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Application News

No. GCMS-2202

Gas Chromatography Mass Spectrometry

An Automated Workflow for Quantitative Analysis of Microplastics in Environmental Samples via Pyrolysis-GC/MS

■ **Abstract**

With plastic use on the rise, screening for microplastics (MPs) has become a pressing issue. In response, the world has called upon the scientific community to develop analytical methods for identification and measurement of these pollutants. While many of these methods have been successful in their identification, several lack a fast and automated workflow to quantitate MPs in complex matrices.

This work represents the second phase of a study to identify and measure MPs in waterbodies. In this phase of the study, a Shimadzu GCMS-QP2020 NX coupled to a Frontier Py-3030D pyrolyzer was used to demonstrate the quantitative analysis of twelve selected MP polymers, which have been chosen based on their prevalence in the environment [1]. A five-point external calibration curve showed linearity for all compounds with coefficient of determination (r^2) > 0.9959. A repeatability test was conducted both on the lower and upper ends of the calibration range using 0.2 and 3 mg standard weight (n=7). Percent relative standard deviation (%RSD) for the various polymers at 0.2 mg ranged from 3.6-23.6, while at 3 mg %RSD was 2.3-12.1. An accuracy evaluation was also conducted, and results showed that the 0.2 mg standard weight had recoveries of each polymer that ranged from 66.4 – 145.1% and at 3 mg recoveries ranged from 92.2 – 102.8%.

A lower limit of quantitation (LLOQ) experiment determined the lowest concentration that the target polymer can be measured. The LLOQ in this study was the lowest point of the calibration curve for each polymer and ranged from 0.1 - 7.29 µg (0.2 mg standard weight). In addition, a continuing calibration check (CCC) test was conducted to ensure that there were no calibration drifts during the experiment. The results showed percent drift for all compounds was less than 20%.

These results demonstrated that the instrument quality control (QC) parameters traditionally evaluated during the development of new analytical methods were within acceptable levels. The method validation results in this application note act as a Py-GC/MS method condition guidance for ongoing MPs studies being used by ASTM Committee D19 [2] and ISO TC147 SC2 JWG 1 [3] to draft and validate new methods for the analysis of MPs in water. In addition, the results can be used as a reference for environmental scientists working on the analysis of MPs in environmental samples.

■ **Introduction**

The ubiquitous occurrence and persistent nature of plastics in the environment may present potentially adverse issues that are not yet well understood. Large plastic debris not only clogs stormwater pipes but can be broken down into micro and nanoparticles that can be a sorption site for other pollutants, including chemicals such as pesticides and microorganisms [4]. These micro and nano-plastics can be ingested by water organisms and bioaccumulate up the food chain into humans. As a result of the need to mitigate plastic pollution, researchers are working to understand the effects of plastics in the environment and develop monitoring and mitigation programs.

Pyrolysis-GCMS (Py-GC/MS) is an innovative technique that is an accurate and precise alternative for the identification and quantitation of microplastics, and provides a fast workflow by shortening many conventional laboratories sample preparation steps, as highlighted by Pipkin et. al. (2021). Some traditional MPs methods require sample preparation, matrix isolation and solvent extraction [4]. Polymers such as polyethylene (PE) and polypropylene (PP) are not easily dissolved in solvents and recovery from initial material may be poor. Therefore, MP analytical challenges may occur with these traditional methods.

Since Py-GC/MS directly analyzes solid samples, sample preparation is easier than with traditional MP methods [4]. In addition, solvent extraction steps may be eliminated from the workflow, since solids can be taken as is and placed into a sample cup for analysis. Thus, using a Py-GC/MS may minimize solvent consumption, time for analysis and discrepancies in methods targeting a broad range of plastics. Prepared samples are placed in sample eco cups, analyzed, and reported in mass per volume, such as ug/L, unlike spectroscopic methods, which count particles and must infer mass concentration. The faster workflow of Py-GC/MS is further enhanced with specialized software, Frontier MP-search, that uses retention indices to readily identify characteristic pyrolyzates of the target compound.

