

**Sample preparation**

## Extraction and analysis of plastics in rice samples using accelerated solvent extraction and pyrolysis-gas chromatography-mass spectrometry

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**Keywords**

Accelerated solvent extraction (ASE),  
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Extractor, microplastics, nanoplastics

**Goal**

To describe a new method for the extraction and analysis of microplastics using accelerated solvent extraction

**Introduction**

Increased production and use of plastics have resulted in growth in the amount of plastic debris accumulating in the environment. This plastic debris can potentially fragment into smaller pieces, with particles <5 mm and <0.1 µm defined as microplastics (MPs) and nanoplastics (NPs), respectively.<sup>1</sup> Over the past decades, an increasing number of studies have reported the occurrence of MPs/NPs in the aquatic and terrestrial environments, including oceans, rivers, lakes, air, soil, and dust.<sup>2-6</sup> Most of the previously reported studies have typically relied on visual inspection and spectroscopic imaging approaches, reporting data on the size, shape, color, number, and polymer type of particles. These measurements may not reflect the total mass concentration of polymers in samples because the approaches are typically limited by size. The number of studies now reporting mass-based concentrations of common plastics is growing, as these methods can report the total plastic content of samples, including plastics in both the

MPs and NPs size ranges. This is particularly important for assessing plastic contamination of food, because the extent of human exposure is relatively unknown, particularly exposure through the diet.<sup>7</sup>

The present study uses the extraction and analysis methods previously reported by Okoffo et al.<sup>8</sup> using a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor (ASE system) coupled with pyrolysis gas chromatography/mass spectrometry (Pyr-GC/MS) to extract and quantify the six (6) most common polymers from rice food samples. Extraction and quantification of the polymers polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), polycarbonate (PC), and polyvinyl chloride (PVC) were validated using matrix-spiked samples of store-bought rice, and a selection of common rice products from major supermarket chains was assessed for total plastic content.

Specifically, this application note describes the suitability of using the Dionex ASE 350 system to efficiently and quantitatively extract MPs/NPs to allow their measurement by Pyr-GC/MS to determine MPs/NPs contamination in consumer rice products, as previously reported.<sup>9</sup> The advantage of this methodology is that it is particle size independent and provides concentrations as a total mass of plastic per sample.

## Experimental

### Accelerated solvent extraction of MPs/NPs

Rice samples were subjected to ASE extraction using a validated method previously reported.<sup>8</sup> Samples were freeze-dried and milled to fine powder with a commercial grinder (Extech equipment Pty. Ltd., Victoria, Australia) for 30 min.<sup>8</sup> Following this, 1.0 g of the homogenized sample was transferred into a pre-cleaned 10 mL stainless steel ASE extraction cell with high temperature Viton™ O-rings (Thermo Scientific™ Dionex™ ASE™ extraction cell O-Ring, P/N 056325) and glass fiber filters (Thermo Scientific™ Dionex™ 100/150/200/300/350 extraction cell filters, P/N 068092) in the endcaps and cell base, and the void volume filled with Hydromatrix™ (inert diatomaceous earth sorbent, pre-cleaned with DCM). The cells were then spiked with 40 µg of deuterated polystyrene (d<sub>5</sub>-PS) internal standard and extracted for MPs/NPs on a Dionex ASE 350 system using the parameters listed in Table 1. Samples were extracted with dichloromethane (DCM) at 180 °C and 1,500 psi with a static time of 5 min using two extraction cycles (Table 1). Immediately after extraction, 80 µL of the extract were transferred into a pyrolysis cup, evaporated for 30 min at room temperature in a fume hood, and loaded onto an autosampler for Pyr-GC/MS analysis. It was previously shown that the six polymers investigated remained sufficiently dissolved for over 2.5 hours post extraction<sup>8,10</sup> thus allowing sufficient time to aliquot into the pyrolysis cups for analysis.

