

Environmental

Identification of microplastic particles in environmental water and food using pyrolysis GC with high resolution Orbitrap mass spectrometry

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Keywords

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Goal

The objective of this technical note is to demonstrate the utility of pyrolysis-gas chromatography-Orbitrap™ mass spectrometry for the detection and identification of common polymers in food and environmental samples.

Introduction

Plastics comprise a wide range of synthetic and semi-synthetic polymers, with unique physical and chemical characteristics, and are used in products found in every aspect of day-to-day life. Large-scale production of synthetic polymers started in the 1950s, and by the end of the last decade, it was estimated that the global plastic production had surpassed 400 million tons per year, of which, approximately 85% was not recycled.¹

Microplastics are small particles made from synthetic polymers with a diameter typically ranging between 5 mm and 1 µm, whereas nanoparticles cover particles sizes of sub 1 µm. Two sources of microplastics can be recognized. The primary source is cosmetic and medical products, where microparticles—typically polypropylene, polyethylene, and polystyrene—were added deliberately. The secondary source is debris formed through the fragmentation of larger items made from synthetic polymers that typically enter the environment through inadequate disposal. The fragmentation occurs due to mechanical stress and atmospheric conditions.² Some legal steps have been taken to limit the usage of microplastics in cosmetic products; however, secondary sources are considered the major contributor to microplastic pollution.³

Today, microplastics are present in the terrestrial and aquatic environment. Because of their small size they can easily migrate from the environment into the food chain.⁴ Microplastics may consist of not only the pure synthetic polymer but also include residuals of the monomer, plasticizers, flame retardants, and many other toxic additives that can have a negative impact on human health.⁵ Over time, microplastics may incorporate environmental contaminants such as trace metals.

Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy, and microscopy-based techniques are commonly applied to screen samples for the presence and identification of the chemical backbone of microplastic particles. However, especially for microscopy-based analysis, the number of samples that can be screened is limited. Pyrolysis gas chromatography-mass spectrometry (py-GC-MS) presents a promising alternative for surveillance and identification of microplastics where throughput is critical. Furthermore, this analytical approach enables time-saving detection of bulk amounts of micro- and nanoplastics below the lower size limit of the microscopy techniques.

Experimental

Sample preparation

Two sample types were investigated in this study, covering potential contamination in environmental waters and food-related matrices. For the stormwater analysis, the sample (1 L total volume) was spiked with deuterated polystyrene (D_5 -PS). The sample was filtered sequentially through Whatman™ 1 and 0.7 μm glass fiber filters (GFFs) to collect particulates (47 mm, GF/A and GF/F, Rowe Scientific, Wacol, Australia). The GFF was wrapped in aluminum foil (precleaned with acetone), dried in an orbital incubator at 50 °C (Thermoline Scientific, Wetherill Park, Australia), weighed in a pyrolysis cup (Eco-Cup LF, Frontier Laboratories, Japan)⁶ to which deuterated polystyrene (D_5 -PS) was added. The milk and steak samples were freeze dried and milled with a grinder for 30 min (Extech Equipment Pty. Ltd., Victoria, Australia) using an overhead shaker at 140 rpm for 2 h to homogenize. After that, 1 g of each sample was spiked with

D_5 -PS and extracted by pressurized liquid extraction in pre-cleaned 5 mL ASE cells on a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor. Extraction was performed with dichloromethane at 180 °C and 1,500 psi with a heat and static time of 5 min using three extraction cycles. The extracts were weighed and 80 μL transferred to a pyrolysis cup. At the end, the solvent was evaporated in a fume hood at room temperature for 30 min prior to analysis.²

Instrumental analysis

A pyrolyzer (Multi-Shot Pyrolyzer™ and Auto-Shot Sampler, Frontier Laboratories) was mounted on a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph coupled with a Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer (Figure 1). The pyrolyzer was connected to an iConnect™ Split/Splitless (SSL) Injector via a hot injection adapter. A Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m \times 0.25 mm I.D. \times 0.25 μm film capillary column (P/N 26096-1425) was used to separate the products of the pyrolysis.