This application note demonstrates the quick, accurate, sensitive, and precise analysis of 12 MPs using a Shimadzu GCMS-QP2020NX coupled to a Frontier PY-3030D pyrolyzer system.
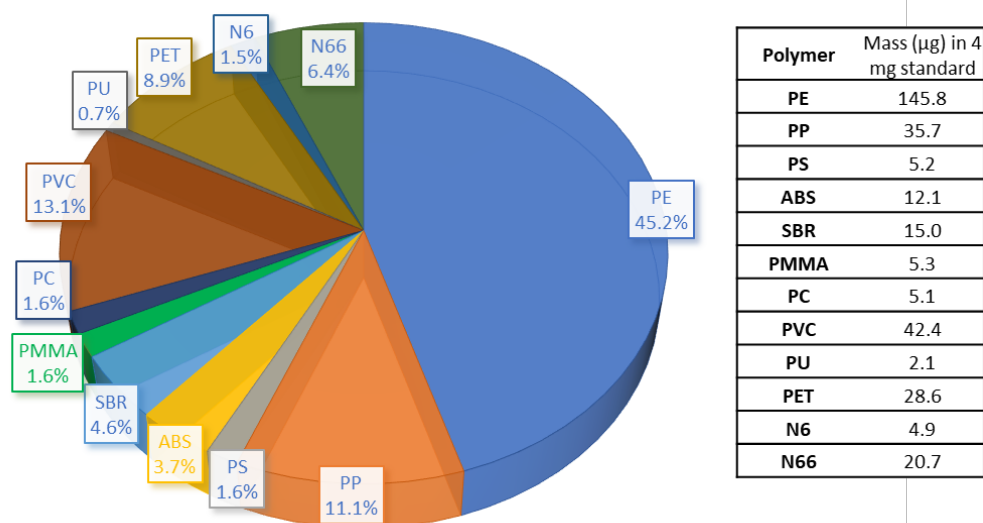
■ **Experimental Approach**
A Frontier lab multi-shot Pyrolyzer (Py) was interfaced with a Shimadzu GCMS (Figure 1). The system configuration for this application consisted of a Shimadzu GCMS, model QP2020 NX, Frontier multi-shot Pyrolyzer, model EGA/PY-3030D, an auto-shot sampler, model AS-1020E, a Frontier Lab ultra-alloy microplastics (UAMP) column, a vent-free GC/MS adapter, and a F-Search MP library software. Consumables consisted of a calibration standard mixture of 12 polymers, eco-cup LF and a packed inlet liner.

Figure 1: Shimadzu GCMS-QP2020 NX and Frontier Multi-Shot Pyrolyzer EGA/PY-3030D.

The polymers analyzed in this study were polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC) polycarbonate (PC), polyethylene terephthalate (PET), poly (methyl methacrylate) (PMMA), nylon-6 (N-6), polystyrene (PS), acrylonitrile-butadiene-styrene copolymer (ABS), styrene-butadiene rubber (SBR), nylon-6,6 (N-66) and MDI-polyurethane (PU). A workflow to determine a characteristic pyrolyzate for seven of the above polymers is described in Shimadzu application news GCMS-2201 [5].

A five-point calibration curve was prepared using a Frontier MPs-CaCO$_3$ standard [1]. The percentage distribution of polymers in the MPs-CaCO$_3$ standard as well as the mass of each analyte in a 4 mg standard within a CaCO$_3$ diluent is shown in Figure 2.

Polymer	Mass (µg) in 4 mg standard
PE	145.8
PP	35.7
PS	5.2
ABS	12.1
SBR	15.0
PMMA	5.3
PC	5.1
PVC	42.4
PU	2.1
PET	28.6
N6	4.9
N66	20.7

Figure 2: Percent polymer composition as well as mass of each analyte in 4 mg MPs-CaCO$_3$ analytical standard with CaCO$_3$ diluent.

