Application Note

Environmental

Food & Beverage Testing

Py-GC/MS/MS Microplastic Analysis of Samples Containing Interfering Substances

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Abstract

Plastics are widely used in every aspect of our lives and can be found throughout the environment. In this application note, the analysis of microplastics—defined as plastics with a diameter of 5 mm or less—is demonstrated using pyrolysis (Py)-GC/MS/MS. This approach provides a sensitive and selective alternative to existing spectroscopic techniques such as infrared (IR) or Raman.

Analysis of a mixed reference material containing 11 types of microplastics, performed under optimized Py-GC/MS/MS (multiple reaction monitoring [MRM] mode) conditions, yielded linear calibration curves with R^2 values generally above 0.99 for each microplastic type across a concentration range of several hundred ng to several µg.

We present a comparison between Scan and MRM data acquisition modes, illustrating the enhanced selectivity of MRM mode and associated lower noise and higher signal-to-noise (S/N) ratios. Additionally, we demonstrate that more accurate quantitative results can be obtained using MRM mode for samples with matrix interference. For such complex matrix samples and quantification of trace amounts of microplastics, Py-GC/MS/MS (MRM mode) can be very useful.

Introduction

Microplastics, defined as plastic fragments with a particle size of 5 mm or less, originate from various products, such as cosmetics, clothing, food packaging, and plastic waste. Microplastics have been detected in water, soil, and air environments,[1] as well as in food and within living organisms.[2] Consequently, numerous analytical techniques are being developed to investigate the extent of microplastic contamination and its impacts.

Microplastics can be seen with the naked eye or a microscope when the particle size is large, but observation is difficult when the size is small, and quantitative analysis by type of plastic is difficult. Techniques using spectroscopic instruments, such as IR or Raman, or thermal analysis instruments such as Py-GC/MS or thermal extraction desorption (TED)-GC/MS have been widely used.[3, 4, 5, 6] IR and Raman spectroscopy can be used to identify the type, shape, size, and count of microplastics on filters after sample preparation and filtration. These techniques rely on spectroscopic analysis of the microplastics retained on the filter. For Py-GC/MS or TED-GC/MS, the microplastics collected on the filter are thermally decomposed, and the resulting compounds are analyzed with GC/MS, allowing for the quantification of each type of microplastic[7] (by weight). In particular, Py-GC/MS has recently been recognized as an ASTM International method[8] and is widely applied as an analytical method for analyzing microplastics in drinking water.[9] In the case of environmental samples or food samples with complex matrices, additional matrix effects can negatively impact analytical performance or result in thermal decomposition products that cannot be separated from microplastic thermal decomposition product peaks, causing interference and overlapping with target ions.[10]

In this application, we applied Py-GC/MS/MS (MRM mode) to microplastic analysis to determine whether accurate quantitative analysis of microplastics is possible even in samples containing complex matrices.

Experiment

Reference materials and samples

The reference material[11,12] used in this experiment was a microplastics calibration standard (Py1-4940, from Frontier Laboratories, Fukushima, Japan) containing 11 types of microplastics (PMMA, Nylon-6, Nylon-6,6, SBR, PP, PET, PVC, PC, PE, ABS, and PS) diluted to a specific concentration in deactivated SiO_2. To generate calibration curves, 0.05, 0.1, 0.5, and 1.0 mg of the reference material were placed into individual pyrolyzer sample cups and analyzed with Py-GC/MS/MS.

The real samples used were sea salt. Sea salt samples measuring 100 g were each dissolved in distilled water, then filtered through a 20 µm SUS filter. The filter was folded multiple times and placed into a pyrolyzer sample cup, then analyzed with Py-GC/MS/MS.

Instrument and analysis conditions

The instruments used were a Frontier Lab pyrolyzer (Py-3030D) and an Agilent 8890 GC coupled to a 7000E triple quadrupole GC/MS system. The temperature of the pyrolyzer was set to 600 °C, as shown in Table 1, and the components produced by thermal decomposition were passed through the GC inlet (320 °C, split 50:1), cryo-focused by liquid nitrogen applied to the front end of deactivated metal capillary column (UA-5, 30 m x 0.25 mm x 0.25 µm) in the Microjet Cryo Trap, separated by the GC oven temperature program after about 3 minutes, and transferred to the mass spectrometer. The analysis conditions are shown in Tables 1, 2, 3, and 4.

Table 1. Frontier Lab 3030D pyrolyzer analysis conditions.