The Orbitrap Exploris GC 240 mass spectrometer was tuned and calibrated in under one minute using perfluorotributylamine to achieve optimal ion transmission and sub-ppm mass accuracy. The mass spectrometer was operated in full-scan mode using 60,000 mass resolving power (measured as FWHM at m/z 200). Lock mass corrected data was processed using Thermo Scientific™ Compound Discoverer™ software and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. Additional details regarding the pyrolysis, GC, and MS conditions are given in Table 1.

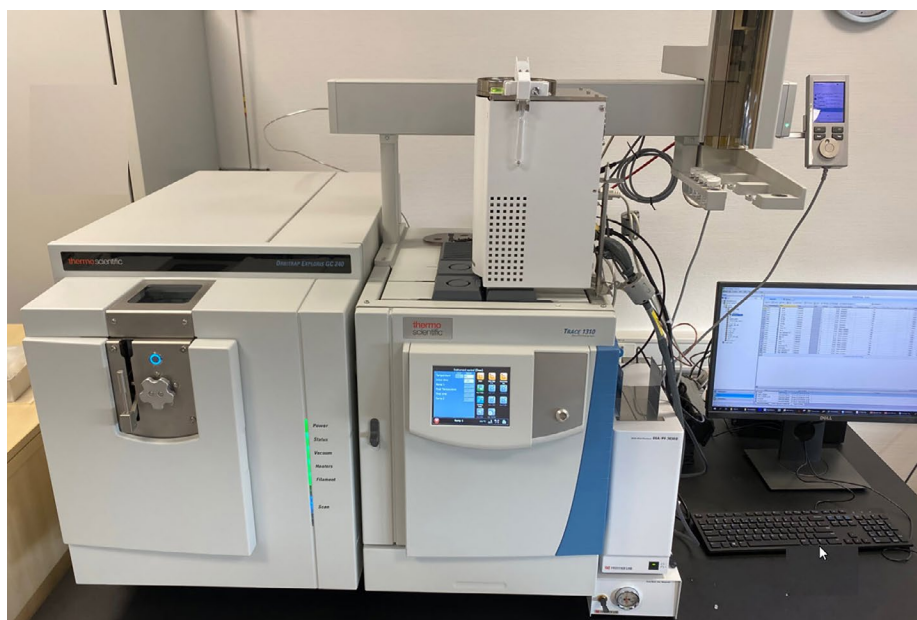


Figure 1. Instrumental setup: Multi-Shot Pyrolyzer (Frontier EGA/PY-3030D) with Auto-Shot Sampler (AS-1020E) coupled to an Orbitrap Exploris GC 240 mass spectrometer

Table 1. py-GC-MS conditions for environmental and food samples

Multi-Shot Pyrolyzer EGA/PY-3030D parameters	
Analysis type	Double-shot analysis
Thermal desorption	
Initial (°C)	100
Initial (min)	0
Rate (°C /min)	20
Final (°C)	300
Final (min)	1
Total time (min)	11
Pyrolysis	
Initial (°C)	650
Initial (min)	0.2
Interface temperature °C	320
Trace 1310 GC System parameters	
Injector type	SSL with an adapter kit for gas injection
Injection mode	Split
Temperature (°C)	300
Split ratio	200:1
Carrier gas (mL/min)	He, 1
Oven temperature program	
Temperature 1 (°C)	40
Hold time (min)	2
Rate (°C /min)	20
Temperature 2 (°C)	320
Hold time (min)	14
Orbitrap Exploris GC 240 MS parameters	
Transfer line temperature (°C)	300
Ionization type	EI
Ion source temperature (°C)	280
Electron energy (eV)	70
Emission current (μA)	50
Acquisition mode	Full scan
Mass range (<i>m/z</i>)	40–600
Resolving power setting	60,000
Lock masses (<i>m/z</i>)	133.01356; 207.03235; 225.04292; 281.05114; 299.06171; 355.06993

Results and discussion

A double-shot method was used for the analysis of food and environmental samples. In this kind of analysis, the pyrolysis is preceded by a thermal desorption (TD). Double-shot methods are useful when very complex samples are analyzed, as the TD step eliminates a significant part of the matrix; therefore, the chromatogram (pyrogram) obtained after the pyrolysis is less complicated—in other words it contains fewer interferences.

