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Quantification of Microplastics in Environmental Samples Using Pyrolysis and GC/MSD

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Abstract

There is growing interest in quantifying microplastics in environmental samples. This application note presents a robust pressurized liquid extraction (PLE) with pyrolysis-gas chromatography-mass spectrometry (pyr-GC/MS) method for quantitation of microplastics like polyethylene (PE), polypropylene (PP), and polystyrene (PS) at low concentrations in environmental matrices using the Agilent 5977B GC/MSD, Agilent 7890B GC, and Agilent MassHunter workstation software. Linearity, limits of quantitation (LOQs), and reproducibility for real environmental samples were evaluated. The GC/MSD addressed the insufficient limits of detection that have challenged previous methods. PE, PP, and PS microplastics were quantified down to 0.005 mg/g. Excellent linearity (R^2 >0.97) for calibration samples from 0.005 to 1 mg/g was obtained. Relative standard deviations (RSDs) for both spiked and environmental samples were <10% or lower, demonstrating excellent system reproducibility and reliability.

Introduction

Microplastics are commonly defined as plastic particles and fragments smaller than 5 mm in length. These particles enter the environment from many sources and persist over hundreds of years or more, thus increasing the chances of their ingestion and accumulation in organisms. As a result, there is increased interest in quantifying microplastics concentrations in environmental samples such as soils, water, and organic matter.

Due to their very high molecular weights and poor solubility in most solvents, quantification of microplastics in environmental samples using traditional GC/MS or LC-tandem MS methods has been challenging. Microplastics can be measured using spectroscopic techniques like infrared spectroscopy (IR), which provide particle numbers, chemical identity, and size and area distributions. Complementary to these techniques, the use of thermal extraction coupled to GC/MS gives information on mass concentrations that could be essential for regulatory and monitoring purposes.[1,2] Though pyrolysis-gas chromatography-mass spectrometry (pyr-GC/MS) approaches have shown promise, traditionally they have suffered from insufficient limits of detection, matrix-related background interferences, and inhomogeneous distributions of microplastic particles in samples.

Dierkes et al. have proposed that reliable determination of microplastics in environmental samples requires limits of quantitation of below 1 mg/g.[3] To overcome analytical limitations, Dierkes et al. developed a robust method that used pressurized liquid extraction (PLE) with pyr-GC/MS for quantitation of microplastics at low concentrations in complex sediments, soil, and sewage sludge matrices.[3] The authors designed the method to provide relatively high-throughput analysis of frequently encountered microplastics such as polyethylene (PE), polypropylene (PP), and polystyrene (PS). This application note describes the suitability of the Agilent 5977B GC/MSD with the Agilent 7890B GC and Agilent MassHunter workstation software to perform the method. In particular, the GC/MSD was evaluated in terms of linearity, limits of quantitation (LOQs), reproducibility, and detection and quantification of PE, PP, and PS in real environmental samples. Besides these most abundant microplastics, other plastic types like nylon polymers, polyurethane (PUR), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), and fluorocarbon polymers can be analyzed using this technique.

Experimental

Standards and sample preparation

Due to the poor solubility of PE and PP in most solvents, preparing calibration concentrations from a stock solution by dilution is nearly impossible. Direct weighing of polymers is also unsuitable due to the errors associated with weighing the very small amounts needed to prepare the lower calibration levels. Therefore, calibration samples were made by serially diluting a stock mixture with an inert matrix (calcined sea sand.) PE, PS (PSS Polymer), and PP (Sigma-Aldrich) were ground in a cryo mill and 250 mg of each were mixed into calcined sea sand to obtain a 10 g stock mixture. The mixture was then homogenized for 20 minutes in a planet mill. The stock mixture was serially diluted by mixing 1 to 2 g with sand to obtain a polymer concentration for each calibration point from 0.005 to 10 mg/g. The mixture was homogenized after each dilution step.