Parameter	Value
Instrumentation	Frontier Lab 3030D pyrolyzer
Mode	Single shot
Furnace Temperature	600 °C

Table 2. Agilent 8890 GC analysis conditions.

Parameter	Value
Instrumentation	Agilent 8890 GC system
Inlet	Split injection mode, split ratio 50:1
Inlet Temperature	320 °C
Column	UA-5 (30 m x 250 µm x 0.25 µm)
Column Flow Rate	1 mL/min (constant flow, He)
Oven	40 °C for 5 minutes 20 °C/min to 320 °C (hold for 6 minutes)
Transfer Line Temperature	300 °C

Table 3. Agilent 7000E triple quadrupole GC/MS system analysis conditions.

Parameter	Value
Instrumentation	Agilent 7000E triple quadrupole GC/MS
Ion Source	Electron ionization (EI)
Electronic Energy	70 eV
Source Temperature	230 °C
Quadrupole (1, 2) Temperature	150 °C
Detector Settings	Gain 1
Mode	MS1 Scan (*m/z* 29~300) and MRM

Table 4. MRM conditions.

Polymer in Reference Material	Compound (Indicator Peak)	RT (min)	Scan Mode	MRM Mode (Transitions)		
			Target Ion *m/z*	Precursor Ion *m/z*	Product Ion 1 Main *m/z* (CE, eV)	Product Ion 2 Support *m/z* (CE, eV)
PMMA	Methylmethacrylate	3.228	99	99	43 (10)	71 (10)
Nylon-6,6	Cyclopentanone	5.595	84	84	55 (5)	41 (30)
SBR	4-Vinylcyclohexene	6.833	54	54	39 (10)	-
PP	2,4-Dimethyl-1-heptane	7.045	126	126	83 (5)	55 (20)
PET	Benzoic acid	11.197	122	122	105 (10)	77 (20)
PVC	Naphthalene	11.523	128	128	102 (20)	78 (20)
Nylon-6	Caprolactam	11.987	113	113	85 (5)	56 (10)
PC	p-isopropenylphenol	12.319	134	134	119 (10)	91 (20)
PE	1-Tetradecene	12.938	84	84	55 (10)	69 (5)
ABS	2-Phenethyl-4-phenylpent-4-enenitrile (SAS)	17.427	170	170	128 (20)	143 (10)
PS	5-Hexene-1,3,5-triyltribenzene (SSS)	18.604	117	117	115 (15)	91 (20)

Results and discussion

Chromatogram

Chromatograms resulting from the analysis of the microplastics calibration standard (MPCS) that includes 11 types of microplastics are shown in Figure 1 for both Scan and MRM modes. The Scan mode total ion chromatogram (TIC) can be processed into extracted ion chromatograms (EIC) to confirm the target ion (*m/z*) for each indicator peak, as seen on the left side of Figure 1. The MRM TIC and EIC are shown on the right side of Figure 1. As can be seen, MRM mode can reduce matrix interference and enhance selectivity. Calibration curves can be created using either Scan or MRM mode using concentration of the MPCS.

