A flow-based platform hyphenated to on-line liquid chromatography for automatic leaching tests of chemical additives from microplastics into seawater

Kateřina Fikarová[1,2], David J. Cocovi-Solberg[2], María Rosende[2], Burkhard Horstkotte[1], Hana Sklenářova[1] and Manuel Miró[2*]

[1]Charles University, Faculty of Pharmacy in Hradec Králové, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

[2]FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa km 7.5, E-07122 Palma, Spain

Abstract

An automatic flow-based system as a front end to liquid chromatography (LC) for on-line dynamic leaching of microplastic materials (polyethylene of medium density and poly(vinyl chloride)) with incurred phthalates and bisphenol A is herein presented. The microplastic particles were packed in a metal column holder, through which seawater was pumped continuously by resorting to advanced flow methodology. Each milliliter of the leachable (bioaccessible) fraction of chemical additives was preconcentrated on-line using a 10 mm-long octadecyl monolithic silica column placed in the sampling loop of the injection valve of a HPLC system that served concomitantly for analyte uptake and removal of the seawater matrix. After loading of the leachate fraction, the LC valve was switched to the inject position and the analytes were eluted and separated using the monolithic column (Onyx C18HD 100×4.6 mm) applying an optimized acetonitrile/water gradient and UV detection at 240 nm. The automatic flow method including dynamic flow-through extraction, on-line sorptive preconcentration, and matrix clean-up was synchronized with the HPLC separation, which lasted ca. 9 min. The only two currently available multi-component certified reference materials (CRM) of microplastics (CRM-PE002 and CRM-PVC001) were used for method development and validation. Out of the eight regulated phthalates contained in the two CRMs, only the 2 most polar species, namely, dimethyl phthalate and diethyl phthalate as well as bisphenol A, were leached significantly by the seawater in less than 2 h, with bioaccessibility percentages of 51-100%. The leaching profiles were monitored and modeled with the first-order kinetic equation so as to determine the rate constants for desorption in a risk assessment scenario. Intermediate precision values of bioaccessibility data for three batches of CRMs were for the suite of targeted compounds ≤ 22%.

*Corresponding author: E-mail: manuel.miro@uib.es

This work for the first time reports a fully automatic flow method with infinite sink capacity (i.e., using a surplus of extracting solution) for the target species able to mimic the leaching of additives from plastic debris across the water body in marine settings under the worst-case extraction conditions.

Keywords: microplastics, phthalates, bisphenol A, seawater, dynamic leaching, automation

1 Introduction

With growing production of plastics over the past decade exceeding 350 million tons [1], the problem with plastic litter worldwide grows ever faster. Only a part of the plastic waste is recycled while the rest is stored in the landfill and it is estimated that up to 10% of plastic production ends up in the ocean [2].

In the early 2000s, micrometric particles of plastic debris, called microplastics, have firstly been found in ocean waters [3]. Throughout recent years, research on microplastics in environmental settings has attracted a great deal of interest, which is well documented by many reviews published recently [2,4-12] and a rising environmental problem addressed in media of all kinds [13,14]. The size range of microplastics is not uniformly defined; however, most sources set the upper limit to 500 µm, beyond that the term mesoplastics is recommended [4]. Over time, particles degrade to even smaller sizes (so-called nanoplastics, up to a few µm) [4]. Microplastics are divided into 2 groups: (i) Manufactured microscale particles and virgin plastic pellets that are called primary microplastics and used directly in personal care products and cosmetic formulations such as body scrubs, toothpaste, and deodorants or indirectly (e.g., virgin plastic pellets) as precursors of polymer consumer products [2,7]. (ii) Those resulting from the biological or chemical breakdown and weathering of macroplastic debris into small fragments in the beach environment that enter via rivers, wind, or ship litter, or originating directly from abrasion processes, e.g. car tires [2,4], and are called secondary microplastics. Leaching or UV-induced breakdown of plasticizers makes the secondary microplastic debris even more brittle [4,15].

Because of the micro-sized dimension of the plastic particles, the probability of ingestion by various types of marine biota including zooplankton, worms, mussels fish, mammals, and seabirds increases [7]. Beside chemical toxicity and mechanical damage [2], a current concern occurs about translocation into tissues as one of the most important toxicity pathways of microplastics in fish and aquatic invertebrates [9]. Notwithstanding the fact that the role of microplastics as vectors of chemical contaminants adsorbed on the hydrophobic surfaces or bound to their inner structure by physical interactions is still debated [2,10,16,17], plastic debris are regarded as potentially having deleterious effects on marine organisms on account of the leaching of plastic additives, such as plasticizers, antioxidants, colorants, lubricants, flame retardants as well as residual monomers under environmental conditions [2,18,19]. All of the above organic chemicals can accumulate in tissues of animals and by this affect the food chain detrimentally. Moreover, plastic additives can be released

easily during the generation of secondary microplastics by weathering breakdown of beached plastic debris. Another problem is that the sedimentation speed of microplastics is low, hindering their elimination in wastewater treatment plants and allowing them to remain a long time in the upper, biologically active ocean layer [20].

Phthalates are esters of phthalic acid, which possess a wide range of polarity. High molecular mass phthalates, such as diethylhexyl phthalate (DEHP), diisononylphthalate (DINP), di-n-octyl phthalate (DNOP) and diisodecyl phthlate (DIDP) are used mainly as plasticizers. On the other hand, low molecular mass phthalates, like dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-n-butyl phthalate (DnBP) are frequently found as constituents of cosmetics and adhesives [21-23]. Phthalates are considered endocrine disruptors already at the low ng/L and µg/L levels [24]. The European Parliament Directive 2005/84/EC prohibit adding DEHP, DnBP, and benzyl butyl phthalate (BBP) in a concentration higher than 0.1% by mass of the plasticized material to all toys and childcare articles due to their potential reproductive toxicity. For higher molecular mass phthalates, namely, DINP, DIDP, and DNOP, the Directive restriction only applies to toys that can be placed in the mouth by children [25].

Bisphenol A (BPA) is another common organic species in polymer manufacturing and is used as antioxidant or monomer for polycarbonate plastics. Its high solubility in water makes BPA easily leachable to the aquatic environment or into the food from polymer packaging. BPA is known estrogen agonist and androgen antagonist with a variable effect on the human reproductive system. Thus, it is currently regarded as an emerging contaminant [2]. Despite this, regulation EU No 10/2011/EU and Commission Regulation (EU) 2018/213 of 12 February 2018 still allows using BPA in plastic food contact materials, excepting polycarbonate drinking containers intended for infants and toddlers [26].

The above chemical classes of additives are commonly encountered in packaging materials, often made of polyethylene (PE) and polypropylene (PP), which represent one of the largest material fraction of microplastics and marine litters [15]. As for polyvinylchloride (PVC), up to 50% of the weight might be composed of phthalates [27].

