

The Challenge of Matrix Interference in Quantitative Analysis of PM_{2.5} Microplastics Using Pyrolysis–Gas Chromatography–Mass Spectrometry

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Abstract

We evaluated the effect of ammonium sulfate, a major component of airborne particulate matter, in the quantification of airborne micro- and nanoplastics (AMNPs) by analytical pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS). Analytical pyrolysis has shown promising potential in providing mass-based information on AMNPs, which are compatible with established standard protocols to monitor airborne particulate matter. Py-GC/MS can be performed with little to no sample preparation, minimizing the risk of polymer loss or sample contamination. However, reactive components of particulate matter, such as inorganic salts, can interfere with the Py-GC/MS measurement of polymers, leading to over/underestimation of the polymer content and instrument contamination. In this study, we have shown that ammonium sulfate can generate matrix interference in the quantification of AMNPs in PM_{2.5}. We have provided a solution to this issue based on water rinsing of the particulate matter directly inside the pyrolysis crucible, avoiding sample loss and preventing instrument contamination.

Keywords: particulate matter; microplastics; analytical pyrolysis; mass spectrometry; matrix interferences

1. Introduction

Humans have long been exposed to airborne particles such as soot, smoke, tire wear particles, dust, dander, pollen, NO_x and SO_x condensation droplets, carbon black, and other aerosols visible to the naked eye or perceived through the refraction of sunlight. References to particles in the air and the inconveniences of poor air quality in densely populated areas can be found throughout human history, from Seneca's Letters in the 1st century AD to the 17th century's pamphlet *Fumifugium* on the quality of London's air [1–4]. The legislation and regulation of particulate matter air pollution came into greater effect in the mid-1900s. Health concerns about 10 µm and 2.5 µm particulates have catalyzed implementation of >50,000 monitoring stations worldwide [5,6]. Fowler and co-workers provide a good chronology of the global air quality [7].

The recent worldwide media impact of microplastic pollution brought an interest to particulate matter (PM) that has been in the air since the advent of synthetic



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polymers—airborne micro- and nanoplastics (AMNPs). AMNPs are estimated to constitute between 0.1% and 10% of the total suspended PM [8–10], with higher concentrations in indoor environments compared to outdoor environments [11,12]. The main sources of AMNPs are the degradation of textiles and packaging, the wear of vehicle tires, and emissions from industrial activity [8,11,13,14]. AMNPs in PM₁₀ and PM_{2.5} particulate size fractions are of great interest because of their ability to be ingested into the body. While in vitro and animal studies have demonstrated oxidative stress, inflammation, and metabolic disturbances, the health impacts on humans remain under investigation. It is yet to be determined whether these effects result from microplastics themselves or from chemicals associated with the microplastics [15–28].

A search of journals using Mendeley and Scopus has shown an exponential increase in articles about micro- and nanoplastics in the air. Most of the available studies used spectroscopic methods, such as Raman or Fourier transform infrared (FT-IR), to provide particle numbers and size distribution. Only recently has pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) been used to quantify AMNPs [29–39]. Although complementary to other spectroscopic methods, Py-GC/MS can provide mass information, making it more compatible with already established methods to monitor air PM [40,41]; size fractionation during sampling can be used to obtain mass information for dimensional ranges of high interest, such as PM₁₀ and PM_{2.5} [37,42]. In addition, newly released forced splitless pyrolysis technology has enabled Py-GC/MS measurement of trace microplastics in PM filters without the extraction and concentration steps [43].

Minimizing sample pretreatment is generally advisable to reduce the risk of polymer loss or sample contamination. However, this can increase the risk of matrix interference. Such risk is especially relevant in environmental monitoring campaigns, as the sequential analysis of numerous samples can lead to severe instrumental issues with a loss in the reliability of the results [44]. Inorganic species have been known to act as catalyzers, altering the pyrolysis mechanism of polymers and, therefore, the instrumental response provided by Py-GC/MS [45]. The pyrolysis mechanism can also be altered by intentionally added species that are not components of the matrix, such as internal standards [46].

In this study, we have addressed matrix interference in the quantitative analysis of AMNPs using Py-GC/MS. We focused on the effect of ammonium sulfate on instrumental performances, and we developed a method to remove such interfering species.