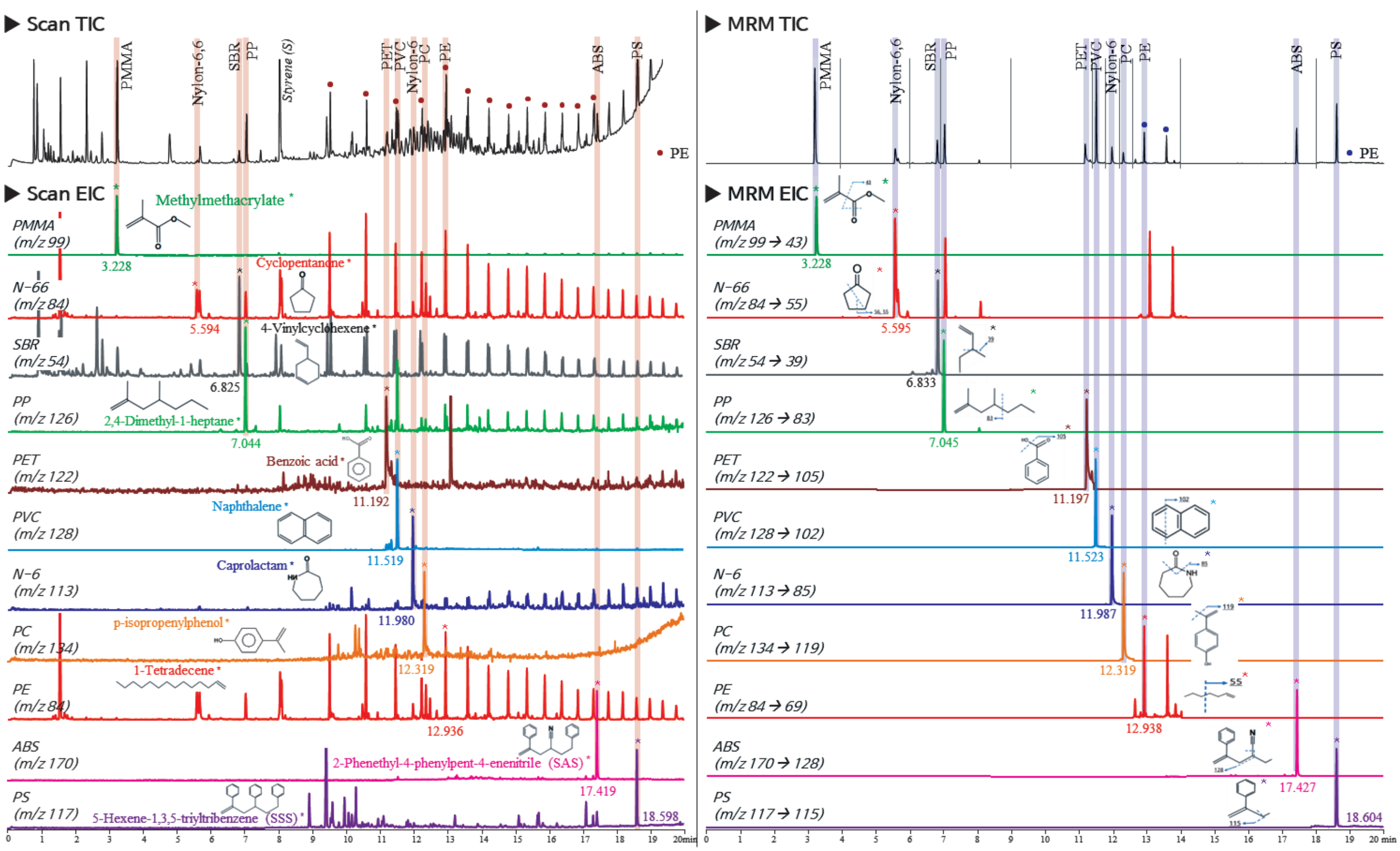

Figure 1. Chromatograms (TIC and EIC) analyzed in Scan mode and MRM mode using microplastics calibration standard (MPCS).

Calibration curve

The MPCS was accurately weighed at 0.05, 0.1, 0.5, and 1.0 mg using an ultra micro balance, placed in a pyrolyzer sample cup, and analyzed as a standard sample for calibration, as shown in Figure 2.

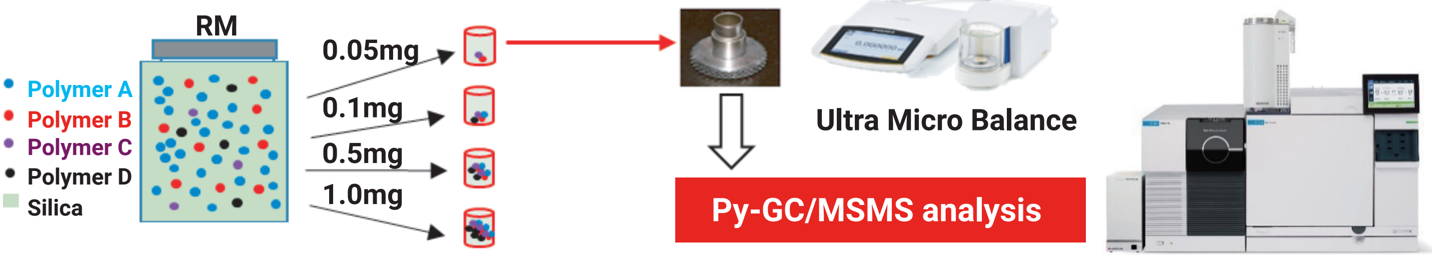

Figure 2. Workflow for creating microplastic calibration curves.

Calibration curves were created using Scan mode and the target ion (*m/z*) peak area for each indicator peak by concentration. The calibration curves for 11 types of microplastics are as shown in Figure 3.

