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Pyrolysis-GC-Orbitrap MS - a powerful analytical tool for identification and quantification of microplastics in a biological matrix

Authors

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Keywords

Exactive GC, pyrolysis, microplastics, high resolution, Orbitrap technology, gas chromatography, fishmeal

Goal

The purpose of the experiments described in this work was to assess the applicability of pyrolysis-gas chromatography-Orbitrap™ mass spectrometry for the qualitative and quantitative analysis of plastic polymers in complex biological matrices.

Introduction

Plastics are synthetic organic polymers, commercially introduced on a large scale starting in the 1950s. Single-use plastics (grocery bags, food packaging, bottles, utensils) are persistent pollutants making up approximately 40% of beach litter[1]. This litter eventually ends up in the marine environment, with an estimated 8 million metric tons of plastic waste entering the oceans worldwide every year[2]. Most plastics have a very long degradation time, and for a timespan up to centuries they end up as macro-, micro- and nanoplastics through weathering. Due to their characteristics and additional content (monomeric residue, plasticizers, flame retardants etc.), micro- and nanoplastics can have complex toxicological effects on marine life through direct ingestion[3-5] and/or leachates[6]. This might represent a hazard for ecosystems and for human exposure through consumption and inhalation[7]. As this is an emerging field, there are limited studies on the identification

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of plastic polymers, and stringent quantification requirements remain to be developed. Estimates of plastic loads in the oceans range six orders of magnitude, while no comprehensive data exist for microplastics in soils despite considerable agricultural use[8].

Among the analytical techniques used for the analysis of microplastics are Fourier Transform Infrared (FTIR), Raman spectroscopy and microscopy and also pyrolysis-gas chromatography mass spectrometry (py-GC-MS). Py-GC-MS presents a promising approach for surveillance where throughput is critical. Furthermore, this analytical approach would enable time-saving detection of bulk amounts of micro- and nanoplastics below the lower size limit of the microscopy techniques. Therefore, low detection limit, dynamic range, and linearity as well as high compound selectivity and measurement uncertainty are crucial.

In this study, the efficiency of pyrolysis GC coupled to high-resolution, accurate-mass spectrometry was investigated for the qualitative and quantitative analysis of microplastics, as opposed to single quadrupole, which has been used in previously published work[9]. The sample pyrolyzer was connected to a bench top, high-resolution accurate-mass Orbitrap-based GC-MS system that facilitates the detection and quantification of low level compounds against a complex chemical background. The experiments described here focused on preliminary assessment of the power of accurate mass for the characterization of plastic polymers as well as the quantitative performance of this analytical configuration.

Experimental

Sample preparation

Custom-made known plastics standards were obtained through participation in the BASEMAN research project, which is financed as part of Joint Programming Initiative (JPI) Oceans, through the Norwegian Research Council (NFR). Aliquots of polymethyl methacrylate (PMMA) and polystyrene (PS) standards, dissolved in ethyl acetate, were transferred to pyrolyzer cups with the final weight of each polymer in each cup approximately 0.05, 0.5, 5, and 50 µg. To investigate whether these polymers could be detected in a more complex matrix easily, fishmeal was decomposed using 10% KOH (w/w) at 50 °C followed by 30% H_2O_2 (w/w) at 40 °C. Between 0.3 and 0.5 g fishmeal resulted in about 5–10 mg decomposed material, which was spiked with 2.5 µg PMMA and 2.7 µg PS. To evaluate the qualitative properties of the py-GC-MS, samples of solid polymers of polyamides (PA), polycarbonate (PC), polyethylene (PE), PMMA, polypropylene (PP), PS, polyvinyl chloride (PVC), and poly(ethylene terephthalate) (PET) and mixtures thereof (10–100 µg of each polymer) were weighed into pyrolyzer cups. To all samples, 10 µL tetramethylammonium hydroxide (TMAH; Sigma-Aldrich; 25%, v/v) was added as a methylating agent before analysis.

Instrumental analysis

The Frontier Lab's Multi-Shot Pyrolyzer™ (Frontier EGA/PY-3030D) with Auto-Shot Sampler™ (AS-1020E) was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N 26096-1420). The GC system was then coupled to a Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer (Figure 1). The Exactive GC system was tuned and calibrated in under one minute using PFTBA to achieve the best ion transmission and sub-ppm mass accuracy. The mass spectrometer was operated in full-scan mode using 60,000 mass resolution (measured as FWHM at *m/z* 200). Lockmass corrected data was processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the pyrolysis, GC, and MS conditions are given in Tables 1 and 2.

