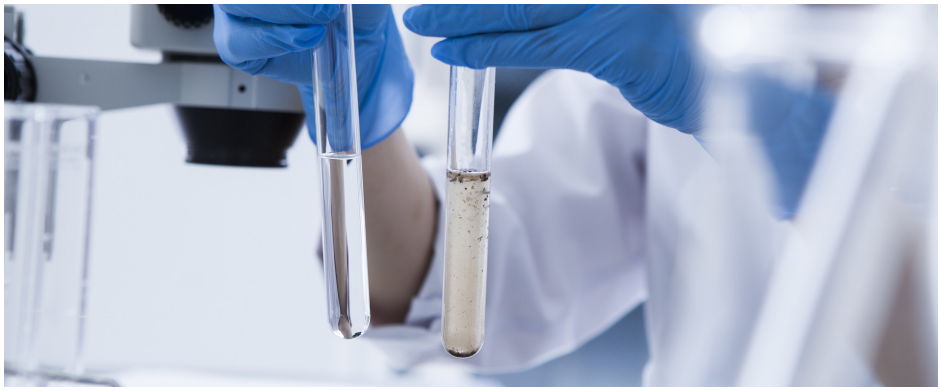
Technical Overview

From Collection to Analysis: A Practical Guide to Sample Preparation and Processing of Microplastics

Essential laboratory setup, sample preparation steps, and analytical methods for analyzing microplastics



Authors

Subharthe Samandra and Bradley Clarke
Australian Laboratory for Emerging Contaminants, School of Chemistry, Faculty of Science, The University of Melbourne, Victoria, Australia

Wesam Alwan
Agilent Technologies, Inc.

Introduction

Microplastics are defined as any polymer that is between 1 and 5,000 µm. They can further be separated into small (1 to 1,000 µm) and large (1 to 5 mm) microplastics. Additionally, they can be distinguished as primary or secondary microplastics. Primary microplastics are plastic particles that have been intentionally manufactured for personal care products, such as microbeads, or for industrial applications, such as pellets. Secondary microplastics are small plastic particles created through larger plastic products that undergo weathering and degradation. Current research has shown that secondary microplastics are more prominent than primary microplastics.[1]

The analytical process for extracting microplastics from various matrices typically involves sample collection, sample preparation, and instrumental analysis.[2] Each of these steps presents an opportunity for the introduction or loss of microplastics. This highlights the importance of robust sample preparation methods, quality assurance, and quality control (QA/QC) measures. QA/QC practices allow researchers to assess the reliability of data.[2-4]

The success of any spectroscopic analysis of microplastic particles relies on the effectiveness of the sample preparation procedure. Inadequate or improper preparation can introduce errors into the analysis, resulting in unreliable data. Whether using infrared (IR) or Raman microscopy, it is essential to isolate microplastic particles and distribute them discretely on a substrate for analysis. The more complex the matrix, the more extensive the preparation required. Standardizing sample preparation methods is crucial for enabling the comparison of results across different studies.

This guide covers key aspects of sample preparation to ensure accurate and standardized characterization of microplastics relevant to the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system. The guide focuses on three main areas:

- Essential tools and considerations for setting up a microplastics laboratory
- The sample preparation process
- Microplastics analysis methods using LDIR in various matrices, including bottled drinking water, environmental water, sand and sediment, and infant formula

Figure 1. The Agilent 8700 LDIR chemical imaging system allows high-speed, routine analysis of microplastics, including the number of particles present in the sample, their size, and their chemical composition.

Essential tools and considerations for setting up a microplastics laboratory

Sample preparation is one of the main challenges in microplastics analysis and the consequences of this challenge can be minimized by managing several aspects of the laboratory. Microplastics are everywhere in the environment, and it is no different in a laboratory setting. Therefore, to minimize contamination, several precautions must be taken.[4]

- Due to the size and weight of microplastics, these particles can be carried through the air. Therefore, it is important to maximize air purity and minimize the levels of airborne contaminants. This is possible using a laminar fume hood when extracting samples.
- As microplastics are not heavily monitored, all reagents, including Milli-Q water, will contain microplastics, therefore, all reagents must be filtered and checked for contamination before use.
- Glassware can also pose a contamination risk and at a minimum must be rinsed with filtered Milli-Q water before use. If possible, before rinsing, glassware should be placed into a furnace.
- Another source of contamination could be personal protective equipment such as laboratory coats. It is therefore recommended to wear 100% cotton laboratory coats and to use a lint roller when removing small fibers.

