**, *393*, 1745–1753.
(40) Pyrzynska, K.; Pobozy, E. *Crit. Rev. Anal. Chem.* **2002**, *32*, 227–243.
(41) Oliferova, L.; Statkus, M.; Tsysin, G.; Shpigun, O.; Zolotov, Y. *Anal. Chim. Acta* **2005**, *538*, 35–40.

Table 1. Analytical Performance of the SI–BI Setup Hyphenated to LC for Determination of Trace Level Concentrations of Chlorotriazines at Varied Sample Volumes

procedure	compound	retention time (min)	normalized regression equation	correlation coefficient (r)	linear range (ng mL^{-1})	enrichment factor	absolute recovery (%)	RSD (%)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
10 mL sample loading[a]	DIA	4.6	$y = 0.1830x - 0.0282$	0.9993	0.12–10	46	79	5.5	0.04	0.12
	DEA	6.9	$y = 0.2370x - 0.0232$	0.9997	0.1–10	49	83	2.1	0.03	0.1
	simazine	10.9	$y = 0.2378x - 0.0125$	0.9990	0.1–10	48	81	1.4	0.03	0.1
	atrazine	14.1	$y = 0.2332x - 0.0093$	0.9994	0.1–10	46	79	1.6	0.02	0.07
	propazine	17.1	$y = 0.1932x + 0.0060$	0.9998	0.1–10	47	80	3.5	0.03	0.1
1 mL sample loading[b]	DIA	4.6	$y = 0.0187x - 0.0016$	0.9984	5–100	4.0	68	6.0	0.5	1.7
	DEA	6.9	$y = 0.0249x + 0.0037$	0.9999	5–100	3.8	64	5.1	0.4	1.3
	simazine	10.9	$y = 0.0243x + 0.0288$	0.9998	5–100	3.1	52	4.3	0.5	1.5
	atrazine	14.1	$y = 0.0246x + 0.0395$	0.9995	5–100	3.0	51	5.0	0.3	1.1
	propazine	17.1	$y = 0.0210x + 0.0175$	0.9999	5–100	2.8	47	4.7	0.5	1.7

[a] Intended for water assays. [b] Intended for soil extract assays. The analytical method comprises in-line cleanup with toluene. Analytical wavelength: 220 nm. LOD and LOQ were calculated as S/N = 3 and 10 on the basis of the analytical signals at the 5 ng mL^{-1} level. Calibration standards: (a) 0.1, 0.5, 1.0, 2.0, 5.0, and 10 ng mL^{-1}; (b) 5.0, 10, 20, 50, and 100 ng mL^{-1}.

(S1) in a 1:1 water/eluent ratio (see Figure 1), thereby rendering a final eluate plug composition of 50% (v/v) MeOH/water, which satisfied the requirements for the LC separation.

Online coupling of noncovalent MIP with LC might be also troublesome as a result of remains of organic solvent from sorbent cleanup within the packed reactor, which might deteriorate further LC separation of target species. Method development experiments should thus include the exploration of appropriate washing solvents and in-line cleanup procedures as described below.

Analytical Performance. The beauty of the hyphenated hybrid flow system furnished with the tandem BI column lies in the fact that both the selectivity and sensitivity of analytical methods might be tuned at will attending the analysis needs. Detectability of triazine residues can be greatly enhanced by loading large sample volumes onto *N*-vinylpyrrolidone–divinylbenzene, yet analytes are retained primary by hydrophobic interactions and sorption is thus fairly nonspecific in nature. Further cleanup for removal of concomitantly adsorbed interfering substances might be accomplished via solvent switch to disrupt the hydrophobic interactions and in-line retention of chlorotriazines within the complementary imprinted sites of the bottom MIP. In order to change the retention conditions of target species to the selective normal-phase mode, a weakly polar and aprotic solvent is needed. Dichlomethane or toluene are the solvents of choice in noncovalent MIP-based triazine assays.[25,37,38,42] The former was proven inappropriate in our configuration because residual traces of solvent leftover after the washing step overlapped severely with simazine and interfere with other analyte peaks as well. On the other hand, possible remains of toluene after 10 min of drying as detailed previously did not interfere with either of the target peaks. Toluene was therefore selected for promoting hydrophilic, hydrogen-bonding-type interactions of chlorotriazines and metabolites thereof with MIP sites.