For PLE, 1 g sample was placed into 10 mL stainless steel extraction cells and extracted using a solvent extraction instrument. Pre-extraction with methanol was used to reduce matrix effects. Microplastics extraction used tetrahydrofuran at 185 °C and 100 bar. The extracts were collected in 60 mL vials containing 200 mg calcined silica gel. After extraction, 10 µL of polystyrene-d5 (270 µg/mL in dichloromethane, Polymer Source) was spiked as internal standard. The solvent was next evaporated with the precipitating polymers captured on the silica gel. Microplastics adhering to vials were rinsed with dichloromethane at least three times. The silica gel was subsequently homogenized for 5 minutes.

Details about the standard and sample preparation steps are provided in the publication by Dierkes et al.

Calibration, method validation (except for particle size experiments), blank (containing only sand), and environmental samples were extracted using the same procedure.

Pyrolysis GC/MS analysis and instrumentation

Pyrolysis GC/MS analysis of all samples was performed using a Multi-Shot Pyrolyzer EGA/PY-3030D and Auto-Shot Sampler AS-1020E (Frontier Laboratories) with the 7890B gas chromatograph and 5977B GC/MSD. For each sample analysis, 20 mg of the homogenized silica gel were weighed into an 80-µL pyrolysis cup (Eco-Cup LF, Frontier Laboratories) and pyrolyzed at 600 °C.

The 7890B gas chromatograph was equipped with a 30 m × 0.25 mm id, 0.25 µm film thicknesses column with an (5%-Phenyl)-methylpolysiloxane Phase (e.g. HP-5ms UI, 19091S-433UI). The Agilent inert GC/MS Interface flow path is designed to provide enhanced performance for analysis of

low-level analytes, extending the range of quantitation and detection. The 5977B GC/MSD was configured with an extractor EI source and operated in selected ion monitoring (SIM) mode to maximize sensitivity. The Extractor EI source maximizes the number of ions created and transferred out of the source body into the quadrupole mass analyzer. The GC/MS run time was 29 minutes. Tables 1 and 2 summarize the GC/MS system configuration and parameters used to carry out the method.

Table 1. Agilent 7890B gas chromatograph parameters.

Parameter	Value
Inlet	Split/splitless (SSL)
Mode	Split
Split Ratio	20:1
Total Flow	28.2 mL/min
Carrier Gas	Helium
Inlet Liner	4 mm id, split, wool
Inlet Liner Part Number	5183-4711
Inlet Temperature	320 °C
Oven	
Initial Oven Temperature	40 °C
Initial Oven Hold	2 min
Ramp Rate 1	20 °C/min
Final Temp 1	320 °C
Final Hold 1	13 min
Total Run Time	29 min
Column	
Type	5% Phenyl methylpolysiloxane phase
Length	30 m
Diameter	0.25 mm
Film Thickness	0.25 μm
Control Mode	Constant flow
Flow	1.2 mL/min
Inlet Connection	Split/splitless inlet (SSL)
Outlet Connection	MSD

Table 2. Agilent 5977B GC/MSD parameters.

Parameter	Value
Model	5977B
Source	XTR EI 350
Tune File	EI-Atune.u
Mode	SIM
Solvent Delay	2 min
EM Voltage Gain Mode	1
Trace Ion Detection	Off
Quad Temperature	150 °C
Source Temperature	230 °C
Transfer Line Temperature	280 °C
Dwell Time	100 ms

Data acquisition and quantification method

MassHunter workstation software revision B.07.00 was used for data acquisition and analysis. Pyrolysis products specific to each polymer were monitored in SIM mode for quantification by MassHunter Workstation software. Table 3 shows the ions and retention times monitored for the polymer pyrolysis products.

For quantification, calibration curves were fitted using 1/x weighting with the MassHunter software Quantitative Analysis tool.