To facilitate microplastics analysis, Table 1 lists the essential tools and materials for setting up a microplastics laboratory.

Table 1. Essential tools and reagents of microplastics analysis.

	Item Description
Filtration	Vacuum filtration apparatus. The filtration apparatus is used to filter extracted microplastics. It features a neck diameter of 27 mm, and accommodates 25 mm gold- or aluminum-coated filters.
	Polyester (PETG) gold-coated membrane filters, 0.8 µm, 25 mm (p/n M7300-68009)
	Polyester (PETG) aluminum-coated membrane filters, 0.8 µm, 25 mm (p/n M7300-68011)
	Vacuum pump equipped with a manual vacuum regulator – Free air displacement, 80 L/min – Motor speed: 1,440 rpm – Oil capacity: 380 mL
	MS Multiple Vacuum Filtration Unit
	Clamps
	Sieves stacks for grouping particles based on size range
Air Purity	Laminar flow hood
	HEPA filters
	Air purifier
	Vacuum cleaner
	Cotton laboratory coats
Sample Preparation and Analysis	Ultrapure water sources for reagent preparation
	Balance
	Hot plate
	Glassware
	Storage boxes
	Low-e slides (p/n M7300-68010)
	Tweezers
	Microspheres (essential for particle size validation)
	Polymer sample kit. Used for library validation and generation.
	Agilent 8700 LDIR for microplastics detection and identification (20 to 500 µm)
	Agilent Cary 630 FTIR for microplastics identification (> 500 µm)

The sample preparation process

Analysis of microplastics involves several key steps: sample collection, digestion, isolation, and instrument analysis (Figure 2). This section focuses on the practical aspects of sample digestion, microplastic isolation, and filtration, and particularly, how to effectively isolate microplastics from a dirty matrix (for example, environmental or protein-rich samples). Removing organic matter effectively ensures accurate microplastics analysis, eliminating potential interference.

Sample digestion (removal of organic matter)

Samples collected from the natural environment contain high concentration levels of organic matter (for example, animal tissue, plant debris, and other micro-organisms). These materials can interfere with microplastics analysis and hinder accurate identification and quantification in all spectroscopic techniques (IR, Raman, or LDIR). Therefore, effective sample digestion should be used to reduce matrix effects and obtain a clean sample. Table 2 summarizes the common chemicals used in sample digestion, including alkaline and acidic solutions, and enzymes, along with their reasons for use.

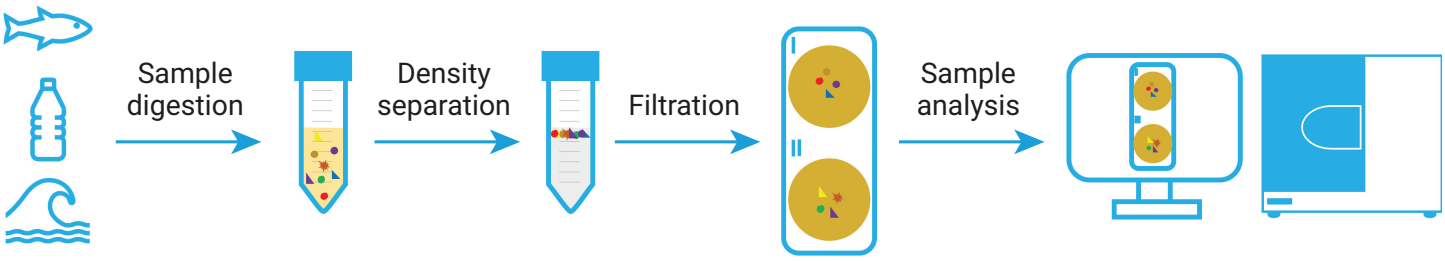


Figure 2. Sample preparation of microplastics and analytical steps.

Table 2. Common sample digestion reagents and their uses.