2. Materials and Methods

Materials and reagents—Ammonium sulfate (NH₄)₂SO₄ (AS, Hayashi Pure Chemicals Co., Ltd., Osaka, Japan) was used to prepare spiked samples. Water (industrial grade, Monotaro, Hyogo, Japan) was used for rinsing samples. Hexamethyldisilazane (HMDS, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was used as a derivatizing agent. Deuterated chrysene (Chrysene-d₁₂, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was used as an internal standard. Dichloromethane (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was used as a solvent for the internal standard. The Microplastics Calibration Standard with SiO₂ as a diluent (MPs-SiO₂-L, Frontier Laboratories, Koriyama, Japan) was used for the polymer recovery tests. This standard contained 11 polymers ranging from 4.63 µg/mg (polyethylene) to 0.13 µg/mg (nylon-6), depending on the number of pyrolyzate peaks in their respective fragmentation patterns [47]. Quartz filters (QMA Ø47 mm × 475 µm, Cytiva/Whatman, Buckinghamshire, UK) were used to collect atmospheric samples and to prepare simulated AS-spiked samples. Before use, all quartz filters were conditioned at 650 °C overnight in a KBF794N Koyo muffle furnace (Koyo Thermo Systems, Nara, Japan). After conditioning, the filters were stored in metal containers wrapped in folded aluminum foil and stored in a refrigerator until use. Refriger-

ation was not necessary but it can minimize background contamination caused by thermal cycling of the storage containers. Conditioned quartz fiber filters have often been used by researchers for collecting microplastics, especially during Py-GC/MS analysis [36,37,48,49]. The US-EPA recommends PM sampling with a polytetrafluoroethylene (PTFE) filter [50]. However, PTFE filters can provide a disruptive background signal when analyzed by Py-GC/MS. Sullivan et al. described the complications that arise when using PTFE filters for microplastic analysis via Py-GC/MS [51].

Airborne particulate samples—Sampling followed the standard procedure for collection of particulate matter as specified by the United States Environmental Protection Agency (US-EPA) [50]. PM sampling was performed using a multiple cascade impact (MCI) sampler (Tokyo Dylec Corp., Tokyo, Japan) or with a Virtual Dichotomous Impact (VDI) sampler (Kimoto Electric, Osaka, Japan). The MCI sampled at 20 mL/min for 168 h, providing PM₁₀, PM_{10-2.5}, and PM_{2.5} fractions. The VDI sampled at 16.7 mL/min for 168 h providing PM₁₀ and PM_{2.5} fractions. Although the total volume of the air samples was different between these samplers, the PM_{2.5} results, expressed in µg/m³, were comparable. The samplers, collocated in Osaka, Japan, were operated simultaneously.

Simulated samples—Samples were prepared to optimize the water rinsing process. The preparation of these simulated samples is shown in Figure 1. A 4 mm punch of 1 µm Dutch weave wire mesh (Frontier Laboratories, Koriyama, Japan) was first inserted at the bottom of an 80 µL flow-through pyrolysis cup (PY1-EC80HF, Frontier Laboratories, Koriyama, Japan). Three 4 mm punches were then cut from a blank quartz filter using a Harris Uni-Core punch (QIAGEN, Venlo, the Netherlands) and placed on top of the wire mesh. Spiking was then performed by adding 2 µL of a 30 g/L AS solution in water and evaporating the solvent by heating at 80 °C under a nitrogen atmosphere. The spiked µg amounts of AS ($M_{AS, Py-GC/MS}$) for this study were based on the work of Ma and co-workers [52], who found that this salt can exceed 40% by weight (f_{AS}) of the particulate matter captured in urban air PM_{2.5} filters.

