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TRACE ANALYSIS OF POLYSTYRENE MICROPLASTICS IN NATURAL WATERS

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ABSTRACT

The development of quantitative and qualitative analytical methods to assess microplastics (MPLs) and nano-plastics (NPLs) content in the environment is a central issue for realistic risk assessment studies. However, the quantitative analysis continues being a critical issue, in particular for MPLs from 100 μm down to the nano-sized range in complex environmental samples.

This paper evaluates the potential of mass spectrometry for the analysis of MPLs and NPLs. The performance of different techniques including matrix-assisted laser desorption ionisation (MALDI) coupled to time-of-flight mass spectrometry (TOF-MS), liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), and the ambient ionization approaches as desorption electrospray ionisation (DESI) and direct analysis real-time (DART), were assessed for the study of polystyrene (PS) MPLs and NPLs in natural waters.

A method based on LC-HRMS, equipped with an atmospheric pressure photoionization source (APPI), operated in negative conditions for the quantitative analysis of PS MPLs and NPLs in natural waters, was developed. The chromatographic separation was achieved using an advanced polymer chromatographic (APC) column using toluene isocratic as the mobile phase. The optimal analytical method showed an instrumental limit of detection (ILOD) of 20 pg and methods limits of detection and quantification around 30 pg L^{-1} and 100 pg L^{-1} , respectively. And, recoveries of 60 and 70% in samples from rivers and the marine coast, respectively. The performance of the new method was proved by the analysis of fortified samples and natural seawater samples.

Keywords: microplastics, nanoplastics, polystyrene, LC-MS, DESI, DART

1. Introduction

The occurrence of plastic litter in the environment is an emerging global threat¹⁻², that has been found distributed ubiquitously in both freshwater³⁻⁵ and marine systems^{1, 6-7}. Among plastic litter, microplastics (MPLs) are the particles of less than 5 mm that reach the environment by different processes: i) the degradation of larger plastic fragments, ii) by direct release of micro-particles used as abrasives, iii) by accidental industrial losses of raw materials, and iv) discharged in sewage sludge. Besides, under environmental conditions, MPLs can be degraded subsequently into nano-sized particles or nanoplastics (NPLs)⁸. In the aquatic environment, due to their size, the floating MPLs and NPLs hamper the oxygen exchange and the transmission of light through the water column, which overall leads to eutrophication processes⁹, and since are similar in size to phytoplankton, are more prone to be ingested by the aquatic organisms¹. Furthermore, the high specific areas of MPLs can lead to the accumulation and transport to the biota of other contaminants¹⁰⁻¹³ and pathogens¹⁴. Besides, non-floating MPLs are deposited on the seafloor and river sediments¹⁵, affecting the biota communities living there. During recent years, different studies highlighted their effects in biota¹⁶⁻¹⁷, such as accumulation¹⁸, transport of co-contaminants^{13, 19-20}, functional diversity of enzymes, impairing living stages²¹⁻²² and behavioural changes. Various factors can influence their effects such as composition, size, ageing of the particles and shape among others. The ecological impact of NPLs and MPLs pollution is still unknown, and there is an urgent need to develop analytical methods to quantify and identify their composition in complex environmental samples as well as characterise the plastic particles.

Among the different analytical techniques currently in use for MPLs characterisation, scanning electron microscope (SEM) can provide high-resolution images of a sample with surface details and resolutions inferior to 0.5 nm. One of the main advantages of SEM is the characterisation of the surface morphology and the weathering processes of MPLs in the environment²³. Moreover, the combined use of SEM and energy-dispersive X-ray spectroscopy (SEM-EDS) can provide detailed information about the elemental composition of MPLs and the inorganic additives composition differentiating MPLs particles from other impurities²⁴. But, the main limitations of SEM-EDS are