**Table 1. Accelerated solvent extraction (Dionex ASE 350 system) settings**

Parameter	Extraction parameters
Cell type	Stainless steel
Extraction solvent	Dichloromethane (DCM)
Extraction/Oven temperature (°C)	180
Static time (s)	5
Cycles	2
Rinse volume (%)	80
Purge time (s)	75
System rinse volume (mL)	9
Heating time (min)	9
Pressure (psi)	1,500

## Pyr-GC/MS parameters

The extracts were analyzed for MPs/NPs using the double-shot component of a multi-shot micro-furnace pyrolyzer equipped with an auto-shot sampler and coupled to a GC/MS single quad system.<sup>8,9</sup> The first pyrolysis shot (ramped from 100 to 300 °C) of the double-shot method was used as a clean-up step to thermally desorb/remove the potentially interfering volatile and semi-volatile organic materials co-extracted from the rice samples. The second pyrolysis shot (at 650 °C) was used to quantitatively measure MPs/NPs identified in samples as previously described.<sup>8,9</sup> To improve selectivity in the quantification of PET, a second aliquot of the extracted sample was derivatized by adding 10 µL of tetramethylammonium hydroxide (TMAH; 25%

in methanol, Sigma-Aldrich) to an additional aliquot of the sample in a pyrolysis cup, prior to analysis as previously described.<sup>11</sup>

## Recoveries of spiked MPs/NPs in rice samples

Recoveries of target MPs/NPs from rice were assessed through extraction of basmati rice samples (1 g each) spiked with 2 mg/g each of the six plastics (equating to 5 µg/injection). The spiked rice samples were ASE extracted as described above. Three replicates of spiked rice samples were prepared and analyzed to identify the error in the quantification. In addition, three rice samples without spiking were analyzed as blank controls to determine the background of the spiked polymers. Acceptable ideal recoveries of the target MPs/NPs in rice samples were between 81% and 130% (Table 4).

**Table 2. Conditions for pyrolysis-GC/MS measurements**

Parameter	Setting
<b>Micro-furnace Multi-Shot Pyrolyzer™ (Double-Shot analysis) EGA/PY-3030D (Frontier Lab)</b>	
First-shot furnace temperature (thermal desorption)	Ramped from 100 °C → 20 °C/min → 300 °C (1 min)
Second-shot furnace temperature (pyrolysis)	650 °C
Interface temperature	320 °C
Pyrolysis time	0.20 min (12 s)
<b>GC</b>	
Column	Ultra Alloy™ 5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Frontier Lab)
Injector port temperature	300 °C
Column oven temperature program	40 °C (2 min) → (20 °C/min) → 320 °C (14 min)
Injector mode	Split/splitless (split 50:1)
Carrier gas	Helium, 1.0 mL/min, constant linear velocity
<b>MS</b>	
Ion source temperature	250 °C
Ionization energy	Electron ionization (EI); 70 eV
Scan range	40 to 600 <i>m/z</i>

**Table 3. List of target MPs/NPs and their respective specific ions used for quantification**

Plastic type	Pyrolysis product	Indicator ions ( <i>m/z</i> )	Molecular ion ( <i>m/z</i> )	LOQ (µg/g)
PP	2,4-dimethyl-1-heptene	70, 83, <b>126</b>	126	1.25
PS	5-hexene-1,3,5-triyltribenzene (styrene trimer)	<b>91</b> , 117, 194, 312	312	0.94
PET	Dimethyl terephthalate* Vinyl benzoate	194, <b>163</b> <b>105</b> , 77, 148, 51	194 148	2.86
PC	Bisphenol A (BA)	<b>213</b> , 119, 91, 165, 228	242	1.73
PE	n-alkene (C <sub>10</sub> , C <sub>12</sub> , C <sub>14</sub> )	<b>83</b> , 111, 140	140, 196	3.95
PVC	Naphthalene	<b>128</b> , 132, 146, 116, 102	128	3.97
<b>Internal standard</b>				
Polystyrene-d <sub>5</sub>	Styrene monomer	<b>109</b> , 82, 54, 107, 108		