There is a wealth of literature on sampling protocols for micro and nanoplastic sampling in sediments and water columns [28-30] and analytical methods for characterization of the polymers by either FTIR, Raman spectrometry or pyrolysis GC/MS [5,31-34]. Nevertheless, only a limited number of studies concerned leaching of the plastic additives from the plastic debris into marine settings [27,35,36]. To the best of our knowledge all of the reported methods for simulating the potential transfer of additives to the marine environment are based on manual/batchwise protocols encompassing either end-point leaching with seawater using gentle shaking [37] or kinetic desorption methods but with low temporal resolution [35,36,38,39].

We herein report for the first time an automatic flow-based method for investigation of the leachability of chemical additives (i.e., phthalate esters and BPA) from microplastics under dynamic extraction conditions mimicking realistic environmental scenarios, namely, the desorption of littoral plastic debris entering the marine environment for which the liquid (seawater) to solid ratio tends to infinite. In fact, dynamic extraction using flow setups has been demonstrated to simulate environmentally and physiologically changing conditions more accurately than its batch counterparts [40-44]. Our flow system hyphenates in-line leaching of microplastics with seawater as contained in a metal mini-column for ascertaining bioaccessible pools of plastic additives under worst-case scenarios in marine settings. Not only extraction but sample preparation using on-line microsolid-phase extraction (μSPE) is automated for matrix clean-up and analyte preconcentration followed by switching-valve liquid chromatographic separation. Analytical method validation was accomplished by employing the two only available certified reference materials (CRM) of PE and PVC in the market containing micro-sized particles in all instances down to 300 μm. Those samples can be categorized correctly as CRM of microplastics with a known content of phthalates and BPA.

2 Materials and methods

2.1 Reagents and materials

HPLC grade methanol, acetonitrile, and 2-propanol were purchased from Fisher Scientific (Madrid, Spain). Ultrapure water (Millipore, Bedford, USA) with resistivity ≥ 18 MΩ·cm was used throughout. Analytical standards of BPA, DMP, DEP, BBP, DnBP were purchased from Sigma Aldrich/Merck KGaA (Darmstadt, Germany) and diluted or dissolved with methanol (MeOH) to a concentration level of 10,000 mg/L. Standard stock solutions of bis (2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP) and diisodecyl phthalate (DIDP) at a concentration level of 1000 mg/L in methanol and diisononyl phthalate (DINP) in acetone were purchased from SPEX CertiPrep (Metuchen, New Jersey, US). A mixed standard stock solution of 1000 mg/L of methylparaben (MP, Sigma Aldrich) and of 50 mg/L of benzyl benzoate (BB, Sigma Aldrich,) was used as internal standard (IS)/quality control (QC) after 100-fold dilution.

A multianalyte stock solution in MeOH at 1000 mg/L of DMP, BPA, and DEP and 50 mg/L of BBP and DnBP was prepared for calibration of the on-line system aimed at the determination of the leachable microplastic additives. The stock solution was stored at 4°C. Working solutions were prepared by appropriate dilution of the multianalyte stock solution of standards and IS in 25% (v/v) of 2-propanol: water containing 0.025% (v/v) acetic acid (AcOH). The blank solution contained internal standards and organic solvent at the same concentration levels than those of the calibration solutions. For the initial optimization of the LC-based separation method, a mixed working solution was prepared to contain all of the analytes at the 10 mg/L level in MeOH.

Surrogate sea water was prepared by dissolving the following salts in Milli-Q water according to [~~40~~45,~~41~~46]: 3.0 mg/L NaF, 20 mg/L $SrCl_2 \cdot 6H_2O$, 30 mg/L H_3BO_3, 100 mg/L KBr, 700 mg/L KCl, 1470 mg/L $CaCL_2 \cdot 2H_2O$, 4,000 mg/L Na_2SO_4, 10,780 mg/L $MgCl_2 \cdot 6H_2O$, 23,500 mg/L NaCl, 20 mg/L $Na_2SiO_3 \cdot 9H_2O$, and 200 mg/L $NaHCO_3$.

Two standard reference materials (CRM) of PE and PVC microplastics (CRM-PE002 and CRM-PVC001, Spex CertiPrep, Metuchen, New Jersey, USA) were used for analytical method validation. Polyethylene CRM with average particle size down to 110 µm (see Fig. S1A) contained DIDP and DINP at a concentration level of 30,000 µg/g, and DMP, DEP, DEHP, DnBP, DnOP, and BPA at a concentration level of 3,000 µg/g; and polyvinyl chloride CRM with average particle size down to 140 µm and density of ca. 1.40 g/mL (see Fig. S1B) contained DIDP and DINP at a concentration of 30,000 µg/g, and DMP, DEP, DEHP, DnBP and DnOP at a concentration of 3,000 µg/g. PVC and PE microplastics (CRM-PVCBLK and CRM-PEBLK, Spex Certiprep) without incurred analytes (blank CRM) were used for QC/QA purposes.

The CRM microplastics were packed in a stainless-steel holder (50 mm long, 4.6 mm ID) that was incorporated in the flow setup in upright position. One of the ends of the column, in the direction of the flow, was furnished with a plug of melamine foam (3 mm width) acting as a stopper of the plastic microparticles on the basis of its intricate 3D porous structure with pore diameters as small as 40 µm. A metered amount of 50 mg of the PE or PVC CRM was weighted directly in the metal holder.

A C18 monolithic guard column (Chromolith, 10x4.6 mm) from Merck KGaA (Darmstadt, Germany) was selected as a solid-phase preconcentration column (PrC) for on-line preconcentration of leachable species and matrix clean-up.

A sonifier device (Model S-450A, Branson Ultrasonics Corporation, Danbury, USA, 400 W) equipped with a 3.2 mm titanium microtip (Branson Ultrasonics Corporation) and an ultrasonic bath (JP Selecta, code 3000683, generator power of 100 W, Abrera, Spain) were employed for quantitative extraction of the most polar species from CRM-PE and PVC particles, respectively. In the case of probe sonication, the sample was placed in an ice-water bath to avoid solvent evaporation.

2.2. Flow Setup

The flow analyser (MicroSIA, FIALab Inc., Seattle, US) was composed of an automatic bi-directional syringe pump (SP1, Cavro, Tecan, Sunnyvale, USA) equipped with a 0.5 mL glass syringe (Hamilton, Bonaduz, Switzerland) and a low-pressure 10-position Cheminert stream selection valve (VICI AG International, Schenkon, Switzerland) in combination with an auxiliary stand-alone syringe pump (XCalibur, Cavro) furnished with a 0.5 mL glass syringe (SP2) and filled with surrogate seawater as a carrier as schematically shown in Fig. 1. Syringe pump 1 (SP1), containing water as a carrier, was connected via a 90 cm long polytetrafluoroethylene

tubing (so-called holding coil (HC), 1/16'' ID) to the central port of the selection valve, which by computer control allowed the aspiration of the organic diluent (25% (v/v) isopropanol containing 0.025% AcOH), calibration solutions, and cleaning solutions (water, MeOH) toward the PrC. SP2 was connected to the CRM filled extraction column (OUT position) for in-line generation of leaching fractions, which entered into the flow system via a two T-junction connector (see Fig. 1). Alternatively, SP2 served (in Up position) for matrix-matched calibration using seawater, thus bypassing the microplastic containing extraction column. The outlet of the T-junctions communicated with via a stainless-steel mixing coil (200 µL, 0.8 mm id) with the PrC placed in the injection loop of an HPLC valve (see below) by stainless steel tubing (0.5 mm ID, 200 µL). The overall fluidic components of the flow-through system were controlled by the user-friendly CocoSoft 4.5 freeware [47].