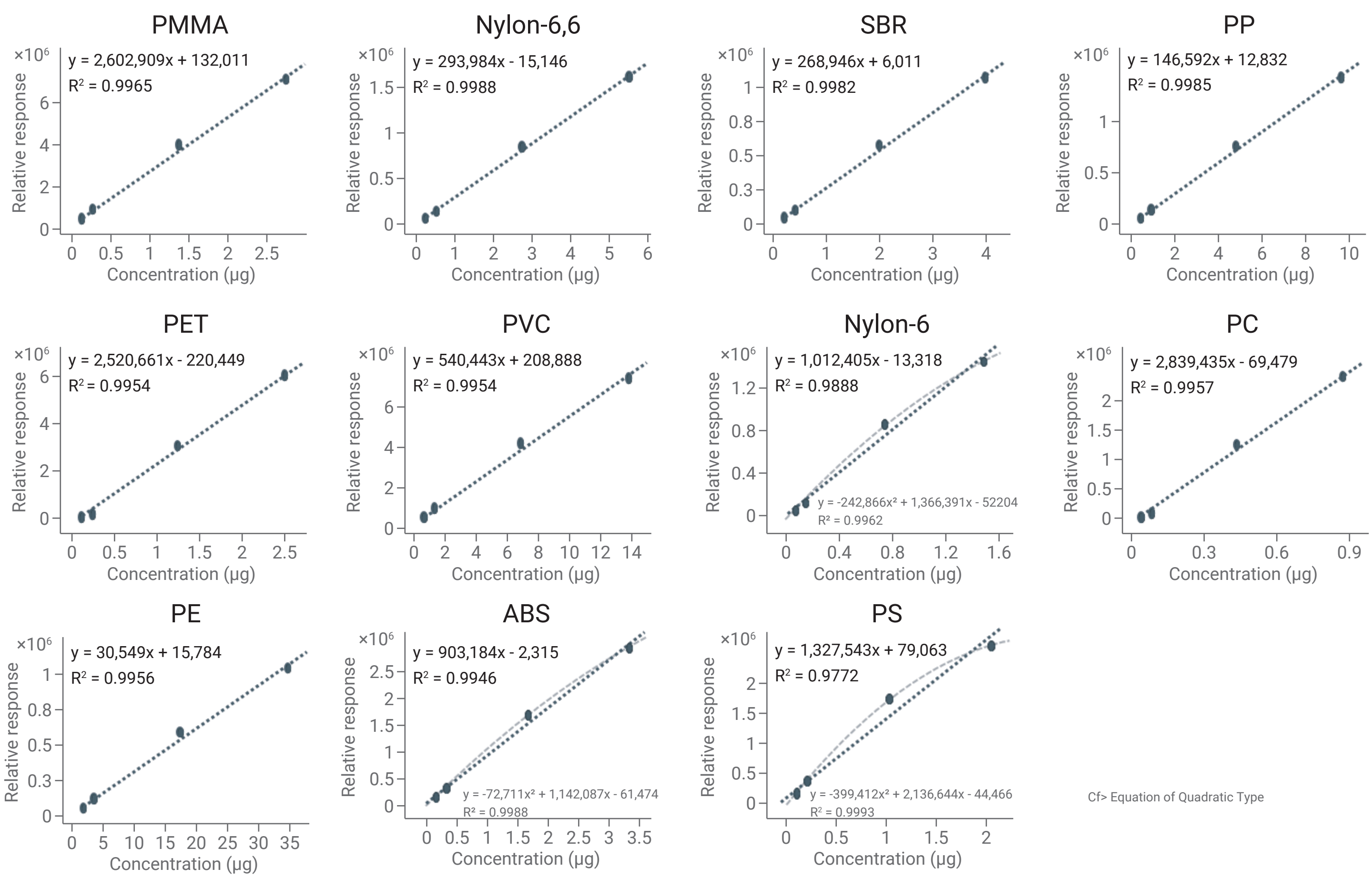

Figure 3. Scan mode (Target EIC) calibration curves for 11 types of microplastics.

In addition, the calibration curves were also created by analyzing in MRM mode, as shown in Figure 4.

