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# A Comprehensive Approach for Successful Microplastics Analysis

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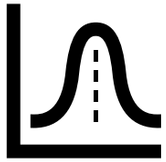
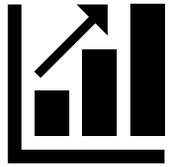
# ASTM D19 has developed and is developing several guides, practices, and methods for microplastics in water



Reference materials D8402

Sample Collection D8332

Sample Preparation D8333



Analysis

DIA D8489

PY-GCMS D8401

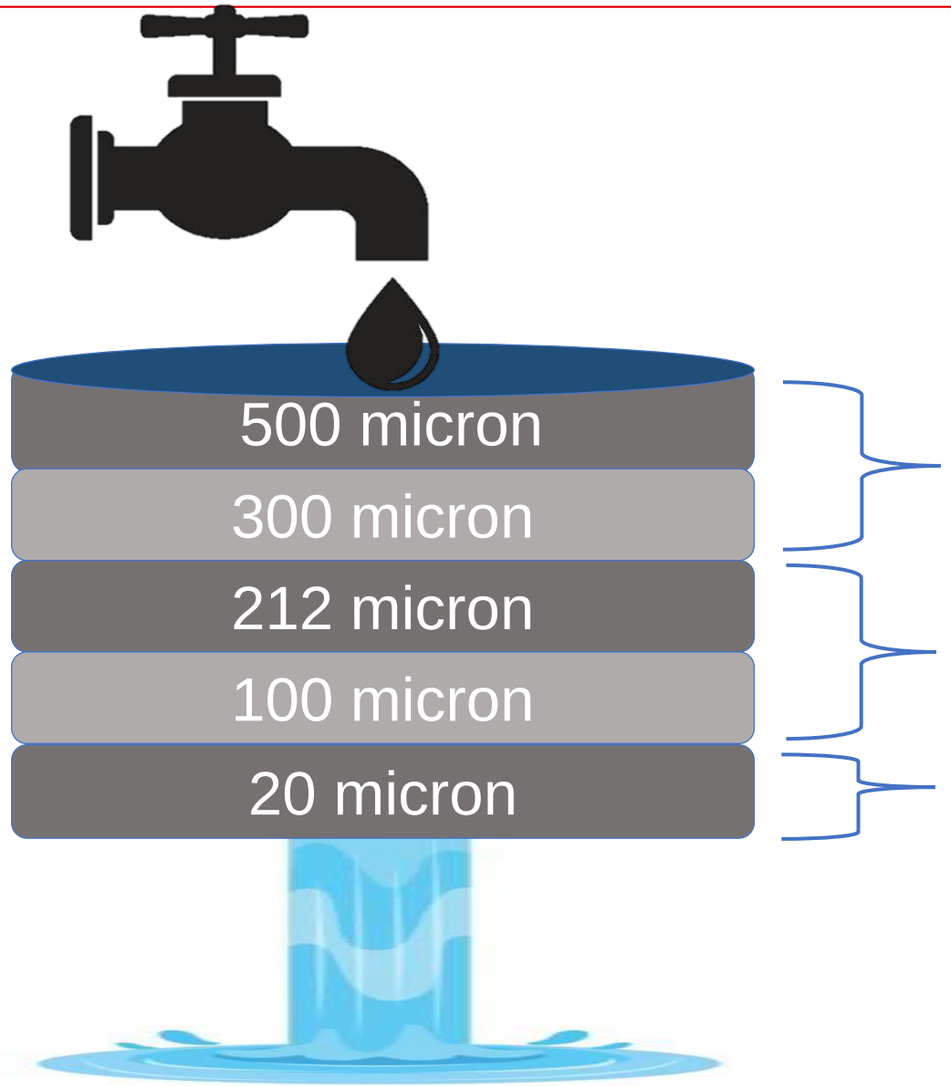
IR Microscopy WK87463

# D8332 describes how large volumes of the entire water column can be sampled

The key is a large volume through sieves

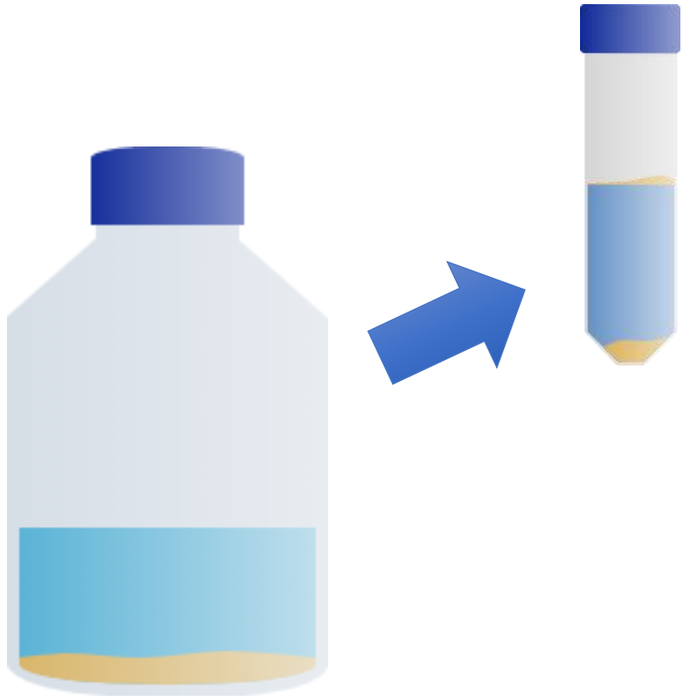
Jeremiah Bryksa ∗ ,Patric McGlashan ,Nadia Stelck ,Jon Wong , Andrew Anderson-Serson,Matthew Hart,Trace Malcom,Bob Battle,Paolo Mussone; High throughput application of ASTM D8332: Detailed prototype design and operating conditions for microplastic sampling of riverine systems; https://methods-x.com/article/S2215-0161(24)00134-1/fulltext

# In addition, sample collection by D8332 presorts particles by sieve fraction




Separate sieve fractions can be digested and analyzed separately

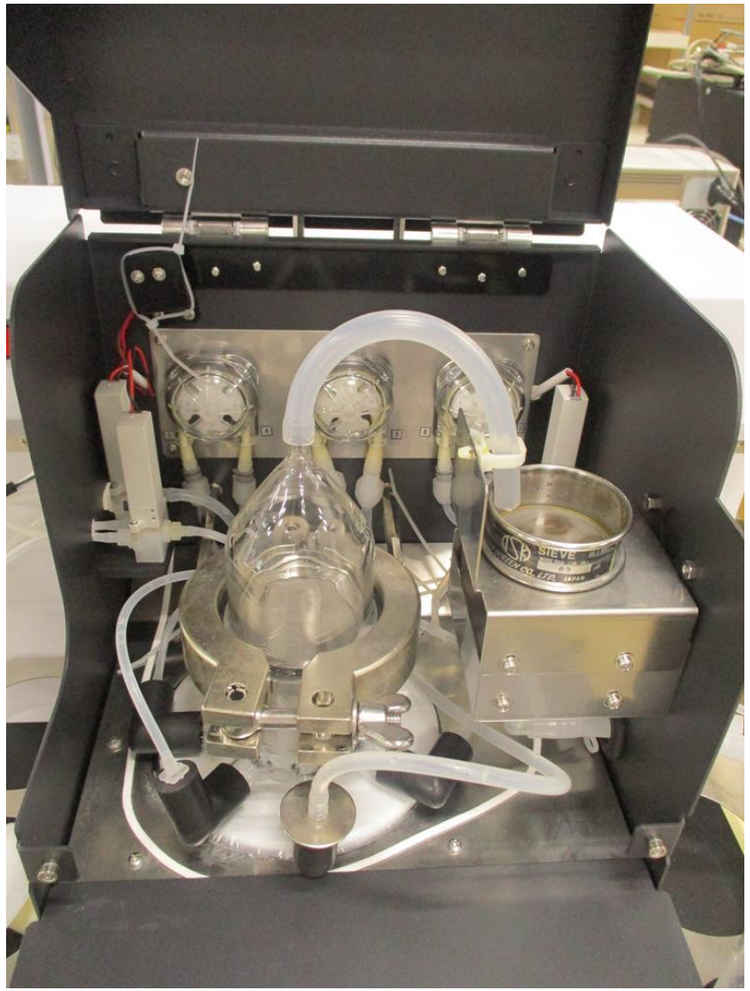
# Organic and other material (interferences) removed from each sieve fraction by sequential digestion using D8333 (only digest when needed)