This assembly was modified for the off-line evaluation of the sorptive capacity of the PrC and the potential breakthrough of analytes in seawater containing organic diluent (viz., a final concentration of 12.5% (v/v) isopropanol or a mixture of 15% (v/v) MeOH and 5%(v/v) 2-propanol). The PrC (0.5 cm or 1 cm) was placed directly to the end of the stainless-steel tubing (0.5 mm ID, 200 µL). The column was cleaned with 0.5 mL of MeOH, then with 1 mL of water. Afterwards, the column was loaded with a mixture of 1 mL of seawater on-line diluted with 1 mL of standard solution at 20 mg/L of DMP, BPA and DEP and 1 mg/L of BBP and DnBP containing IS (10 mg/L of MP and 0.5 mg/L BB) in tested organic diluent and 0.025%(v/v) of AcOH. Then, the column was cleaned with 1 mL of water and eluted with 1.5 mL of MeOH. The eluent was collected in a vial and 10 µL were injected into the HPLC.

2.3 HPLC instrumentation and hyphenation with the flow setup

An LC-4000 HPLC system (Jasco, Tokyo, Japan), consisting of a quaternary high-pressure pump (PU-4180), an autosampler (AS-4050) equipped with a high-pressure injection valve (IV), which was connected to the low-pressure flow setup, and a photodiode array detector (MD-4017), was used for separation of the bioaccessible pools of chemical additives. A chromatographic monolithic column (Onyx C18HD 100x4.6 mm) preceded by a monolithic guard-column (Onyx C18HD 5x4.6 mm), both heated at 30°C (Gecko, Jasco), was used for separation of phthalates and BPA.

For off-line studies of leaching, PrC sorptive capacity and optimization of HPLC separation parameters, the HPLC injection valve was furnished with a loop of 1/32'' ID PEEK tubing and programmed to inject 10 µL. Analytes were detected at 200 nm for maximum sensitivity. For on-line dynamic leaching, the injection loop contained a high-resolution C18 monolithic PrC (Chromolith, 10×4.6 mm) packed in an aluminum holder (Phenomenex, Torrance, CA, USA) for on-valve trapping of the analytes from the seawater leachate fractions and matrix removal.

Switching-valve elution from PrC and chromatographic separation of analytes (DMP, DEP, BPA, BBP, and DnBP) were performed in gradient mode using water (A) and acetonitrile (B) for modulating the mobile phase composition. The HPLC method consisted of the following steps: (i) 0-14 min delay time, during which the bioaccessible analytes (in seawater) were loaded onto the PrC. During this delay, the HPLC pump was stopped for preventing wasting the mobile phase. At minute 13 (t=0 for the HPLC run), the flow-rate of the HPLC pump was smoothly increased up to 1 mL/min and 40 % of B for column equilibration. At minute 14, the linear gradient started from 40% B to 90% B in 5 min. Afterwards, the gradient returned to 40% B in 1 min and the PrC and analytical column were re-equilibrated with the initial conditions of the gradient for 3 min.

On account of the higher concentrations of target species in the first leachate fractions, the detection wavelength was set to 240 nm to guarantee signal linearity over a wide concentration range while improving detection selectivity. Peak area was used as analytical signal throughout. Operational control, chromatogram and spectra acquisition, and data processing was done by ChromNAV software.

2.4 Operational protocol for flow-through dynamic leaching and automatic calibration

In the course of the delay time of the HPLC method (0-14 min), the flow analyzer was synchronized with on-line leaching of microplastics and analysis of a given subfraction of the extraction profile for determination of bioaccessible pools as indicated as follows: i) the two SP were programmed to get initialized, ii) the HC was cleaned with MeOH to prevent carry-over (from IS), iii) the PrC was rinsed with 0.5 mL of MeOH and 0.5 mL of Milli-Q water, iv) 1 mL of seawater was brought to the extraction column (SP2 in position Out) at 0.25 mL/min and the leachate merged on-line with 1 mL of organic diluent containing the two IS in 25% (v/v) isopropanol and 0.025% AcOH, also at 0.25 mL/min, whereupon the stream was loaded onto the C18-PrC via the mixing coil, and v) the PrC was cleaned with 1 mL of Milli-Q water to remove the salt content of the leachates, thus being ready to start the switching-valve HPLC elution method. The organic diluent added on-line to the seawater extract served to decrease the pH of seawater from ca. 8 to 4.7 prior to the retention of the bioaccessible target analytes onto the monolithic PrC.

The above protocol was repeated a number of times until the leaching profiles reached the baseline (usually ≤ 30 fractions) or the increase of extractability in 5 consecutive subfractions for the overall analytes was < 10% of the cumulated bioaccessible amounts. The operational program for on-line leaching (using the flow system) and HPLC separation lasted 23 min per fraction.

For on-line calibration purposes, the very same hyphenated flow system than that depicted in Fig. 1 was utilized. It should be noted that the standard solutions of phthalates and BPA should be prepared in 25% (v/v) isopropanol for minimization of losses by adsorption onto the glassware (see below). The only methodological difference was that the IS-contained calibration standards were aspirated from the multiposition selection

valve (see Fig. 1) and merged on-line with seawater pumped simultaneously by SP2 through the bypass tubing (position Up) so as to perform a matrix-matched calibration to offset the effect of seawater onto the PrC sorptive efficiency.

2.5. Synchronization of the HPLC separation with the automatic on-line leaching procedure

The HPLC system was first initiated, with an injection delay of 14 minutes, that is, the HPLC injection valve turned to position 'Load' and waited 14 minutes for sample (extract) loading onto the C18-PrC. The flow system was manually started at the time that the HPLC valve turned to the 'load' position (see Fig. S2). The sample preparation method lasted almost 13.5 minutes, and, therefore, the HPLC injection delay was well synchronized with the sample preparation time. Sample preparation finished ca. 30 s before the HPLC delay ended, and the flow setup was programmed to wait for the valve turning back to the 'inject' position. When the valve was turned and the HPLC started to run the separation method of the first leachate fraction, the electrical signal from the HPLC autosampler was captured by Cocosoft and this caused the flow system to enter a delay matching the LC separation time (i.e., 9 min). In the very same moment that the LC method finished and passed to the next sample by turning the injection valve to 'load', CocoSoft was programmed to perform the next extraction and processing of the next leachate fraction. The signal generated by the HPLC and captured by CocoSoft allowed resynchronizing both fluidic systems in every fraction to offset the operational time variability of the mechanical components thus enabling a long term unsupervised operation of the hyphenated setup.