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

Table 1. GC and injector conditions

TRACE 1310 GC System Parameters	
Injector:	Thermo Scientific™ Instant Connect Thermospray (TSI)
Inlet:	270 °C
Carrier Gas:	He, 1.2 (mL/min)
Split Flow:	200 mL/min
Oven Temperature Program	
Temperature 1:	50 °C
Hold Time:	1 min
Temperature 2:	320 °C
Rate:	15 °C/min
Hold Time:	5 min
Multi-Shot Pyrolyzer EGA/PY-3030D Parameters	
Oven Temp.:	600 °C
Interface Temp. :	300 °C

Table 2. Mass spectrometer conditions

Exactive GC Orbitrap Mass Spectrometer Parameters	
Transfer Line:	320 °C
Ionization Type:	EI
Ion Source:	280 °C
Electron Energy:	70 eV
Emission Current:	20 µA
Acquisition Mode:	Full-scan, centroid
Mass Range:	50-650 Da
Resolving Power:	60,000 FWHM at *m/z* 200
Lockmass, Column Bleed:	207.03235 *m/z*

Data processing

Data was acquired in full-scan centroid mode using TraceFinder software, version 4.1. This is a single software platform that allows instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder software also contains spectral deconvolution and spectral matching functionality.

Results and discussion

The applicability of the Exactive GC Orbitrap GC-MS system in combination with pyrolysis for qualitative and quantitative assessment of microplastics was tested using both standards and fishmeal that were spiked with known amounts of plastic polymers. Indicator exact masses, characteristic for the plastic compounds, were extracted using different *m/z* windows to demonstrate advantages of high-resolution, accurate-mass capabilities for this application.

Sensitivity, selectivity and linearity

Linearity was tested using PS and PMMA standards with the concentration points of 0.05, 0.5, 5, and 50 µg, which is a range that corresponds to amounts found in real samples analyzed with pyrolysis-GC-MS (Kögel et al. unpublished). Excellent linear responses were obtained for both compounds with coefficient of determinations R^2 >0.999 and %RSD for residuals <15% (Figure 2-1). Using the GC-MS conditions described in Tables 1 and 2, the number of scans/chromatographic peak exceed 25 scans for a 3.5-second-wide peak for PS, and 20 scans for PMMA, allowing for and enabling accurate peak integration and compound quantification (Figure 2-2).