requirements of sample preparations, and qualitative data is only provided. Another technique that has been employed for MPLs characterisation is Fourier transform infrared (FTIR) spectroscopy. FTIR give an infrared spectrum for a specific type of chemical bond. This technique can accurately identify the polymer types and also can be used to characterise the weathering processes by analysing their oxidation degree²⁵. But, one of the main limitations of this approach is that FTIR is only capable of identifying the polymeric composition of MPLs with a size bigger than 10-20 μm ²⁶. Recently, different derivative technologies have been successfully used for MPLs such as FTIR micro-spectroscopy (micro-FTIR), that facilitates the detection of particles smaller than 10 μm , attenuated total reflectance-FTIR (ATR-FTIR) used for the analysis of particles bigger than 500 μm without a sample preparation²⁷, and focal plane array FTIR spectroscopy (FPA-FTIR) that can offer high throughput analysis of particles bigger than 20 μm ²⁸. Both FPA-FTIR and ATR-FTIR have been successfully applied for the analysis of complex samples. For example, ATR-FTIR has been used to identify aged and surface contaminated plastics within environmental samples without the use of clean-up processes combining the use of ATR-FTIR with a chemo-metric approach²⁹. In another example, FPA-FTIR has been used for the identification and quantification of MPLs in wastewater. In this case, a sample pre-treatment using 30% hydrogen peroxide was employed followed by FPA-FTIR. The method identified different types of MPLs of polyethylene, polypropylene, nylon-6, polyvinyl chloride, and polystyrene²⁸. Another prominent technique for the MPLs polymers composition identification in complex samples is Raman spectroscopy³⁰. In this case, identification is undertaken by irradiating a monochromatic laser beam onto a sample resulting in a different frequency backscattered that identifies a specific molecular structure and atomic composition²⁴. The Raman shift can produce a unique spectrum for each polymer. Moreover, Raman spectroscopy is a non-destructive chemical characterisation technique³¹. Some of the main advantages are the high reliability of this technique, the possibility for high throughput analysis, and low sample amount requirements³². A combination of Raman spectroscopy with microscopy (micro-Raman) makes it possible to identify MPLs down to 1 μm in size, which is an advantage. Raman spectroscopy has also being used combined with confocal laser scanning microscopy to locate MPLs in biological tissues³. However, the main limitation of Raman based techniques is that MPLs additives, pigments and other chemicals can interfere with the accuracy of the analysis²⁶.

Pyrolysis–gas chromatography–mass (Pyr-GC-MS) spectrometry that has also been successfully employed in the chemical identification of MPLs in environmental samples³³. This is a destructive technique consisting in the analysis of MPLs by their thermal degradation products in comparison with reference pyrograms generated by known pure polymers. Pyr-GC-MS can provide detailed information about the chemical composition of the polymer and the organic additives of the MPLs³⁴. However, this technique does not give information on shape and size³⁵, and only particles that are large enough (>100 µm) to be manually inserted into the pyrolysis tube are suitable to be analysed³⁵. In this regard, different variants have been developed to overcome these limitations, such as thermal extraction and desorption–gas chromatography–mass spectrometry (TED-GC-MS)³⁶, that combines thermal extraction with thermogravimetric analysis (TGA) and thermal desorption gas chromatography–mass spectrometry (TD-GC-MS). Very recently, TGA-MS approach was also applied for the study of poly(ethylene terephthalate) (PET) in soil³⁷. Also, liquid chromatography coupled with mass spectrometry (LC-MS) was applied to quantifying polymer MPLs by depolymerization³⁸.

In spite of the efforts to develop practical analytical procedures for the detection of NPLs in the environment is not yet possible³⁹, at least using a single technique. There is still lacking standardized analytical procedures, there are very few test materials⁴⁰, and there is a need of analytical approaches able to identify and quantify MPLs and NPLs with enough sensitivity to assess it in the environment. The main difficulty starts with sampling approaches. For example, to evaluate the presence of MPLs and plastics at the surface layer, neuston nets are frequently used. After collection, the identification of the polymer composition of MPLs can be achieved by FTIR, Raman, and TED-GC-MS techniques. However, these approaches are not suitable for quantitative results, only qualitative or in some cases, semi-quantitative measures of enough large particles can be achieved, and in any case, these are methods not suitable for the investigation of NPLs. Other separation techniques based on filtration, nanofiltration or field-flow fractionation (FFF) combined with microscopy (TEM, SEM, AFM, and SRS) can be used for semi-quantitative assessment of NPLs, but then the polymer composition later is not identified. It should be highlighted that the analysis of NPLs is of high interest because, while MPLs cannot be digested, in the case of NPLs, it cannot be discarded due to their size translocation to different tissues. Therefore, combined analytical

approaches should be considered to obtain the maximum of information including the study of NPLs.

In this regard, the first objective of this work was to develop a quantitative analytical method able to determine the chemical composition of the polymers of NPLs and MPLs in natural waters. As a proof of concept, the method was developed explicitly for polystyrene (PS) determination, one of the most commonly used materials, largely found in costal environment as debris⁴¹. Additionally, the negative effects of polystyrene microplastics on marine organisms⁴²⁻⁴³ and its capacity to adsorb and transport other contaminants⁴⁴⁻⁴⁵ have been already reported in several studies. The analytical method is based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), equipped with an atmospheric pressure photoionisation source (APPI), operated in negative conditions. The performance of the method was evaluated by spiking experiments and finally by assessing the occurrence of PS MPLs in 17 natural water samples. The potential of other mass spectrometric techniques for the rapid screening was as well explored such as desorption electrospray ionization (DESI) and direct analysis real-time (DART) as well as the performance of more classical approaches such as matrix-assisted laser desorption ionisation (MALDI) coupled to time-of-flight mass spectrometry (TOF-MS).

