Technical Overview

Collecting and Preparing Microplastics for Analysis by the Agilent 8700 LDIR

Applying the sample preparation methodology specified in ASTM D8333 and SCCWPR

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Introduction

The success of any spectroscopic analysis of microplastic particles depends on the effectiveness of the sample preparation procedure. Inadequate or improper sample preparation can introduce errors into the analysis, leading to unreliable data. Whether using infrared (IR) or Raman microscopy, microplastic particles need to be isolated so they can be discretely distributed on a substrate ready for analysis. The more complex the matrix, the greater the amount of preparation will be required. Standardization of sample preparation methodology is also important to enable the comparison of results from different studies.

Analysts typically use the guidelines specified in standard practice documents published by industry bodies such as ASTM International when collecting and preparing environmental waters for microplastics analysis. ASTM D8332 provides protocols for the collection of water samples in "Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers".[1] ASTM D8333 provides guidance in "Standard Practice for Preparation of Water Samples with High, Medium, or Low Suspended Solids for identification and quantification of Microplastic Particles and Fibers Using Raman Spectroscopy, IR Spectroscopy, or Pyrolysis-GC/MS".[2]

The D8333 standard describes the steps for microplastic sample preparation depending on the level of suspended solids present in the water. Irrespective of the complexity of the samples, which may require enzymatic digestion and extraction, all samples will eventually require filtration. The least complex samples with the lowest levels of suspended solids, e.g., drinking water, should only require filtration. ASTM D8333 and the guidance document issued by the Southern California Coastal Water Reclamation Project (SCCWRP)[3], "Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water", describe steps for the filtration of suspended solids from drinking water samples. Per both guidance documents, the method for the extraction of microplastics from drinking water samples requires several successive sieving and vacuum filtration steps.[2,3]

Sample collection

ASTM D8333 assumes that samples are size-fractioned, as detailed in ASTM D8332 and summarized in this paper. For drinking water (with low suspended solids content), procedures noted in ASTM 8332 specify that 1,500 L of water is passed through two sieves with pore sizes of 500 and 20 µm, in turn. The water flow through each sieve is metered to record total volume, so that numbers of particles/fibers per unit volume or mass per unit volume can be calculated and reported. SCCWRP prescribes an additional fractionation of drinking water samples using a 212 µm sieve, as well as the 500 and 20 µm stated in ASTM D8332. If analysts follow the SCCWRP protocol, the 20 to 212 µm fraction would typically be analyzed separately from the 212 to 500 µm fraction, doubling the analysis time.

According to the ASTM D8333 or SCCWRP methods, the sieve fractions should then be transferred (rinsing with a minimal amount of microplastic analysis grade (MAG) water) to a single 0.250 L bottle or petri dish ready for sample preparation. For waters with medium and high levels of suspended solids, the ASTM documents specify similar procedures that involve the use of additional sieves.

Sample preparation

Per the ASTM D8333 sample preparation guidance, the sieve contents of the 0.250 L flask (or petri dish) should be transferred to a 50 mL centrifuge tube, rinsed with 10 mL of methanol, and centrifuged for three minutes at 5,000 rpm. At this point, the contents are ready to be transferred to a filter or microscope slide for characterization using visible and IR microscopy.

As mentioned in the previous section, SCCWRP prescribes that an additional fractionation of the 20 to 500 µm portion be performed with a 212 µm sieve, providing two fractions (20 to 212 and 212 to 500 µm) for analysis. It also includes a series of detailed steps to be followed for size fractioning and manual wet-sorting to be completed before visible and, in some cases, spectroscopic characterization of particles can proceed.[3]

Laser direct infrared (LDIR) chemical imaging system

The default and fully automated microplastic Particle Analysis workflow of the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system measures particles in the size range of 20 to 500 µm. Therefore, LDIR can process the whole 20 to 500 µm fraction of microplastics in one go, removing the need to analyze the 20 to 212 and 212 to 500 µm fractions separately, simplifying the SCCWRP protocol.

The contents of the 0.250 L flask (or petri dish) containing the 20 to 500 µm fraction of microplastics from the water sample may be poured directly through a gold-coated, 0.8-micron-pore-size, 25 mm diameter filter, which is attached to a vacuum filtration system (Figure 1). The filter is transferred to a two-position filter holder (Figure 2), which is then inserted into the 8700 LDIR for characterization of microplastics.[4]

Figure 1. Vacuum filtration apparatus.

Figure 2. Two-position filter holder.

The Particle Analysis workflow within the Agilent Clarity software allows the analyst to locate, describe, and identify the microplastics particles on the filters. For every particle detected by LDIR, the 8700 method automatically:

- Measures the physical properties of the particle
- Classifies the particle by size
- Takes IR and visible images
- Collects an IR spectrum of each particle
- Compares the particle spectrum to the Agilent-supplied microplastics spectral library for identification purposes.

Conclusion

Sample preparation is the key to collecting high-quality data on the microplastic content of environmental waters. Following ASTM and SCCWRP guidance, the complexity of the sample matrix dictates the number of steps that will be required to prepare a sample for analysis.

However, as outlined in this white paper, using the Agilent 8700 LDIR microplastic "on-filter" workflow significantly simplifies the final filtration stage of the sample preparation procedure, minimizing the risk of sample contamination.

Reducing the complexity of sample preparation can enhance efficiency, cut costs, and improve data accuracy—all desirable outcomes in environmental laboratories with a large sample load. Once samples have been prepared for analysis, the 8700 LDIR direct on-filter method provides comprehensive data on microplastic particles extracted from environmental waters.

Additional information

Download the white paper: Best Practice for On-Filter Analysis of Microplastics Using the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System

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DE14220605

Printed in the USA, January 16, 2024
5994-6999EN

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