The temperature in the TD stage is lower than the temperature applied in the pyrolysis step. During the analysis, two data files are created, one is the chromatogram of the thermal desorption products and the other contains the pyrolysis products. Here, only the pyrolysis data were processed for the identification of the polymers. The chromatogram generated during the TD could contain potentially interesting compounds, although it was not screened for compounds in this study. However, it is worth highlighting the importance of TD as a clean-up technique. An overlap of the TD total ion current chromatogram of the standard mix and the milk sample is shown in Figure 2. This comparison demonstrates that the TD stage removes a considerable quantity of chemical background from the sample data.

In the first step of this study, a series of polymer standards were subjected to pyrolysis to find characteristic fragmentation products that can be used for polymer identification in real samples. For data processing, the resulting pyrograms were screened with Compound Discoverer software to find the pyrolysis products known from the literature^{2,7}. Compound Discoverer software can use both nominal as well as high-resolution accurate mass (HRAM) spectral libraries.

To simplify further data treatment during the analysis of samples, a targeted processing method was created using Chromeleon software. The processing method included all compounds previously identified, with the presence of a particular compound confirmed using a minimum of three representative ions extracted from the TIC using a mass extraction window of ±5 ppm around the exact mass.

Figure 3 shows examples of identification with the NIST Tandem Mass Spectral Library, 2020 release (nominal mass) and Thermo Fisher Scientific environmental contaminants library (exact mass). Table 2 shows the pure polymers and their pyrolysis product(s) found in the screening. In the case of polystyrene, six polymer pyrolysis products were detected which only serves to emphasize the complexity of this analysis. Some of these compounds can be formed by the pyrolysis of matrix components and thus, are not necessarily indicative (or characteristic) of the presence of a particular polymer. For example, styrene the major pyrolysis of polystyrene can be formed during the pyrolysis of chitin, wood, and fish protein. Similarly, the presence phenylalanine can result in the detection of styrene.² In this case, it is important to carefully select pyrolysis compounds such as styrene dimers and trimers to avoid incorrect identifications. A similar case is PVC, where the most typical pyrolysis products are aromatic hydrocarbons. These compounds are common environmental contaminants; thus, it is safer to confirm the polymer identification using more than one of the pyrolysis products to avoid a false positive result.

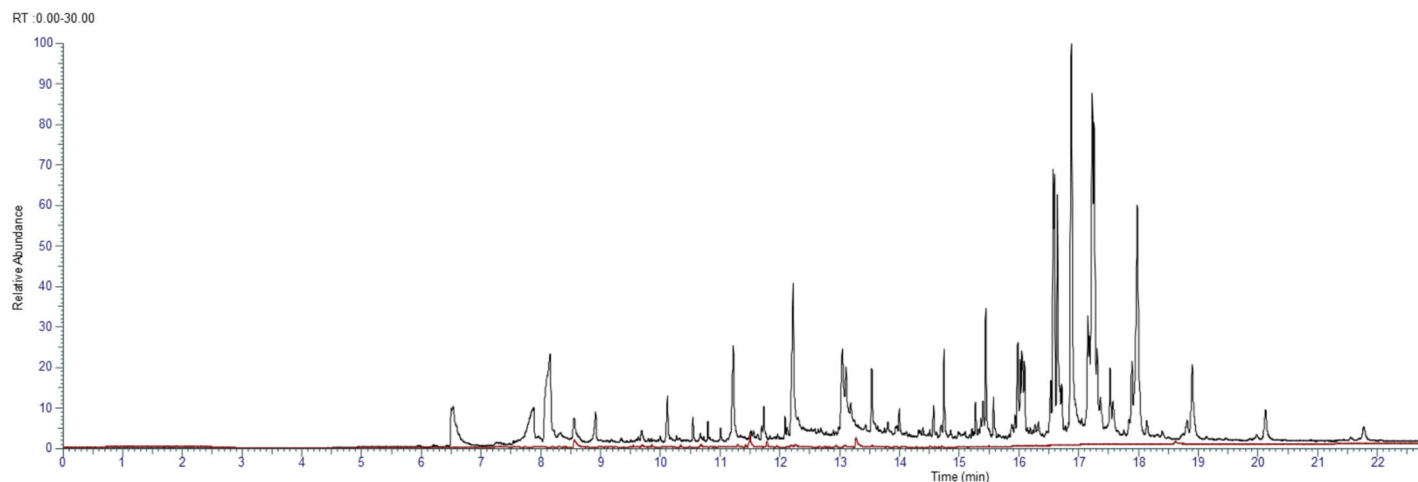


Figure 2. Total ion current chromatogram (m/z 40–600) obtained for a milk sample (black chromatogram) compared with a solvent standard of a mix of polymers (red chromatogram) after the TD step