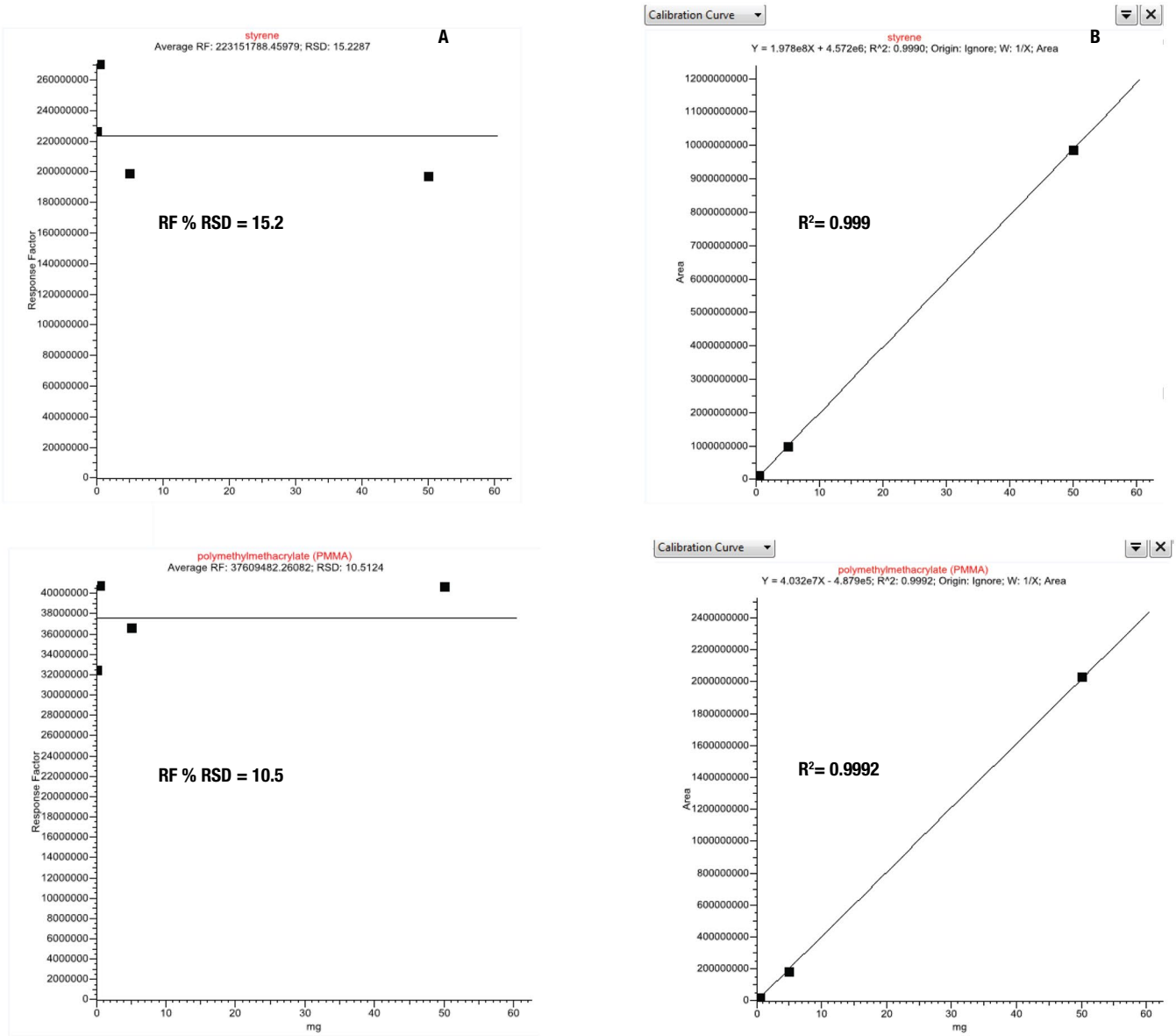

Figure 2-1. Chromatography and linearity of styrene (top) and methyl methacrylate (bottom) in a 0.05 µg standard. Extracted ion chromatograms of styrene (*m/z* 104.0621) and methyl methacrylate (*m/z* 99.0441) were used to assess the linearity of response (R^2 and RF %RSD residuals) over four concentration points 0.05–50 µg (A and B).

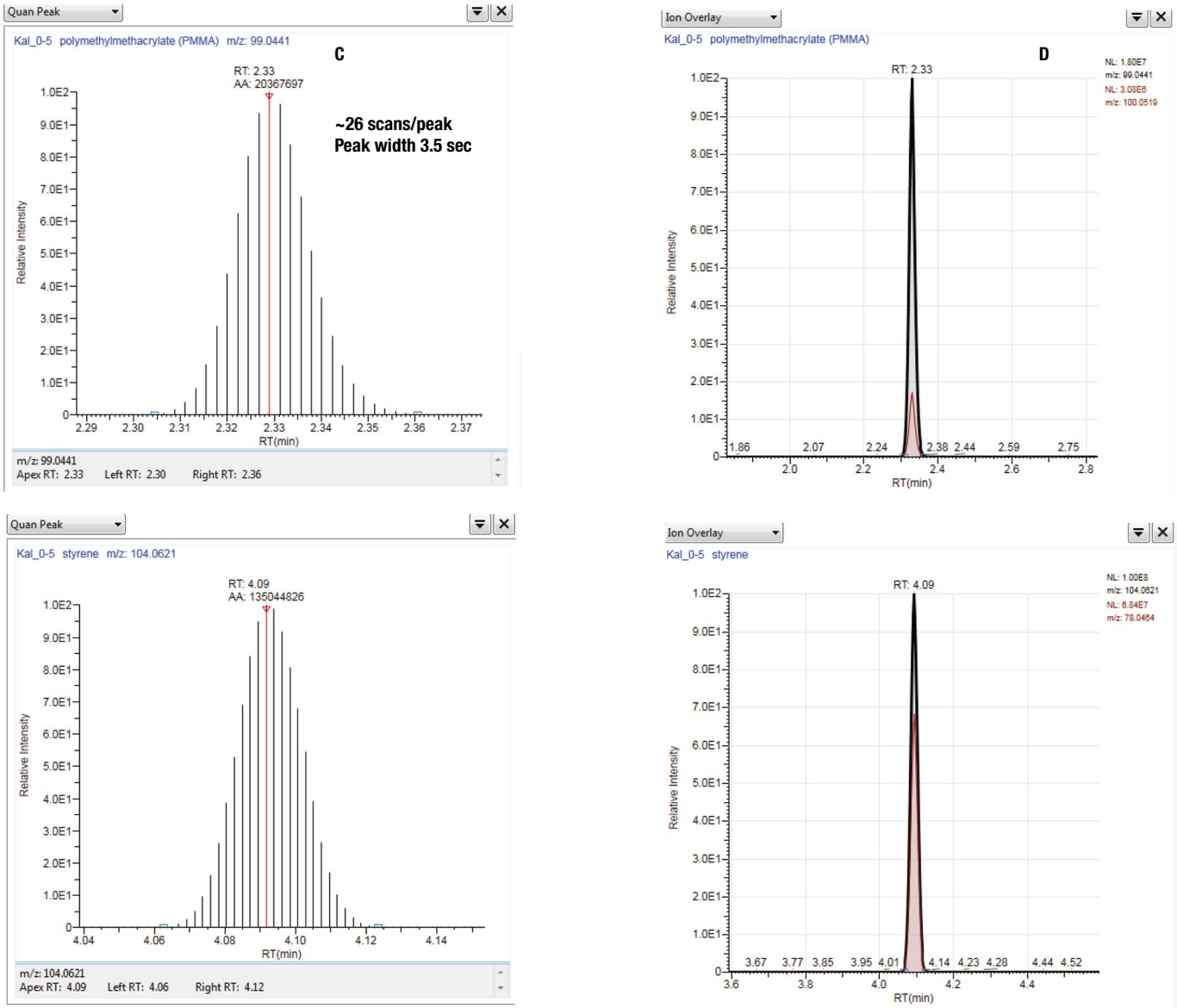

Figure 2-2. Chromatography and linearity of methyl methacrylate (top) and styrene (bottom) in a 0.05 µg standard. C shows integrated peak area of the quantification ion with corresponding scans/peak, and D shows an overlay of the quantification ion and the confirmation ion. Data were acquired in full-scan at 60,000 resolution (FWHM at *m/z* 200). Peak retention time (RT) as well as peak area counts (AA) are annotated. Peak smoothing (5× moving average was applied).