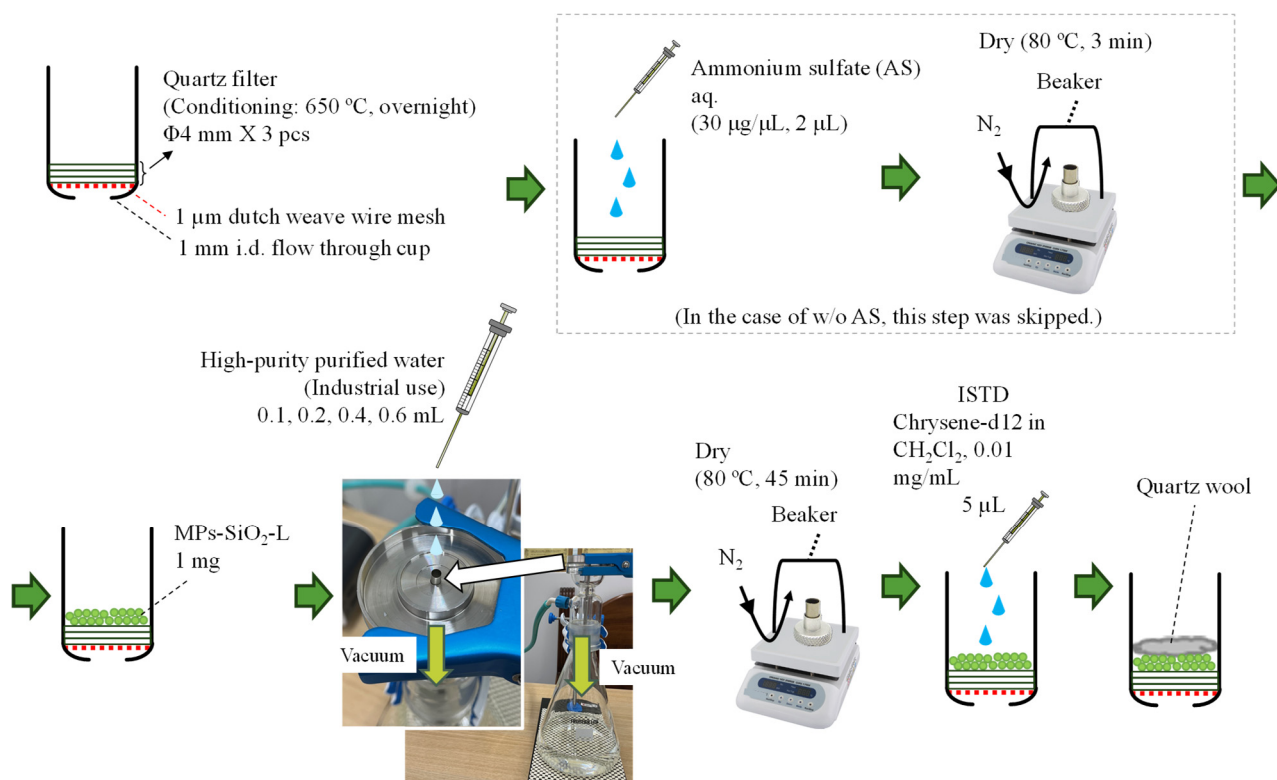


Figure 1. Sample handling steps to test removal of ammonium sulfate and recovery of MPs-SiO₂-L.

Calculations were performed as follows: the average PM concentration ($C_{\text{pm}2.5}$) for the PM_{2.5} filters sampled in this study was 8 µg/m³; 200 m³ of air was sampled (V_{air}) through a filter area of 1735 mm² (A_{filter}); and 3 × 4 mm punches represented 2.2% (A_{punches}) of the filter area.

$$\text{Total PM } (T_{\text{PM}}) = C_{\text{pm}2.5} \times V_{\text{air}} = 8 \text{ µg/m}^3 \times 200 \text{ m}^3 = 1600 \text{ µg}$$

$$M_{\text{AS, filter}} = T_{\text{PM}} \times f_{\text{AS}} = 1600 \text{ µg} \times 0.40 = 640 \text{ µg}$$

$$A_{\text{punches}} = (3 \times (2 \text{ mm})^2 \times \pi) \div (23.5 \text{ mm})^2 \times \pi = 37.7 \text{ mm}^2 \div 1735 \text{ mm}^2 = 0.022$$

$$M_{\text{AS, Py-GC/MS}} = M_{\text{AS, filter}} \times (A_{\text{punches}}) = 640 \text{ µg} \times 0.022 = \sim 14 \text{ µg}$$

The World Health Organization has estimated that many low- and middle-income countries have urban PM_{2.5} averages above 30 µg/m³ [5]. Using these higher PM loading amounts from the WHO suggests >52 µg AS should be added to the pyrolysis vessels to accommodate PM_{2.5} measurements in areas of higher pollution.