Type	Examples	Use
Alkaline	Hydrogen peroxide (H_2O_2)	Widely used for its high efficiency in removing organic matter; less damaging to microplastics[5-7]
	Fenton reagent (H_2O_2 + Fe (II))	Effective for water and sediment samples; temperature-dependent, can potentially affect polymers if not controlled[8,9]
	– Sodium hydroxide (NaOH) – Potassium hydroxide (KOH) – Sodium hypochlorite (NaClO)	Suited for the maceration of animal tissue. Large carbohydrates (such as cellulose) are resistant to alkaline hydrolysis[10-11]
Acids	– Nitric acid (HNO_3) – Hydrochloric acid (HCl) – Perchloric acid ($HClO_4$) – Sulfuric acid (H_2SO_4)	Effective at removing organic matter, but may degrade microplastics; often used in combination with alkaline digestion[12]
Enzymes	– Cellulase – Lipase – Protease	Mild digestion methods, targeting specific organic compounds without affecting microplastics; often followed by oxidation treatment for complete removal[13]

Density separation

After sample digestion, microplastic particles can be extracted efficiently using density separation.[9] In theory, when a matrix solution containing microplastics is suspended in a saturated, dense salt solution, microplastic particles will float to the top and the heavier inorganic matter will sink to the bottom (Figure 3). An appropriate density separation reagent can be selected based on the cost, environmental impact, and densities of the most commonly observed plastic polymers in the environment (0.85 to 1.45 g/cm^3), (Figure 4 and Table 3).[14,15]

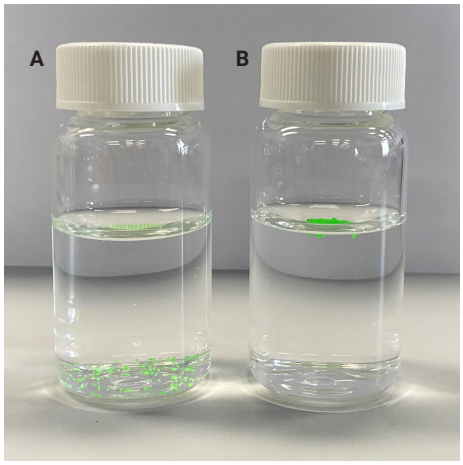


Figure 3. Green polyethylene microspheres (diameter: 250 to 300 µm) suspended in (A) distilled water and (B) calcium chloride ($CaCl_2$) solution. Particles clearly float to the top when a dense salt solution is used.