Consistent mass accuracy

In addition to the quantification performance, the mass accuracy of target compounds was assessed across all the concentrations. Obtaining accurate mass information is critical to avoid misidentification and erroneous quantification. For all compounds targeted, the mass accuracy was < 1 ppm irrespective of matrix complexity or concentration level. Figure 3 shows an example of consistently high mass accuracy maintained for all ions in PS spectra measured in the lowest (0.05 µg) and at the highest standard (50 µg).

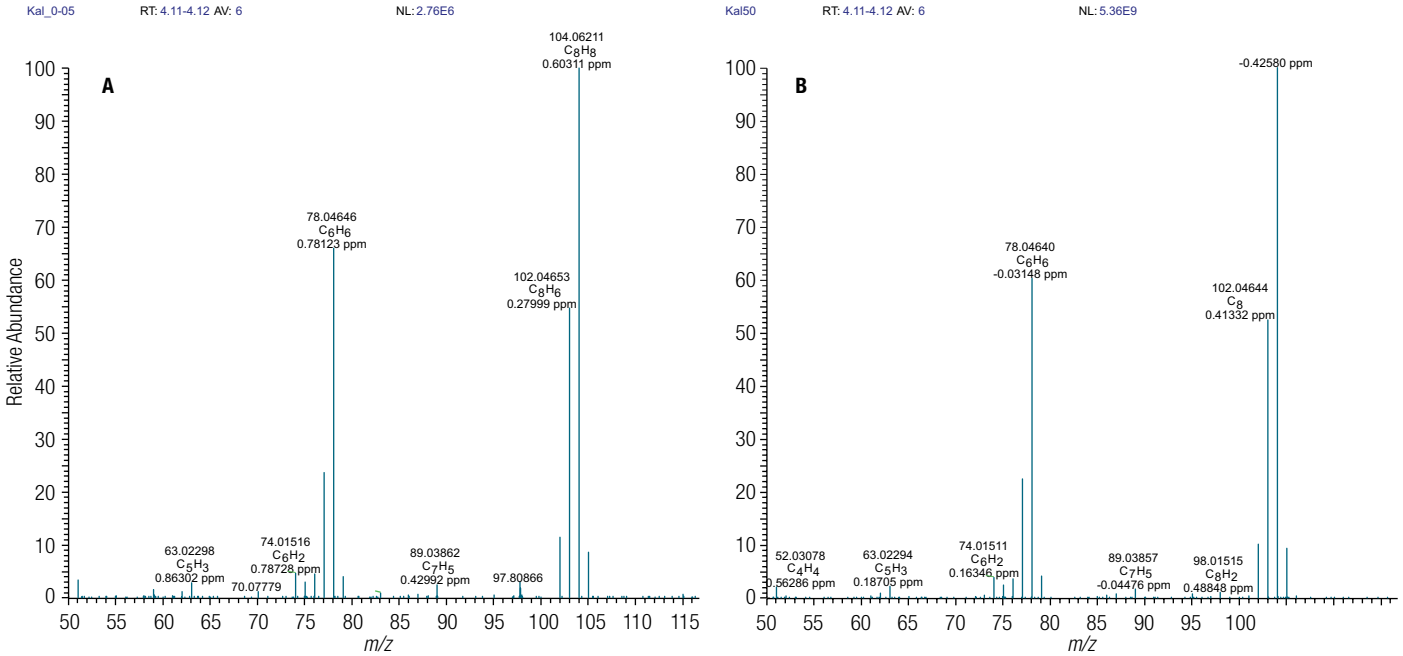

Figure 3. Stability of spectral fidelity and mass accuracy irrespective of compound concentration demonstrated for PS (styrene monomer) at 0.05 µg (A) and at 50 µg level (B)

Quantification of microplastics in a decomposed fishmeal sample spiked with PS and PMMA

To assess the accuracy of quantification of microplastics using the Exactive GC Orbitrap py-GC-MS approach, a fishmeal sample was spiked with known amounts of PS and PMMA (Table 3) and back calculation of these amounts was performed using individual PS and PMMA external standard calibration curves over the range of 0.05 to 50 µg. Even without using internal standard correction, the average % deviation from the expected results was good (-2.3% as average deviation calculated for PS and PMMA) for analysis in complex matrixes (Table 3).