The results of this study demonstrated that MS techniques can be an excellent complementary analytical approach for target quantification of MPLs and NPLs in environmental samples.

2. Experimental section

2.1. Chemicals and Reagents

Polystyrene analytical standard for GPC/SEC (MW~1200Da) was supplied by Polymer Standard Service GmbH (PSS, Mainz, Germany). Industrial-grade polystyrene (MW~180kDa) was purchased from Repsol[®] (Madrid, Spain) and expanded polystyrene (EPS) with HMW derived from common packaging. Toluene ($\geq 99.7\%$ purity), and HPLC-grade water were purchased from J.T.Baker[®]-VWR (Radnor, PA, USA). Nitrogen used as drying gas with 99.995% purity was acquired from Air Liquide (Spain). For the MALDI analysis, tetrahydrofuran (THF), matrices (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB), dithranol (DIT) and

salicylic acid (SA)) and silver trifluoroacetate salt ($\geq 99.99\%$) were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Standard solutions

To optimise LC-HRMS, DESI, and DART analytical approaches, a stock standard solution of polystyrene was prepared by dissolving 100 mg in 10 mL of toluene to obtain a stock solution of 10 g L^{-1} and stored at -20°C . Standard working mixtures, required for calibration curve and spiking tests, were prepared daily, at concentrations from 100 ng L^{-1} to 10 mg L^{-1} in toluene. In the case of MALDI-TOF-MS, a standard solution (100 mg L^{-1}) of PS 1200 Da was prepared in THF.

2.3. Sampling and Sample Preparation

To assess the good performance of the optimised analytical method, 17 samples from the Ebro Delta (Catalonia, North-East of Spain) were taken during winter 2017 in order to characterize MPLs. Ebro Delta is the 3rd largest delta in the Mediterranean Sea, and it is an area under the pressure of industry, agriculture and tourism. Because of this, the investigation of the presence of MPLs in this area is of high interest.

In the specific case of MPLs, water samples were collected from the upper water column (top 5 cm) where the presence of low-density PS is mainly expected. 10 L samples were taken in amber glass bottles (previously cleaned in the laboratory with toluene, methanol and water successively) cleaned with sample water 3 times before taking the final sample. Afterwards, the samples were transported to the laboratory at 4°C . Then, 2 L were filtered using a dried and weighted glass fiber filters $0.70 \mu\text{m}$ (GF/F, WhatmanTM, Maidstone, United Kingdom). The filters were, successively, dried overnight at 60°C until constant weight, and stored at -20°C until their extraction. All the analyses were carried out working in triplicates. The extraction of PS from filters was achieved by ultrasonic assisted extraction (UASE) with toluene ($3 \times 10 \text{ mL}$) during 10 minutes each cycle. Finally, the extracts were dried under a gentle stream of N_2 near to dryness and reconstituted up to 1.5 mL of toluene in LC-vial for their analysis.

2.4. Instrumental analyses

2.4.1. LC-HRMS

Chromatographic separation was achieved using an Acquity LC (Waters, Milford, MA, USA) chromatograph system equipped with an advanced polymer chromatography

(APC) column (Acquity APC™ XT45 1.7 µm). The mobile phase consisted of toluene 100% working with isocratic conditions. The flow rate was kept at 0.5 mL min⁻¹ throughout the run, and the sample volume injected was 20 µL. The total chromatographic run had a duration of 5 min. The LC system was coupled to a Q-Exactive (Thermo Fisher Scientific, San Jose, CA) hybrid quadrupole–Orbitrap mass spectrometer, equipped with an atmospheric pressure photoionisation source operating in negative ionisation mode ((-) APPI). The acquisition was performed in full scan mode (m/z 500–3000) with a resolution of 17500 full widths at half maximum (FWHM). The optimal APPI source conditions were as follows: sheath gas at 60 a.u., auxiliary gas at 35 a.u., S-lens RF at 100 and the capillary and probe temperatures were maintained at 400 °C.

One of the main issues in MS of polymers remains in the data mining since the mass spectra are often complex because of the composition of technical blended polymers, isotope distribution, adducts, multiply charged and fragment ion series⁴⁶. For these reasons, for identification purposes and data mining of mass spectra, the Kendrick Mass Defect (KMD) analysis was applied as proposed by Sato et al.⁴⁷. The m/z obtained by LC-HRMS were converted to Kendrick Mass (KM) according to eq. 1:

$$\text{KM (ion)} = \frac{m}{z}(\text{ion}) \cdot \frac{\text{round}(R/X)}{R/X} \quad (\text{eq. 1})$$

Where Nominal Kendrick Mass (NKM) and Correct Nominal Kendrick Mass (CNKM) are represented in eq. 2 to eq. 4:

$$\text{NKM (ion)} = \text{round (KM(ion))} \quad (\text{eq. 2})$$

$$\Delta\text{KMD (ion)} = \text{NKM(ion)} - \text{KM(ion)} \quad (\text{eq. 3})$$