$$\text{Total PM } (T_{\text{PM}}) = C_{\text{pm}2.5} \times V_{\text{air}} = 30 \text{ µg/m}^3 \times 200 \text{ m}^3 = 6000 \text{ µg}$$

$$M_{\text{AS, filter}} = T_{\text{PM}} \times f_{\text{AS}} = 6000 \text{ µg} \times 0.40 = 2400 \text{ µg}$$

$$A_{\text{punches}} = (3 \times (2 \text{ mm})^2 \times \pi) \div (23.5 \text{ mm})^2 \times \pi = 37.7 \text{ mm}^2 \div 1735 \text{ mm}^2 = 0.022$$

$$M_{\text{AS, Py-GC/MS}} = M_{\text{AS, filter}} \times (A_{\text{punches}}) = 2400 \text{ µg} \times 0.022 = \sim 53 \text{ µg}$$

After the AS spiking, 1 mg of MPs-SiO₂-L was put into the pyrolysis cups. Water rinsing to remove the AS was then performed by placing the cup on a custom-made steel funnel connected to a vacuum, and known volumes of water were added. After rinsing, the cups were dried again. Finally, 5 µL of a 0.01 mg/mL chrysene-d12 solution in dichloromethane was added as an internal standard, and the solvent was removed by drying at room temperature. Before Py-GC/MS analysis, the cups were plugged with quartz wool to prevent spilling.

Instrumental setup—Data were generated using a Py-GC/MS system equipped with a Multi-Shot Pyrolyzer (EGA/PY-3030D), an Auto-Shot Sampler (AS-2020E), a MicroJet Cryo-Trap (MJT-2030E), a Multi-Functional Splitless Sampler (MFS-2015E), and a Vent-free GC/MS Adapter (Frontier Laboratories, Koriyama, Japan). The MFS enabled splitless pyrolysis as well as backflushing of higher molecular weight pyrolyzates that were not used for identification or quantitation, thus shortening the GC runtime and facilitating a cleaner GC/MS system. Table 1 shows the Py-GC/MS experimental conditions. The GC was equipped with an SMC (smart precolumn) to improve PE pyrolyzate peak shape, and a UAMP Column Kit specifically designed for microplastics analysis (Frontier Laboratories). The initial oven temperature was 40 °C with a hold time of 2 min, after which the oven was first ramped at 20 °C/min to 280 °C and held for 10 min, before being ramped again at 20 °C to reach a final temperature of 300 °C and held for 5 min. F-Search MPs 2.1 software (Frontier Laboratories) was used for the identification and quantification of microplastics.

Table 1. Py-GC/MS conditions.

Furnace temperature	600 °C
Pyrolyzer interface temperature	300 °C
Pyrolysis mode	F-Splitless
Pyrolysis time	0.5 min
Backflush start time	10 min
GC injector temperature	300 °C
Carrier gas	He, constant pressure, 150 kPa
Smart precolumn	UASMC-M20, 2 m × 0.25 mm, deactivated
Precolumn	UA+-50, 1 m × 0.25 mm, film thickness 1.0 µm
Column	UA+-5, 30 m × 0.25 mm, film thickness 0.5 µm

Table 1. Cont.

GC/MS transfer line temperature	300 °C
MS scan range and rate	m/z 29–350, 4 scans/s

3. Results and Discussion

Challenges in airborne microplastics analysis—Inconsistencies in the responses provided by the control samples of MPs-SiO₂-L reference were detected during a continuous 3-week long Py-GC/MS analysis sequence of over 60 real PM_{2.5} filters. These inconsistencies were ascribed to a significant loss of instrumental sensitivity and to poor analyte transfer from the pyrolysis furnace to the GC/MS system. The compromised performances of the Py-GC/MS can be clearly seen in Figure 2, showing a decrease in the signal of bisphenol A (BPA), the pyrolysis marker for polycarbonate (PC), at four different points during the 3-week long analysis sequence. This decrease in instrumental response was ascribed to the presence of condensed matter evolved from the PM_{2.5} filters and deposited in the pyrolysis furnace or the injector, as well as partial degradation of the GC stationary phase functional groups. These deposits constituted active sites that could interact with pyrolysis products of polymers and hinder their GC elution and MS detection. This interaction was especially strong for moderately polar pyrolyzates quantitation targets such as BPA, benzoic acid from polyethylene terephthalate (PET), and ϵ -caprolactam from nylon-6 (N-6). The hypothesis of active site formation was supported by injecting HMDS into the Py-GC/MS system. HMDS derivatized the active sites, making them inert towards eluting pyrolysis products. After HMDS treatment, the BPA's response was restored to 83% of its original value (ion count 17,000 vs. original 20,600), thus illustrating active sites as the cause of a BPA response loss.