Table 3. Accuracy of quantification demonstrated for PS and PMMA spiked into a decomposed fishmeal sample. Quantification of PS was done using the sum of *m/z* 104.06211 + *m/z* 91.05418, whereas PMMA was quantified using the sum of *m/z* 99.04407 + *m/z* 100.05188.

Compound	Spiked Amount (µg)	Measured Amount (µg)	% Deviation
Polystyrene (PS)	2.7	2.9	+7.4
Polymethyl methacrylate (PMMA)	2.5	2.2	-12.0

Selectivity

By using the Exactive GC Orbitrap py-GC-MS operated at routine 60,000 resolution, it is possible to selectively isolate *m/z* values corresponding to pyrolysis products of various polymers. Examples of selectivity for PA, PC, PE, PMMA, PP, PS, PVC, and PET are shown in Figures 4 and 5. Typical fragment masses used to identify and quantify the different plastic materials differ from *m/z* 78 (PVC) to *m/z* 228 (PC).[9] The minimum mass difference that is meaningful to use when extracting the quantifier ions is a function of the resolution used.

Figure 4 shows extracted quantifier ions; the ions are extracted at ±5 ppm, just above the smallest mass difference that can be resolved with a resolution of 60,000. The many characteristic peaks of the x-meric pyrolytic products of the polymer types with very small monomers (PP and PE) are clearly visible. Another important feature is the clearly peaking multimers of PS, which are necessary for selective quantification of PS, excluding the monomeric styrene, which is a pyrolysis product also deriving from natural marine chitin[9].

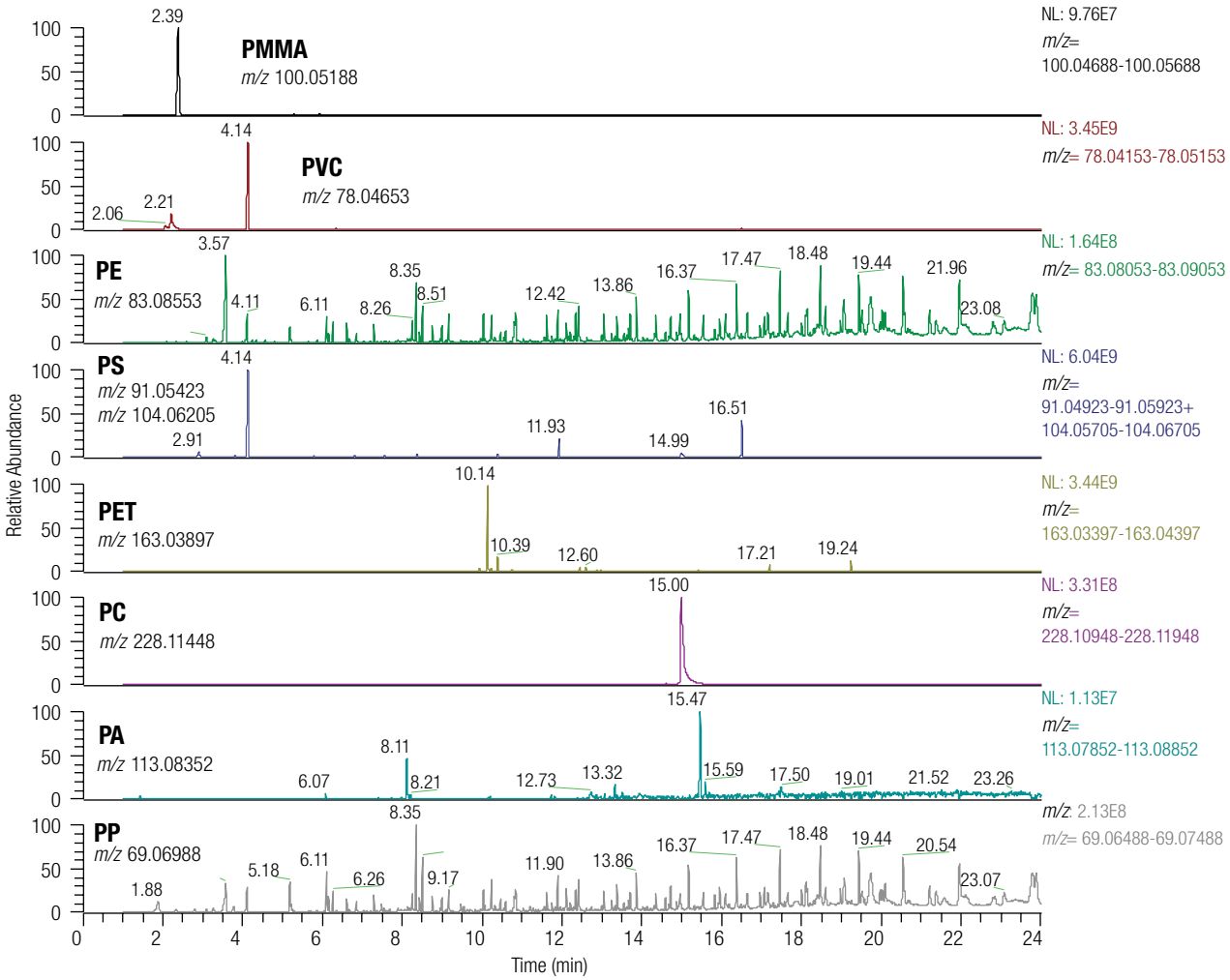

Figure 4. Selectivity for PMMA, PVC, PE, PS, PET, PC, PA, and PP polymers demonstrated as extracted ion chromatograms of each polymer using a ±5 ppm extraction window