Using an electronic semi-micro balance, calibration standards of 0.2, 0.4, 0.8, 2.0 and 4.0 mg were weighted in eco-sample cups in replicates (n=4). Subsequently, one to two mg of quartz wool was placed in each of the sample cups, which were placed onto the auto-shot sampler carousel. Blank eco cups followed by samples were analyzed with the lowest concentrations first. Each sample cup was released into the pyrolyzer furnace, resulting in thermochemical decomposition of the standard. Figure 3 illustrates the pyrogram of a 4 mg weight standard.

Data processing was conducted using both GCMSsolution and the Frontier MP-search software. GCMSsolution was used to determine the instrument's LLOQ and Frontier MP-search was used to process the other method validation parameters such as calibration curve linearity, precision and accuracy. In the process, the target polymers were identified based on a characteristic pyrolyzate (Figure 3). A primary quantitative ion for the pyrolyzate was used for quantitative analysis while reference ions were used for qualitative confirmation of each polymer.

A calibration curve was automatically created by the MP-search software based on average peak area, from replicate analysis, versus a respective measured average mass.

A short-term repeatability test was conducted by analyzing seven replicates at the lower end (0.2 mg) and seven replicates at the upper end (3 mg) of the calibration range. The percent relative standard deviation (%RSD) of each was calculated. The accuracy or recovery of each standard was calculated using theoretical concentration for each component within the 0.2 mg and 3 mg levels. The method LLOQ was experimentally obtained by conducting five injections at 0.1, 0.2, 0.4, 0.6 and 1 mg total standard mass. LLOQ was established at the 0.2 mg standard mass where the Signal-to-Noise ratio (S/N) was above 10 for the least sensitive compounds. A continuing calibration check standard, consisting of a mid-range calibration standard, was analyzed to evaluate the validity of the initial calibration curve in the analysis. The experimental parameters for both GC-MS and Py systems when operated in single shot mode are listed in Table 1.
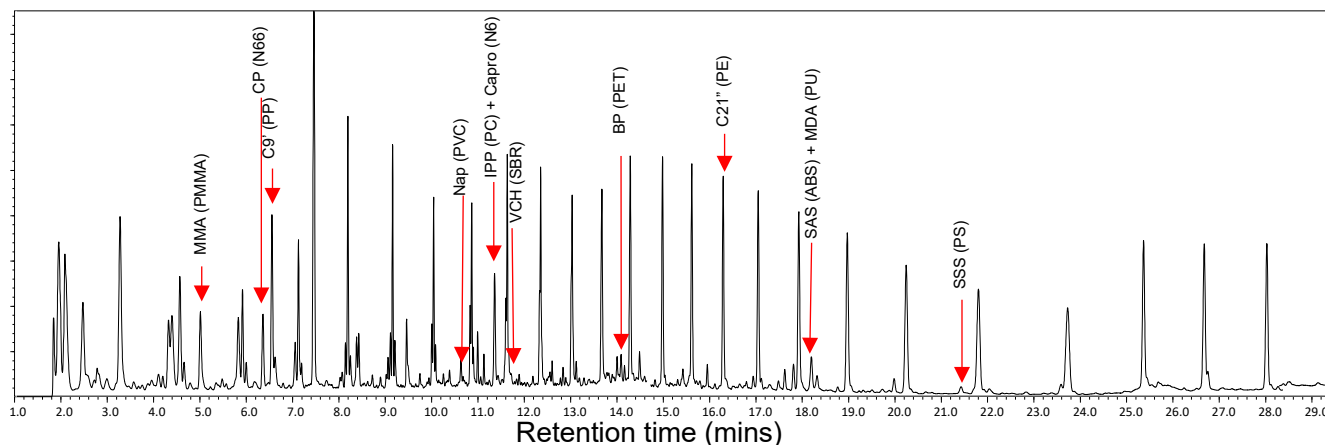

Figure 3: Pyrogram of 4 mg standard showing elution order of the 12 characteristic pyrolyzates