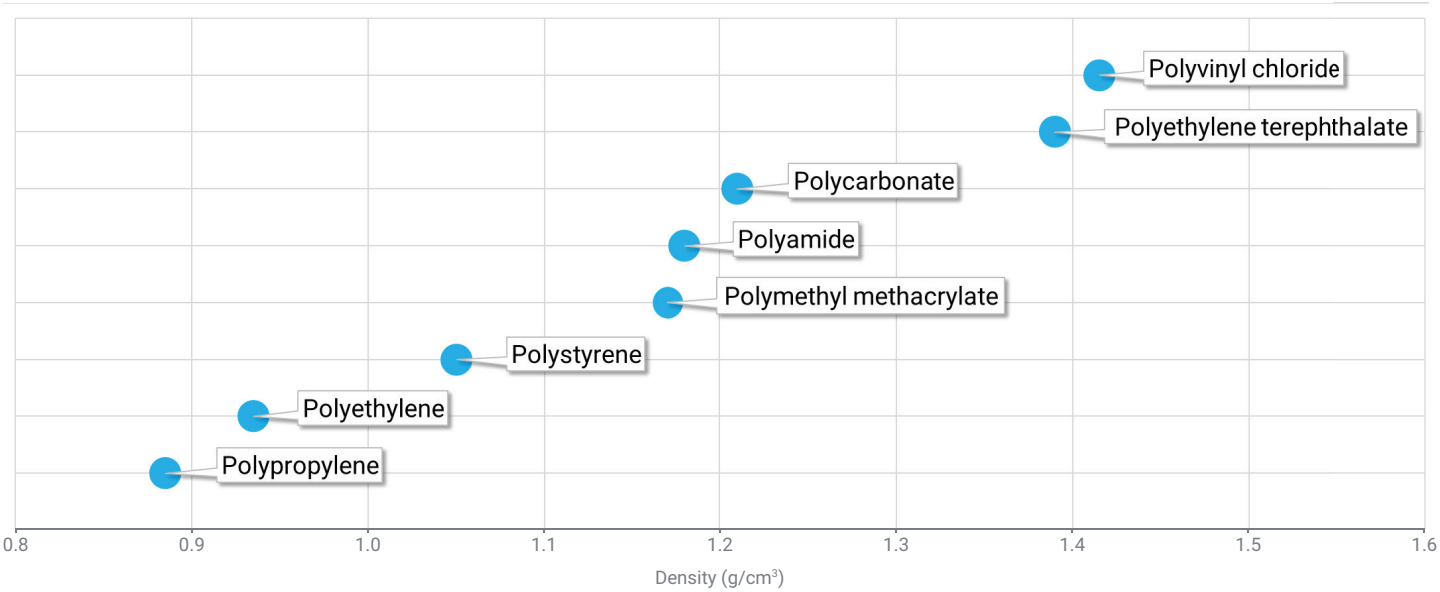


Figure 4. The average densities of common polymers.

Table 3. Common density separation reagents and their uses.

Chemical	Solution Density (g/cm^3)	Use and Limitations
Sodium Chloride (NaCl)	1.15 to 1.30	– Widely used due to low-cost, nonhazardous nature, and effective for many common plastics – Ineffective for high-density polymers (such as PET or PVC)
Calcium Chloride ($CaCl_2$)	1.30 to 1.35	– Low cost – Residual $CaCl_2$ may remain in the final sample, interfering with microplastic detection
Sodium Iodide (NaI)	1.55 to 1.80	– Good for all types of microplastics – More expensive
Zinc Chloride ($ZnCl_2$)	1.50 to 1.80	– Very high density, effective for all plastics – Very expensive and environmentally hazardous

Filtration

Regardless of the sample complexity, which may involve digestion and density separation, all samples will eventually need filtration to capture the suspended microplastics. Filtration can occur during sample preparation or directly on the filter for analysis. Following are some considerations:

General filtration during sample preparation

- Glass fiber membranes are not recommended for IR spectroscopy analysis due to their tendency to shed fibers.
- Cellulose or polycarbonate filters can be used as long as they effectively retain microplastics. Be mindful of microplastic shedding from polycarbonate filters.
- Test the chemical compatibility of filters with the reagents before filtration.

Direct on-filter analysis

- A suspension containing microplastics can be filtered directly through gold- or aluminum-coated filters with a 0.8 µm pore size and a 25 mm diameter, attached to a vacuum filtration system (Figure 5). The filter can then be transferred to a two-position filter holder and inserted into the 8700 LDIR for direct microplastic characterization.
- Alternatively, the microplastic suspension can be deposited onto a low-e IR slide, allowing the solvent to evaporate before transferring it into the LDIR for analysis.