In addition, the importance of high-resolution accurate-mass mass spectrometry for selectivity is demonstrated in Figure 5 for PA and Figure 6 for PS. Using an extraction window of >±100 mmu to simulate a low-resolution mass spectrometer, crowded extracted ion chromatograms are seen. When taking advantage of the accurate mass measured by using an extraction window of ±2 ppm, the chromatographic peaks selected for quantification are more evident.

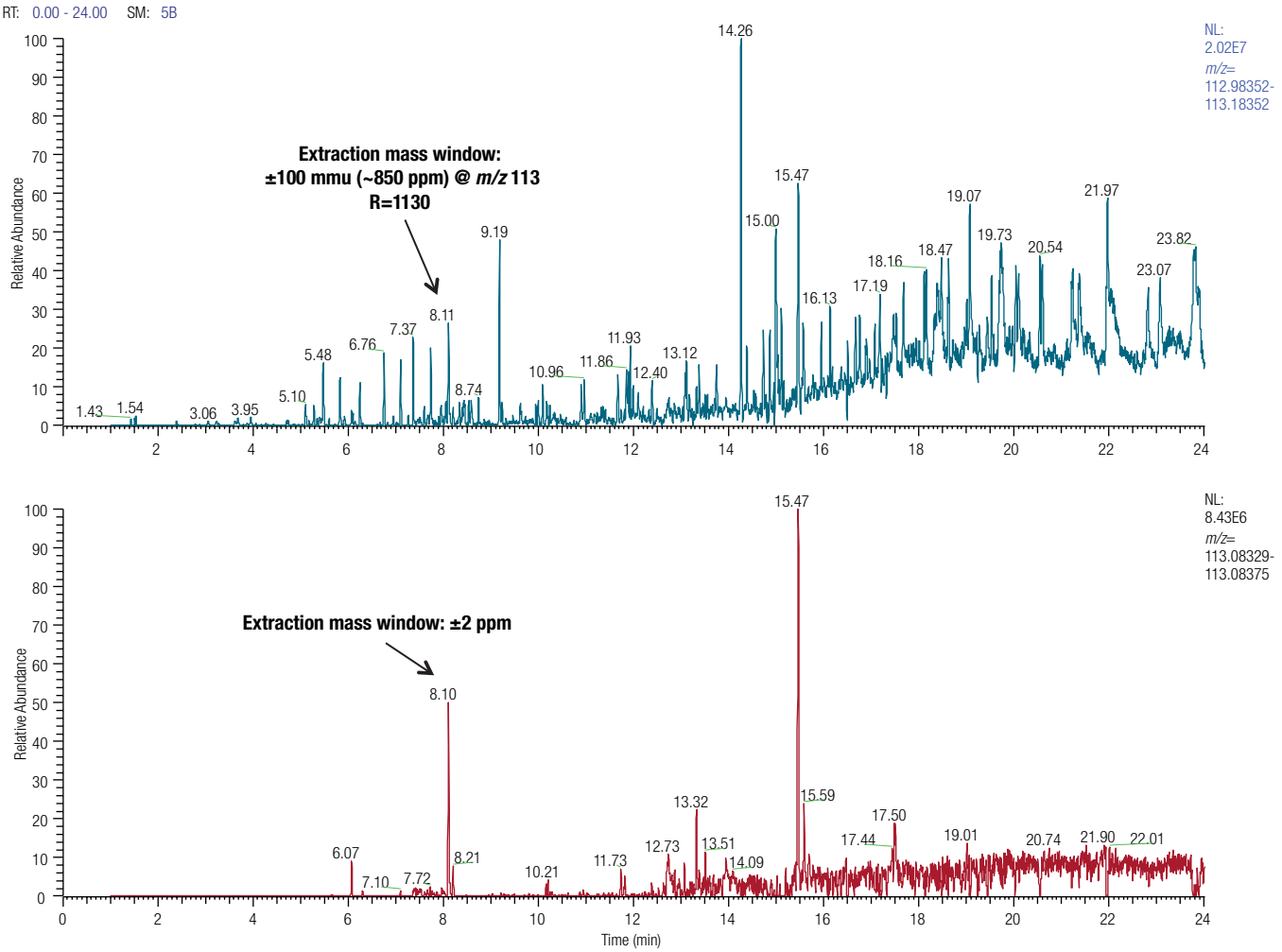

Figure 5. Selectivity for PA fragments, RT = 8.10 min, using an extraction window for *m/z* 113.08352 of ±100 mmu (equivalent to ~850 ppm) simulating a mass resolution of ~1100 (top) and ±2 ppm taking advantage of the accurate mass measured (bottom)