Table 1: GCMS and Pyrolyzer operating conditions

Gas Chromatography	Nexis GC-2030
Injection Port Mode	Split mode; 50:1 split ratio
Injector Pressure	150 kPA
Carrier Gas	Helium
Injection Port Temperature	300 °C
Column: Frontier Lab UAMP Column	UA precolumn 50: Ultra Alloy-50 (2 m x 0.25 mmID x 1.0 μm) and separation column: Ultra Alloy-5 (30 m x 0.25 mmID x 0.5 μm)
Flow Control Mode	Constant pressure
Oven Temperature	40 °C (2 mins. hold), 20 °C /mins. to 280 °C (10 mins. hold), 40 °C/min to 320 °C (60 mins. hold)
Mass Spectrometer	**QP2020 NX**
Interface Temperature	300 °C
Ion Source Temperature	230 °C
Detector Voltage	Relative to Tune 0 kV
Threshold	0
Scan Range	*m/z* 29 to 350; Event time 0.2 s
Pyrolyzer	**EGA/PY-3030D**
Single Shot Furnace Temp	600 °C
Interface Temp	300 °C
Auto Sampler Flushing Gas	Helium
Auto Sampler Purge Time	10 sec

■ Results and Discussion

Initial Demonstration of Low System Background
As a quality control measure, an initial demonstration of low system background was conducted before developing the calibration curve. Blank sample cups were analyzed, and the system was deemed to be free of contamination.

Initial Calibration
A five-point initial external calibration plot was generated across a linear range for a mixed polymer analysis (Figure 4).

Four replicates at each concentration level were analyzed and the average of the four points was calculated, plotted as a calibration curve, and the coefficient of determination was determined. Calibration results showed good linearity for all compounds. Figure 5 illustrates calibration curves for the least sensitive polymers, i.e., polymer signal with a S/N ratio > 10. Despite the lower sensitivity for these compounds, coefficient of determination (r^2) for all 12 polymers was > 0.9959 (Table 2).
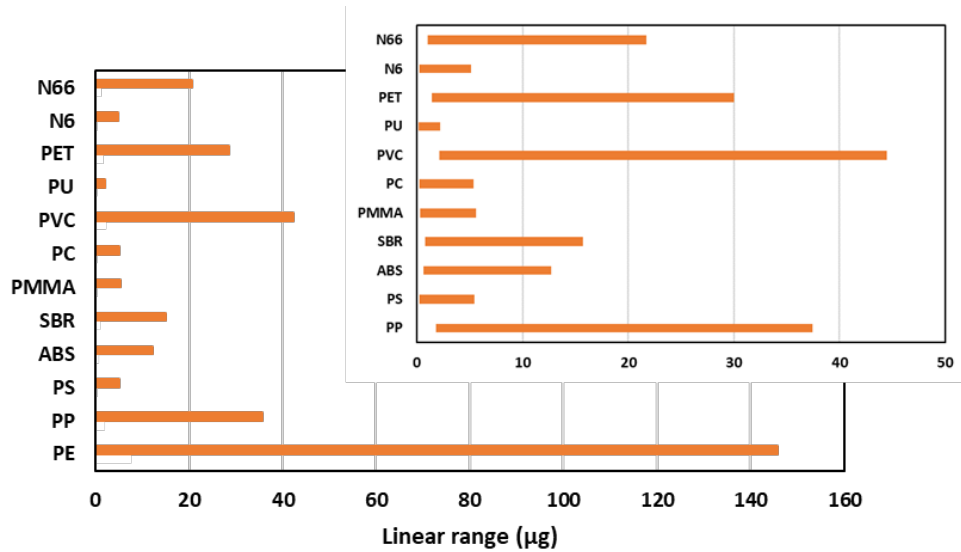