\*Only after TMAH treatment

**Table 4. Summary of recovery (%) results**

	PE	PP	PET (VB)	PET*	PS	PVC	PC
Spike 1	95	96	99	91	86	80	128
Spike 2	75	75	97	66	96	73	131
Spike 3	86	78	97	87	90	93	132
<b>Avg</b>	<b>85</b>	<b>83</b>	<b>97</b>	<b>81</b>	<b>90</b>	<b>82</b>	<b>130</b>
<b>St Dev</b>	<b>10</b>	<b>11</b>	<b>1</b>	<b>13</b>	<b>5</b>	<b>10</b>	<b>2</b>

\*After TMAH treatment, VB: quantification using vinyl benzoate

**Table 5. The concentrations of MPs/NPs detected among the different rice treatments and samples**

Sample	Concentration of polyethylene (µg/g dw)				Concentration of polypropylene (µg/g dw)				Concentration of polyethylene terephthalate (µg/g dw)				Overall (µg/g dw)				
	Not shaken		Shaken		Not shaken		Shaken		Not shaken		Shaken		Not shaken		Shaken		
	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	Not washed	Washed	
1	317	143	207	166	3	3	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	320	146	217	166
2	314	132	260	209	3	<LOD	3	<LOD	4	<LOD	8	17	322	132	272	226	
3	64	47	55	51	105	<LOD	<LOD	3	<LOD	<LOD	<LOD	<LOD	<LOD	168	47	55	54
4	94	51	56	51	14	10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	108	61	56	51	
5	60	45	56	45	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	64	45	56	45	
6	57	48	67	53	3	<LOD	7	<LOD	<LOD	<LOD	<LOD	<LOD	61	48	74	53	
7	55	58	55	54	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	59	58	55	54	
8	60	46	57	46	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	63	46	57	46	
9	60	54	57	54	3	<LOD	3	<LOD	<LOD	<LOD	<LOD	<LOD	63	54	61	54	
10	63	53	54	50	0	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	63	56	54	50	
11	79	51	47	45	3	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	82	54	47	45	
12	68	52	63	56	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	71	52	63	56	
13	51	51	48	60	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	54	51	48	60	

dw = dry weight

### Plastic contamination in commercial rice

Rice samples purchased from a supermarket chain in southeast Queensland, Australia, were extracted using the above method for the target MPs/NPs and in previously published work.<sup>9</sup> Different processing conditions were also tested, including shaking the rice in the package in which it was bought (simulating rough transport) or comparing washing the rice and not washing the rice before sample processing. All rice samples contained MPs/NPs (Table 5). Polyethylene was detected in all the samples at concentrations ranging from 45 to 317 µg/g dw. 40% of samples contained polypropylene at a maximum concentration of 105 µg/g dw. Polyethylene terephthalate was quantifiable in 6% of samples, with the highest concentration of 17 µg/g dw. Polycarbonate, polystyrene, and polyvinyl chloride were not detected in any sample. The predominant polymer by mass was polyethylene (95%), followed by polypropylene (4%) and polyethylene terephthalate (1%). Plastic concentrations in rice samples were differentiated by whether the type of rice was washed/unwashed and shaken/not shaken.

### Conclusion

This application note demonstrates that accelerated solvent extraction can extract a variety of MPs/NPs including PS, PP, PE, PP, PC, and PVC from rice samples. Combined with Pyr-GC/MS, the Dionex ASE 350 system provides an improved alternative for the identification and mass quantification of MPs/NPs in rice samples. This reduces processing and labor time needed to pre-treat and isolate MP/NP particles from samples before identification or quantification. The approach described here was applied to rice samples that were found to contain between 17 and 317 µg/g dw of MPs/NPs and has been applied elsewhere.<sup>7,8,10-15</sup> Accelerated solvent extraction shows high potential for effective extraction of the ever-growing list of MPs/NPs from a wide range of environmental samples. It should be noted that the method as presented in this application note is applicable for samples with low (<3%) lipid content when quantifying PE. However, an ASE extraction and analysis of PE in samples with medium to high lipid content (>3%) is optimized elsewhere.<sup>7</sup>

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