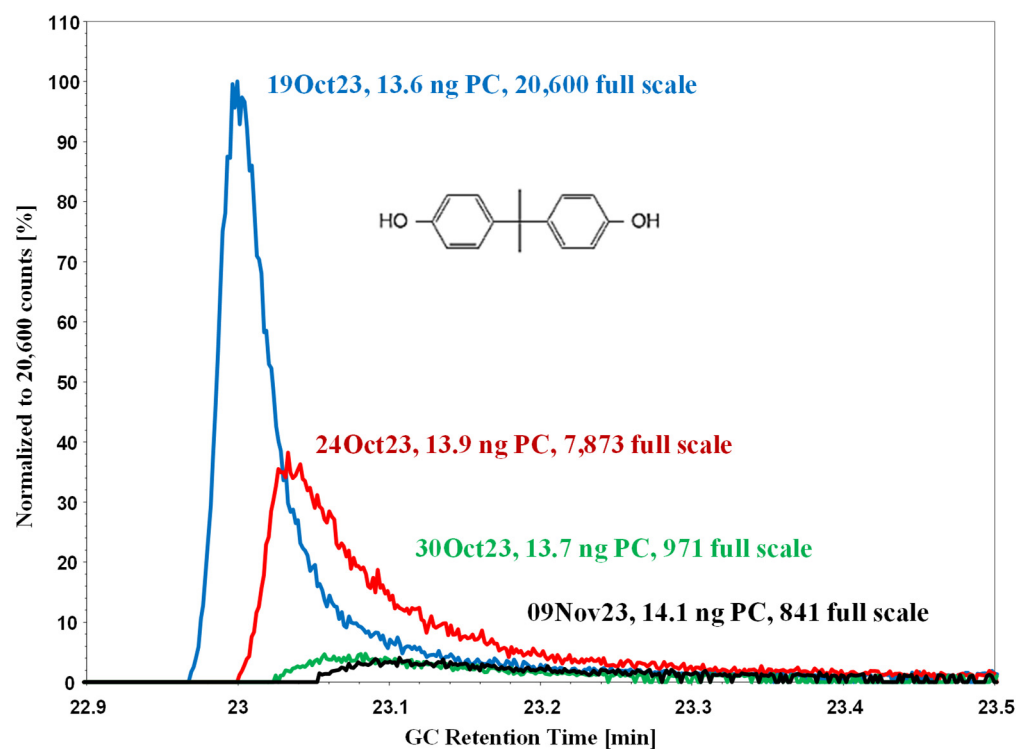


Figure 2. Bisphenol A Py-GC/MS response decay over the analysis of 66 PM_{2.5} filters, as measured by ~300 μ g MPs-SiO₂-L interspersed throughout the sequence. Full-scale ion abundance is 20,600 counts.

While conditioning of the Py-GC/MS system could be used to restore instrumental sensitivity, it can be time-consuming and requires either the use of reagents such as HMDS

or, in the worst cases, turning off the instrument to replace consumable parts. In addition, AS amounts may vary significantly between the PM samples collected in different environments. This could make instrumental contamination unpredictable and indicates the need for constant performance checks, further increasing the time-intensiveness of the workflow. Therefore, although a simple filter-punch-pyrolysis analysis schema would be preferred, the detrimental effects of PM inorganics require the development of an additional sample pretreatment step.

Developing pretreatment—The first step in developing this pretreatment was pinpointing which species in PM could be the source of instrumental contamination. AS, which displays acid-base properties, is a major component of PM. It was therefore investigated as most likely to be the cause for the loss of instrumental performance. Hodan and Barnard elucidated the creation of AS from SO_x and NO_x precursors facilitated by ozone [53]. Figure 3 shows the TIC pyrogram of an MPs-SiO₂-L reference with AS added and without AS added. It is easy to see the baseline interference of the AS early in the pyrogram.