In order to assess the efficiency of the μMISPE column for selective uptake of triazines against chlorinated herbicides with similar chemical structure, polarity, and molecular size, diuron (*N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea) was selected as a model compound for cross-reactivity studies. A mixed standard solution of target chlorotriazines at the 30 ng mL^{-1} each was doped with the interfering species at the same concentration level. Following loading of 1 mL standard onto the multimodal microcolumn, increasing volumes of toluene ranging from 0 to 1000 μL were percolated through the tandem microcolumn to foster the selective redistribution of the analytes from the Oasis HLB top sorbent to the imprinted sites of the bottom MIP. Experimental results revealed that the higher the volume of washing solvent, the better was the removal of diuron interference, yet concomitantly the most significant were the losses of the more hydrophobic triazines, which might jeopardize the sensitivity requirements of the assays. These results are in good agreement with earlier findings when handling noncovalent MIP in a batchwise MISPE mode.[18] By employing a rinsing volume of 800 μL toluene, diuron interference (peak area) was removed >80% and propazine and simazine were washed off ≤10%. Quantification of the parent structural analogues was accurately performed in the presence of diuron with deviations <10%.

Under the optimized chemical and physical variables detailed in the foregoing sections, the figures of merit of the hybrid flow system are summarized in Table 1, including retention times, calibration graphs, dynamic linear ranges, enrichment factors, absolute recoveries, repeatability, reproducibility, and sensitivity for further determination of the suite of herbicides in either untreated water samples or crude extracts. In the former, 10-mL samples doped with 5 ng mL^{-1} internal standard (prometon) were perfused through the packed beads and analyzed without solvent switch (see the section on real sample analysis). In the latter, 1-mL samples spiked with 50 ng mL^{-1} prometon were processed in the flow setup and analyzed by LC following bidimensional BI-μSPE with toluene washing.

For quantification of the parent triazines and dealkylated metabolites, five-level calibration plots were exploited in the entire set of assays with determination coefficients >0.9984. Concentration ranges spanned over 2 orders of magnitude, that is, 0.1–10 or 5–100 ng mL^{-1}, in accordance with the standard/sample volume percolated. Absolute recovery percentages were calculated as the ratio of the peak areas in the online SI–LC methods and those from 100 μL direct chromatographic injection of an equivalent mass in a medium matching the initial chemical composition of the LC gradient elution. Enrichment

(42) Turiel, E.; Martín-Esteban, A.; Fernández, P.; Pérez-Conde, C.; Cámara, C. *Anal. Chem.* **2001**, *73*, 5133–5141.

Table 2. Concentrations and Recoveries of Target Chlorotriazines and Metabolites in Untreated Environmental Waters[a]

	tap water		ground water		creek water	
compound	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)	2.0 ng mL^{-1} spiked (recovery, %)	0.5 ng mL^{-1} spiked (recovery, %)
DIA	1.95 ± 0.07 (98)	0.54 ± 0.02 (108)	2.07 ± 0.07 (104)	0.59 ± 0.02 (119)	2.05 ± 0.09 (103)	0.56 ± 0.01 (112)
DEA	1.89 ± 0.03 (95)	0.47 ± 0.03 (94)	2.06 ± 0.02 (103)	0.52 ± 0.05 (104)	1.97 ± 0.04 (98)	0.44 ± 0.01 (88)
simazine	1.92 ± 0.04 (96)	0.46 ± 0.07 (92)	2.00 ± 0.04 (100)	0.53 ± 0.01 (106)	1.88 ± 0.03 (94)	0.46 ± 0.02 (92)
atrazine	1.96 ± 0.04 (98)	0.57 ± 0.05 (114)	1.96 ± 0.03 (98)	0.51 ± 0.01 (101)	1.93 ± 0.02 (96)	0.47 ± 0.03 (94)
propazine	1.75 ± 0.02 (88)	0.48 ± 0.06 (96)	1.91 ± 0.01 (96)	0.50 ± 0.01 (100)	1.91 ± 0.04 (96)	0.43 ± 0.01 (86)

[a] Sample volume: 10 mL.

Table 3. Concentrations and Recoveries of Target Chlorotriazines and Metabolites in Crude Extracts of Agriculture Soils[a]

	soil 1		soil 2	
compound	50 ng g^{-1} spiked (recovery, %)	20 ng g^{-1} spiked (recovery, %)	50 ng g^{-1} spiked (recovery, %)	20 ng g^{-1} spiked (recovery, %)
DIA	55.7 ± 4.8 (111)	22.8 ± 5.5 (114)	55.8 ± 2.1 (112)	24.0 ± 1.4 (120)
DEA	46.3 ± 3.4 (93)	16.8 ± 2.8 (84)	52.7 ± 1.0 (105)	22.3 ± 1.6 (112)
simazine	49.9 ± 2.4 (100)	20.6 ± 3.5 (103)	50.9 ± 0.6 (102)	23.9 ± 4.7 (120)
atrazine	44.7 ± 1.4 (89)	18.2 ± 3.3 (91)	46.8 ± 0.4 (94)	20.1 ± 1.9 (100)
propazine	45.0 ± 1.0 (90)	21.2 ± 2.8 (106)	43.3 ± 0.5 (87)	21.1 ± 0.4 (106)

[a] Sample volume: 1 mL.

factors were calculated as the ratio of the linear range sensitivity of the proposed SI−preconcentration procedures and that obtained by direct injection of 170 μL standard solutions into LC.