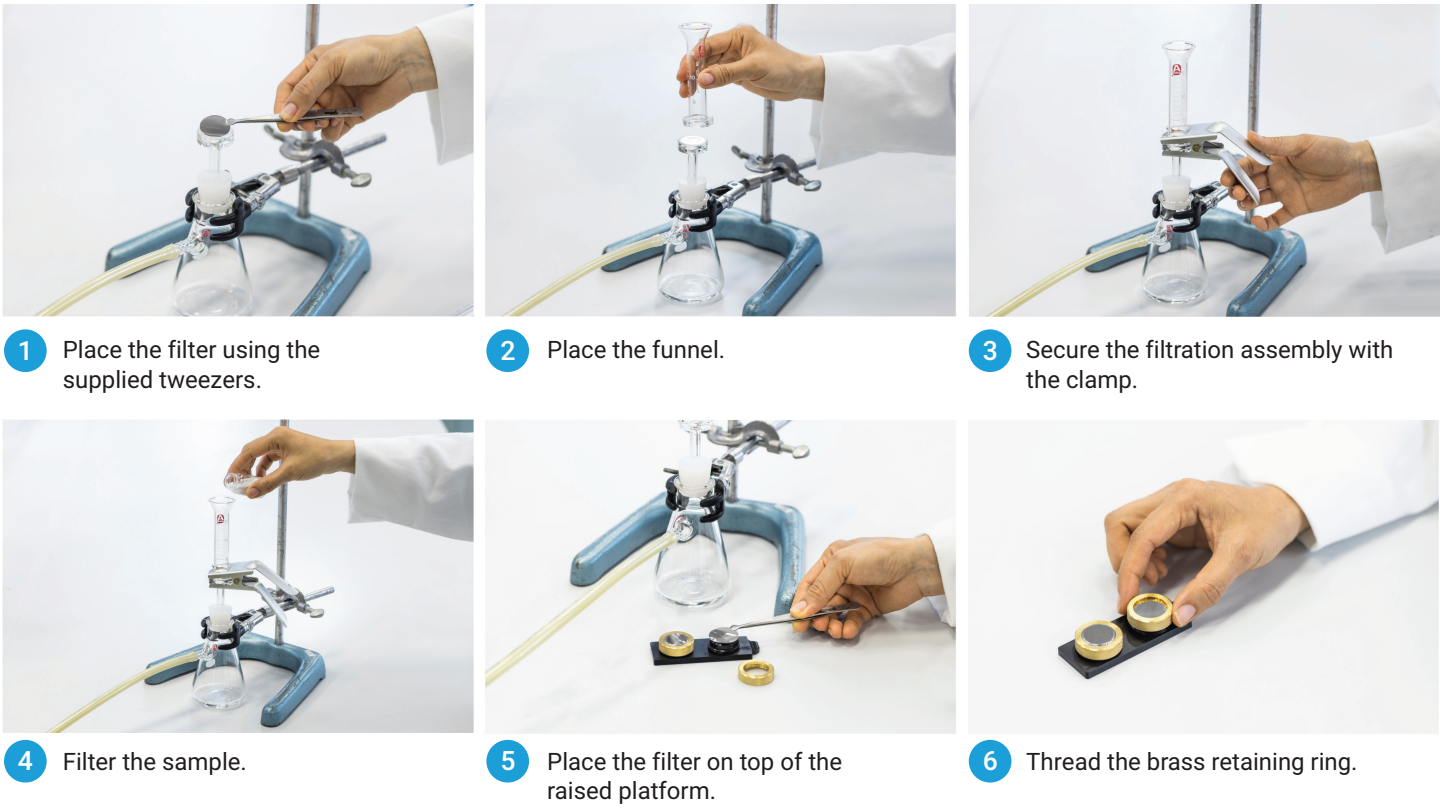


Figure 5. Sample filtration equipment and steps for the preparation of microplastic samples on aluminum-coated filters, ready for analysis using the Agilent 8700 LDIR chemical imaging system.

Analysis methods for microplastics using LDIR in various matrices

Research groups around the world have used LDIR as an integral tool for the analysis and characterization of microplastics in various matrices, including groundwater, bottled water, air deposition samples, and fish intestines.[16-19]

This section summarizes several sample preparation methods used to isolate microplastics from common matrices, which were then analyzed using LDIR.

Bottled drinking water

Sample preparation for bottled water is minimal, as it is considered a clean matrix. Sample digestion and density separation are not necessary for this matrix; only direct filtration is required. The entire bottle of water (600 to 1,000 mL) can be filtered through a polyester (PETG) gold- or aluminum-coated membrane (0.8 µm, 25 mm).

1. Set up the filtration flask, place the filter paper onto the glass frit, and position the funnel on top.
2. Securely clamp the frit and funnel together. Slowly pour the water through the filter paper.
3. After filtering the water, wash the inside of the funnel three times to ensure that any microplastics stuck to the sides are collected on the filter paper.
4. Finally, place the filter on the raised platform and tighten it with the brass ring to ensure a flat surface, which will not affect the line profiling of the sample. The sample can then be analyzed directly on the filter using LDIR. To read the full application note, click here.[20]

Environmental water

Environmental water usually contains high levels of organic matter, requiring an initial digestion step.

1. First, samples are filtered through a membrane with a pore size appropriate for the particle size of interest, typically between 5 to 20 µm.
2. The membrane can then be transferred to a 30% H_2O_2 solution to eliminate all organic matter. Generally, H_2O_2 is shaken and heated to 55 °C for 12 to 24 hours to ensure that most (or all) organic matter is removed.
3. The solution is filtered again, and the glass vial is washed three times with filtered Milli-Q water.
4. Once all organic matter is removed, the filter is transferred into a solution of $CaCl_2$ or $ZnCl_2$ to isolate microplastics through density separation.
5. The top layer, containing isolated microplastics, is then transferred onto a low-e slide for analysis using the 8700 LDIR.[16]

Soil and sediment

There are multiple methods for isolating microplastics from soil and sediment, but most follow a standard sample preparation approach (sample digestion followed by density separation), as described in Figure 6.