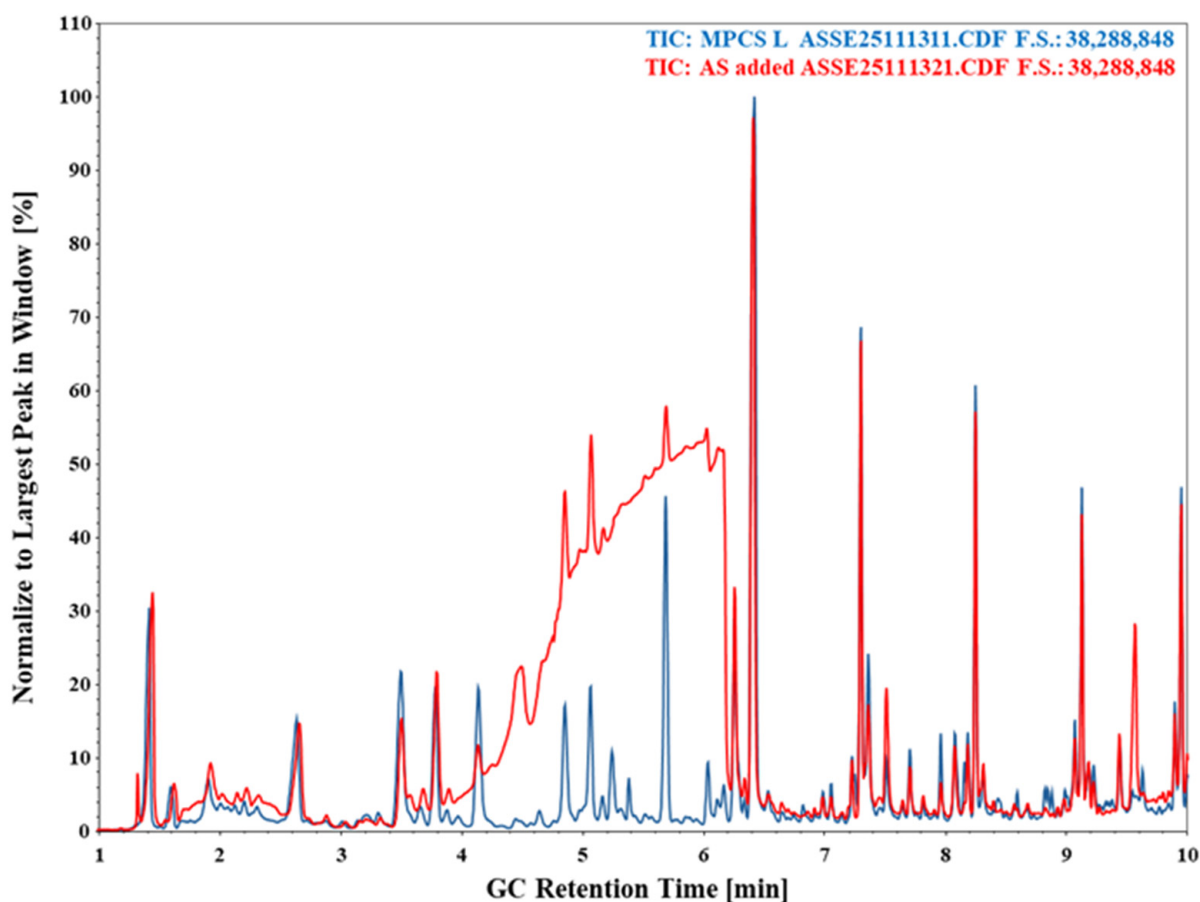


Figure 3. TIC MP's-SiO₂-L pyrograms with AS (red) and without (blue).

AS and other water-soluble inorganics can be easily removed by rinsing the filter punches with water while in the pyrolysis cup. The first rinsing test was carried out using an AS-spiked MP's-SiO₂-L sample with 200 μL of water. This volume was sufficient to return the m/z 64 signal to normal abundance in the pyrogram, as shown in Figure 4. Comparing the total area under the curve for AS before rinsing (red line) and after rinsing (blue line) yielded a 99.9% removal of AS. Note that calculating the % removal of AS with m/z 64 may be underestimated due to the saturation of the detector response.