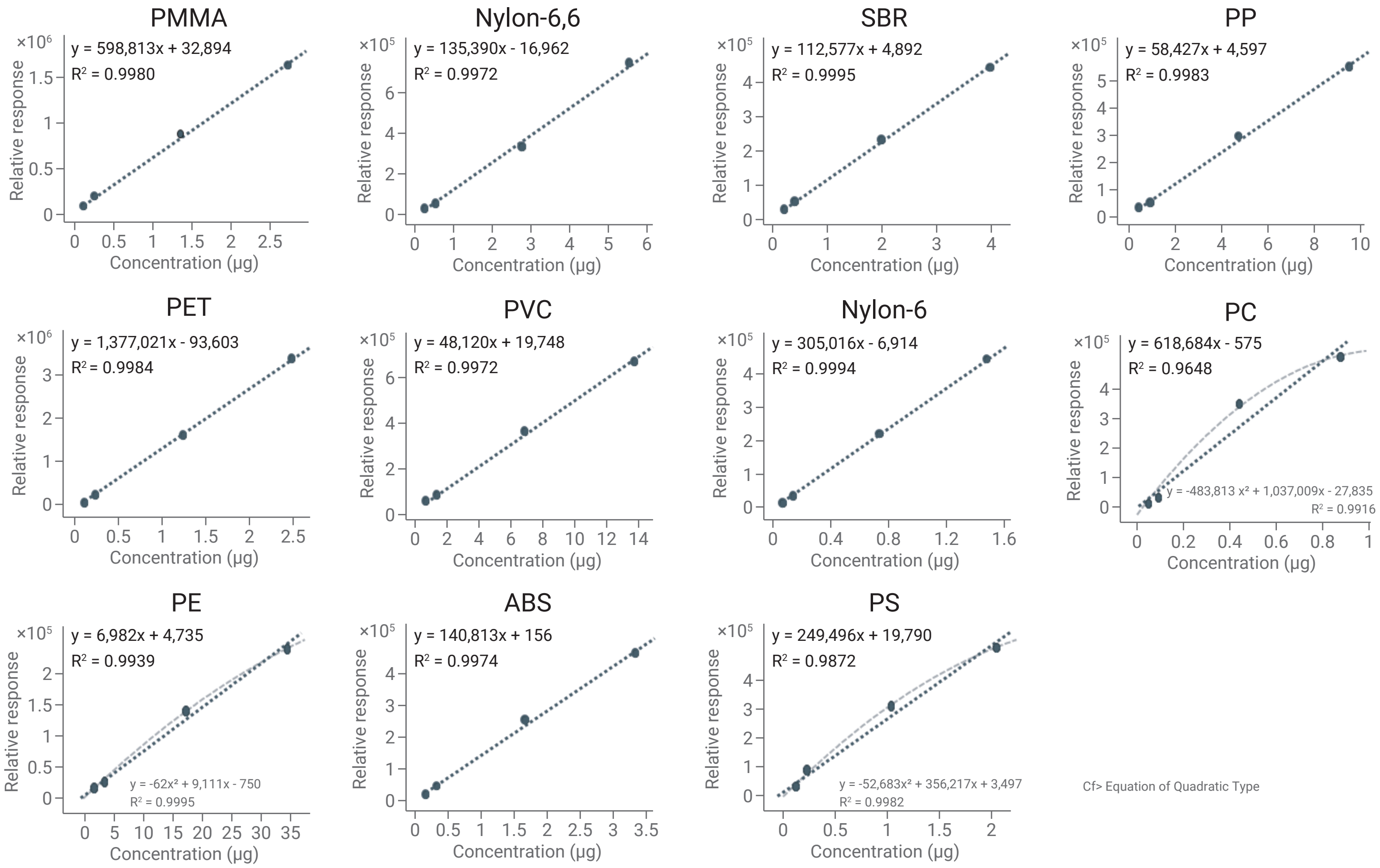

Figure 4. MRM mode calibration curves for 11 types of microplastics.

As shown in Figures 3 and 4, it was possible to simultaneously generate calibration curves for all 11 microplastics, using mixed reference material analysis across a concentration range of several hundred ng to several µg. A few compounds demonstrate quadratic-type behavior, resulting in slightly lower but still effective linearity. The calibration curve equations for both Scan mode and MRM mode are presented in Table 5.

Table 5. Summary table of calibration curves for Scan and MRM mode analysis of 11 types of microplastics.