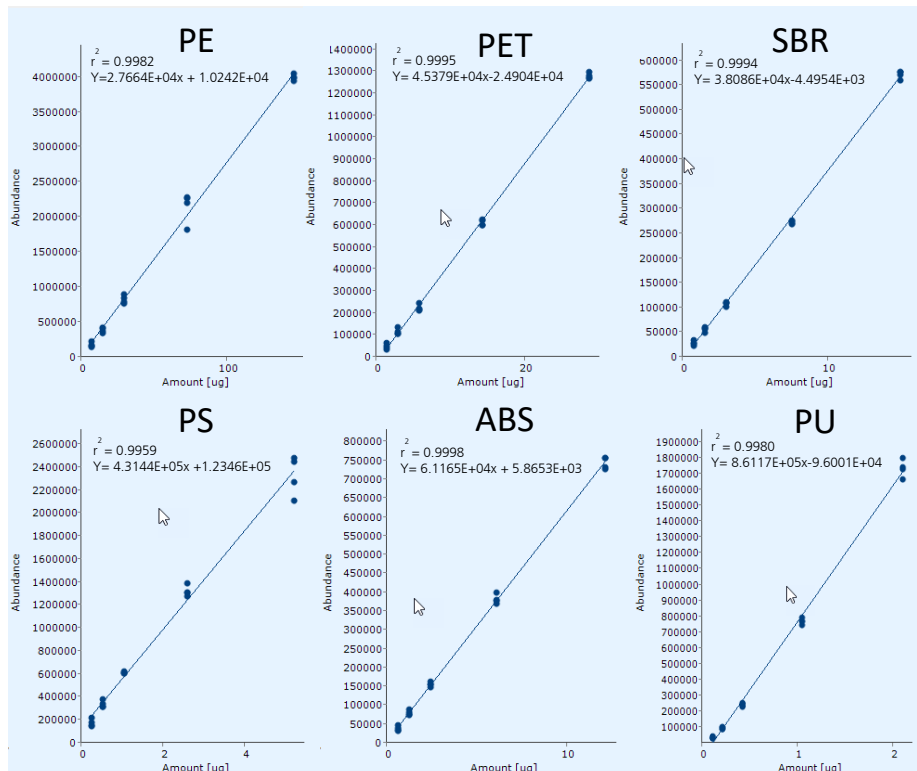**Figure 4**: Calibration curve linear range of each microplastic compound.

**Figure 5**: Calibration curves for the least sensitive polymers.

Table 2: Statistical analysis of calibration curves

Polymer	Characteristic pyrolyzates	Quant ion (*m/z*)	Reference ions (*m/z*)	Linearity (r²)
PE	1,20-Heneicosadiene	82	41, 55,97	0.9982
PP	2,4-Dimethyl-1-heptene	126	43,55,70	0.9997
PS	Styrene trimer	91	117,207,312	0.9959
ABS	2-Phenethyl-4-phenylpent-enenitrile	170	91,115,118	0.9998
SBR	4-Phenylcyclohexene	104	158	0.9994
PMMA	Methyl methacrylate	100	69,41,99	0.9988
PC	4-Isopropenylphenol	134	91,119	0.9999
PVC	Naphthalene	128	102	0.9999
PU	4,4'-Methylenediabiline	198	106,182,197	0.9980
PET	Benzophenone	182	51,77,105	0.9995
N6	ε-Caprolactam	113	30,55,85	0.9999
N66	Cyclopentanone	84	39,55,56	> 0.9999

Initial Demonstration of Precision

A repeatability test was done on the lower and upper end of the calibration curve. Both 0.2 and 3 mg of MPs-CaCO₃ standard mass were placed into seven individual cups and analyzed in order within the sequence, i.e., injection 1 through 7. The concentration of each analyte in each replicate was calculated using the initial calibration curve.

Percent RSD for the polymer replicates at 0.2 mg and 3 mg standard weights, respectively, ranged from 3.6-23.6 and 2.3-12.1 (Figure 6). Most compounds were within a RSD of 10%.
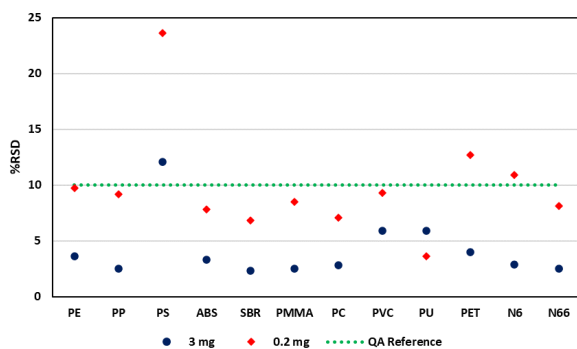
Figure 6: Precision test %RSD (n=7).