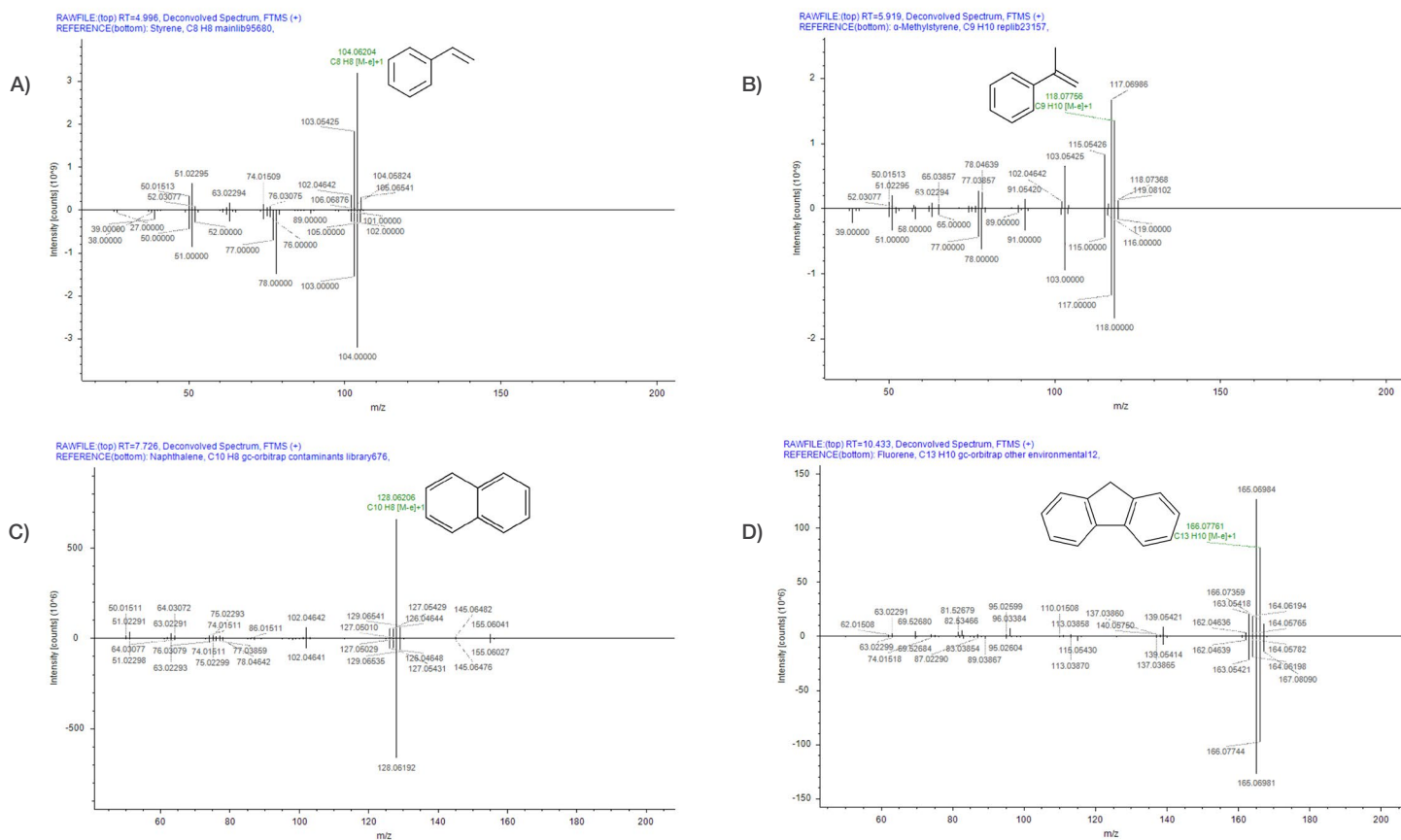


Figure 3. Mix of polymer standards; examples of identification with NIST library (nominal mass) and Thermo Fisher Scientific contaminants library (exact mass library). The top spectrum is the deconvoluted spectrum, whereas the bottom one comes from the library; the molecular ion is marked in green. A) styrene (nominal mass library); B) α -methylstyrene (nominal mass library); C) naphthalene (exact mass library); D) fluorene (exact mass library)

In the second step of this study, real samples (prepared as described above) were pyrolyzed to confirm the presence of microplastic particles and identify the polymer types if particles are present. During the data processing in Chromeleon software, benzene, naphthalene, and fluorene were found in the stormwater sample. As can be seen in Table 2, these compounds are formed during the pyrolysis of PVC. Moreover, the proportions between them were similar to the proportions in the standard. Figure 4 shows molecular ions of benzene, naphthalene, and

fluorene in the standard mix and in the stormwater sample. It was concluded that PVC was present in the sample. Styrene, allylbenzene, α -methylstyrene, and toluene were detected in the pyrolysis chromatograms of milk and beef, indicating the possible presence of polystyrene. However, the most indicative polystyrene pyrolysis products, styrene dimer and styrene trimer, were not found (Figure 5). Therefore, the contamination with polystyrene could not be confirmed.

Table 2. Polymers and their pyrolysis products identified

Polymer	Pyrolysis products
Polystyrene (PS)	Styrene; styrene dimer; styrene trimer; allylbenzene; α -methylstyrene; toluene
Polypropylene (PP)	2,4-dimethyl-1-heptane; 3-5-dimethyl-1-hexane
Polyvinyl chloride (PVC)	Benzene, naphthalene, fluorene
Polymethyl methacrylate (PMMA)	Methyl methacrylate
Polycarbonate (PC)	Bisphenol A
Polyethylene terephthalate (PET)	Vinyl benzoate