Material	Mode	Regression Equation	R^2	Quantitative Range (µg)
PMMA	Scan	y = 2,602,909x + 132,011	0.9965	0.14 ~ 2.73
	MRM	y = 598,813x + 32,894	0.9980	
Nylon-6,6	Scan	y = 293,984x - 15,146	0.9988	0.28 ~ 5.53
	MRM	y = 135,390x - 16,962	0.9972	
SBR	Scan	y = 268,946x + 6,011	0.9982	0.20 ~ 3.98
	MRM	y = 112,577x + 4,892	0.9995	
PP	Scan	y = 146,592x + 12,832	0.9985	0.48 ~ 9.55
	MRM	y = 58,427x + 4,597	0.9983	
PET	Scan	y = 2,520,661x - 220,449	0.9954	0.13 ~ 2.50
	MRM	y = 1,377,021x - 93,603	0.9984	
PVC	Scan	y = 540,443x + 208,888	0.9954	0.68 ~ 13.68
	MRM	y = 48,120x + 19,748	0.9972	
Nylon-6	Scan	y = 1,012,405x - 13,318	0.9888	0.07 ~ 1.48
	MRM	y = 305,016x - 6,914	0.9994	
PC	Scan	y = 2,839,435x - 69,479	0.9957	0.044 ~ 0.88
	MRM	y = 618,684x - 575	0.9648	
PE	Scan	y = 30,549x + 15,784	0.9956	1.73 ~ 34.60
	MRM	y = 6,982x + 4,735	0.9939	
ABS	Scan	y = 903,184x - 2,315	0.9946	0.166 ~ 3.33
	MRM	y = 140,813x + 156	0.9974	
PS	Scan	y = 1,327,543x + 79,063	0.9772	0.10 ~ 2.05
	MRM	y = 249,496x + 19,790	0.9872	

In Scan mode, except for Nylon-6 and PS, and in MRM mode, except for PC and PS, the linearity R^2 values of the calibration curve for the 11 types of microplastics showed 0.99 or higher in the respective calibration ranges.

S/N ratio comparison

The noise and S/N ratios of each quantifier peak were compared for 11 types of microplastics in Scan and MRM modes at the lowest calibration level (reference material 0.05 mg).

Table 6. S/N details of the quantifier ion/transition at calibration level 1.

Polymer in Reference Material	Reference Material in SiO_2 0.05 mg	Scan Mode (× mark)		MRM Mode (◯ mark)	
	Each Amount (µg)	Noise*	S/N Ratio**	Noise*	S/N Ratio**
PMMA	0.14	155.5	1583.3	3.3	15618.8
Nylon-6,6	0.28	73.7	2399.8	2.2	4511.7
SBR	0.20	171.3	147.8	88.3	184.6
PP	0.48	556.1	77.7	10.9	1791.3
PET	0.13	94.5	733.6	1.1	8611.6
PVC	0.68	875.6	476.8	105.9	394.2
Nylon-6	0.07	202.5	97.4	26.0	284.6
PC	0.04	343.8	41.2	34.0	148.0
PE	1.73	221.3	228.4	111.2	122.6
ABS	0.17	824.2	87.8	62.6	226.5
PS	0.10	216.6	426.9	39.3	570.4

* Noise was defined to RMS (root-mean-square) noise algorithm.

** S/N ratio is a signal for a specific range noise calculated by RMS.

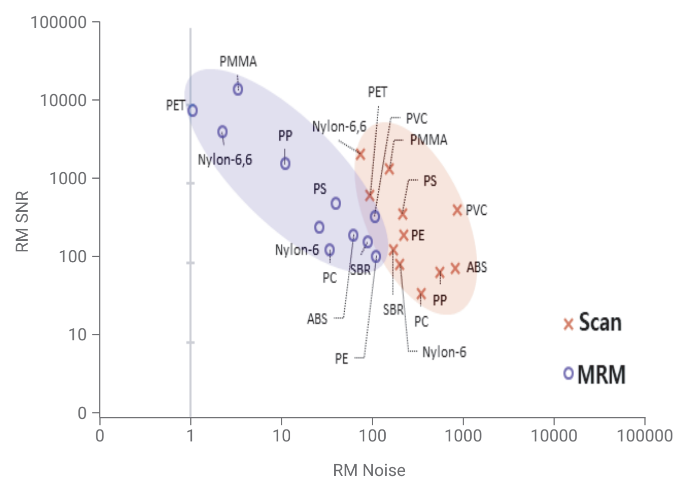

Figure 5. Comparison of noise and S/N ratio of quantifier peak at level 1.

As shown in Fgure 5 and Table 6, it was observed that the noise level was approximately two to several tens of times lower when analyzing in MRM mode compared to Scan (Target EIC) mode. Accordingly, the S/N ratio increases when analyzed in MRM mode, allowing for better selectivity in the detection of microplastics. Additionally, when there is matrix interference during real sample analysis, these differences in noise and S/N ratio can become even greater, and the application of MRM mode enables excellent sensitivity and accurate quantitative results.

As shown in Figure 6, in cases where there was minimal matrix interference during the actual sample analysis, the quantification results for PE analyzed using the Scan (Target EIC) mode and the MRM mode were similar. However, in cases of significant interference, it was confirmed that the values analyzed using the Scan (Target EIC) mode were overestimated compared to those analyzed using the MRM mode.