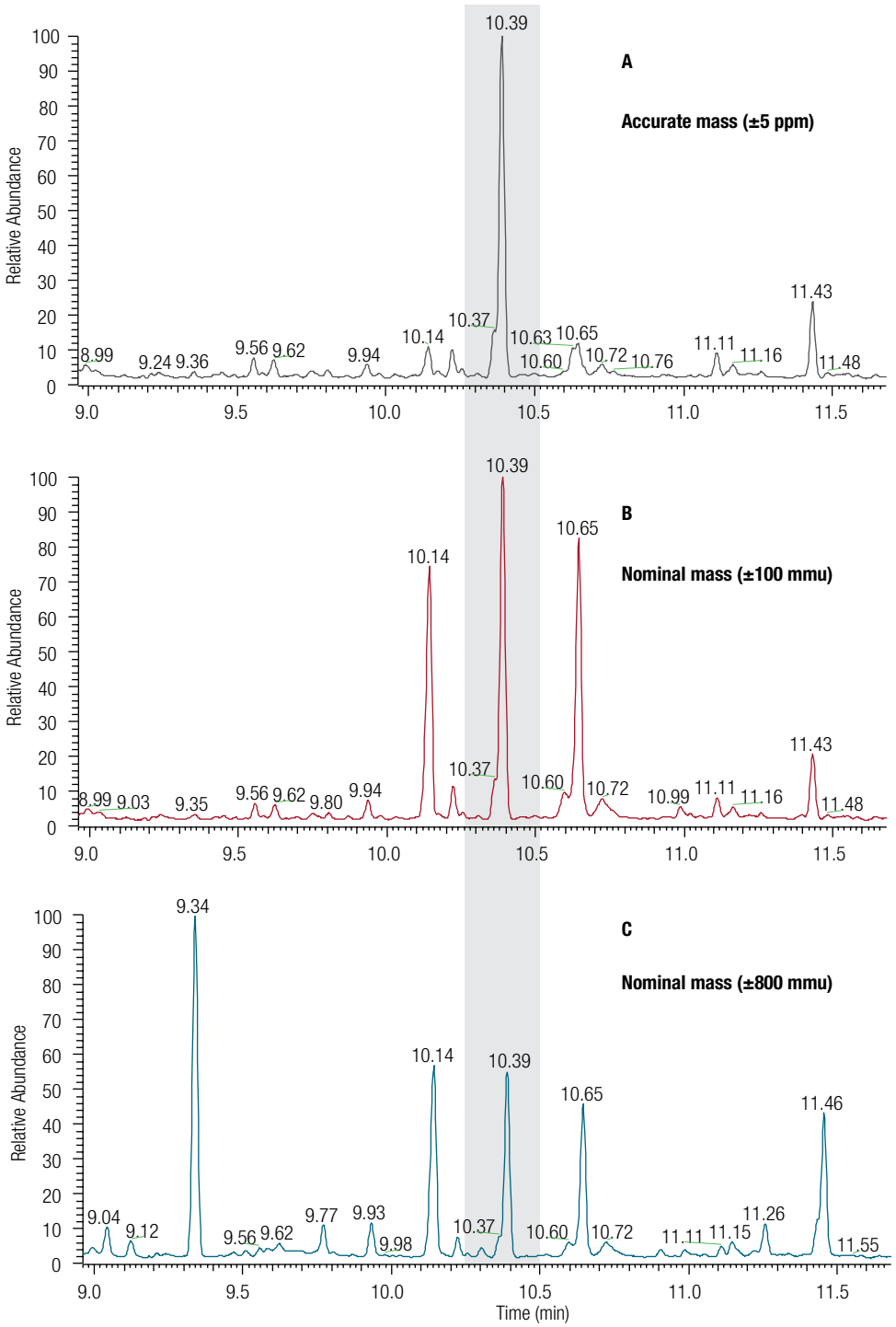

Figure 6. Full-scan accurate mass selectivity demonstrated for PS in a mixed sample containing PS, PA, PC, PE, PMMA, PP, PVC, and PET. Accurate mass measurements enable confident detection (±5 ppm, A), whereas at nominal mass acquisitions additional interfering compounds can be detected (±100 mmu, B and ±800 mmu C).

Additional non-targeted unknown compounds identification

In addition to the targeted quantification, TraceFinder software allows for untargeted analysis of samples that were acquired on GC-Orbitrap systems. This represents a distinct advantage of Orbitrap GC technology due to its routine full-scan, high-resolution mode of operation. This way the analyst can screen the raw data from the quantitation experiment to search for additional chemicals resulting from the pyrolysis process. An example of this is shown in Figure 7 for α-methylstyrene (2-phenylpropene), a known degradation product formed as a consequence of PS pyrolysis[10].