- $H_2O_2$
- Schweizer 5 minutes
- Ammonium Hydroxide 5 minutes
- Buffer
- Protease, buffer, lipase
- React 20 hours

Final sample = plastics suspended in methanol

# Further prep may be needed after D8333, depending on the analysis method



# Once the final sample is prepared each sieve fraction is ready for measurement

Pyrolysis GCMS

D8401

Dynamic Imaging

D8489

FTIR Microscopy

Manual IR Spectrometry

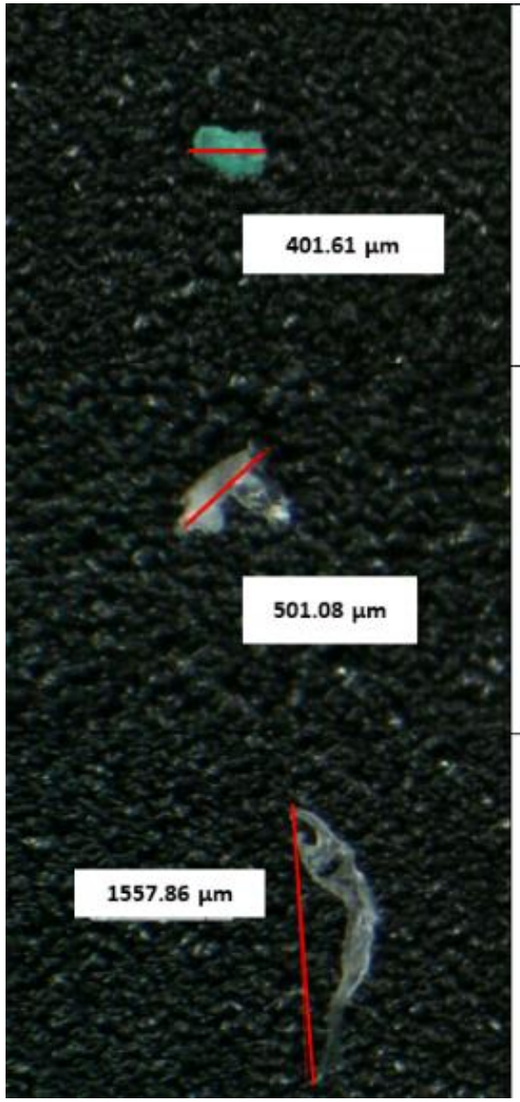
# We developed ASTM D8489, a dynamic imaging method, to rapidly count particles in the < 100 µm sieve fractions

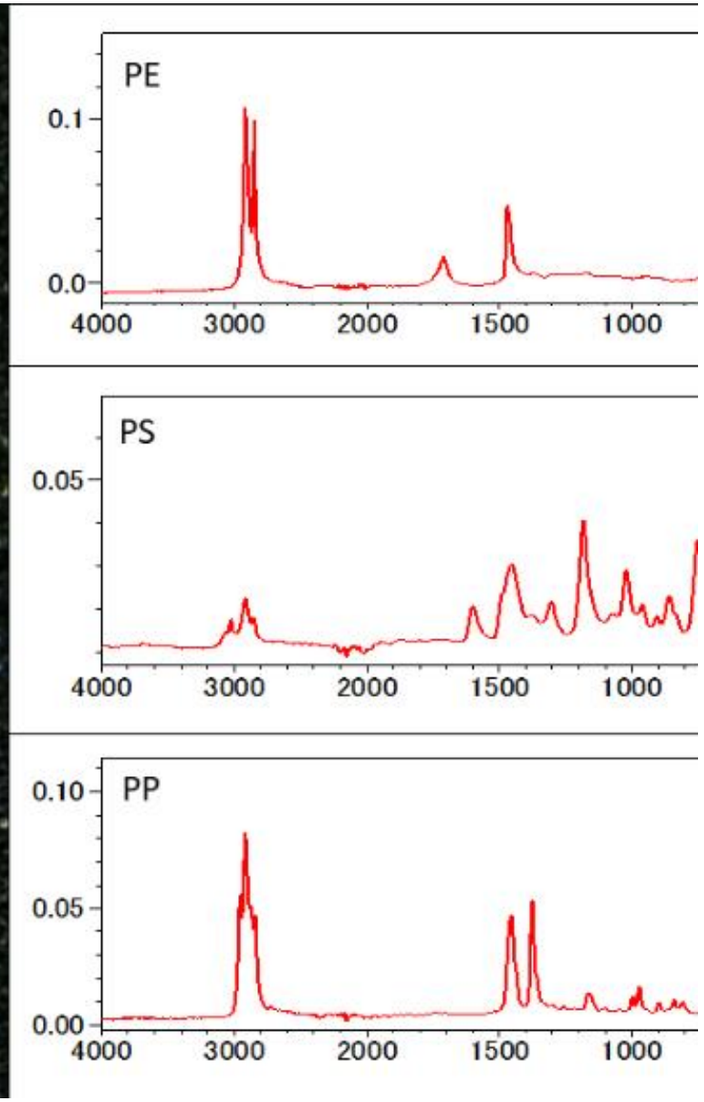
- The total number of particles, and size distribution is quickly obtained
- Data shows aliquots from the D8333 sample prep step are reproducible.
- You also get a size distribution
- Counting is much faster than by IR