Correct Nominal Kendrick mass:

$$\text{CNKM(ion)} = \text{NKM(ion)} - \text{ceiling(NKM(ion)} - m/z(\text{ion})) \quad (\text{eq. 4})$$

where R is the mass of a repeat unit and X is a positive integer used as a base unit for the calculation of KM(ion). Normally, X = 1 for a standard KMD analysis. However, the concept of fractional base unit was recently introduced by Fouquet *et al.* (2017) to increase the resolution of KMD plots. The KMD plot present in the paper is the result of

the application of this concept in order to obtain a distinct separation of ^{12}C and $^{13}\text{C}_x$ isotopes of polystyrene for each adduct generated by LC-HRMS (Figure S-1). Finally, the best option was when ($X \sim \text{round (R)}$) or $X=102$ which offer a clear visualisation of the isotope distribution (^{12}C and $^{13}\text{C}_x$ isotopes) of polystyrene.

In the specific case of polymers analysed by LC-HRMS, just the high-resolution mass spectra followed by KMD analysis can be used to achieve the minimum number of identification points, since the retention time (Rt) by APC column depends on molecular weight (MW) and not on polymer type. In this context, a conversion of concentration was required due to the possible difference in terms of MW between the polymer used as standard and the polymer present in samples. As an example, the area of the highest peak of the mass spectra profile in samples was used to quantify a polymer with MW different from 1200 Da. The concentration obtained by calibration curve $[\text{PS}]_{\text{CC}}$ was converted to equivalent concentration $[\text{PS}]_{\text{eq}}$ according to eq. 5. The equivalent factor (F_{eq}) depends on the number of monomers present in the standard ($(n.\text{mon.})_{\text{std}}$) and in the sample ($(n.\text{mon.})_{\text{sample}}$) (eq. 6). The $n.\text{mon}$ are the rounded values obtained by dividing the mass of the highest peak of the polymer's profile by 104, the mass of PS-monomer (eq. 7).

$$[\text{PS}]_{\text{eq}} = \frac{[\text{PS}]_{\text{CC}}}{F_{\text{eq}}}$$

$$F_{\text{eq}} = \frac{(n.\text{mon.})_{\text{sample}}}{(n.\text{mon.})_{\text{std}}}$$

$$(n.\text{mon.}) = \frac{(\text{mass})_{\text{highest peak}}}{104}$$

2.4.2. Ambient ionisation techniques-MS

The applicability of ambient ionisation techniques to obtain qualitative information of the polymer composition of MPLs in environmental samples was explored. The advantage of these techniques is the possibility to obtain a mass spectrum without sample pre-treatment or with minimal treatment. Two different techniques were employed: direct analysis in real time (DART) and desorption electrospray ionisation (DESI).

DART-SVP ion source (Ion Sense, Saugus, MA, USA) interfaced to an LTQ-Velos Orbitrap dual-cell linear ion trap mass spectrometer (Thermo Fisher Scientific, Bremen,

Germany) with a Vapur[®] API interface (IonSense, Saugus, MA, U.S.A.) was used. The optimised DART settings were as follows: DART ion source was positioned 47 mm away from the sample cards and 1.5 cm away from the mass spectrometer inlet; gas temperature of 450 °C; and grid electrode voltage of +100 V; helium gas at a flow rate of 3.0 L min⁻¹; and a needle potential of 3 kV. 3 µL of the standard solutions of PS1200, industrial PS and EPS in toluene were deposited on a transmission module stainless steel wire mesh and left to dry. A constant speed of 0.2 mm s⁻¹ was used for the transmission module rail system. Polymer mass spectra were acquired in full scan mode for the mass range of 200-3000, and the summed mass spectra were stored at a rate of 5 spectrum s⁻¹. Xcalibur 3.1 (Thermo Fisher Scientific) software was used for instrument control and data processing.

DESI ion source (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) with a 1D moving stage and interfaced to a quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific, San José, CA, USA) was used. A red permanent marker that contains rhodamine 6G (ion at m/z 443.2329, positive ion mode), purchased from Fine Sharpie (Stanford Corp., Oak Brook, IL), was used to establish the DESI geometric source conditions. The optimised DESI settings were as follows: acetonitrile:water (80:20, v:v) was used as DESI solvent at a flow rate of 3.5 µL min⁻¹; sprayed solvent was directed onto the sample surface at a nebulization capillary angle of 55°; spray tip-to-mass spectrometry inlet distance was 9.2 mm; nitrogen (99.95% pure, Air Liquide, Madrid, Spain) at a pressure of 8 bars was used as nebulizer gas; electrospray voltage was set at 4.5 kV in positive ion mode and 5 kV in negative ion mode; transfer capillary temperature was 250°C. Q-Exactive mass spectrometer operated in positive and negative modes in full-scan at a mass resolution of 70,000 FWHM (full width at half maximum) at m/z 200, with a maximum injection time of 300 ms and a scan range from m/z 200 to 3000. Automatic gain control (AGC) values were set at 1×10^6 . Omni Spray ion source software v2.0 (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) was used to control the DESI source, while Xcalibur software v2.2 (Thermo Fisher Scientific, San José, CA, USA) was used for data acquisition and data processing. For sample analysis, 3 µL of a liquid sample was deposited onto microscope glass slides of 7.1 mm polytetrafluoroethylene (PTFE) (Teflon; McMaster-Carr, Santa Fe, CA, USA) printed spots.