Conclusions

The results of this study demonstrate that:

- The Exactive GC Orbitrap GC-MS system in combination with pyrolysis has proven to be a very promising analytical technique that opens new possibilities with respect to the analysis of microplastic polymers in biological matrices.
- The Exactive GC Orbitrap GC-MS system demonstrates excellent linear response over a concentration range of 0.05 µg to 50 µg absolute weight for each plastic material with accurate quantitative estimation of microplastic polymers in real samples.
- The high resolving power of the Exactive GC Orbitrap GC-MS system facilitates sub-ppm mass accuracy at low and high concentrations, essential for achieving enough selectivity to confidently separate and identify pyrolysis products and reduces detection limits (ex: PS and PP).
- Full-scan acquisition enables the detection and identification of additional compounds produced during the pyrolysis process of microplastics. Putative identifications require confirmation using analytical standards.

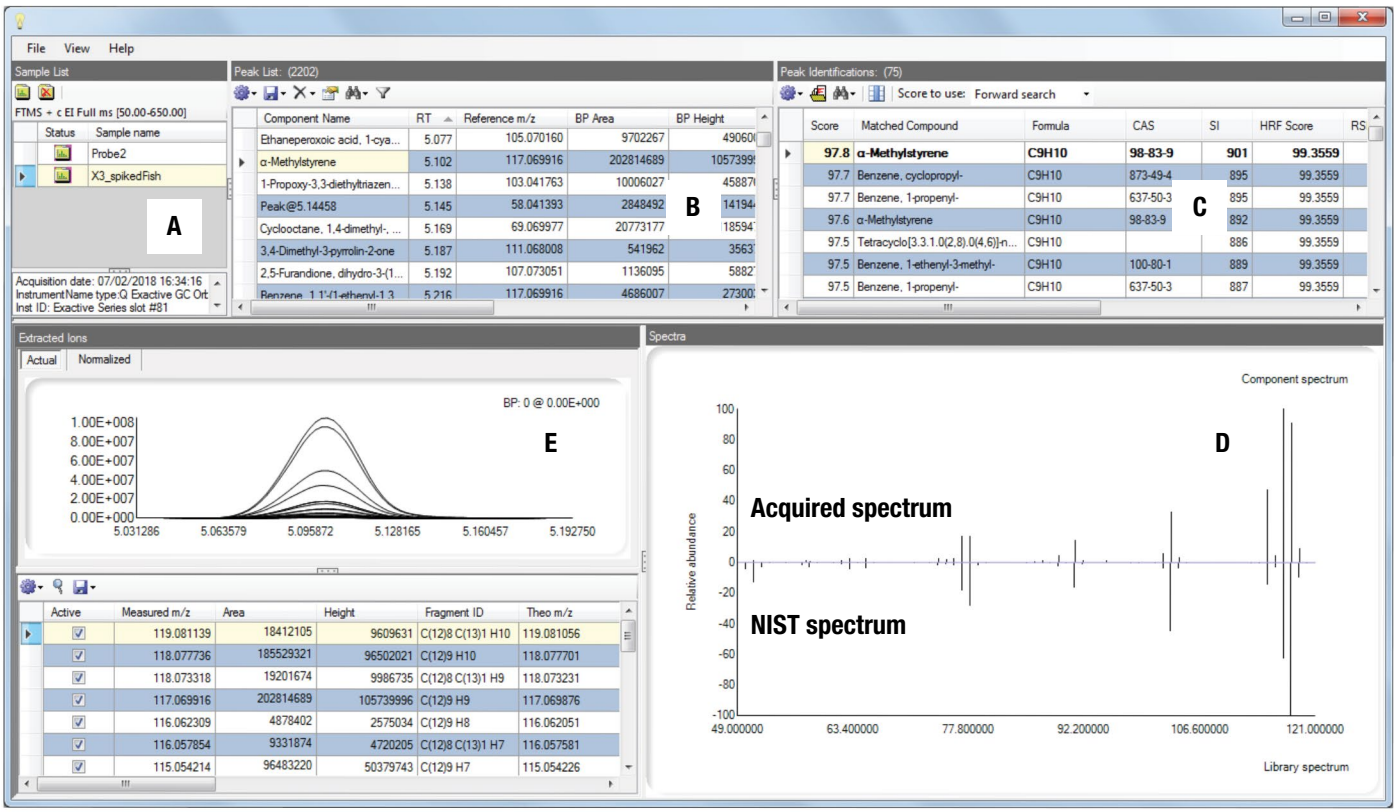

Figure 7. TraceFinder deconvolution browser showing α-methylstyrene (RT=5.1 min) tentative identification based on library (NIST) match (reverse search index, SI 901), fragment rationalization with a confidence score > 97% and mass accuracies of measured fragments (e.g., base peak *m/z* 117.069, Δ_{ppm} = 0.3). Samples processed (A), peaks detected (B), identified chemicals (C), acquired versus library spectra (D), and deconvoluted mass spectra for α-methylstyrene (E) are indicated.

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