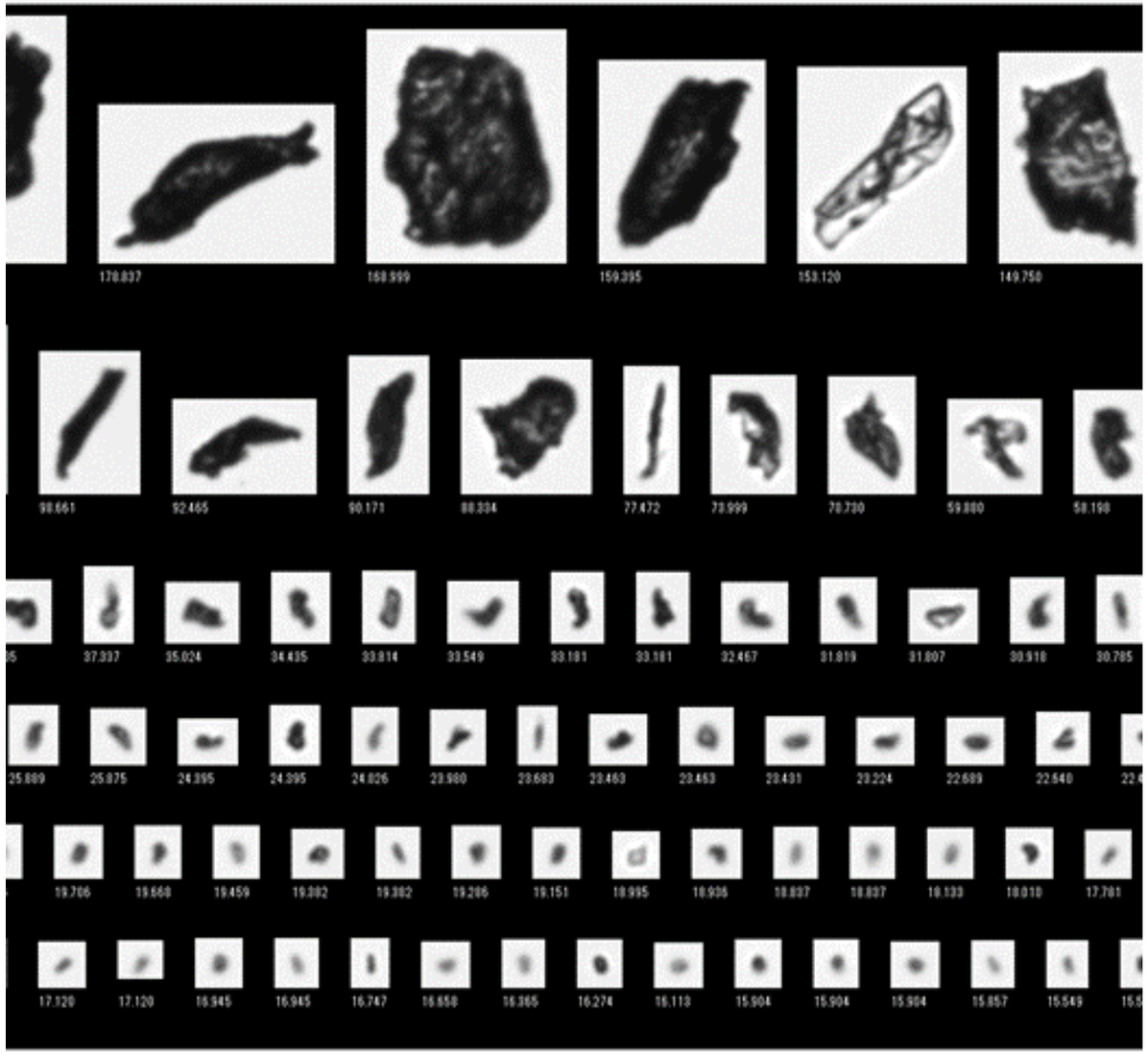
| Replicate number | Concentration (count/mL)* | | | | | |
|---|---|---|---|---|---|---|
| | All | 5-10 µm | 10-25 µm | 25-50 µm | 50-100 µm | > 100 µm |
| 1 | 3253 | 1623 | 1250 | 214 | 83 | 83 |
| 2 | 3667 | 2051 | 1140 | 283 | 69 | 124 |
| 3 | 3764 | 2037 | 1271 | 290 | 90 | 76 |
| 4 | 3640 | 2141 | 1140 | 228 | 76 | 55 |
| 5 | 3419 | 1837 | 1167 | 235 | 111 | 69 |
| 6 | 3508 | 2051 | 1105 | 242 | 76 | 35 |
| 7 | 3101 | 1761 | 1050 | 186 | 62 | 41 |
| Average | 3479 | 1929 | 1160 | 240 | 81 | 69 |
| S.D. | 238.4 | 190.1 | 77.9 | 36.7 | 15.8 | 30.1 |
| RSD | 6.85% | 9.86% | 6.72% | 15.31% | 19.54% | 43.59% |

* Concentration is calculated from Number of Particles, Number of Frames and flowcell volume.

# Using ASTM D8489, you also get some morphology data that could be correlated with later IR analysis.



# The D8489 data also shows that you can pipet the D8333 digestion within reasonable precision for individual measurement methods