2.4.3. MALDI-TOF-MS

This analysis was performed using an AutoFlex III MALDI-TOF/MS instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 200 Hz Smart beam laser and were controlled using Flex Analysis 3.4 and PolyTools 1.0 (Bruker Daltonik GmbH, Bremen, Germany).

The acquisition was conducted in positive reflectron and linear ion modes for low and high-molecular-weight polymers, respectively. The voltage of the ion source 1 was set at 19.2 kV, and for the ion source 2 at 16.2 kV, the lens voltage was set at 8.5 kV, and mass spectra were collected in a m/z range between 500-3000. The mass spectra were acquired by averaging 500 laser shots. Laser irradiance was maintained slightly above threshold.

As sample matrix a solution of Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) in tetrahydrofuran (THF) at 100 mg mL⁻¹ was used. While silver trifluoroacetate (Ag-TFA) in THF was used as ion pairing agent at 10 mg mL⁻¹. Finally, 100 mg L⁻¹ of PS 1200 Da in THF was used for tests. Volumes ratio 1:10:1 V/V of the polymer solution, matrix and salt solution were used. The same mix was used for the study of the behaviour using industrial origin polymers and EPS. Then, 1 µL of the mixture was deposited onto the stainless-steel target plate, which was then dried in air before insertion into the ion source chamber.

2.5. Precautions and safety considerations

In order to minimise the potential losses by adsorption, complete dryness was prevented during the evaporation of the extract. The instrumental analysis was always carried out within the 12 h after extraction. All manipulation of standard solutions and extracts were performed under a fume hood. Since toluene was used as mobile phase, the LC system was vented into a fume hood.

3. Results and discussion

3.1. Sample preparation

PS is soluble in toluene, tetrahydrofuran (THF) and acetone, and partially in methanol. Therefore, according to their solubility, toluene, THF, and acetone, can be selected as PS solvents. However, THF and acetone were discarded since they were not compatible with the instrument pumps, and toluene was chosen as the optimal one.

3.2. Liquid Chromatography-High Resolution Mass Spectrometry analysis

The following parameters were optimised: i) selection of the filter type; ii) UASE extraction time; iii) UASE extraction volume, and iv) number of cycles. Regarding filter type, cellulose nitrate 0.45 μm (NC45, Whatman™, Maidstone, United Kingdom) and glass fibre 0.70 μm (GF/F, Whatman™, Maidstone, United Kingdom) were tested because their chemical composition would not produce cross-contamination effects. In the case of extraction time, extraction volume, and the number of cycles, 10, 20 and 30 minutes and 10 and 20 mL were tested for each cycle, and up to 3 cycles were tested. The results are summarised in Figure 1. As can be seen, the best recoveries were obtained with 0.70 μm glass fibre filter (GF/F), which was selected.

Regarding extraction time and volume, no significant differences were observed between 10 and 30 min, neither comparing extraction volumes of 10 mL vs 20 mL. The optimum method includes 10 mL of toluene for extraction, with recoveries higher than 80% (Figure 1), with extraction of 10 minutes three times. Three cycles of extraction were finally chosen in fact no significant differences were observed when the number of cycles was increased, but more than two cycles of extraction are normally suggested for the extraction of complex sample⁴⁸⁻⁵⁰. In spite of slightly higher recoveries are obtained with 20 mL and 10 mL were selected since it is more environmentally friendly.

3.3.1. LC-HRMS optimisation

To optimise the mass spectrometry parameters flow injection analysis with PS standard solution (200 $\mu\text{L min}^{-1}$) was carried out. The performance of the following ionisation sources: electrospray ionisation (ESI), atmospheric pressure ionisation (API), atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photoionization (APPI), were tested. ESI was not efficient enough due to the low

polarity of PS, as expected. Finally, APPI was selected because the ionisation was milder than APCI and, consequently, the matrix effect could be reduced (Figure S-2).