2.6. Ultrasound-assisted chemical extraction of additives from microplastics

Two simple analytical methods were developed for quantitative extraction of the most polar additives from the CRM materials with the purpose of mass balance validation. To this end, 50 mg of CRM-PVC microparticles were extracted with 10 mL of ACN by probe ultrasonication for 30 s at 10% power. Afterwards, the suspended microplastics were filtered (0.45 µm, Nylon syringe filter) and the supernatant was made to 25 mL with ACN. The second method involved extraction of 50 mg of CRM-PE microparticles with 10 mL of a 1:1 (v/v) mixture of tetrahydrofuran (THF) and isopropanol assisted by bath ultrasonication for 30 min. Undissolved microplastics were filtered through 0.45 µm Nylon filters and the extract made to 25 mL with 1:1 (v/v) THF and isopropanol. The content of DMP, DEP, and BPA, was determined by HPLC-UV (injection of 10 µL).

3 Results and discussion

3.1 Configuration of the flow system for on-line dynamic leaching of microplastics and automatic processing of the extracts

Preliminary dynamic leaching tests with seawater were performed in a semi-automatic mode by coupling the microplastic-loaded stainless-steel column containing either PE or PVC (with or without incurred analytes) and the C18-PrC in series, followed by elution of the retained plasticizers and BPA from the C18 microcolumn by 100% MeOH and off-line HPLC analysis. The miniaturized SP was proven able to endure the pressure drop caused by the two mini-columns in series. Only five out of nine species from the two CRMs with incurred analytes were identified as bioaccessible across the seawater leachates, that is, DMP, DEP, BPA, DnBP, and BBP, which were thus selected as the target compounds of the automatic flow-through leaching method in the remainder of this work. No detectable concentrations of phthalates and BPA were however found in the leachates of either blank CRM.

The automatic flow system should be devised to concomitantly permit the handling of (i) the CRM microplastics, (ii) the seawater extracts that are subjected to on-line solid-phase extraction, and (iii) the calibrants as well. On-line calibration across the flow system is a common practice in flow-through sorptive microextraction approaches to offset non-exhaustive uptake of targets compounds [48,49]. In our case, aqueous solutions of phthalates and BPA cannot be used as calibrants because of unwanted sorption of the species on glassware and inner surfaces of the fluorinated tubing of the flow system, even for the most polar DMP and DEP species. Thus, the addition of organic modifiers should be considered. However, high content of water-miscible organic solvent might jeopardize the efficient uptake of the most polar species, viz. DMP, by the reversed-phase C18-PrC. The composition of the on-line seawater leachates should be also tailored to contain a given percentage of organic modifier to overcome losses of the bioaccessible plasticizers in the way toward the PrC. The required solvent was therefore added on-line to the leachates by SP1, after aspiration in the HC, at the confluence before the PrC (see Fig. 1). At first, the maximum content of organic diluent (MeOH or 2-propanol) that could be added to the seawater without causing salt precipitation was tested. Indeed, precipitation was observed with ≥ 40% (v/v) of MeOH or ≥ 30% (v/v) of 2-propanol.

Peak areas of mixed standards containing DMP, BPA and DEP at the 1 mg/L level and 1% (v/v), 5% (v/v), and 10% (v/v) of MeOH were compared against those of a mixed standard in 100% MeOH. It was found that BPA and DEP were not stabilized by such low concentrations of MeOH (see Table S1). The experiment was repeated with (i) 2-propanol (5%, 10%, 15% 20% and 25% (v/v)), (ii) increased contents of MeOH (20% and 30% (v/v)) and (iii) a combination of MeOH and 2-propanol (30% and 10% (v/v), respectively). Mixed standards with 5 phthalates and BPA were used in this experiment. The experimental results (see Table S1) showed that the combination of 30% (v/v) MeOH and 10% (v/v) 2-propanol, i.e. 15% and 5% after on-line dilution of the

290 leachates; and 25% (v/v) of 2-propanol (12.5% after dilution) afforded appropriate stability of all of the target
291 species inasmuch as recoveries > 91% for DMP, DEP, DnBP, and BPA were obtained as compared with standards
292 prepared in 100% MeOH. However, selection of the composition of the organic diluent for standards and
293 leachates should be done on the basis of the analyte breakthrough studies of the 5 target species using the
294 on-valve preconcentration system.

295 Two lengths of the preconcentration column (5 mm and 10 mm) and two compositions of the organic diluent
296 (30% MeOH+10% (v/v) 2-propanol and 25% (v/v) 2-propanol) were compared at the 10 mg/L level of DMP,
297 BPA, and DEP and 1 mg/L of BBP and DnBP. The standard solution (1 mL) was on-line mixed 1:1 with Milli-Q
298 water or seawater and loaded onto the preconcentration column. Afterwards, the analytes were eluted with
299 1 mL of MeOH and off-line injected into HPLC (10 µL). For the calculation of the extraction efficiency, the same
300 standard solution (10 mg/L DMP, BPA and DEP and 1 mg/L BBP and DnBP) in 100% MeOH was directly injected
301 into the HPLC (10 µL). Regardless of the column, analyte breakthrough was detected for the most polar species
302 (DMP), yet the longer the column and the lower the percentage of the organic modifier the better was the
303 sorption efficiency for the entire suite of compounds with quantitative recoveries of DEP, BPA, BBP, and DnBP
304 (see Figures S3 and S4). Even though the recovery of DMP was ≤ 40%, the sensitivity was sufficient for analysis
305 of the on-line leachates from microplastics. Therefore, 25% (v/v) isopropanol and the 10 mm PrC were selected
306 for further on-line experiments.

307 The effect of the seawater matrix on the sensitivity of the sorptive preconcentration method was ascertained
308 by comparison of the slopes of the on-line calibration graphs for the five analytes as obtained in MilliQ water
309 against 1:1 diluted seawater, all containing 12.5% (v/v) isopropanol. The *t*-test to compare slopes of regression
310 lines (based on t_{exp} and t'_{exp}) [50] revealed statistically significant differences at the 0.05 significance level for
311 the most polar phthalate species. The feasibility of offsetting matrix effects by two internal standards (10 mg/L
312 MP and 0.5 mg/L BB) was therefore studied. MP and DMP behaved alike in terms of breakthrough and BB alike
313 the rest of analytes (see Fig. S3 and Fig. S4) yet the use of a matrix-matched calibration with seawater
314 throughout the kinetic bioaccessibility assays was proven necessary for reliable measurements. The two IS
315 were, however, added to the organic diluent ("blank" solution in Fig. 1) that merged on-line with the seawater
316 leachates for QC of the operation of the hyphenated flow system and detect potential clogging effects of PrC
317 in the time course of the leaching tests. The flow manifold was finally configured for the seawater contained
318 in SP2 (see Fig. 1) to serve concomitantly as a leaching reagent of the microplastics and matrix modifier of the
319 standards for accurate quantification of bioaccessible pools of plasticizers and BPA.