Pyrolysis GCMS

D8401

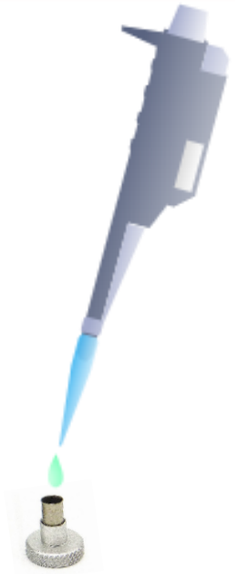
Dynamic Imaging

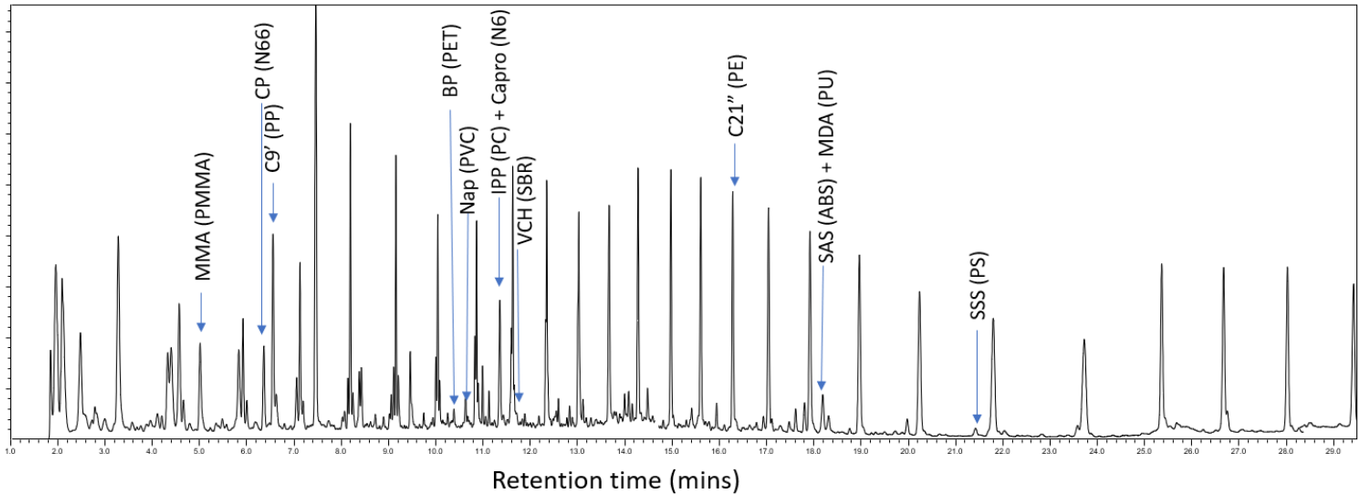
D8489

FTIR Microscopy

Manual IR Spectrometry

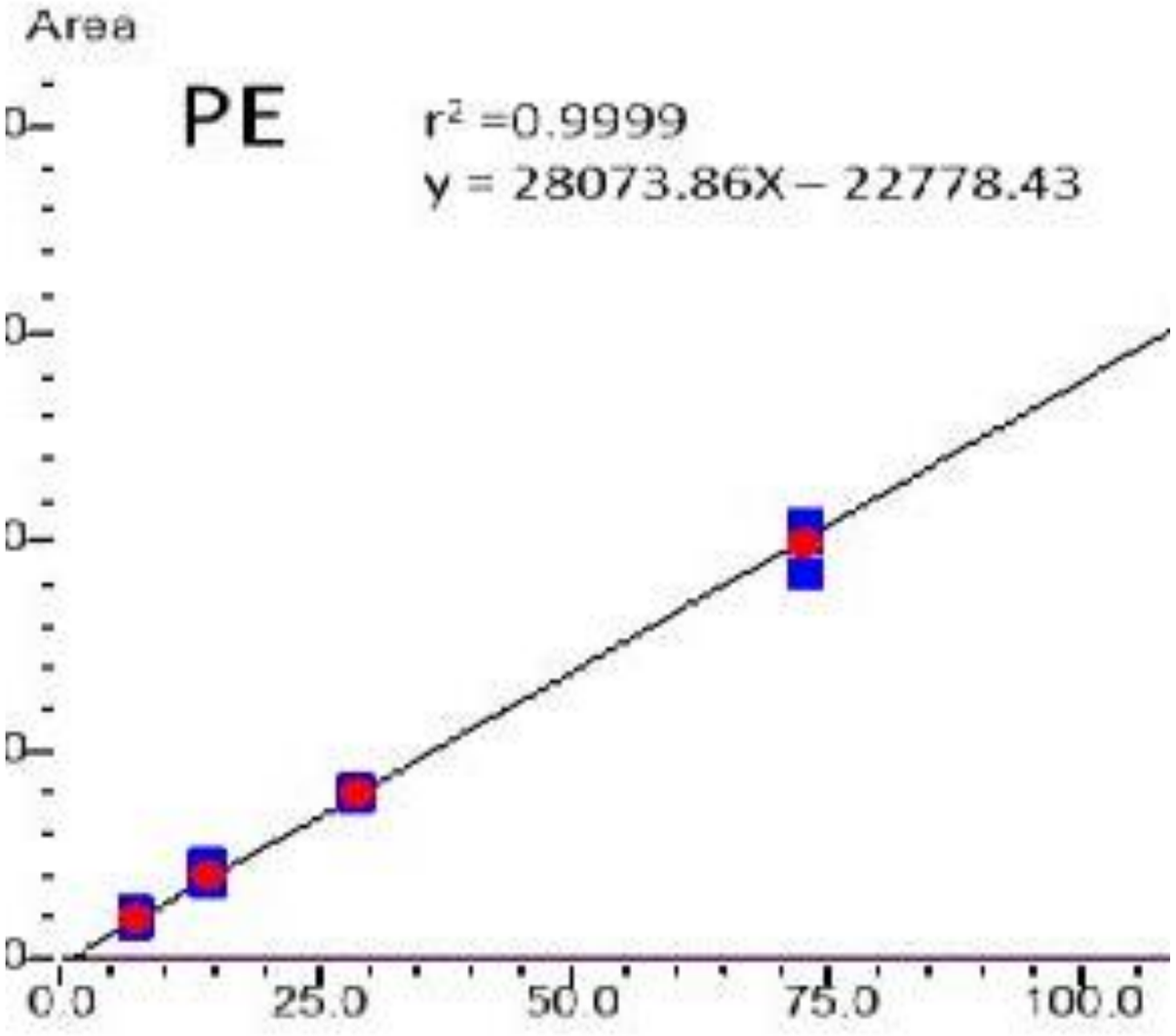
# ASTM D8401 Microplastics by Pyrolysis GCMS