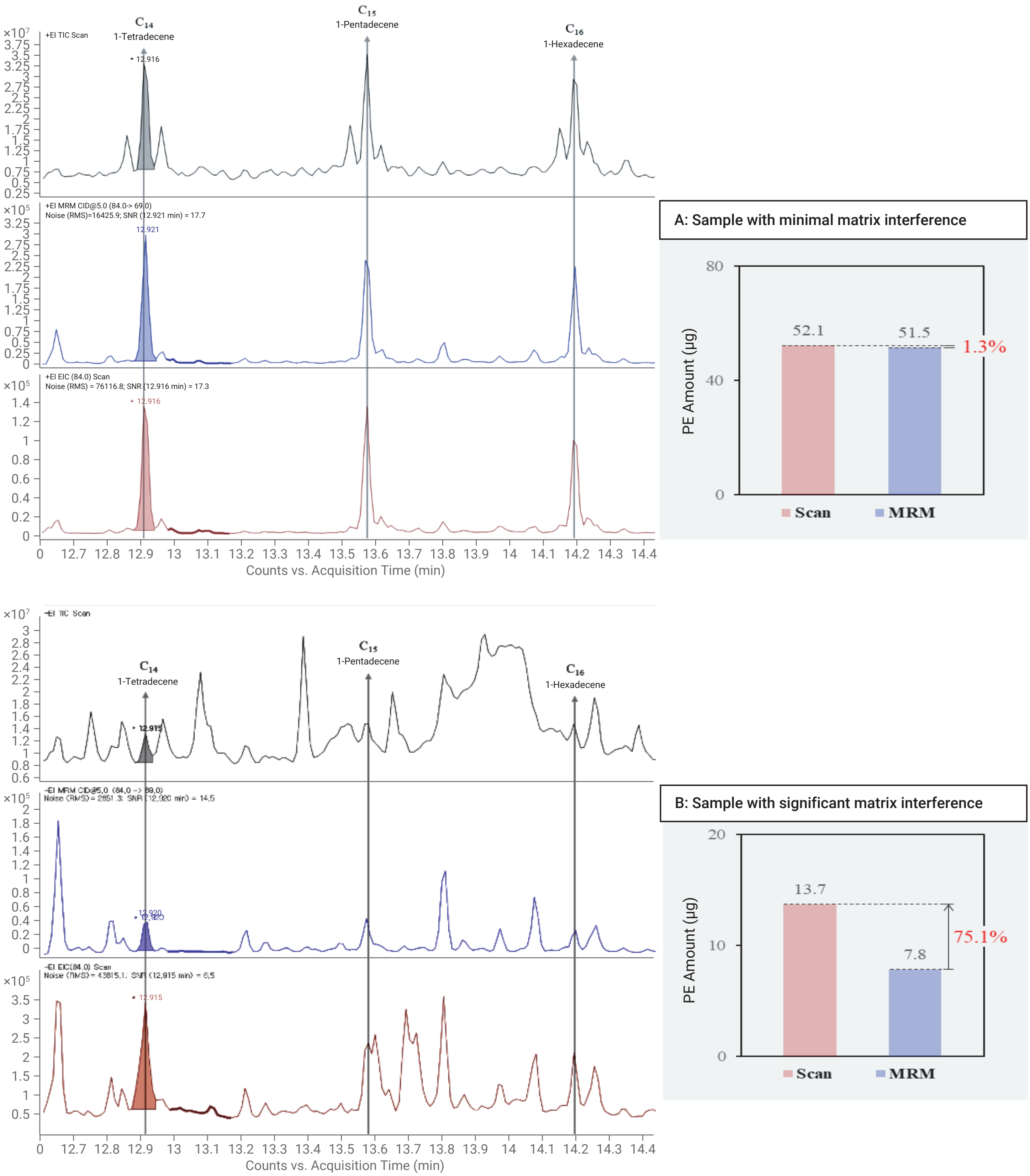

Figure 6. Differences in quantitative results between Scan mode and MRM mode due to interference.