320

3.2 Configuration of the hyphenated system for on-line µSPE-HPLC analysis

Of the various on-line hyphenation modes of µSPE with HPLC [51], heart-cut approaches using pure methanol or acetonitrile as eluent of the reversed-phase PrC were discarded because of band broadening effects across the HPLC column for the most polar species, DMP, for which the sensitivity of the on-line µSPE method is the lowest. A switching valve mode with the C18-PrC integrated into the loop of the HPLC injection valve was used instead. Initially, a bespoke system configuration enabling the loading of the preconcentration column in parallel to the chromatographic separation of the analytes concentrated in the previous run was assembled. To this end, the HPLC IV was left in 'inject' position for 2 min to elute the analytes by the mobile phase containing ≤ 40% ACN. Then, the valve was turned back to 'load' position for further conditioning and loading of the PrC by the low-pressure flow-based system. While there was a clear advantage of time efficiency of the procedure, the elution strength of the mobile phase was proven insufficient for the less polar analytes (BBP, DnBP) resulting in a carry-over effect across consecutive runs. Therefore, the final on-line configuration of the hyphenated system involved the sequential loading of PrC and the analyte elution and separation by the full HPLC gradient. The time of analysis per fraction increased from 14 to ca. 23 min. Nevertheless, the quantitative elution of all 5 target analytes without carry-over effects and the unsupervised control of both flow system and HPLC by user-friendly software throughout the entire dynamic leaching protocol were accomplished. It should be noted that the loading and elution steps were programmed in a forward-flow mode because of the minute dimensions of the PrC that acted as a filter and thus avoided the introduction of potentially interfering species, e.g., precipitated salts at the head of the PrC, into the analytical column.

3.3 Leaching study and method validation

Two standard CRM (see section 2.1) of polyethylene (PE) and polyvinylchloride (PVC) microparticles were selected for investigation of the feasibility of the hyphenated flow system for the automatic analysis of the leachable pools of chemical additives from microplastics, and the recording of the temporal extraction profiles of target species in seawater. The PVC material differed from PE in terms of composition (BPA is absent) and stronger agglomeration of the microparticles (see SEM images in Fig S1). The shape of the PVC particles was irregular, but PE particles were rather spherical. Three leaching profiles consisting of a maximum of 40 leaching fractions (1.0 mL seawater each) were recorded for each material and the average is shown in Fig. 2. The bioaccessible fraction of a given analyte was deemed quantitatively released when the extraction profile reached the baseline or the extracted analyte quantity of five consecutive fractions was less than 10% of the accumulated quantity from all fractions for such analyte. The analytes with lower K_{ow}, i.e., DMP, DEP, and BPA were leached rapidly, with a maximum of 30 fractions for extracting the overall bioaccessible pools from PE and PVC, which corresponded to a maximum extraction time of 120 min (see Fig. 2). Bioaccessibilities of DMP and DEP from PE in seawater were > 84% as compared with values of > 79% in PVC (see Table 1). BPA was

however only leached from PE by 50% using the dynamic flow-through method. In contrast, a minute and virtually steady leaching of the less polar BBP and DnBP (< 8%) was observed throughout. The low solubility in seawater was the limiting factor for the bioaccessibility of species with log K_{ow}>4.5. Multiple chromatograms illustrating the leaching profiles of PE in seawater (fractions 1-25) are overlaid as a representative example in Fig. S5.

The on-line matrix-matched calibration with standards prepared in 25% (v/v) isopropanol and containing the two IS was fully automated by nesting the various standard solutions to the multiposition valve (see Fig. 1) and pumping seawater by SP2 in a 1:1 volume ratio. The dynamic linear range for quantification of bioaccessible species spanned from 0-10 mg/L for DMP, BPA and DEP and 0-0.5 mg/L for DnBP and BBP with correlation coefficients (R^2)> 0.9984, and limits of detection (LOD) and quantification (LOQ) at 240 nm (calculated as 3 times the standard deviation (SD) for LOD and 10 times SD for LOQ of the lowest concentration level of the calibration range divided by the method sensitivity) of 0.03 and 0.1 mg/L for DMP; 0.3 and 0.9 mg/L for BPA; 0.2 and 0.7 mg/L for DEP; 0.03 and 0.1 mg/L for BBP; and 0.01 and 0.03 mg/L for DnBP. The analytical sensitivity of the five target analytes (given as L/mg·area) was 286,000; 628,000; 837,000; 817,000 and 874,000 for DMP, BPA, DEP, BBP, and DnBP, respectively. The relative SD for standard solutions at the lowest concentration levels of the calibration graph, that is 2.5 mg/L for DMP, BPA and DEP and0.125 mg/L for BBP and DnBP (n=7) ranged from 0.8 to 3.6%. The intermediate precision of the overall bioaccessible concentrations of DMP, BPA, and DEP for PE and DMP and DEP as obtained by the summation of the fractions of the kinetic extraction profiles ranged from 7 to 22% (RSD).

Trueness (lack of bias) of the on-line leaching method followed by automatic µSPE for determination of bioaccessible pools was ascertained by the use of mass balance validation (see Table 1). To this end, the sum of the leached quantities of each analyte determined by on-line switching valve-HPLC in each fraction and that remaining in the microplastic particles (immobile fraction) after the leaching study were compared against the total amount as indicated by the certified values and that obtained by ultrasound-assisted solid-liquid extraction. At first, the analytical method for quantitative extraction of DMP, DEP, BPA, DnBP, and BBP from the primary CRM microplastics and the residual (non-bioaccessible) leftovers was investigated. Solvents compatible with HPLC were selected for this study to avoid reconstitution of the extracts. Batch extraction of CRM microplastics with MeOH was tested using vortex mixing and ultrasonic bath-assisted extraction (5 min, 100 W). MeOH appeared to have insufficient eluotropic strength for stripping out of DMP, DEP, BPA, DnBP, and BBP from the microplastic particles. Ultrasonic-probe assisted extraction (30 s, 10% power) using acetonitrile afforded quantitative recoveries (> 96%) for the three analytes in CRM-PVC (See Table S2) but recoveries for PE were only about 50%, and no improvement was observed by increasing the time of sonication. For CRM-PE, ultrasonic-bath assisted extraction with 2-propanol for 20 min afforded quantitative recoveries for all of the analytes except BPA (ca. 60%). To ameliorate the elution strength, mixtures of 2-

propanol: acetone (70:30) and 2-propanol: THF (50:50) were explored instead (Table S2). The 2-propanol: THF mixture using 30 min sonication was finally selected as the extraction solvent for PE (original CRM and residual microplastics) with absolute recoveries ranging from 91 to 103%.

The *t*-tests of comparison of the sum of the bioaccessible concentrations of DMP, DEP or BPA plus their respective residual fractions against the total amounts as determined by ultrasound-assisted extraction, following the F-test, revealed the inexistence of statistically significant differences at the 0.05 level for any of the tested analytes and CRM because the experimental *t* values were in all instances below the t_{crit}=3.18 (see Table 1). Hereto, the dynamic leaching method for DMP, DEP, and BPA from PE and PVC microplastics as a 'front end' to on-line µSPE-HPLC was free from multiplicative and additive matrix interferences. As for DnBP and BB, the low percentages of bioaccessibility from PE and PVC (≤ 8%), which were on a par or even lower than the RSD values of the total concentrations in CRMs, made the validation of the method by mass balance impracticable.