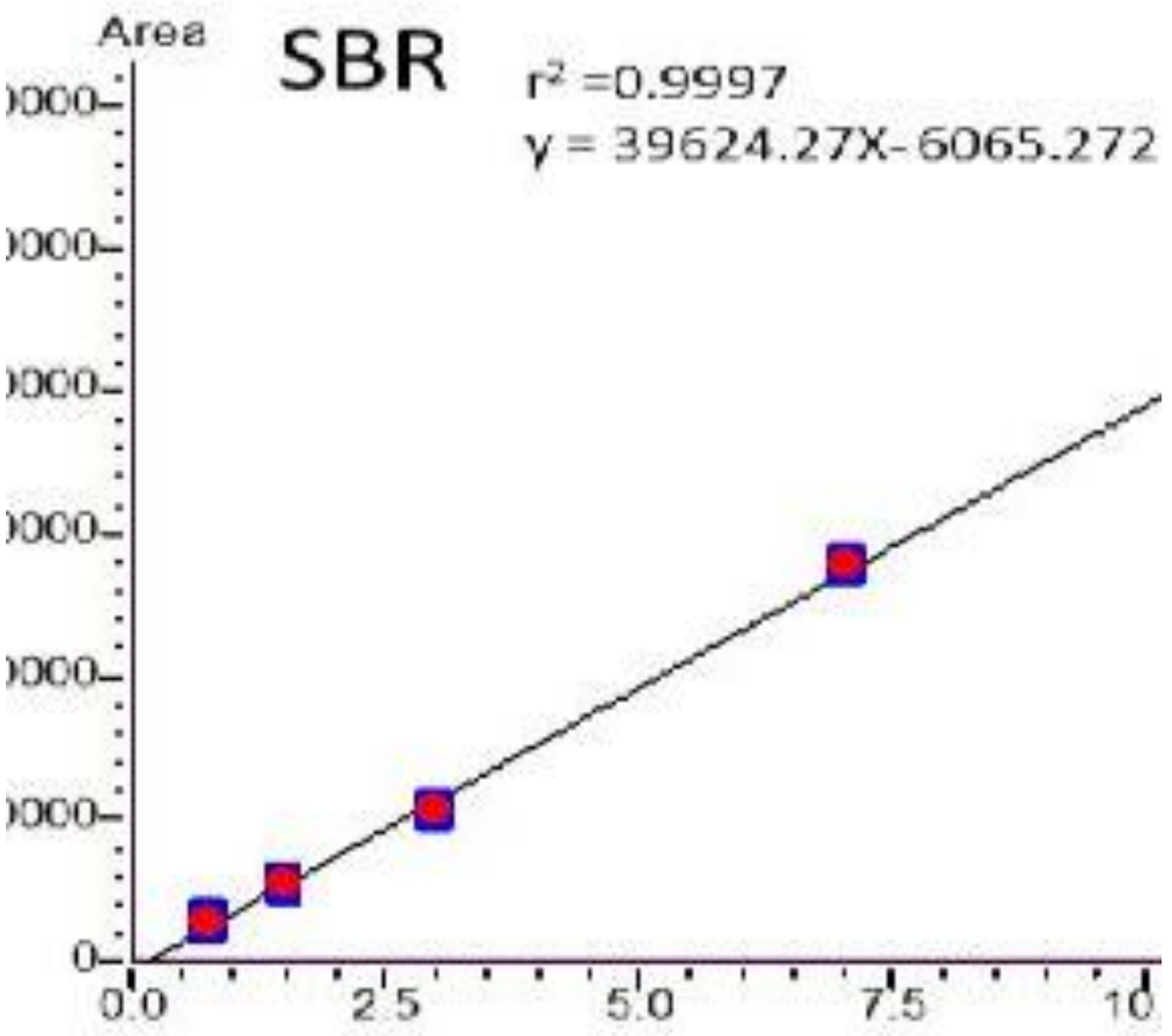




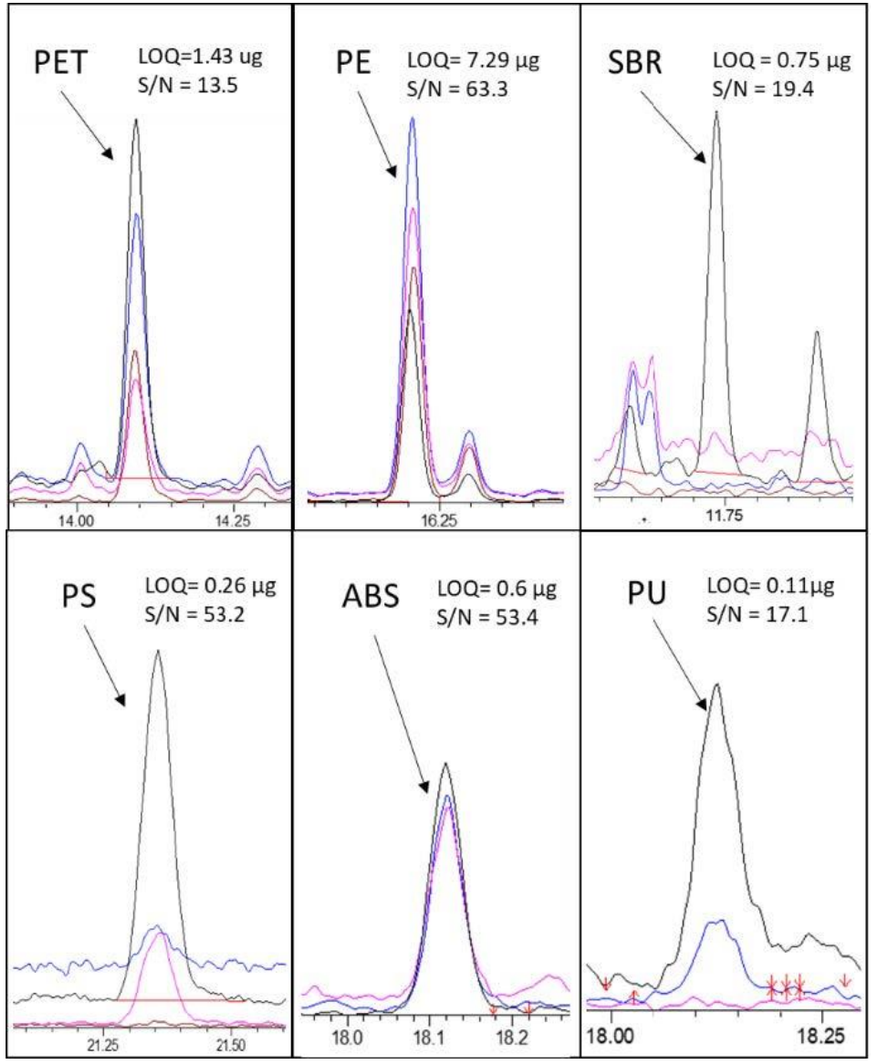

- Plastic particles from D8333 are transferred to PY cup and pyrozylates separated and identified.
- Pipet aliquot and evaporate, or dry crush and weigh
- 30-minute run time

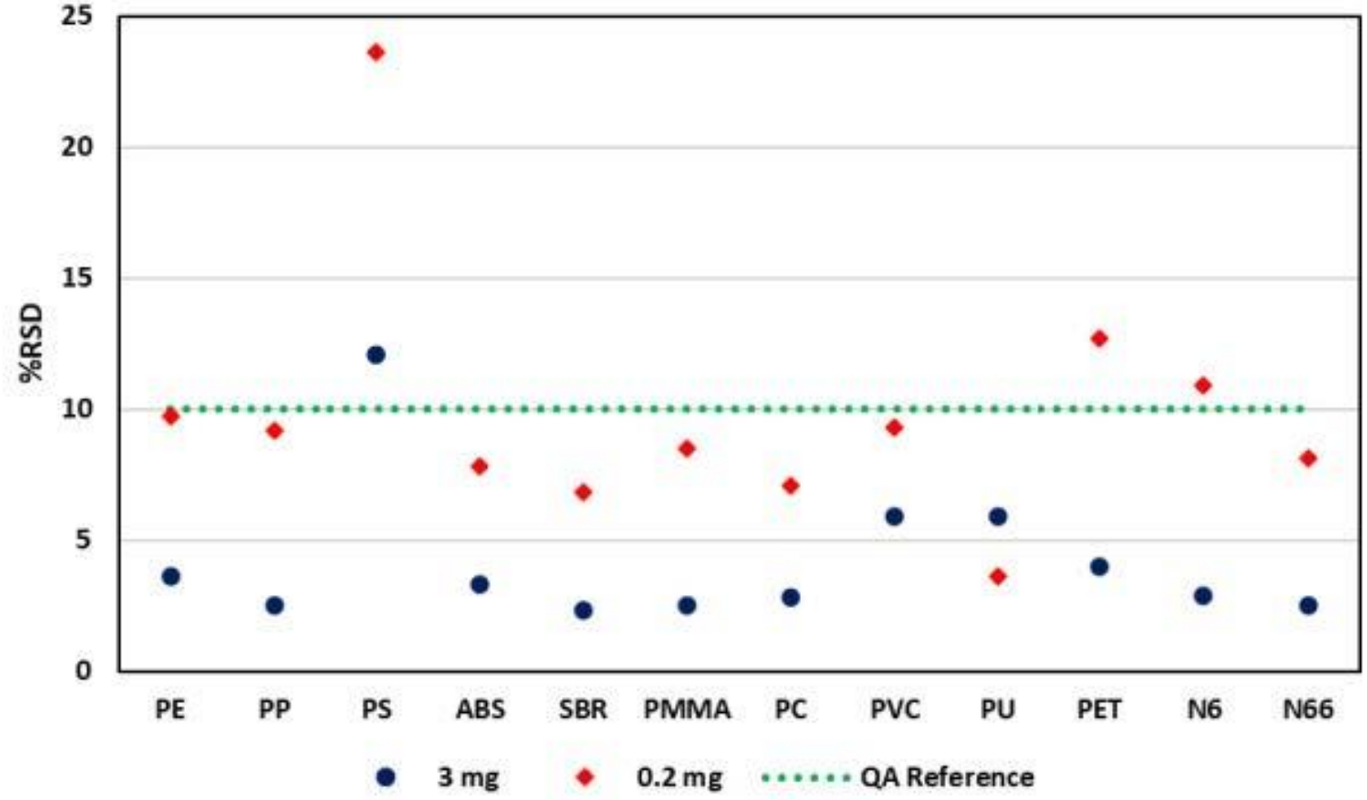
# PY GCMS identifies and quantitates plastics and rubber by mass



# PY-GCMS peaks and precision data



# Single laboratory precision of 12 microplastics in CaCO3

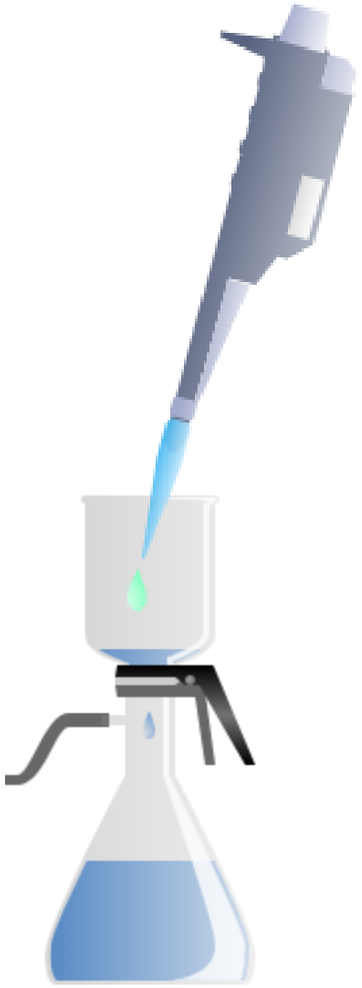
Single Laboratory Precision of 12 microplastics in 0.3 mg of a CaCO3 reference standard