Method evaluation and validation

Method selectivity was evaluated by extracting and analyzing matrices not contaminated with microplastics mixed with sea sand (3 wt. %). The matrices included cherry tree wood, evening primrose leaves, yew needles, humic acids, carp filet, crayfish, motor oil, and filter paper and represented typical ingredients of environmental matrices such as proteins, fats, and polysaccharides.

Method recovery was determined by analyzing 1 g sand containing 3 wt.% of humic acids spiked with 0.05 and 0.75 mg/g of PE, PP, and PS. Method reproducibility was determined using repetitive analyses of five environmental samples each extracted several times.

The influence of microplastic particle sizes on reproducibility and the minimum sample mass needed to obtain representative aliquots were determined by analyzing mixtures of sand and PE of two different sizes (10 to 50 μm and 200 to 400 μm) at two spiked concentrations (1 mg/g and 10 mg/g).

To evaluate method effectiveness for testing real environmental samples, at least 500 g of a variety of matrices including sediments, suspended matter in rivers, roadside soils, potting soil, and sewage sludge were collected, air-dried and then prepared, extracted, and analyzed in at least triplicate using the procedures described. Contamination of the samples from plastic tools and vessels was carefully avoided.

Table 3. Ions and retention times monitored per polymer and pyrolysis product.

Polymer	Pyrolysis Product	Ions Monitored (m/z)	Retention Time (min)
Polypropylene	2,4-Dimethyl-hept-1-ene	126, 70	4.89
Polyethylene	1,14-Pentadeca-diene	81	10.29
Polyethylene	1-Pentadecene	97	10.32
Polystyrene	Styrene	104, 91	5.54
Polystyrene-d5	Styrene-d5	109	5.50

Blue: Quantification ions

Results and discussion

Linearity and limits of quantitation

Figure 1 shows the extracted ion chromatograms of the standard mixture at 0.25 mg/g.

To plot calibration curves, relative peak areas were calculated using the polystyrene-d5 internal standard. Linear calibration curves ($R^2 > 0.97$) down to 0.005 mg/g were obtained for all the polymers. Linear responses were obtained up to 1 mg/g for PP and PS. For PE, response was linear up to 10 mg/g.

False positive detection of microplastics is a concern because of the pervasive use of plastic in most laboratories. Therefore the authors defined the LOQs as the concentration at which 95% of blank samples show a lower signal. Using the average relative responses of 11 blank samples, the blank polymer concentrations with the one-sided confidence interval ($t_{(p = 0.95; n - 1 = 10)} = 1.812$) were calculated as the LOQs (Table 4.)

Table 4. Limits of quantification.