On-line leaching data from PE and PVC of each analyte (Fig. 2) were fitted to a first-order kinetic model for evaluation of the extraction rates in seawater. The best fit for all the target species ($R^2 > 0.82$, see Table 2) was obtained with a single exponential decreasing equation:

$$Y(t)=A\cdot\exp^{(-k\cdot t)}$$

Wherein the parameter A stands for the maximum dynamic bioaccessibility of species per weight unit of microplastic (µg/g) at the initial time, and k (min^{-1}) is the apparent rate constant. The lack of fit test (LOF) with $p > 0.05$ in all instances revealed that the variability of the dynamic leaching data was well accounted for with the parameters specified by the single first-order equation. The theoretical bioaccessibility compiled in Table 2 was calculated as the integral of the exponential equation within the timeframe of t=0 to t=∞ min, which equals to A/k. According to the kinetic constants (see Table 2), DMP and BPA were leached in seawater twice as rapid as DEP from PE, and DMP was extracted thrice as rapid as DEP from PVC, which indicate the potential environmental risks posed by DMP and BPA-laden beached PE and PVC microplastics.

4 Conclusion

This article reported the proof-of-concept applicability of a versatile instrumental setup based on programmable flow for automatic dynamic leaching of plastics additives (viz., DMP, BPA, and DEP) from primary CRM microplastics in seawater, and on-line matrix clean-up and analyte preconcentration from leachates as a 'front-end' to chromatographic separation using monolithic columns. The low-pressure flow system was proven suitable for pumping of seawater through microplastics contained in a mini-column that was connected in series with a short C18-monolith preconcentration column placed in the loop of the high-

pressure HPLC injection valve without flow backpressure effects. On-line fully automatic matrix-matched calibration in seawater to offset the breakthrough of DMP across the monolith preconcentration column was accomplished by resorting to the software-controlled multiposition valve and two synchronized microsyringe pumps. This setup also served for on-line merging of the leachates with 25% (v/v) isopropanol in a 1:1 volume ratio to prevent losses of the extracted species on the tubing surfaces of the flow manifold.

Fast evaluation of the content of bioaccessible additives from PE and PVC was demonstrated with monitoring the extraction profiles at near real-time. Experimental data were fitted to a first-order extraction model for elucidation of the kinetic parameters, including the extraction times and leaching rates of DMP, BPA, and DEP in seawater. Further research is underway in our lab to broaden the applicability of this work for the investigation of the oral bioaccessibility of beached microplastics with incurred organic emerging contaminants to ascertain the potential role of microplastics as vectors for contaminants in marine settings.

Acknowledgments

MM, DJCS and MR acknowledge financial support from the Spanish State Research Agency through projects CTM2017-84763-C3-3-R (AEI/FEDER, EU) and CTM2017-90890-REDT (AEI/FEDER, EU). KF acknowledges financial support from the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF and the support by the Charles University (Project SVV 260 412). The authors are grateful to Prof. František Švec for fruitful discussions. Scanning electron micrographs of the PE and PVC microplastics were kindly taken by Dr. Ferran Hierro from the Scientific and Technical Center (SCT) of the University of the Balearic Islands.

Table 1: Bioaccessible concentrations in seawater and mass balance validation of the automatic on-line leaching method for the determination of DMP, BPA and DEP from PE and PCV microplastics*.

Sample	Target	Fractions	Bioaccessibility [mg/g]	Residue [mg/g]	Sum [mg/g]	Ultrasound-assisted Extraction [mg/g]	Certified value [mg/g]	t_{exp}**
PE	**DMP**	20	2.3±0.3	0.10±0.03	2.4±0.4	2.71±0.08	2.977±0.178	2.83
	BPA	20	1.7±0.3	1.70±0.07	3.4±0.3	3.04±0.05	3.001±0.180	1.38
	DEP	30	3.0±0.7	0.31±0.06	3.4±0.5	2.88±0.03	2.998±0.180	1.38
PVC	**DMP**	15	2.4±0.4	0.6±0.2	3.0±0.5	3.03±0.07	3.005±0.180	0.01
	DEP	25	2.6±0.2	0.4±0.1	2.96±0.08	3.04±0.04	3.001±0.180	1.20

* Results are given as the average of three or four replicates ± standard deviation

** t_{crit} (α=0.05)= 3.18

Table 2: Kinetic and statistical parameters of the first-order mathematical model for the description of the on-line leaching method of chemical additives from PE and PVC microplastics

Sample	Target	Extraction time (min)	R^2	k (min^{-1})	Theoretical bioaccessible (mg/g)	F value	Fcrit (α=0.05)	LOF test p (>0.05)
PE	**DMP**	80	0.910	0.064±0.003	1.66 ± 0.08	0.08	1.59	1
PE	**BPA**	80	0.820	0.056±0.003	1.11 ± 0.07	0.28	1.59	>0.999
PE	**DEP**	120	0.820	0.026±0.001	2.7 ± 0.2	0.11	1.59	1
PVC	**DMP**	60	0.973	0.093±0.003	1.91 ± 0.08	0.13	2.06	>0.999
PVC	**DEP**	100	0.918	0.031±0.002	2.7 ± 0.2	0.38	2.06	>0.995

Figure Captions

Figure 1: Schematic illustration of the flow system configuration for in-line leaching of chemical additives from microplastics as a 'front end' to on-line µSPE and HPLC separation. EC: extraction column, HC: holding coil, HV: head valve, MC: Mixing coil (40 cm, 0.8 mm id), PrC: C18 monolithic preconcentration column (10 × 4.6 mm); SV: selection valve; IV: HPLC injection valve.

Figure 2: Kinetic extraction profiles (real and fitted to a single first-order equation) of DMP, BPA and DEP from CRM-PE and CRM-PVC microplastics as obtained from on-line dynamic leaching coupled to preconcentration and on-line HPLC analysis.

1 **Figure 1**

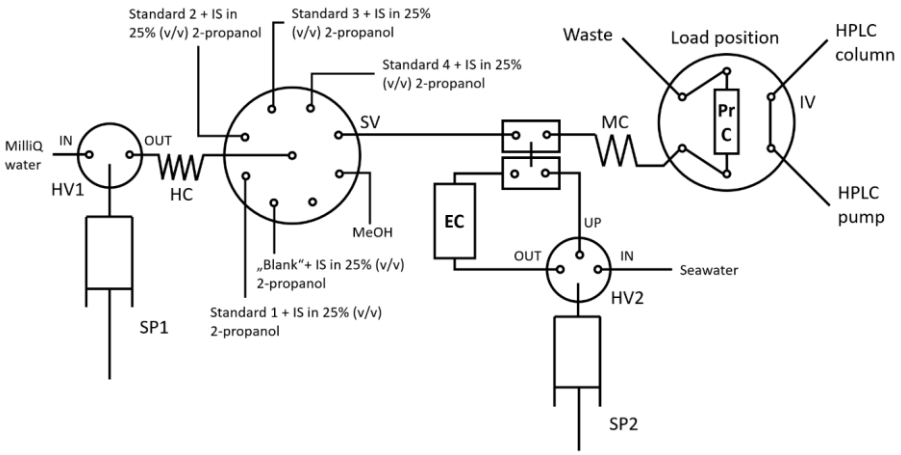
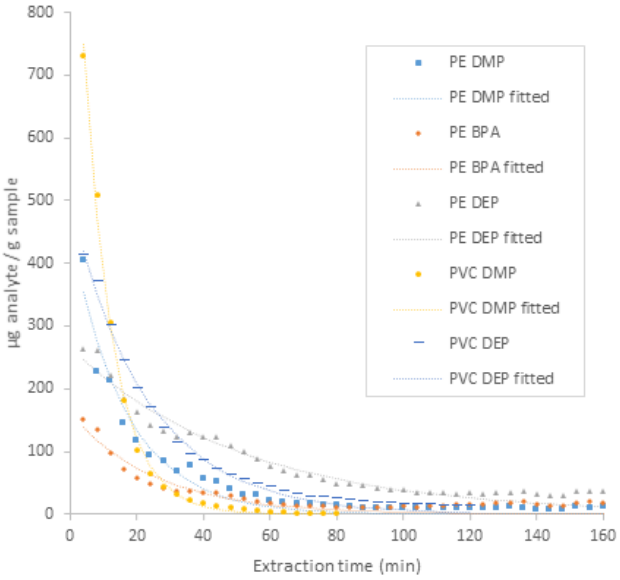

