Quantitative Analysis of Microplastics in Shellfish using Pyrolysis-GC-MS

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Application Note
Environmental

Abstract
This application note demonstrates sample preparation, detection and quantification of six different microplastics in shellfish using Pyrolysis-GC-MS. Sample preparation involves digestion with potassium hydroxide and cryo-milling.

Introduction

Five hundred million tons of plastic was manufactured in 2020 and production keeps ramping upwards. Over 50% of that plastic winds up as environmental pollution [1]. This plastic waste can harbor harmful chemical additives such as phthalates, which have known health implications. Once in the environment, the plastic degrades into particles smaller than 5 mm in size particles, otherwise known as microplastics [2]. Through the food chain, microplastic enters plants and animals, including seafood such as shellfish. Consequently, when we eat, microplastics, along with attached additives, enter our bodies, resulting strong concerns and demands to quantify microplastics in the environment.

Many analytical techniques are capable of quantifying microplastics. Microplastics larger than 20 μm can be analyzed by Fourier transform infrared spectroscopy and Raman spectroscopy on a count-based method, but it is impossible to analyze microplastics smaller than 20 μm by these optical methods. On the other hand, pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) can be an effective solution. Py-GC-MS has no sample-size limit, and it can detect multiple microplastics in a single analysis under 40 minutes, as opposed to days by optical methods. One disadvantage with Py-GC-MS is the loss of microplastic particle size information. Previous work has established that alkaline digestion combined with cryo-milling for Py-GC-MS-MS [3]. In this application note, the established work was inherited for Py-GC-MS, and the cryo-milling was further optimized with a cryo-mill from CDS Analytical.

Experiment Setup

Precautions were taken to minimize contamination during sampling and laboratory sample preparation including using only glass and metal vessels and washing sample handling tools three times with water and ethanol. Analysts wore lab coats made of 100% cotton and performed operations under fume hoods to minimize contamination from microplastics in the air. All solvents (water, ethanol, and potassium hydroxide solution) were pre-filtered on a 0.45 μm PTFE membrane (Sigma-Aldrich P/N JHWP04700), and DISC tubes (CDS Analytical P/N 6201-3004) were pre-cleaned at 1000°C for 30 seconds using the “Clean” function of the Pyroprobe, while holding the top flap of the chamber open.

Four types of shellfish (oysters, Stimpson’s surf clams, Asian clams, and scallops) were purchased, de-shelled, and 1 gram of each sample type were transferred to individual 30 mL glass flasks with glass stoppers for alkaline digestion. Eighty milliliters of a 10% solution potassium hydroxide was added to each flask and then digested at 40°C for 24 hours on a shaker incubator with continuous agitation of 500 rpm. After digestion, the sample was filtered onto glass fiber filters (Whatman® Sigma-Aldrich P/N WHA1825047) under vacuum.

Filters containing particles from alkaline digestion were then ground with a CDS cryo-mill (CDS Analytical P/N 6204-3023). Each filter was placed into the 5 mL grinding vessel of the cryo-mill with a 9.6 mm grinding ball and capped. Next, each vessel was immersed in liquid nitrogen for 5 min, then removed and ground

in the mill for 40s at a 65Hz vibration frequency for a total of 8 repetitions. A 2 mg ground subsample was added to a pre-cleaned DISC tube for pyrolysis.

One microliter of 1% Polystyrene (PS) emulsion of 100 μm particle size from Huge Biotechnology was added to 3 of the samples before sample preparation, introducing 10 μg of PS to study recovery rates.

For the standard calibration, 1 mg each of Polyethylene (PE), polymethyl methacrylate (PMMA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS) and polypropylene (PP), were weighed and added to cryo-mill vessels with 0.15 g of diatomaceous earth and ground as previously described to obtain a 6.67 μg/mg concentration of microplastics as a stock standard. Then, 0.66 mg, 0.97 mg, 1.48 mg, 1.97 mg and 2.44 mg, corresponding to polymer masses of 4.4 μg, 6.47 μg, 9.87 μg, 13.13 μg, and 16.27 μg were placed in pre-cleaned DISC tubes and analyzed for a total of 5 calibration levels.

Pyroprobe 6150

DISC:	700°C 40s
Interface:	300°C
Valve Oven:	300°C
Transfer Line:	325°C

GC-MS

Column:	5% phenyl (30m x 0.25mm)
Carrier:	Helium, 50:1 split
Column Flow:	1.00mL/min
Injector:	320°C
Oven:	40°C for 2 minutes
	10°C/min to 100°C
	50°C/min to 300 °C (3min)
Mass Range:	35-600 amu

Results and Discussion

To tackle the difficulty of weighing insoluble polymers at a microgram level, diatomaceous earth (silica) and homogenization by cryo-mill were used to obtain a diluted standard powder, which could be more easily weighed. A control study performed on 2 mg of the diatomaceous earth yielded a clean blank.