Lower Limit of Quantification (LLOQ)

Various masses of the mix standards were weighted on a semi-micro balance and analyzed on the system until a S/N of > 10 was obtained for the least sensitive polymer. The LLOQ is arbitrarily defined as the lowest calibration point and, in this study, determined as 0.2 mg standard weight of the mix polymer. LLOQ for all polymers ranged from 0.1 - 7.3 µg (Figure 7).
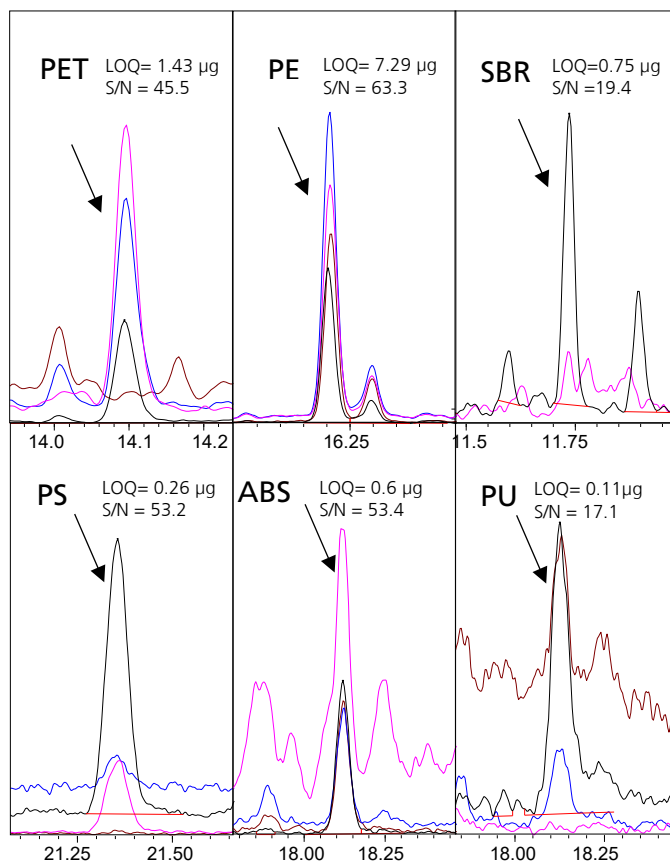
Figure 7: Limit of quantitation for the least sensitive polymers in this study.

Initial Demonstration of Accuracy
The mean concentration of each analyte in all replicates from the repeatability test was determined. This average concentration and the theoretical concentration were used to calculate the percent recovery at 0.2 and 3 mg standard mass (Table 3). The 0.2 mg standard mass had percent recoveries of each polymer that ranged from 66.4 – 145.1 and at 3 mg recoveries ranged from 92.2 – 102.8.

Continuing Calibration Check
A CCC was analyzed at the end of the batch (42 injections) to validate the use of the calibration used during the study. Percent drift of the CCC standard was used to evaluate the suitability of this calibration over the entire batch. The equation below was used to calculate % Drift:

% Drift =

$$\frac{\text{Calculated concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \, x \, 100$$

A mid-point calibration level (0.8 mg) was used to evaluate the percent drift. The peak area of the quantitation ion of the continuing calibration checks was monitored. A maximum drift of 20% was assigned to verify the use of the calibration curve. Results showed percent drift for all compounds in the CCC were less than 20%, satisfying the criteria of the test (Table 3). This result indicates that the initial calibration curve was valid during the entire batch analysis.

■ **Conclusion**
The study demonstrated the satisfactory performance of the Shimadzu GCMS-QP2020NX coupled to a Frontier Py-3030D pyrolyzer for quantitation of selected plastics. The method validation results in this application note act as a Py-GC/MS method condition guidance for ongoing MPs studies.