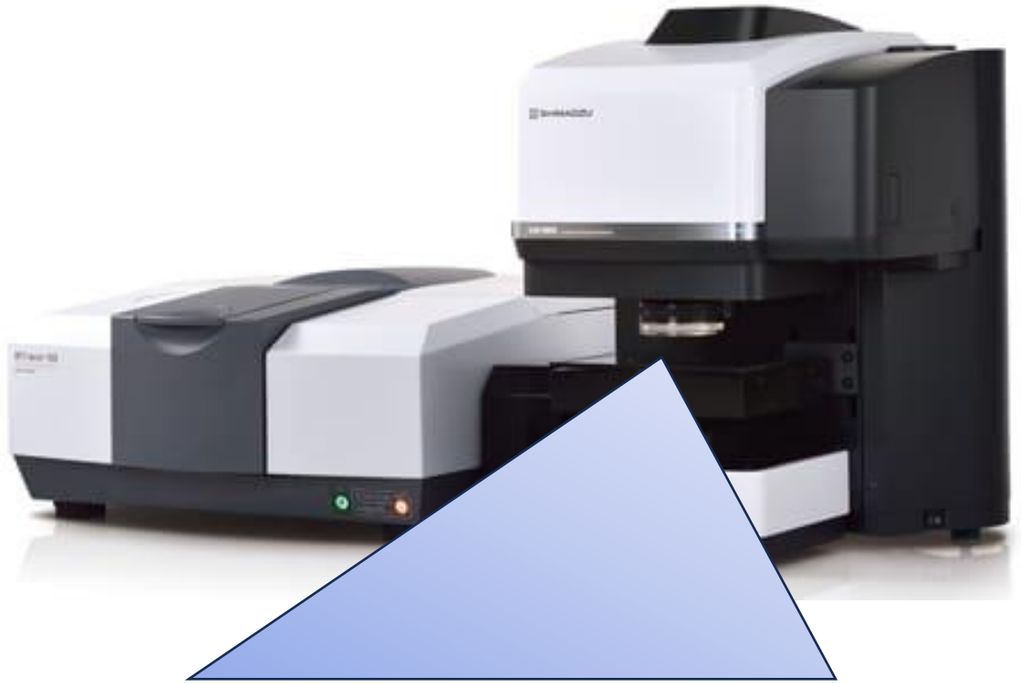
| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | %RSD |
|---|---|---|---|---|---|---|---|---|
| PE | 7.728 | 5.721 | 7.402 | 7.461 | 7.667 | 7.344 | 7.675 | 9.69 |
| PVC | 2.543 | 1.92 | 2.416 | 2.551 | 2.517 | 2.462 | 2.518 | 9.29 |
| PP | 2.074 | 1.643 | 1.976 | 2.104 | 1.959 | 1.947 | 2.227 | 9.17 |
| PET | 1.648 | 1.44 | 1.826 | 1.872 | 2.19 | 1.825 | 1.884 | 12.69 |
| N66 | 1.244 | 1.032 | 1.272 | 1.303 | 1.295 | 1.273 | 1.343 | 8.12 |
| SBR | 0.926 | 0.785 | 0.985 | 0.93 | 0.947 | 0.906 | 0.913 | 6.81 |
| ABS | 0.694 | 0.543 | 0.664 | 0.655 | 0.642 | 0.624 | 0.685 | 7.84 |
| PC | 0.316 | 0.276 | 0.311 | 0.337 | 0.34 | 0.32 | 0.339 | 7.09 |
| PMMA | 0.283 | 0.24 | 0.256 | 0.294 | 0.31 | 0.282 | 0.292 | 8.55 |
| N6 | 0.283 | 0.221 | 0.323 | 0.276 | 0.288 | 0.277 | 0.292 | 10.88 |
| PU | 0.164 | 0.148 | 0.16 | 0.164 | 0.159 | 0.158 | 0.164 | 3.58 |
| PS | 0.142 | 0.107 | 0.216 | 0.178 | 0.224 | 0.18 | 0.161 | 23.64 |

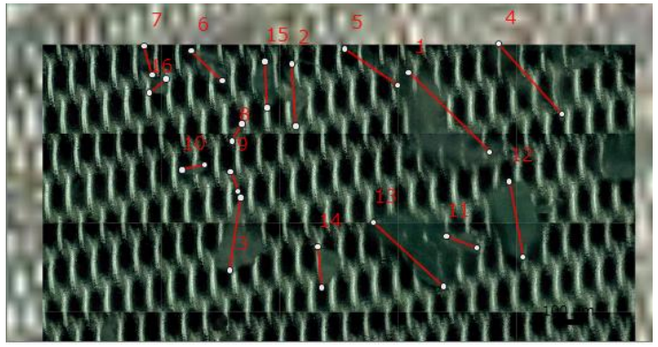
Single Laboratory Precision of 12 microplastics in 4 mg of a CaCO3 reference standard

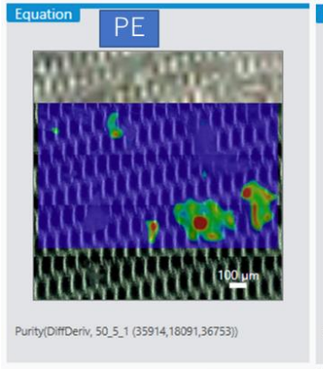
| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | %RSD |
|---|---|---|---|---|---|---|---|---|
| PE | 104.2 | 105.3 | 113.5 | 111.7 | 111.8 | 105.7 | 105.7 | 3.58 |
| PVC | 28.58 | 31.33 | 33.05 | 33.14 | 31.47 | 30.54 | 28.83 | 5.87 |
| PP | 26.36 | 25.92 | 27.37 | 27.72 | 26.82 | 26.34 | 26.1 | 2.52 |
| PET | 19.01 | 20.26 | 21.02 | 21.4 | 20.92 | 19.99 | 19.87 | 4.03 |
| N66 | 15.04 | 15.58 | 16.21 | 15.98 | 15.79 | 15.62 | 15.34 | 2.50 |
| SBR | 10.3 | 10.83 | 11.08 | 10.95 | 10.92 | 10.8 | 10.69 | 2.33 |
| ABS | 8.433 | 8.737 | 8.633 | 8.269 | 8.218 | 8.703 | 8.013 | 3.27 |
| PMMA | 3.923 | 4.006 | 4.257 | 4.076 | 4.104 | 4.127 | 4.109 | 2.54 |
| PC | 3.651 | 3.778 | 3.881 | 3.742 | 3.767 | 3.636 | 3.578 | 2.78 |
| N6 | 3.514 | 3.671 | 3.838 | 3.737 | 3.735 | 3.649 | 3.597 | 2.87 |
| PS | 3.243 | 4.116 | 3.754 | 3.087 | 3.391 | 4.208 | 3.383 | 12.12 |
| PU | 1.419 | 1.569 | 1.536 | 1.633 | 1.466 | 1.389 | 1.452 | 5.85 |

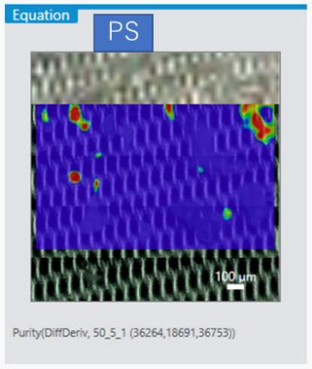
# A portion of the D8333 digest sieve fractions can be filtered for analysis by IR Microscope