As each microplastic component has the possibility of pyrolyzing into hundreds of pyrolysates, a sample containing many microplastics can create complex data. Therefore, to distinguish microplastics from each other, indicator compounds were chosen to both identify and quantify each microplastic (Table 1).

Table 1. Indicator Peaks for 6 microplastics

Microplastic	Indicator Compound	RT	Quant Ion
PMMA	Methyl Methacrylate	3.82	100
PS	Styrene	6.92	104
PP	2,4-Dimethylhept-1-ene	5.99	70
PVC	Naphthalene	10.25	128
PE	1-Undecene	9.95	55
PET	Biphenyl	11.09	154

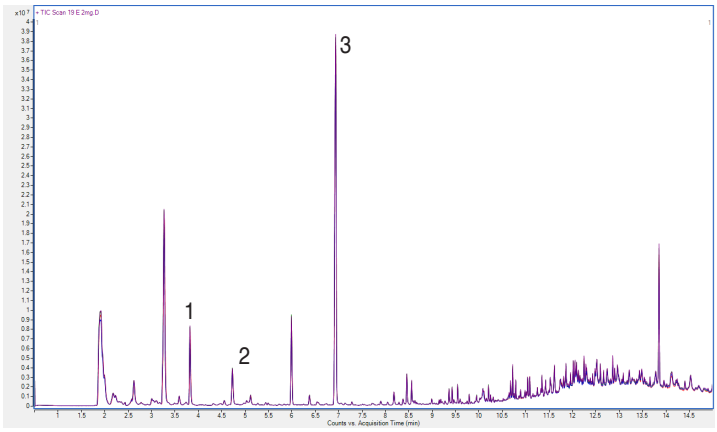
Calibration was performed by plotting polymer weight against quant ion area counts. Each of the six microplastic standards presented a linear calibration with an R^2>0.97 (Table 2). A replicate study on the 13.13 μg level provided RSDs around or under 4% (Table 3). The replicate chromatograms are overlayed in Figure 1. Additionally, the spiked recovery of PS was between 82~85% (Table 4).

Table 2. Calibration Curve Linearity

Polymer	R^2
PMMA	0.999
PS	0.992
PP	0.970
PVC	0.982
PE	0.985
PET	0.987

Table 3. RSD Cryo-grinding Replicates

Polymer	Replicate Area Counts				
	1	2	3	4	RSD
PMMA	930897	968001	887338	970507	4.15%
PS	207426	214896	203695	213600	2.51%
PP	208176	224057	204323	222725	4.67%
PVC	242205	246945	241199	257526	3.02%
PE	87323	83657	87368	89454	2.77%
PET	723135	686987	667420	695292	3.34%



Peak	Identification
1	Methyl methacrylate
2	2,4-Dimethylhept-1-ene
3	Styrene

Figure 1. Overlay of 4 replicate standards

Table 4. PS spiked recovery results

	% Recovery	% Recovery Average
1	85	
2	84	83.7%
3	82	

Quantification of the microplastics in the shellfish was performed by applying the linear equations generated from each calibration plot. It was found that the shellfish contained PE and PP, exceeding 80% of the total microplastic amount, with ranges from 1 μg/g to 15 μg/g (Figure 2). There is a correlation between the amount of plastic in marine organisms and feeding patterns, marine habitats,

or nutritional status[4]. Whether microplastics are transferred from the digestive system to tissues or blood, and whether microplastics only briefly stay in the organism, the mechanism of ingestion, or excretion of plastic particles remains unclear. The main terrestrial microplastic contamination comes from PP and PE, the latter of which usually exceeds 80% of the total microplastic amount. In addition, PE has been reported to dominate microplastic found in marine samples, with an average composition of 42%[5].

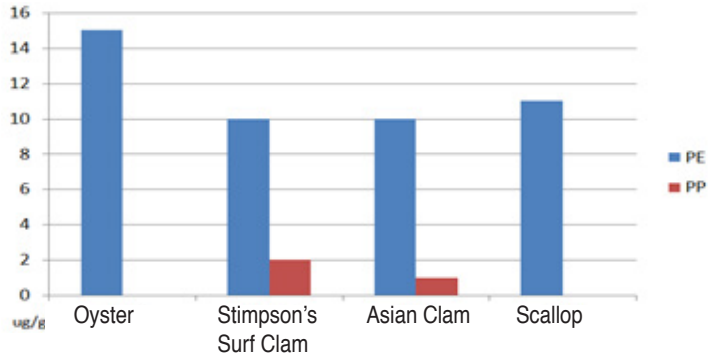


Figure 2. Sample test results

Conclusion

Quantification of microplastics in shellfish was accomplished by sample preparation involving a solid diluent and cryo-milling, followed by pyrolysis-GC-MS. Linear calibrations of R^2>0.97, an RSD <5%, and an average recovery rate of 84% was observed with 6 different microplastic standards. The sampled shellfish contained 10~15 μg/g of PE, and 0~2 μg/g of PP.

References

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