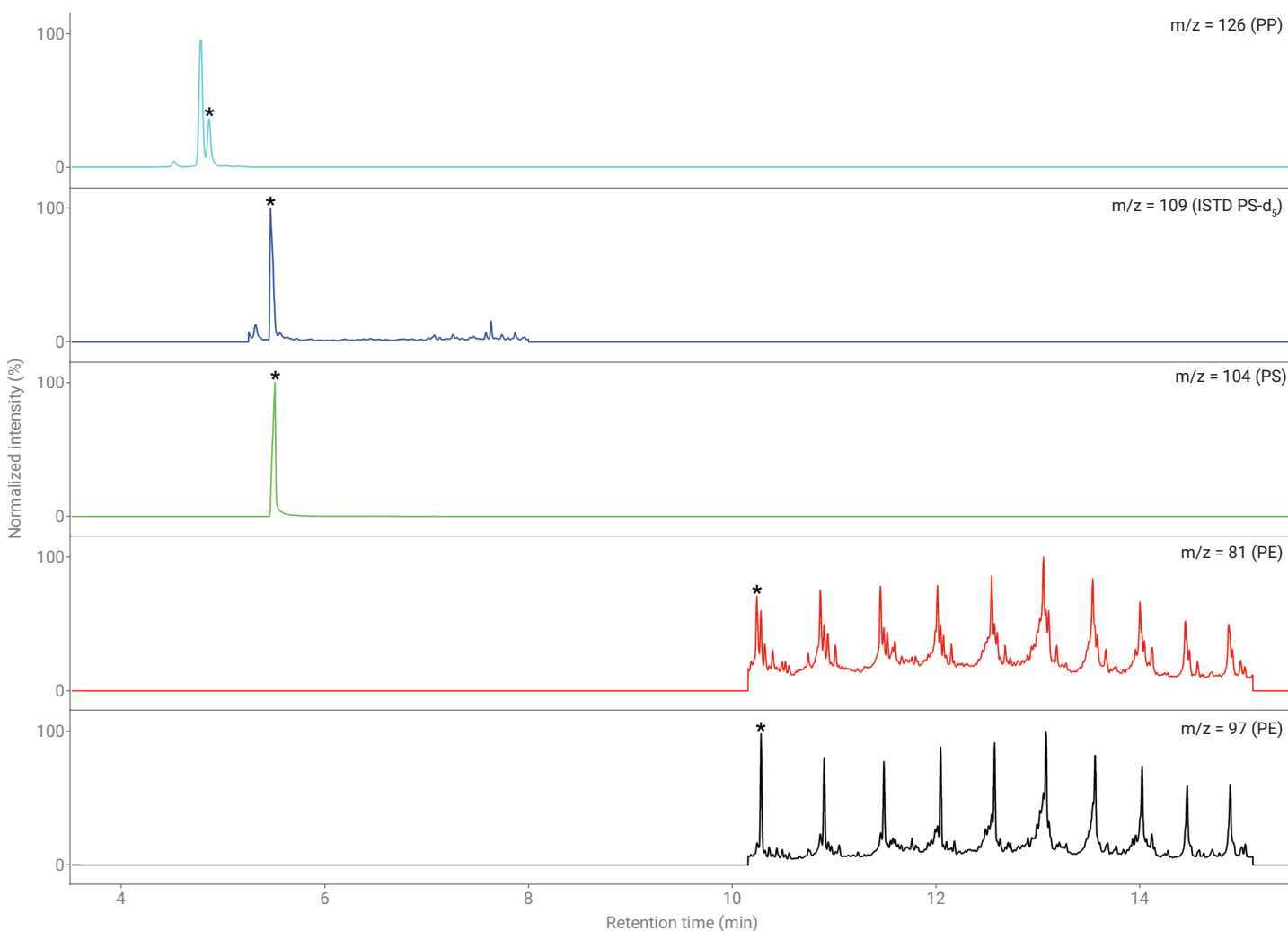| Polymer	LOQ (mg/g)
PP	0.007
PE (*m/z* 97)	0.007
PE (*m/z* 81)	0.16
PS	0.008

Figure 1. Extracted ion chromatograms of the standard mixture at 0.25 mg/g.

Method selectivity

Because the pyr-GC/MS quantitation method is based on indirect quantitation of polymer pyrolysis products (Table 3), interferences from matrix ingredients in the noncontaminated environmental samples were evaluated. The matrices tested showed no concerning interferences for PP. Wood matrix led to an increased background of 0.042 mg/g for PS. Even though the amino acid phenylalanine is a pyrolysis precursor for styrene, protein-rich fish matrices did not produce interferences. Proteins were eliminated during the pre-extraction with methanol. Thus it is unlikely that pyr-GC/MS based quantification of PP and PS in environmental samples is significantly biased by the presence of naturally occurring substances as long as there is not a high proportion of wood present. However, quantitation of PE was subject to interferences. For example, 30 mg engine oil produced ion intensities similar to the 1.1 mg PE standard. This result could present a problem when testing environmental samples contaminated with oil or gasoline. Overall, pre-extraction with methanol, solvent extraction with THF, and the indicator ions used for quantification resulted in high method specificity, and ensured method suitability for microplastics quantification in matrices containing high quantities of organic compounds.