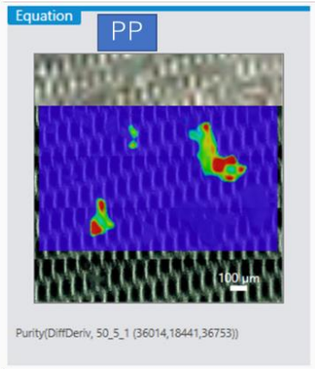






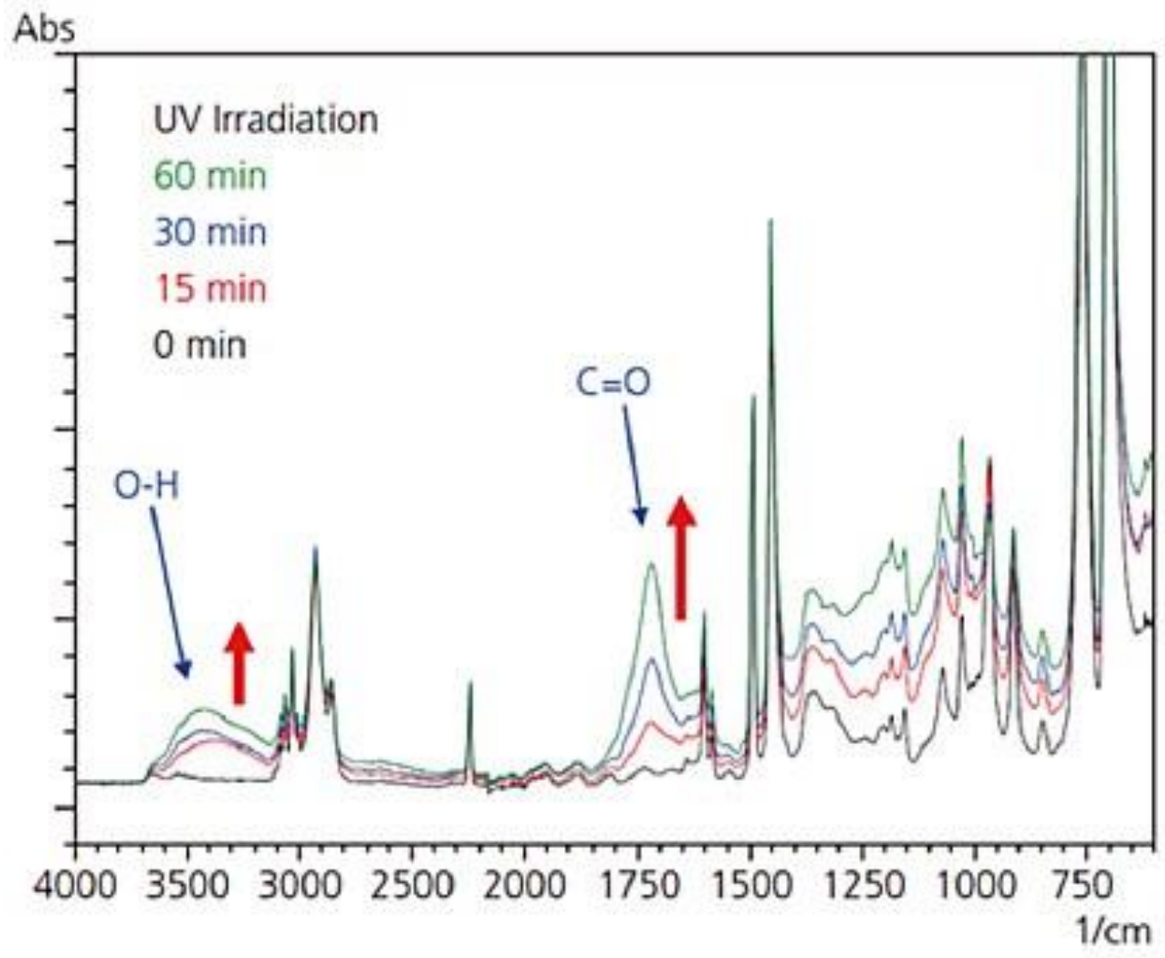



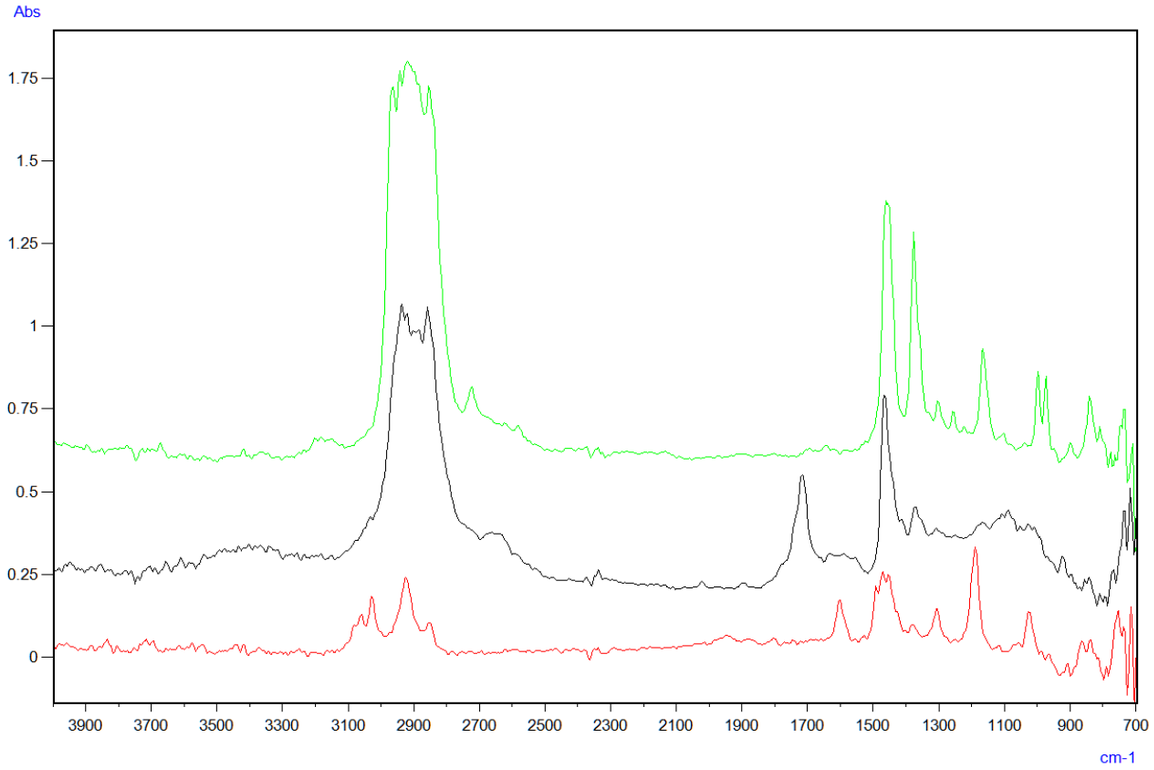



# There are Interferences with microscopy and IR methods, so sample digestion more important than with PY-GCMS

- Undigested organic matter and inorganics obscure view
- Extra digestion or removal after D8333 may be needed
- May be limited to particles > 20 µm in diameter
- Surface contamination, such as oxide coatings
- Thermal and UV damage