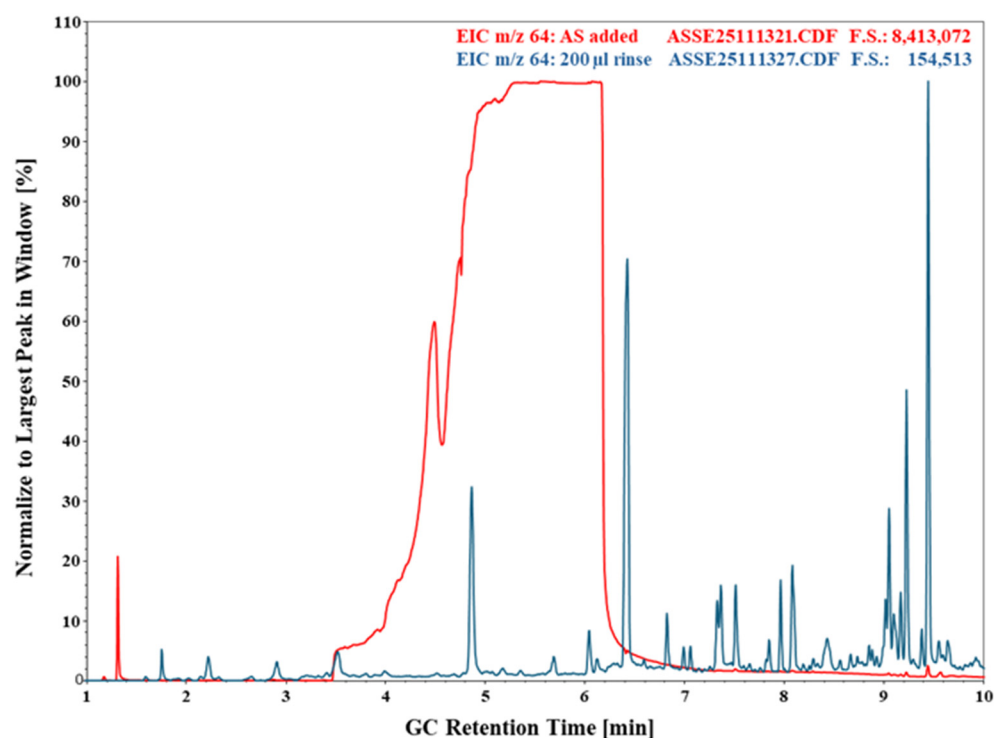


Figure 4. EIC m/z 64 from MPs-SiO₂-L with ammonium sulfate added (red) and after 200 μ L water rinse (blue).

Validation of water rinsing—After assessing its efficacy, the water rinsing step was validated for the polymer loss. MPs-SiO₂-L samples spiked with AS were prepared, and four rinsing volumes (100–600 μ L) were tested. The polymer recoveries after rinsing were assessed by Py-GC/MS, and the results are shown in Table 2. The amount of MPs-SiO₂-L added was 1.01 mg (± 0.03 mg) for each analysis. Values for each polymer were normalized to their MPs-SiO₂-L value of 1 to visualize the effects on the target pyrolyzates more clearly. The addition of AS suppressed the response of the pyrolyzates, with significant suppression of PP, PET, N66, PS, N6, and PMMA. This effect was relevant for polyethylene terephthalate (PET) and nylon-6 (N6), which are susceptible to the presence of inorganic species with acid/base properties such as AS during pyrolysis [45]. Some polyaddition polymers, such as polypropylene (PP), polystyrene (PS), and polymethyl methacrylate (PMMA), were also significantly affected. These results were likely due to a catalytic effect of AS upon pyrolysis, acting on the most reactive sites of these polymers such as the ester bond in PMMA and the tertiary carbon atom in PP. The removal of AS by water rinsing returned most pyrolyzates' response and reproducibility to nominal values.

Table 2. Effect of AS addition and removal on target microplastic pyrolyzates. Values are averaged over two replicates and normalized to 1.

	MPs-SiO ₂ -L	60 μ g AS Added	100 μ L Rinse	200 μ L Rinse	400 μ L Rinse	600 μ L Rinse
PE	1	0.90	1.00	1.01	1.05	1.03
PP	1	0.52	0.95	0.98	1.01	0.97
SBR	1	0.91	0.85	0.74	0.71	0.87
PVC	1	0.99	1.08	0.99	0.96	1.03
ABS	1	0.87	0.95	0.99	1.03	0.98
PET	1	0.58	0.91	0.96	0.98	0.95
N66	1	0.60	0.91	1.11	1.10	1.02
PS	1	0.62	0.83	1.02	1.02	1.01

Table 2. Cont.