Method repeatability was expressed as the precision obtained from six consecutive measurements of a 10 mL mixed standard solution at the 2 ng mL^{-1} level using a permanent multimodal sorbent column. Relative standard deviations ranging from 1.4% to 5.5% (see Table 1) were better than repeatability values previously reported in batchwise MISPE procedures for determination of chlorotriazines where RSDs varied from 9 to 18%[25] or 4 to 8%[42] and batchwise MISPME where RSDs of 5.7%−10.6%[43] and 4−10%[44] were reported. The overall procedure reproducibility was expressed as the RSD of six consecutive analysis of 1 mL mixed standard at the 20 ng mL^{-1} level exploiting renewable surfaces. The automatic SI−tandem column BI method rendered improved RSDs for triazines (4.3−6.0%) as compared to manual MIP-based extraction protocols with reproducibility values of 7−15%[44] or 5−10%.[42]

The LODs and LOQs, calculated at a peak-to-peak signal-to-noise ratio (S/N) of 3 and 10, respectively,[45,46] for analysis of 10 mL-spiked water at the 0.5 ng mL^{-1} level ranged from 0.02−0.04 to 0.07−0.12 ng mL^{-1}, respectively (see Table 1). Therefore, the automatic μSPE procedure fully meets the requirements endorsed by the current EU Water Framework Directives[27,28] for determination of triazines at environmentally relevant levels, where the maximum allowed concentrations of atrazine and simazine in surface waters are set to 2.0 and 4.0 ng mL^{-1}, respectively, and <0.1 ng mL^{-1} in tap waters. In fact, LODs are even better than those earlier reported in off-line MISPE of 100 mL water samples prior to LC separation (0.05−0.2 ng mL^{-1}).[25] Compared to alternative preconcentration/separation procedures for triazine assays in aqueous media, e.g, in-line MISPE−capillary electrophoresis with LODs within 0.2−0.6 μg mL^{-1},[47] electrochemical sensing with LOD of 0.2 μg mL^{-1},[48] or MISPME coupled to GC/MS with LODs from 0.02 to 0.09 μg mL^{-1},[49] the proposed flow-through procedure features 3−4 orders of magnitude better LODs.

Applicability to the Analysis of Untreated Water Samples and Crude Extracts. To assess the reliability and ruggedness of the flow-based analytical method, real-life samples of variable matrix complexity and nature, that is, environmental waters and soil extracts, were processed with minimum prior sample treatment. Due to the lack of certified reference materials containing both triazines and dealkylated metabolites thereof, surface and ground waters and soils were doped with the overall analytes at environmentally relevant levels (see Tables 2 and 3).

Untreated Environmental Waters. Preliminary assays demonstrated that preconcentration and cleanup of tap, surface, and ground waters onto *N*-vinylpyrrolidone−divinylbenzene sufficed for the accurate determination of chlorotriazines at the concentration levels specified by EU directives for water bodies. Thus, 10 mL of untreated water samples was processed in the proposed SI assembly without solvent switch to ensure appropriate sensitivity. In addition, the in-valve μSPE column could be reused up to six injections without deterioration of the analytical performance. Relative recoveries of analytes in spiked water (tap, creek, and well) samples at the 0.5 and 2.0 ng mL^{-1} levels are summarized

(43) Hu, X.-G.; Hu, Y.-L.; Li, G.-K. *J. Chromatogr. A* **2007**, *1147*, 1–9.
(44) Turiel, E.; Tadeo, J. L.; Martín-Esteban, A. *Anal. Chem.* **2007**, *79*, 3099–3104.
(45) Boqué, R.; Vander Heyden, Y. *LC−GC Eur.* **2009**, *22*, 82–85.
(46) Vial, J.; Jardy, A. *Anal. Chem.* **1999**, *71*, 2672–2677.
(47) Lara, F. J.; Lynen, F.; Sandra, P.; García-Campaña, A. M.; Alés-Barrero, F. *Electrophoresis* **2008**, *29*, 3834–3841.
(48) Pardieu, E.; Cheap, H.; Vedrine, C.; Lazerges, M.; Lattach, Y.; Garnier, F.; Remita, S.; Pernelle, C. *Anal. Chim. Acta* **2009**, *649*, 236–245.
(49) Djozan, D.; Ebrahimi, B. *Anal. Chim. Acta* **2008**, *616*, 152–159.