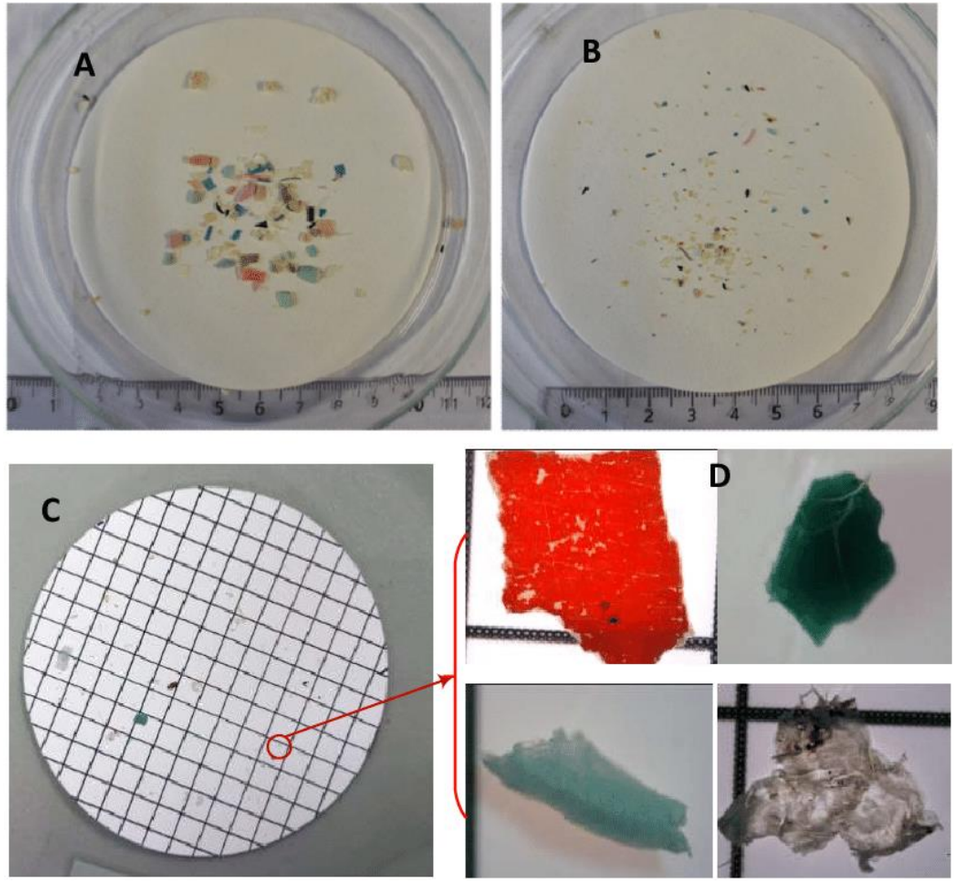
# Spectra are obtained and microplastics identified by comparing to databases




| | Sample 1 | | | | Sample 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | PE | PS | PP | Total | PE | PS | PP | Total |
| Average Particles per measurement area | 4.3 | 3.3 | 2.3 | 10.7 | 5.3 | 1.3 | 1.7 | 8.3 |
| Total Particles per filter | 1725 | 1327 | 929 | 4246 | 2110 | 517 | 663 | 3317 |

- The identified plastics are counted, and total particles are calculated based on size of the filtered aliquot

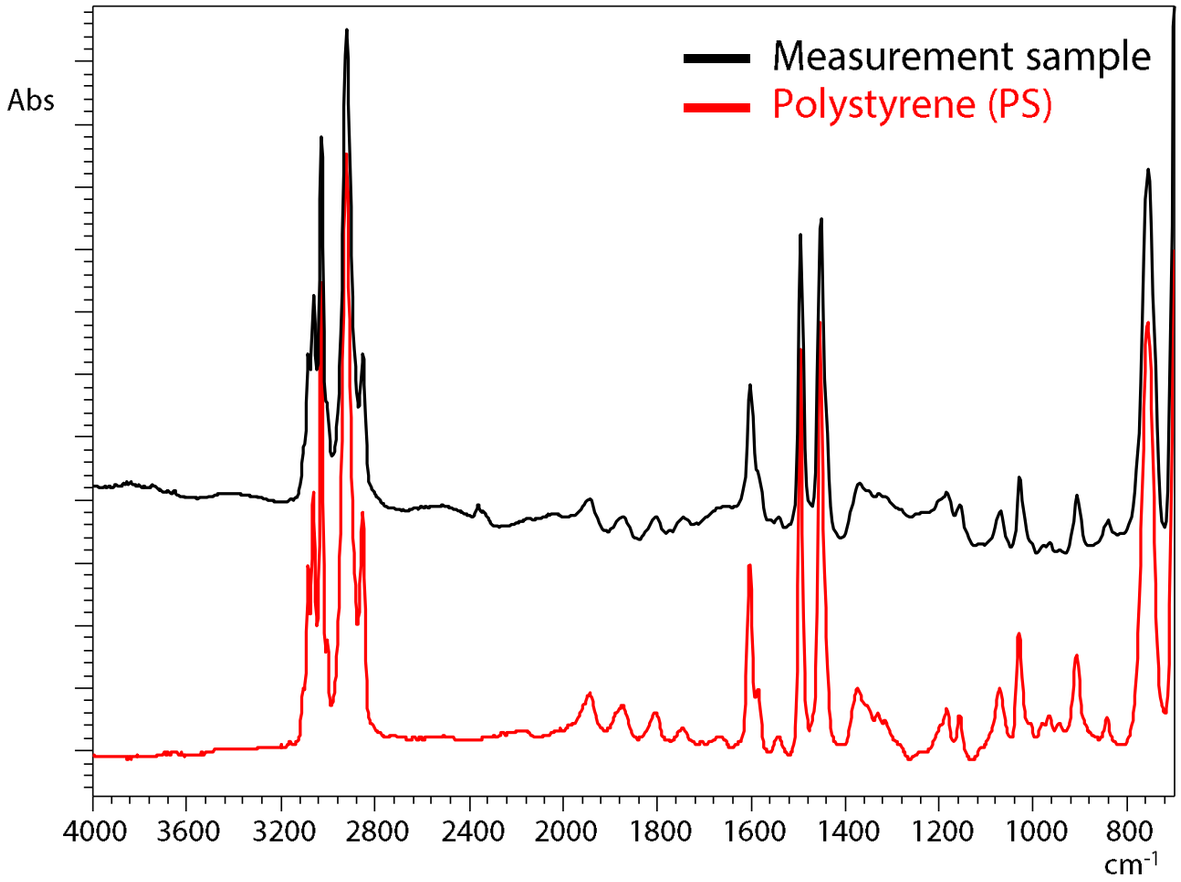
# Microplastics > 212 µm sieve fractions can be analyzed with a stereo scope and bench top IR




- The identified plastics are counted, and total particles are calculated based on size of the filtered aliquot
- Spectra are collected per particle and compared to database.

Microplastics Contamination in a High Population Density Area of the Chao Phraya River, Bangkok
June 2020Journal of Engineering and Technological Sciences 52(4):534-545
DOI:10.5614/j.eng.technol.sci.2020.52.4.6

# Identify the various plastics by comparing spectrum to a library




- Generate a spectrum of each of the above selected particles.
- Use a commercial or laboratory generated library to compare spectrum with reference spectrum. Positive identification is made with a ≥60 % match.
- Less than 60 % may mean interferences are present.

# Data from each technique can be used to fully characterize the plastics in the sample

- **D8332** samples large volumes and “pre-sorts” into various sieve fractions
- **D8333** removes interferences and maintains “pre-sorted” fractions
- Each digested fraction analyzed by pipetting aliquots
  - **D8489** rapidly count and obtains size distribution for smaller particles. Morphology can be correlated to optical and IR analysis.
  - **D8401** rapidly determines up to 12 microplastics, including rubber, and can be calculated as µg (mass).
  - IR Microscopy automates IR identification and counting of the smaller particles. Shapes and size distribution information is collected.
  - Optical characterization followed by manual IR Spectroscopy counts, sorts, and identifies the larger plastic particles.
  - The optical methods (including IR Microscopy) can estimate volume of the particles. With volume and identity, the mass of the particles can be estimated.
  - Mass from the IR and Microscopy data can be compared to mass obtained by pyrolysis GCMS

# Questions?

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