Method recovery and reproducibility

Table 5 shows the recoveries for PP, PS, and PE in humic acids and sea sand. Considering the ±20% statistical uncertainty, matrix effects were not problematic. Recoveries in other organic materials--fish filet, crayfish, leaves, and needles--were similar. Relative standard deviations (RSDs) were <10% or lower, demonstrating excellent method reproducibility and reliability.

Table 5. Recoveries for different polymers and spiking levels.

Polymer	Spiking level (mg/g)	Recovery average (%)*
PP	0.05	95 ± 9
	0.75	85 ± 15
PS	0.05	77 ± 19
	0.75	118 ± 17
PE (*m/z* = 81)	0.05	<LOQ
	0.75	123 ± 1
PE (*m/z* = 97)	0.05	114 ± 35
	0.75	131 ± 15

* Confidence interval $t_{(0.95; n - 1 = 2)}$ = 4.303

Because direct (not extracted) pyr-GC/MS analyses of 20 mg, the maximum manageable sample in-weight for 80 µL pyrolysis cups, resulted in RSDs of up to 50%, increased sample in-weights of up to 1000 mg for PLE with tetrahydrofuran were used. A 200 mg in-weight for the 10 to 50 µm fractions resulted in RSDs <10%, while for the 200 to 400 µm fractions, even an in-weight of 1000 mg resulted in an RSD of 21%. These results indicate that samples containing MP particles >200 µm should be ground in a cryo mill before PLE to obtain a homogeneous distribution of particles.

Method performance for analyses of environmental samples

Sediments, suspended particulates, soils, and sewage sludge were analyzed for PP, PE, and PS contamination. The results for these analyses are provided in Table 6. Findings of note include:

- In river sediment, PP and PE were detected at concentrations of 0.032 ± 0.001 and 0.028 ± 0.006 mg/g respectively, while PS was not present above the LOQ (0.008 mg/g). PE and PP were detected in the suspended matter in concentrations up to 0.98 ± 0.02 and 0.055 ± 0.010 mg/g, respectively.

- In sewage sludge, all three polymers were detected at concentrations ranging from 0.08 ± 0.02 mg/g (PP) to 3.3 ± 0.3 mg/g (PE).

- Potting soil contained PE in concentrations similar to the sewage sludge, but only traces of PP were detected. PS was not detected.

- In roadside soil, all three polymers were found with concentrations up to 0.85 ± 0.07 mg/g (PE).

Theses results confirm that the 5977B GC/MSD is suitable for pyr-GC/MS detection and quantification of PE, PP, and PS microplastics in different kinds of matrices.

Table 6. Environmental sample results.

Sample	N	PE		PP		PS	
		Average (mg/g)	RSD%	Average (mg/g)	RSD%	Average (mg/g)	RSD%
Soil 1	7	0.79	6.0	0.039	7.4	0.054	9.6
Soil 2	6	0.85	7.3	0.040	9.2	0.057	7.9
Sludge 1	7	3.03	6.8	0.100	12.3	0.61	4.1
Sludge 2	7	3.27	4.2	0.081	10.0	0.50	4.0
Potting Soil	7	2.81	7.1	< 0.007	n.a.	< 0.008	n.a.

Conclusion

Here, the Agilent 5977B GC/MSD with the Agilent 7890B GC and Agilent MassHunter workstation software was determined effective for routine, robust, and reliable quantification of microplastics in the environment using the pyr-GC/MS method described by Dierkes *et al.* In particular, the GC/MSD was able to address the insufficient limits of detection that have challenged previous methods. Specifically, PE, PP, and PS microplastics were successfully quantified at low concentrations (down to 0.005 mg/g) in test matrices and real environmental samples. Excellent linearity (R^2 >0.97) for calibration samples from 0.005 mg/g to 1 mg/g was obtained for PE, PP, and PS. For PE, response was linear up to 10 mg/g. Relative standard deviations (RSDs) for both spiked and environmental samples were <10% or lower, demonstrating excellent system reproducibility and reliability.

References

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