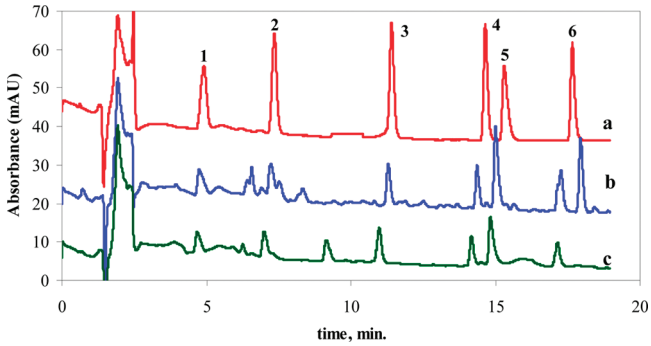

Figure 4. Chromatograms for the determination of chlorotriazines and dealkylated metabolites in (a) 100 μL of 1000 ng mL^{-1} of target compounds manually injected into LC, (b) the eluate of tandem-μSPE column following in-line processing of 1 mL of soil extract without solvent switch, and (c) the eluate of tandem-μSPE column following in-line processing of 1 mL of soil extract with further rinsing with 800 μL of toluene. Soil sample was spiked at the 20 ng g^{-1} level with 1 = deisopropylatrazine, 2 = deethylatrazine, 3 = simazine, 4 = atrazine, 6 = propazine. The internal standard (prometon, peak 5) was spiked at the 50 ng g^{-1} level. Analytical wavelength: 225 nm. Impurities of toluene eluted in part c between DEA and simazine. Shifts of more hydrophobic compounds in c part are a consequence of the remains of toluene in the system, which works as an organic HPLC modifier.

in Table 2. Satisfactory recoveries ranging from 88 to 104% at the 2.0 ng mL^{-1} level and from 86 to 119% at the 0.5 ng mL^{-1} level were encountered for the suite of analyzed samples. The relative recoveries of triazines herein reported are better than those earlier published for batchwise MISPE using large amounts of sorbents.[25,50]

Crude Soil Extracts. As a result of concomitantly extracted interfering components in the soil extracts, a further cleanup of the sample following uptake of the target species onto *N*-vinylpyrrolidone−divinylbenzene or surface moieties of MIP was proven necessary. Thus, molecular recognition of the triazines by the imprinted sites of the MIP material was here fostered via toluene washing. A significant decrease in the number of interfering species, good baseline separation, and accurate quantification of the overall triazines (particularly DEA and propazine in the assayed samples) were attained following solvent switch onto the bidimensional BI column (see chromatograms in Figure 4). The cleanup procedure was distinctly more efficient for removal of the more hydrophobic compounds. Bead materials were automatically renewed after processing each individual sample or replicate. In order to minimize the eventual interfering effects on the dealkylated metabolites, analytical wavelengths were recorded simultaneously at 220 and 225 nm for further data processing. Relative recoveries in the analysis of 1 mL of agriculture soils doped at the 20 and 50 ng g^{-1} levels ranged from 84 to 120% (see Table 3). Similar or even better recoveries than those early reported for soil extracts using SPE/SPME-based methods prior to chromatographic separations[42,43,51,52] were here obtained. Sorbent expenses for single-use tandem columns amounted to <1.9 Euros per assay of soil samples.

CONCLUSIONS

In this paper, the proof-of-concept of renewable MIP as a μSPE reactor in an SI setup prior to chromatographic separations has been demonstrated for processing of crude soil extracts and determination of organic contaminants. Due to the low concentration levels of triazines and the effect of interfering matrix ingredients, multimodal SPE involving two kinds of beads (reversed-phase copolymeric and MIP sorbents) was employed aimed at the concomitant enhancement of selectivity and retention efficiency of target compounds in the sorptive extraction procedure. In-line μSPE with merely *N*-vinylpyrrolidone−divinylbenzene is recommended for environmental water assays in order to simplify the system without compromising the selectivity. After appropriate scrutiny of the various parameters governing the performance of the system, the flow analyzer provides sufficient sensitivity and reliability for long-term assays at concentration levels of herbicides below those endorsed by the current legislations for human water consumption and surface waters.

As an appealing "front-end" to LC, the hybrid flow system herein proposed has proven suitable to not merely handle both aqueous and organic solutions in a single automatic setup encompassing in-line sample pretreatment but effectively solve LC band-broadening effects via in-line dilution of the SPE eluate.

Further research is to be focused on expanding the developed MIP-based flow-through analyzer to the preconcentration, purification, and determination of trace level concentrations of other priority xenobiotics and endogenous organic compounds in a vast number of matrices including environmental and biological samples and foodstuffs as well.

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(50) Almalric, L.; Mouvet, C.; Pichon, V.; Bristeau, S. *J. Chromatogr. A* **2008**, *1206*, 95–104.
(51) Cacho, C.; Turiel, E.; Martín-Esteban, A.; Ayala, D.; Pérez-Conde, C. *J. Chromatogr. A* **2006**, *1114*, 255–262.
(52) Shen, G.; Lee, H. K. *J. Chromatogr. A* **2003**, *985*, 167–174.

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