As can be seen in Figure S-3, working in positive conditions the formation of adducts decreases the signal of the molecular ion significantly, for this reason, negative ionization conditions were selected. The sheath and auxiliary gas were ranging between 5 and 70 a.u., the tube S-lens RF between 50-100 and the capillary and probe temperature were varied from 150 to 500 °C (data not shown). Finally, the optimum APPI conditions were as follows: sheath gas pressure 60 a.u, auxiliary gas 35 a.u, capillary and probe temperatures at 400 °C.

The chromatographic separation was optimised using two size exclusion chromatography (SEC) columns: a conventional gel permeation chromatography (GPC) column (Phenogel™ Phenomenex, 5 µm, 50 Å, 300 x 7.8 mm,) and an Advanced Polymer Chromatography (APC) column (Acquity APC™ XT45, 1.7 µm, 150 x 4.6 mm). In general, SEC is used to characterise the complete molecular weight distribution of a polymer. The use of this type of columns coupled to HRMS offers the possibility not only to determine the polymer type by their unequivocal m/z but also to determine the MW of the polymer since plastics are decomposed in the environment in different MPLs weight' depending on the polymer monomers' and weathering conditions. The columns were compared regarding the retention time, peak resolution and separation efficiency among different MWs. It is important to mention that no gradient ramps with, for example, methanol were tested since selected columns do not afford the use of other solvents than toluene and THF (this last one is not recommended for the pumps of the instrument). In this sense, isocratic conditions with toluene were used in both cases, and this solvent was also used as a dopant for APPI. Different flow rates were tested ranging from 0.3 to 0.7 mL min⁻¹. Finally, the best chromatographic separation in terms of peak resolution, retention time and separation efficiency was achieved with the APC column (Figure S-4).

In Figure 2A, a total ion current (TIC) chromatogram and an extracted ion chromatogram (XIC) of PS is shown. The m/z (1339.8131) is one of the highest signals in the isotopic distribution of the PS-polymer. In Figure 2B the relative mass spectra of PS is shown. The isotopic profile is characterised by the highest peaks referred to oxygenated analyte product ions such as $[M-O_2]^-$ or $[M-H+O]^-$ due to the presence of

high quantities of oxygen in APPI ionization source operating under ambient conditions. The ionization source produces superoxide ion (O_2^-). This ion can react with the analytes generating a negative radical adduct or can do a substitute reaction depending on ionization conditions⁵¹⁻⁵². Finally, the molecular ion distribution of PS ($N=12$) is shown in Figure 2B, where the monoisotopic mass (1338.8178) is the most abundant, followed by the nominal mass (1337.8116) and the average mass (1339.8131). This non-symmetrical profile of PS agrees with the most probable profile when the mass range of polymer is within 1000-3000 m/z ⁵³.

The profile of the PS and its isotopic pattern was confirmed by the KMD analysis, with two PS-polymer chains with different end-group combinations, one end-group negatively charged $[\text{M-O}_2]^-$ and the other one with an adduct of oxygen formed by the substitution reaction previously described $[\text{M-H+O}]^-$. In Figure 2C, these results are summarised.

The good performance of the analytical method was evaluated in terms of linearity, accuracy, precision, and instrumental limit of detection (Table 1). The instrumental quality parameters were calculated using ten points calibration curve in toluene working with two ranges: from 0.5 to 50 $\mu\text{g L}^{-1}$ and from 50 to 1000 $\mu\text{g L}^{-1}$. Instrumental linearity and ranges were estimated using linear regressions with good results in both ranges ($R^2 > 0.995$). Instrumental precision ($n = 5$), expressed as the relative standard deviation (RSD%) of intra-day and inter-day repeatability of three different concentration levels (5, 50, and 500 $\mu\text{g L}^{-1}$) was evaluated. In all the cases the precision was lower than 5% and 25% for intra- and interday evaluations, respectively. The ILOD was estimated from the injection of the lowest calibration curve point (20 pg).

3.3.2. Method validation

The method limits of detection and quantification (MLOD and MLOQ, respectively), recoveries and matrix effects for freshwater and seawater are summarised in Table 1. Regarding extraction method parameters, recoveries of quintuplicates at three spiking concentrations (5, 50, and 500 $\mu\text{g L}^{-1}$) were calculated. The MLOD and MLOQ achieved were of 32.5 and 110 pg L^{-1} for freshwater and 30 and 102.5 pg L^{-1} for seawater, respectively. The method was shown to be sensitive enough for the analysis of polymers in environmental samples. In this work, the matrix effect was estimated by comparing the slopes of standard curves dissolved in extraction solvent and matrix-

matched calibration curves. The results confirm up to 40% of ion suppression for freshwater while in seawater the suppression was much higher with values up to 70%. These results highlight the need to use matrix match standard calibration curves for quantitative analyses.

1. Therefore, the performance of the method was tested as well with an industrial polystyrene (MW > 150 kDa) (HMW), which have impurities compared with standards. The mass spectra are shown in Figure S-5. As can be seen, the profile of PS was clearly identified and the number of chains with a reduced molecular weight form is constant and it is enough for the identification of the polymer.