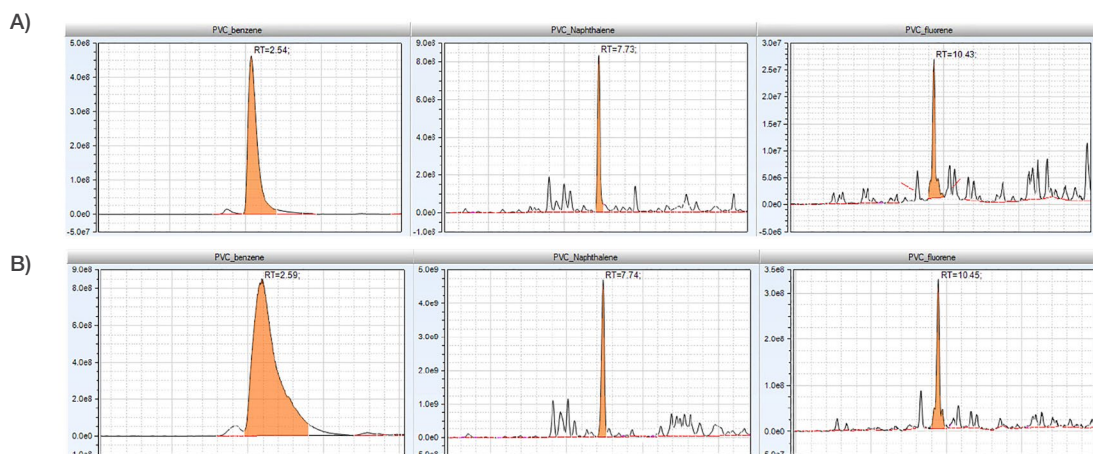


Figure 4. Identification of PVC, A) standard and B) stormwater sample

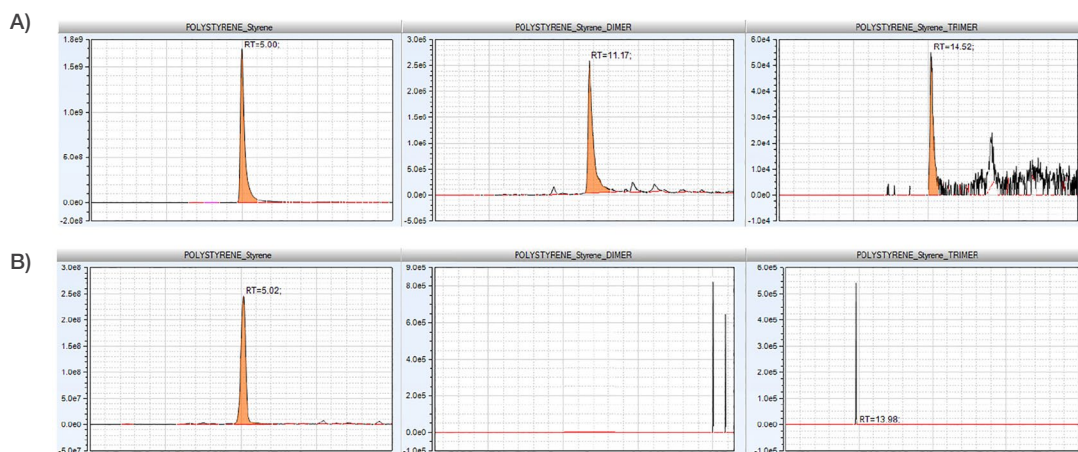


Figure 5. Identification of polystyrene, A) standard and B) milk sample

The analyzed sample matrices in this proof-of-concept study can be considered challenging due to their complexity. The high amount of matrix compounds remaining after TD can be easily deduced from the total ion current chromatogram. Figure 6 depicts an overlap of the stormwater sample TIC and the mixture of standards TIC. In the most interesting retention time range (3–18 min), the sample TIC is considerably higher than the standard mix TIC. For such a difficult sample, nominal mass spectrometry, especially single-quadrupole instruments, may not be selective enough. This clearly demonstrates the advantages of high-resolution mass spectrometry. The difference in the selectivity between high-resolution mass spectrometry and nominal mass spectrometry is demonstrated in Figure 7. The storm water sample was spiked with deuterated polystyrene