1. Before sample treatment, the samples are either air-dried, freeze-dried, or oven-dried to obtain consistent weight across the study.
2. Samples must then be sieved for homogeneity and to remove larger particles, as well as to obtain particles in the desired size range.[21]
3. The sample is then submerged in a 30% H_2O_2 solution or Fenton's reagent at 55 °C to remove organic matter. To effectively eliminate organic materials, it is important to use enzymatic treatment.
4. Afterward, a density separation process is conducted involving NaCl, $CaCl_2$, and $ZnCl_2$ to separate the microplastics.
5. Similar to the procedure for environmental water, the microplastics-containing layer is then transferred onto a low-e slide or filtered through a gold- or aluminum-coated filter for analysis using the 8700 LDIR.[19]

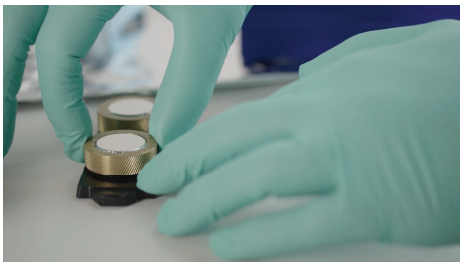
1 Weigh the dry soil sample.

2 Perform sample digestion.

3 Ensure removal of organic matter by leaving the sample over time.

4 Perform density separation.

5 Filter the sample.



6 Place the filter on the filter holder.

Figure 6. Soil sample preparation steps.

Infant formula

Food products are as challenging as environmental samples, but for different reasons. Food products contain various amounts of fats, carbohydrates, vitamins, and protein, all of which need to be removed to perform microplastics analysis. Unoptimized microplastic sample preparation methods could affect the number of particles detected and lead to false positive identification of microplastics. To our knowledge, there have only been two studies that used LDIR for the analysis of infant formula.

For the first study:

1. Infant formula (5 g) was placed into a 100 mL beaker with concentrated nitric acid (68%) to digest the macromolecular substances. It was left alone for 48 hours.
2. After this time, the solution was heated at 95 °C for at least 3 hours to fully digest all protein.
3. After 3 hours, the solution was filtered through 13 µm filter paper before resuspending all the particles. The beaker was then filled with ethanol and sonicated for 30 minutes.
4. Once this solution was filtered, the suspended particles were concentrated into 200 µL and transferred to a low-e slide to be analyzed by LDIR.[22]

Another approach for extracting microplastics is separating the fatty layer and the liquid layer:

1. This is possible using sodium chloride (NaCl) and by centrifuging for 30 minutes at 3,000 rpm.
2. From here, the liquid layer can be filtered through a 47 mm, 14 µm polycarbonate filter. Once dried, the particles should be washed into a tube with ethanol.
3. The fatty layer requires a digestion step, with 0.1 M sodium hydroxide (NaOH) heated for 20 minutes at a temperature of 50 to 60 °C.
4. The digested sample can be filtered through a 47 mm, 14 µm polycarbonate filter paper.
5. From here, the method follows the same process as the lower layer. Both layers are then filtered through a gold-coated filter and analyzed directly using LDIR. To read the full application note, click here.[23]

Conclusion

Isolating microplastics is a complex process. There are several reasons for this, including the multifaceted sample preparation methods, the ubiquitous nature of microplastics, the risk of introducing contaminants, and the heterogeneity of microplastics in samples. As a result, microplastics analysis is challenging and time-consuming. This is true regardless of the technique used for characterization—whether LDIR, FTIR, Raman, or other technologies.

Understanding the health and environmental impact of microplastics is crucial. To facilitate this, global efforts have focused on simplifying the analytical process. These efforts include developing standardized methodologies for isolating microplastics, creating reference materials, and generating comprehensive polymer libraries.

The Agilent 8700 LDIR microplastics analysis workflow plays a key role in this global environmental challenge. The system provides efficient microplastics analysis and saves significant time. The direct-filter LDIR method requires less sample handling, reducing the potential for sample contamination and offering excellent accuracy and higher sample throughput. The automated workflow of the 8700 LDIR aids accurate characterization of microplastics in different matrices that involve high numbers of samples and require fast sample throughput.

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Further information

- Agilent 8700 LDIR Chemical Imaging System
- Agilent Clarity Software
- Microplastics Technologies FAQs
- Microplastics Analysis in Water

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