3.3.3. Analysis of real samples

As a proof of concept, the method was applied to the analysis of 17 samples (8 freshwater and 9 seawater samples) from the Ebro Delta from Catalonia (North-East of Spain). In Figure 3 and Table S-1 of Supporting Information, the main results were summarised. After the analysis, five samples were positive in PS showing a concentration between 1 and 140 ng L⁻¹ (RSD < 5%). The highest concentration of PS (136.74 ng L⁻¹) with a MW range of 1500-2000 Da corresponded to Port d'Illa de Mar, the main industrial harbour of seafood in Fangar Bay. To the authors knowledge, this is the first time that PS ranging from 800 to 2000 Da has been detected in Ebro Delta area.

3.3. DART- Orbitrap-MS analysis

The DART transmission was employed, where the heated ionizing gas flows through a metal screen onto which samples have been deposited, increasing the surface area available for sample deposition, and thus increasing sensitivity and uniform thermal desorption. Helium pressure and heater temperature, distance from the source to the MS, and sample speed through the DART source were optimised using a solution of PS (6 μL). The ionisation conditions were set in positive. The temperature of the helium gas in the DART was optimised in steps of 50 °C (Figure S-6A). Below 200 °C, no signal was observed while at higher temperatures than 450 °C intensity decreased. In regards to the exit grid voltage, the intensity of the signal decreased by the increase of the voltage (Figure S-6B). The optimum results were obtained when the exit grid voltage was set at +100V and the temperature of gas ionization at 450 °C. Finally, the entrance hole was set at 4.6 cm of the DART SVP railing guide.

Once the optimal parameters were set, the analytical performance was explored with a PS standard in toluene ranging from 0.1 to 10 mg L⁻¹ (Figure 4) As can be seen in Figure 4B, the least squares linear regression indicates good linearity ($R^2 = 0.9947$). In Figure 4C, the DART mass spectra are presented. As can be seen, the molecular ion $[\text{M}]^+$ was less intense than the ammonia adduct and the oxidation products such as $[\text{M}-\text{O}_2]^+$. In contrast, adducts with ammonium give up to 5-fold intensity compared to molecular ion.

The use of DART is a promising tool for the rapid identification and quantification of MPLs, and further work will be done in this field. However, it is noteworthy to mention that ambient ionization techniques are not suitable for the ionization of molecules with MW higher than 1500-2000 Da.

Although DESI has been previously used to detect and characterise polar low molecular weight polymers such as polyethylene glycol in a complex sample⁵⁴ this ambient ionization technique was not suitable for the analysis of non polar polymers such as PS, and, consequently, any results were obtained.

3.4. MALDI-TOF-MS

The parameters of MALDI were optimised, and they are presented in Table S-2 of the Supporting Information. Three different matrices (DCTB, DIT and SA) were prepared in THF at different concentrations. Matrix, polymer and cationization agent were mixed at three different ratios (20:30:1, 100:10:1 and 10:1:1). The results of the MALDI

analyses were obtained in positive ionization mode. The AgTFA salt was used because its efficiency to ionise long and short polymer chains⁵⁵⁻⁵⁷. Especially, in the case of polystyrene, the presence of phenyl rings facilitates π -complexation of metal cations such as Ag^+ or Cu^+ . One of the central parameters to a successful characterisation of a polymer is the proportion between the matrix and polymer. Initial experiments were performed using PS with a molecular weight of 1200 Da and low polydispersity ($\text{PDI} < 1.1$). Matrix, polymer and salt were mixed at a ratio of 20:30:1, 1 μL of the mix was put on the target plate and finally air-dried at room temperature. In this analysis, DCTB and DIT matrices were used because they have been previously reported for the analysis of polystyrene by MALDI⁵⁸⁻⁶¹. An example of mass spectra using a reflectron TOF mass spectrometer can be seen in Figure S-7. As it can be observed, the difference between two adjacent mass peaks is of 104 Da (repeating unit of PS $-(\text{C}_8\text{H}_8)-$) for series of single peaks ranging from $800 \pm 2000 \text{ m/z}$, and with values of n between 7 and 18. Comparing the results obtained with different matrices (Figure S-8), the PS distribution showed higher intensities when working with DTCB.

Finally, MALDI-TOF/MS method was applied to HMW polymers (industrial PS and EPS) and samples from Ebro Delta. In this case, the proportion of matrix/polymer/salt was optimised again in order to have the highest response and settled at 10:1:1. It should be noticed the necessity to increase the molar amount of matrix for higher MWs due to the decrease in sensitivity. According to the results shown in Figure S-9, the methodology gives different response depending on the nature of the polymer (i.e., a peak intensity of HMW is lower compared to a standard sample). In the case of Ebro Delta samples, the-sensitivity given by this methodology is lower than LC-HRMS, and none of the analysed samples gave positive in PS.