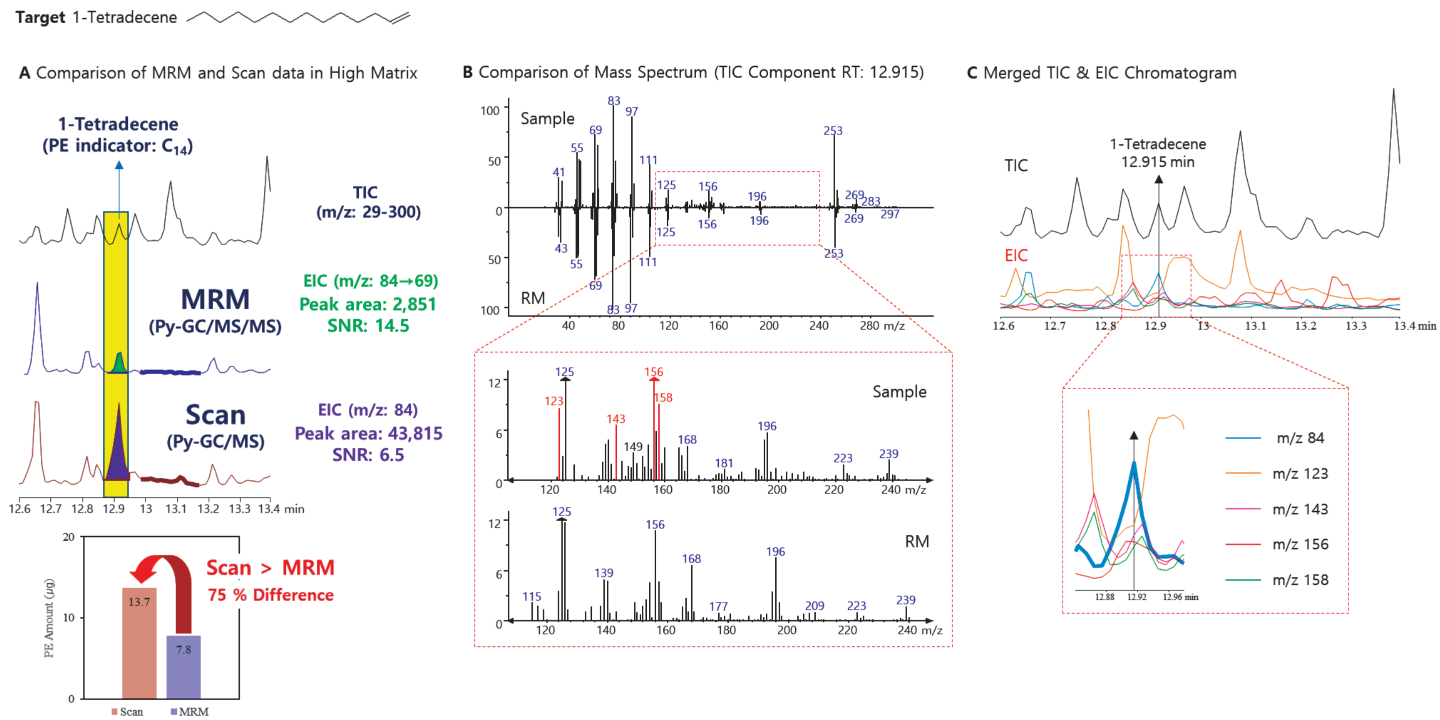

Figure 7. A: Scan and MRM mode chromatograms of sample with matrix interference B: Scan mode TIC spectral comparison with reference material C: Comparison of Scan mode TIC and EIC.

As shown in Figure 7A, by applying optimized compound transitions through MRM analysis, the interferences that affect the PE result in Scan mode are minimized, resulting in a lower noise value and higher S/N ratio. In this way, we were able to perform more accurate quantitative analysis while also providing good selectivity through MRM mode analysis.

The sample fragments identified in red (Figure 7B) demonstrate additional substances coeluting with the target analyte, requiring additional deconvolution steps to confirm presence (Figure 7C). In this way, unlike in the reference material analysis, the presence of interference from compounds other than PE was confirmed by the confirmation of the EIC spectrum being deconvoluted, as in Figure 7C in the actual sample analysis.

Conclusion

We have established a quantitative analysis method using Py-GC/MS/MS (MRM mode) for complex matrix samples and trace levels of microplastics.

Under the established Py-GC/MS/MS (MRM mode) conditions, the linearity of the calibration curves generated for the 11 types of microplastics, ranging from several hundred ng to several µg, was excellent ($R^2 > 0.99$). Compared to Scan (Target EIC) mode, noise is reduced by two to several times when analyzed in MRM mode, and accordingly, the S/N ratio is increased when analyzed in MRM mode, enabling the detection of microplastics with better selectivity. In addition, when there is matrix interference during sample analysis, more accurate quantitative results can be obtained by applying the MRM mode, and Py-GC/MS/MS (MRM mode) can be very useful for quantifying complex matrix samples and trace levels of microplastics.

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