standard (D_5 -PS). The upper part of the figure shows an extracted ion chromatogram of m/z 109.0934 (the exact mass of the deuterated styrene monomer- C_8H_3D) ± 5 ppm. The use of a 5 ppm mass extraction window is the most common approach in HRAM. On the extracted ion chromatogram, there is the deuterated styrene peak ($t_R = 4.98$ min) and only one additional peak at $t_R = 7.47$ min, moreover no baseline is present. In the bottom part of the figure there is the same ion, however extracted with a mass extraction window of ± 0.5 amu, that simulates the resolution of a single quadrupole mass spectrometer. The extracted ion chromatogram contains numerous peaks and an elevated baseline, and the deuterated styrene peak is barely distinguishable from the interfering compounds.

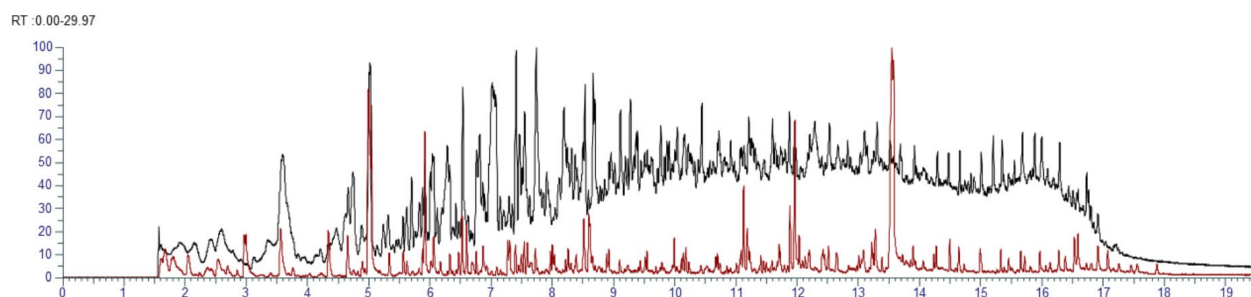


Figure 6. Comparison of the total ion current chromatograms (m/z 40–600). Red - mix of polymer standards; black - storm water sample. Both TICs come from the pyrolysis step.