4. Conclusions

This work showed the suitability of LC-APPI-HRMS for quantitative analysis of small size-range PS-MPLs and NPLs with MW below 3000 Da in the suspended fraction of natural environmental waters. The method presented high sensitivity and robustness, allowing the analysis in real environmental samples. As a proof of concept, the method was applied to the analysis of 17 samples of freshwater and seawater, and the concentration founded were at the ppt level. For the analysis of environmental water samples, it should be necessary to optimize the sampling, also to have more information about the particles size collected. Moreover, follow-up studies will need to show

whether a similar analytical setup could be extended to the analysis of biota samples. Among the different analytical methods assessed in this study (MALDI-TOF/MS, DART-MS, and LC-APPI-HRMS) for the analysis of PS-MPLs/NPLs, LC-APPI-HRMS provided the best sensitivity. The method developed here can be an excellent complement to the existing procedures, to carry out quantitative measures.

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Appendix A. Supplementary data

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Table 1. Instrumental and method quality parameters for the analysis of polystyrene by LC-APPI(-)-HRMS in freshwater and seawater.

Parameters			PS (MW=1.2kDa)	
Instrumental parameters		ILOD (pg)	20	
		Linearity ($\mu\text{g L}^{-1}$), R^2	0.5 – 50, 0.998	
		Linearity ($\mu\text{g L}^{-1}$), R^2	50 – 1000 , 0.996	
		Accuracy (%)	< 20	
	Inter-day (n = 5)	5 $\mu\text{g L}^{-1}$	15.5	
	repeatability (%)	50 $\mu\text{g L}^{-1}$	20.0	
		500 $\mu\text{g L}^{-1}$	25.6	
	Intra-day (n = 5)	5 $\mu\text{g L}^{-1}$	2.4	
	repeatability (%)	50 $\mu\text{g L}^{-1}$	1.9	
500 $\mu\text{g L}^{-1}$		1.8		
Method parameters		Fresh water	MLOD _{FW} (pg L ⁻¹)	32.5
	MLOQ _{FW} (pg L ⁻¹)		110	
	Recovery _{FW} (%) ^b		5 $\mu\text{g L}^{-1}$	63.4
			50 $\mu\text{g L}^{-1}$	57.7
			500 $\mu\text{g L}^{-1}$	57.0
	Inter-day _{FW} (%) ^a			< 10
	Matrix effect _{FW} (%) ^c		-44.8	
	Sea water	MLOD _{SW} (pg L ⁻¹)	30.0	
		MLOQ _{SW} (pg L ⁻¹)	102.5	
		Recovery _{SW} (%) ^b	5 $\mu\text{g L}^{-1}$	60.4
50 $\mu\text{g L}^{-1}$			72.7	
500 $\mu\text{g L}^{-1}$			67.1	
Inter-day _{SW} (%) ^a			< 10	
Matrix effect _{SW} (%) ^c		-71.4		

^aMethod repeatability was assessed in triplicate. ^bRSD value for the recovery is lower than 20% or 25% for freshwater and seawater, respectively. ^cME (%) = (slope matrix / slope solvent -1)*100.

^aMethod repeatability was assessed in triplicate. ^bRSD value for the recovery is lower than 20% or 25% for freshwater and seawater, respectively. ^cME (%) = (slope matrix / slope solvent -1)*100.

Figure 1. Radial graphs of the recoveries of polystyrene with different filters, extraction volumes and times for freshwater (A and A') and seawater (B and B').

Figure 2. A) Separation by APC-APPI-HRMS of polystyrene. The total ion current (grey) and extracted ion chromatogram (red) of one fraction. B) APPI-HRMS mass spectrum of polystyrene 1200. Negative ion mode, solvent/dopant = toluene, T = 400°C, flow rate = 500 μ L min⁻¹. C) KMD plots using PS/102 as the base unit.

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Figure 3. Main results from the sampling sites located in the Ebro Delta area (NE Spain).

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751 **Figure 4.** (+)DART-Orbitrap of polystyrene; heater temperature 450 °C and exit grid
 752 voltage +100V: **A)** analytical response (calibration) of PS in the range of 0.1 – 10 mg L⁻¹
 753 with $R^2 = 0.9947$; **B)** TIC and mass chromatogram of PS at m/z 699.99-700.99
 754 whereby the mass signal was dependent on positioning; **C)** Major ions observed in
 755 (+)DART mass spectra of polystyrene.

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- LC-APPI(-)HRMS method for the quantitative analysis of polystyrene microplastics;
- LC separation was achieved using an advanced polymer chromatographic (APC) column;
- The instrumental limit of detection (ILOD) was 20 pg;