	MPs-SiO ₂ -L	60 µg AS Added	100 µL Rinse	200 µL Rinse	400 µL Rinse	600 µL Rinse
N6	1	0.75	0.98	1.20	1.21	1.16
PMMA	1	0.21	0.64	0.67	0.75	0.70

To assess the statistical relevance of these findings, a parallel set of experiments was performed where the same amounts of MPs-SiO₂-L were rinsed with the same water volumes, but no AS was added. Then, one-way repeated measures ANOVA tests were run individually on both the datasets. The tests were run considering each polymer as a separate subject, and the columns in Table 2 show the levels of treatment. Within-polymer tests in the dataset with AS spiking provided an F-value of 4.75 ($p = 0.001$) and an η^2 value of 0.53. On the contrary, within-polymer tests in the dataset without AS spiking provided $F = 0.15$ ($p = 0.961$) and $\eta^2 = 0.02$. These results indicated that the different levels of water rinsing have a significant effect on polymer recoveries when AS is present, but not when AS is absent.

Two anomalies were observed: N6 increased with increasing rinse volume and PMMA did not return to nominal values with the removal of AS. The experimental results excluded the most intuitive explanations for these trends. The hydrolysis of N6 bonds could lead to leaching of monomers/oligomers during water rinsing, which would lower their recovery instead of increasing them. The hydrolysis of the methyl carboxylate moieties in PMMA could lead to methacrylic acid being generated upon pyrolysis, but no peak of this compound was detected in the chromatograms. Oligomers of PMMA could be present in the MPs-SiO₂-L which was then lost during rinsing. However, such a loss was not observed when the samples were rinsed in the absence of AS. Further study into this result is merited.

4. Conclusions

We showed that ammonium sulfate, a major inorganic component of PM_{2.5}, can provide significant signal loss when analyzing airborne micro- and nanoplastics by analytical pyrolysis–gas chromatography–mass spectrometry. Water rinsing of the samples in dedicated pyrolysis crucibles can prevent this signal loss, with satisfactory recoveries for most of the polymers that are commonly found in airborne particulate samples.

This study shows that identifying specific, problematic matrix components and removing them by developing targeted pretreatment strategies is a more promising approach in microplastic analysis by analytical pyrolysis, as compared with untargeted, multi-step pretreatment processes, these might lead to polymer loss or sample contamination.

Future studies should be aimed at improving our knowledge on the nature of the interactions between ammonium sulfate and single polymers, and how these interactions depend on the relative amounts of the two. Exploring other potential components of the particulate matter that could constitute a source of matrix interference, as well as their removal through water rinsing, are also critical aspects that should be further investigated.

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Conflicts of Interest: Author William Pipkin was employed by the company ATRq. Authors Ai Shiono, Makoto Niwa and Atsushi Watanabe were employed by the company Frontier Laboratories Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AMNP	Airborne Micro- and Nanoplastics
PM	Particulate Matter
Py-GC/MS	Pyrolysis–gas chromatography–mass spectrometry
WHO	World Health Organization
US-EPA	United States Environmental Protection Agency
CFR	Code of Federal Regulations
MCI	Multi-stage Cascade Impact Sampler
VDI	Virtual Dichotomous Impact Sampler
PTFE	Polytetrafluoroethylene
MPs-SiO ₂ -L	Microplastics Calibration Standard with Silica Diluent, Low Concentration
AS	Ammonium Sulfate
SMC	Smart Precolumn
UAMP	Ultra-Alloy Microplastics Capillary Column
HMDS	Hexamethyldisilazane
TIC	Total Ion Chromatogram
EIC	Extracted Ion Chromatogram
PE	Polyethylene
PP	Polypropylene
PS	Polystyrene
ABS	Acrylonitrile–Butadiene–Styrene Copolymer
SBR	Styrene–Butadiene Rubber
PMMA	Polymethyl Methacrylate
PC	Polycarbonate
PVC	Polyvinyl Chloride
PET	Polyethylene Terephthalate
N-6	Nylon–6 (polycaprolactam)
N-66	Nylon–6,6 (poly(hexamethylene adipamide))

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