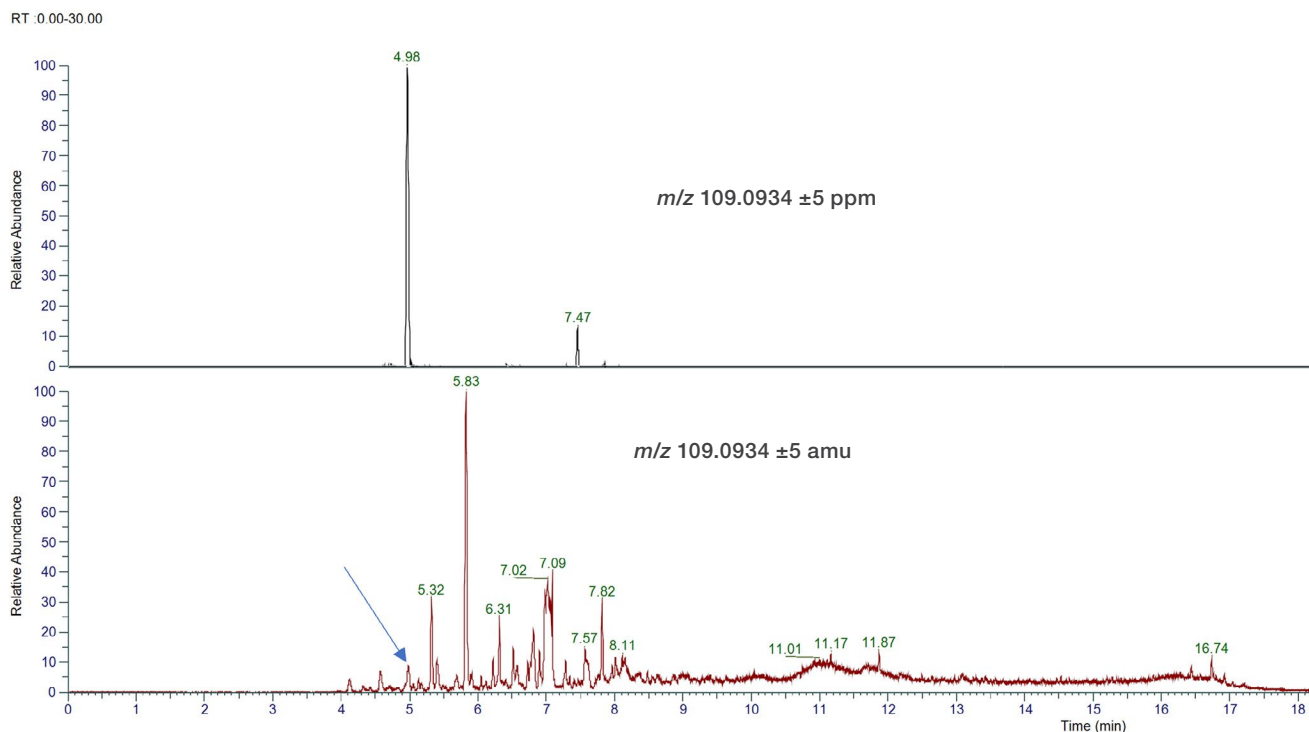


Figure 7. Extracted ion chromatogram of m/z 109.0934 for a spiked storm water sample. The upper chromatogram was obtained with a mass extraction window of ± 5 ppm (HRAM approach); the bottom chromatogram was obtained with a mass extraction window of ± 0.5 amu (simulation of a single quadrupole mass spectrometer). The blue arrow points to the deuterated styrene peak.

Conclusion

The work presented demonstrates the following:

- py-GC-Orbitrap MS is an excellent tool for the confirmation of the presence and identity of microplastics in different sample types.
- High selectivity and sensitivity were achieved by using the unique characteristics of the Orbitrap mass spectrometer, in combination with a targeted screening approach using both Compound Discoverer software and Chromeleon software.
- The combination of automated sample analysis using the pyrolyzer and targeted data processing enables an automated analysis of environmental samples.

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