Standard 2 + IS in 25% (v/v) 2-propanol
Standard 3 + IS in 25% (v/v) 2-propanol
Standard 4 + IS in 25% (v/v) 2-propanol
Waste
Load position
HPLC column
IV
Pr C
SV
MC
MilliQ water
IN
OUT
HV1
HC
EC
UP
MeOH
OUT
IN
Seawater
HV2
„Blank"+ IS in 25% (v/v) 2-propanol
Standard 1 + IS in 25% (v/v) 2-propanol
SP1
SP2
HPLC pump

Figure 2


800
700
600
500
400
300
200
100
0
µg analyte / g sample
0
20
40
60
80
100
120
140
160
Extraction time (min)
PE DMP
PE DMP fitted
PE BPA
PE BPA fitted
PE DEP
PE DEP fitted
PVC DMP
PVC DMP fitted
PVC DEP
PVC DEP fitted

5 References

[1] Plastic Europe. Plastics – the Facts 2018. An analysis of European plastics production, demand and waste data. http://staging-plasticseurope.idloom.com/en/resources/market-data. (Accessed April 03 2019).

[2] M. Cole, P. Lindeque, C. Halsband, T.S. Galloway, Microplastics as contaminants in the marine environment: A review, Mar. Pollut. Bull. 62 (2011) 2588-2597.

[3] R.C. Thompson, Y. Olsen, R.P. Mitchell, A. Davis, S.J. Rowland, A.W.G. John, D. McGonigle, A.E. Russell, Lost at Sea: Where Is All the Plastic?, Science 304 (2004) 838-838.

[4] A.L. Andrady, Microplastics in the marine environment, Mar. Pollut. Bull. 62 (2011) 1596-1605.

[5] A.B. Silva, A.S. Bastos, C.I.L. Justino, J.P. da Costa, A.C. Duarte, T.A.P. Rocha-Santos, Microplastics in the environment: Challenges in analytical chemistry - A review, Anal. Chim. Acta 1017 (2018) 1-19.

[6] N.P. Ivleva, A.C. Wiesheu, R. Niessner, Microplastic in Aquatic Ecosystems, Angew. Chem. 56 (2017) 1720-1739.

[7] H.S. Auta, C.U. Emenike, S.H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions, Environ. Int. 102 (2017) 165-176.

[8] F. Ribeiro, J.W. O'Brien, T. Galloway, K.V. Thomas, Accumulation and fate of nano- and micro-plastics and associated contaminants in organisms, TrAC-Trends Anal. Chem. 111 (2019) 139-147.

[9] R. Triebskorn, T. Braunbeck, T. Grummt, L. Hanslik, S. Huppertsberg, M. Jekel, T.P. Knepper, S. Krais, Y.K. Müller, M. Pittroff, A.S. Ruhl, H. Schmieg, C. Schür, C. Strobel, M. Wagner, N. Zumbülte, H.-R. Köhler, Relevance of nano- and microplastics for freshwater ecosystems: A critical review, TrAC-Trends Anal. Chem. 110 (2019) 375-392.

[10] S.-A. Strungaru, R. Jijie, M. Nicoara, G. Plavan, C. Faggio, Micro- (nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology, TrAC-Trends Anal. Chem. 110 (2019) 116-128.

[11] M. Oliveira, M. Almeida, I. Miguel, A micro(nano)plastic boomerang tale: A never ending story?, TrAC-Trends Anal. Chem. 112 (2019) 196-200.

[12] A. Cincinelli, T. Martellini, C. Guerranti, C. Scopetani, D. Chelazzi, T. Giarrizzo, A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea, TrAC-Trends Anal. Chem. 110 (2019) 321-326.

[13] J.R. Jambeck, R. Geyer, C. Wilcox, T.R. Siegler, M. Perryman, A. Andrady, R. Narayan, K.L. Law, Plastic waste inputs from land into the ocean, Science 347 (2015) 768-771.

[14] S. Connor, Microplastic waste: This massive (tiny) threat to sea life is now in every ocean. https://www.independent.co.uk/news/science/microplastic-waste-this-massive-tiny-threat-to-sea-life-is-now-in-every-ocean-9602430.html. (Accessed 03 March 2019).

[15] A. ter Halle, L. Ladirat, M. Martignac, A.F. Mingotaud, O. Boyron, E. Perez, To what extent are microplastics from the open ocean weathered?, Environ. Pollut. 227 (2017) 167-174.
[16] A.A. Koelmans, A. Bakir, G.A. Burton, C.R. Janssen, Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies, Environ. Sci. Technol. 50 (2016) 3315-3326.
[17] J.P. Rodrigues, A.C. Duarte, J. Santos-Echeandía, T. Rocha-Santos, Significance of interactions between microplastics and POPs in the marine environment: A critical overview, TrAC-Trends Anal. Chem. 111 (2019) 252-260.
[18] L. Hermabessiere, A. Dehaut, I. Paul-Pont, C. Lacroix, R. Jezequel, P. Soudant, G. Duflos, Occurrence and effects of plastic additives on marine environments and organisms: A review, Chemosphere 182 (2017) 781-793.
[19] J.N. Hahladakis, C.A. Velis, R. Weber, E. Iacovidou, P. Purnell, An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling, J. Hazard. Mater. 344 (2018) 179-199.
[20] W.J. Shim, R.C. Thomposon, Microplastics in the Ocean, Arch. Environ. Contam. Toxicol. 69 (2015) 265-8.
[21] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices, Environ. Sci. Technol. 49 (2015) 4019-4035.
[22] J.D. Meeker, S. Sathyanarayana, S.H. Swan, Phthalates and other additives in plastics: human exposure and associated health outcomes, Phil. Trans. R. Soc. B 364 (2009) 2097-2113.
[23] P. Ventrice, D. Ventrice, E. Russo, G. De Sarro, Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity, Environ. Toxicol. Pharmacol. 36 (2013) 88-96.
[24] J. Oehlmann, U. Schulte-Oehlmann, W. Kloas, O. Jagnytsch, I. Lutz, K.O. Kusk, L. Wollenberger, E.M. Santos, G.C. Paull, K.J.W.V. Look, C.R. Tyler, A critical analysis of the biological impacts of plasticizers on wildlife, Phil. Trans. R. Soc. B 364 (2009) 2047-2062.
[25] European Plasticisers, Plasticizers information centre. https://www.plasticisers.org/applications/toys/. (Accessed March 17 2019).
[26] Europeam Food Safety Authority, Bisphenol A. https://www.efsa.europa.eu/en/topics/topic/bisphenol. (Accessed March 17 2019).
[27] M. Rani, W.J. Shim, G.M. Han, M. Jang, N.A. Al-Odaini, Y.K. Song, S.H. Hong, Qualitative Analysis of Additives in Plastic Marine Debris and Its New Products, Arch. Environ. Contam. Toxicol. 69 (2015) 352-366.
[28] J.C. Prata, J.P. da Costa, A.C. Duarte, T. Rocha-Santos, Methods for sampling and detection of microplastics in water and sediment: A critical review, TrAC-Trends Anal. Chem. 110 (2019) 150-159.