In this application, a fast and precise workflow was developed for quantitation of twelve polymers. Calibration results showed linearity for all compounds and coefficient of determination (r^2) > 0.9959 were obtained. Using seven replicates of standards at 0.2 and 3 mg, a precision experiment was conducted. Percent RSD for the polymer replicates at 0.2 mg and 3 mg, respectively, ranged from 3.6-23.6 and 2.3-12.1. An accuracy evaluation showed that the 0.2 mg standard mass had percent recoveries of each polymer that ranged from 66.4 – 145.1 and at 3 mg recoveries ranged from 92.2 – 102.8. The LLOQ for all polymers ranged from 0.1 - 7.3 (0.2 mg).

In addition to the above, a CCC test was conducted. After 42 injections, the percent drift for all compounds was less than 20%.

Table 3: Summary of method performance.

Polymer	Recovery % (n=7)		Retention Time (min)	LLOQ (µg)	% Drift (at 0.8 mg)
	3 mg	**0.2 mg**			
PE	99.0	99.9	16.2	7.3	-16.9
PP	99.6	111.2	6.6	1.8	-11.4
PS	92.2	66.4	21.3	0.3	-9.3
ABS	92.9	107.3	18.1	0.6	-18.1
SBR	96.0	121.8	11.7	0.8	-8.7
PMMA	102.8	103.6	5.0	0.3	-6.5
PC	97.2	123.0	11.4	0.3	-10.8
PVC	97.5	114.1	10.6	2.1	-9.6
PU	94.9	145.1	18.1	0.1	-19.8
PET	95.0	126.7	14.1	1.4	-18.2
N6	100.1	112.0	11.4	0.3	-11.0
N66	100.8	121.5	6.4	1.0	-7.2

■ **References**

1. Ishimura, T.; Iwai, I.; Matsui, K.; Mattonai, M.; Watanabe, A.; Robberson, W.; Cook, A.; Allen, H.L.; Pipkin, W.; Teramae, N.; Ohtani, H.; Watanabe, C. Qualitative and quantitative analysis of mixture of microplastics in the presence of calcium carbonate by Pyrolysis-GC/MS. Journal of Analytical and Applied Pyrolysis, **2021** (157): 105188.

2. WK67788 - New Test Method for Identification of Polymer Type and Quantity (Mass) Measurement of Microplastic Particles and Fibers in Waters with High-to-Low Suspended Solids Using Pyrolysis-Gas Chromatography/Mass Spectrometry: Py-GC/MS.

3. Joint ISO/TC 147/SC 2 - ISO/TC 61/SC 14 WG: Plastics (including microplastics) in waters and related matrices.

4. Pipkin, W.; Belganeh, R.; Robberson, W.; Allen, H.L.; Cook, A.; Watanabe, A. Identification of Microplastics in Environmental Monitoring Using Pyrolysis-GC-MS Analysis. LCGC North America. **2021** April 01; 39(4):179-186.

5. How to Kickstart Your Micro- and Nanoplastic Py-GC/MS Analysis. Shimadzu Application News. GCMS-2201.

■ **Consumables**

Part Number	Item Name	Item Description
UAMP-K01	UAMP Column Bracket	UA Precolumn 50: Ultra Alloy-50 (2 m x 0.25 mmID x 1.0 μm) and separation column: Ultra Alloy-5 (30 m x 0.25 mmID x 0.5 μm)
PY1-3346	Packed GC glass insert	Packed GC glass insert for Shimadzu
220-90906-00	Eco-Cup LF	PY-2020 Eco-Cup LF
220-94824-18	Quartz Pyrolysis Tube	Pyrolysis tube, 120MM PY
220-90917-00	Graphite Vespel ferrule	Graphite Vespel Ferrule for Py-2020 (pk of 5)
220-94824-14	Needle Set	Syringe, deactivated needle (PY) (3/pk)
PY1-4940	MP calibration standard set	MP calibration standard consisting of 12 polymers
MS402280	Vent-free GC/MS Adapter	Vent-free GC/MS Adapter N-50
220-94792-03	Quartz wool	Phthalate-free quartz wool
220-94792-00	Septa	Septa for Pyrolysis
221-49662-91	O-ring	O-ring for sealing glass liners when using a Pyrolyzer

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com