[29] F. Stock, C. Kochleus, B. Bänsch-Baltruschat, N. Brennholt, G. Reifferscheid, Sampling techniques and preparation methods for microplastic analyses in the aquatic environment – A review, TrAC-Trends Anal. Chem. 113 (2019) 84-92.

[30] W. Wang, J. Wang, Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis, TrAC-Trends Anal. Chem. 108 (2018) 195-202.

[31] M.-T. Nuelle, J.H. Dekiff, D. Remy, E. Fries, A new analytical approach for monitoring microplastics in marine sediments, Environ. Pollut. 184 (2014) 161-169.

[32] G. Renner, A. Nellessen, A. Schwiers, M. Wenzel, T.C. Schmidt, J. Schram, Data preprocessing & evaluation used in the microplastics identification process: A critical review & practical guide, TrAC-Trends Anal. Chem. 111 (2019) 229-238.

[33] P.M. Anger, E. von der Esch, T. Baumann, M. Elsner, R. Niessner, N.P. Ivleva, Raman microspectroscopy as a tool for microplastic particle analysis, TrAC-Trends Anal. Chem. 109 (2018) 214-226.

[34] J. Pinto da Costa, V. Reis, A. Paço, M. Costa, A.C. Duarte, T. Rocha-Santos, Micro(nano)plastics – Analytical challenges towards risk evaluation, TrAC-Trends Anal. Chem. 111 (2019) 173-184.

[35] J. Sajiki, J. Yonekubo, Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species, Chemosphere 51 (2003) 55-62.

[36] M. Staniszewska, B. Graca, I. Nehring, The fate of bisphenol A, 4-tert-octylphenol and 4-nonylphenol leached from plastic debris into marine water – experimental studies on biodegradation and sorption on suspended particulate matter and nano-TiO2, Chemosphere 145 (2016) 535-542.

[37] V.M. León, I. García, E. González, R. Samper, V. Fernández-González, S. Muniategui-Lorenzo, Potential transfer of organic pollutants from littoral plastics debris to the marine environment, Environ. Pollut. 236 (2018) 442-453.

[38] E.L. Teuten, S.J. Rowland, T.S. Galloway, R.C. Thompson, Potential for Plastics to Transport Hydrophobic Contaminants, Environ. Sci. Technol. 41 (2007) 7759-7764.

[39] T.J. Suhrhoff, B.M. Scholz-Böttcher, Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics — A lab experiment, Mar. Pollut. Bull. 102 (2016) 84-94.

[40] M. Rosende, M. Miró, Recent trends in automatic dynamic leaching tests for assessment of bioaccessible forms of trace elements in solid substrates, *TrAC-Trends Anal. Chem.*, 45 (2013) 67-78

[41] M. Rosende, M. A. Segundo, L.M. Magalhaes, M. Miró, Automated microdialysis-based system for in-situ microsampling and investigation of lead bioavailability in terrestrial environments under physiologically-based extraction conditions, *Environ. Sci.Techn.* 47 (2013) 11668-11675.

Con formato: Inglés (Estados Unidos)

[42] L.A Souza, M. Rosende, M.G.A. Korn, M. Miró, Flow-through dynamic extraction system for exploring chyme bioaccessibility in food commodities, Anal. Chim. Acta 1026 (2018) 51-61

[43] Y.-L. Zhang, M. Miró, S.D. Kolev, An automatic flow assembly for on-line dynamic fractionation of trace level concentrations of mercury in environmental solids with high organic load, Anal. Chim. Acta 975 (2017) 1-10

[44] M. Rosende, A. Prieto, N. Etxebarria, G. Martorell, M. Miró, Automatic mesofluidic system combining dynamic gastrointestinal bioaccessibility with lab-on-valve based sorptive microextraction for risk exposure of organic emerging contaminants in filter feeding organisms, Anal. Chem. 91 (2019) 5739-5746.

[45] B. Horstkotte, J.C. Alonso, M. Miró, V. Cerdà, A multisyringe flow injection Winkler-based spectrophotometric analyzer for in-line monitoring of dissolved oxygen in seawater, Talanta 80 (2010) 1341-1346.

[46] R.G. Wetzel, G. Likens, Limnological Analyses, 2nd edition, Springer, New York, 1991.

[47] D.J. Cocovi-Solberg, M. Miró, CocoSoft: educational software for automation in the analytical chemistry laboratory, Anal. Bioanal. Chem. 407 (2015) 6227-6233.

[48] M. Miró, S. Jończyk, J. Wang, E. Harald Hansen, Exploiting the bead-injection approach in the integrated sequential injection lab-on-valve format using hydrophobic packing materials for on-line matrix removal and preconcentration of trace levels of cadmium in environmental and biological samples via formation of non-charged chelates prior to ETAAS detection, J. Anal. At. Spectrom. 18 (2003) 89-98.

[49] X. Long, M. Miró, R. Jensen, E.H. Hansen, Highly selective micro-sequential injection lab-on-valve (μSI-LOV) method for the determination of ultra-trace concentrations of nickel in saline matrices using detection by electrothermal atomic absorption spectrometry, Anal. Bioanal. Chem. 386 (2006) 739-748.

[50] J.M. Andrade, M.G. Estévez-Pérez, Statistical comparison of the slopes of two regression lines: A tutorial, Analytica Chimica Acta 838 (2014) 1-12.

[51] K. Pyrzynska, E. Pobozy, On-Line Coupling of Solid Phase Extraction Sample Processing with High-Performance Liquid Chromatography, Crit. Rev. Anal. Chem. 32 (2002) 227-243.

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Con formato: Inglés (Estados Unidos)

Con formato: Fuente: Sin Cursiva, Inglés (Estados Unidos)

Con formato: Fuente: Sin Cursiva

Con formato: Inglés (Estados Unidos)

Con formato: Nivel 1