Released: March 2022

Application Note 161

Addressing multiple challenges of microplastics analysis using versatile TD–GC–MS – Evaluation of salt samples

Summary

Volatile organic compounds (VOCs) from polymer standards were analysed to establish marker compounds for microplastics analysis using direct thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS). The marker compounds enabled quantitation of multiple types of microplastics in a variety of salt samples. Sample introduction into the GC–MS was automated using the TD100-xr™ thermal desorber, which increases the throughput of large sample volumes.

Introduction

Over the last 100 years, plastics have transformed our way of life, but there has been no major push to study the long-term effects of plastic on the environment and on human health until recently. A group of plastics called microplastics – polymer fragments 1 µm to 5 mm in size – can enter the human body from our food and water, and even from the air we breathe. As a result, scientists are focusing on investigating microplastics.

Various analytical techniques are used for the investigation of microplastics. These range from TD/extraction to pyrolysis and spectroscopic identification. These complementary techniques can identify and quantify the plastics present, enabling the composition of samples to be characterised. In addition, TD enables polymeric additives or contaminants that may have become trapped either within or on the surface of the microplastic to be identified.

Here, salt samples, including rock salt and sea salt, were analysed so that comparisons could be made between them. Sea salt is expected to be contaminated with microplastics whereas mined rock salt should be less so. An interesting aspect of sea salt analysis is that testing samples from different geographic locations should allow the distribution of different polymeric pollution sources and types to be mapped out.

Direct TD combined with analysis by GC–MS was applied to the quantitative analysis of microplastic residues in salt samples. Selection and validation of the specific marker compounds for different polymers is described.

Direct thermal desorption – an introduction

In this study, samples extracted onto filters or weighed into empty sample tubes were subjected to a two-stage thermal extraction and concentration process (Figure 1). In the first stage, samples are heated to break down the microplastics, which releases their characteristic marker compounds and generates a distinctive chromatographic profile or ‘fingerprint’ for each type of microplastic. (Note that most common polymers degrade from 300°C upwards.[1]) Alternatively, lower desorption temperatures can be used for selectively extracting and characterising any VOC contaminants adsorbed on the surface of microplastic residues. In either case, the volatile and semi-volatile organic compounds released during sample desorption are swept out of the tube in a flow of inert (carrier) gas and focused on a small, electrically-cooled sorbent trap. When the period of sample desorption is complete (typically 10–15 minutes), the focusing trap is rapidly heated, at rates up to 100°C/sec, in a reverse flow of carrier gas to inject the compounds into the GC capillary column as a sharp, focused band of vapour. Reversing the flow of carrier gas during GC injection is important because it allows the trap to be packed with two or more sorbents thus extending the volatility range of analytes that can be analysed simultaneously. The thermal mass of the trap and the rate of heating are also critical in order to deliver narrow peaks to the head of the GC column thus optimising sensitivity and chromatographic resolution.

The transfer of analytes from the sample tube to the focusing trap and the subsequent injection of focused analytes from the trap into the GC column can be carried out as either split or splitless. Large combined split ratios, for example 100:1 on the way into the trap (by using a minimum flow of 2 mL/min to the focusing trap) and then the same 100:1 split ratio during trap desorption enables the analysis of larger, more representative microplastic samples when required without overloading the analytical system. In the example given, the overall split ratio would be 10,000:1.

Note that all Markes’ TD systems, manual and automated, offer quantitative re-collection of both split flows, allowing repeat analysis for data confirmation. Split re-collection is also used to simplify method validation as recommended in standard methods. Please see Application Note 024[2] and

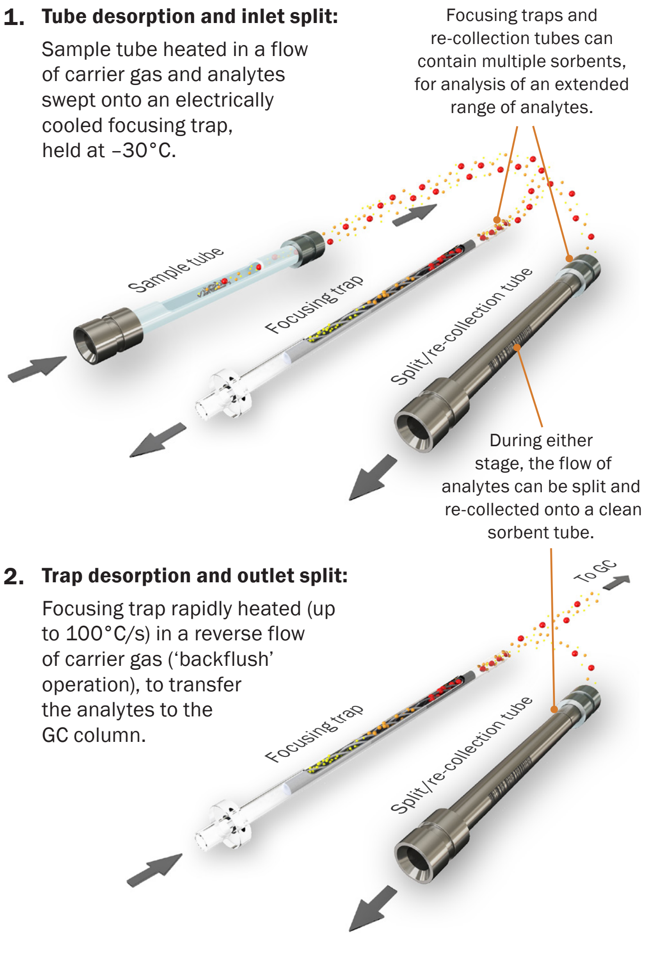

Figure 1: Two-stage thermal desorption process.

Instant Insight 006[3] for more information on this important functionality.

Direct TD offers a number of advantages compared to other GC sample introduction methods. These include:

- **Automation (automated sample introduction):** a TD100-xr has capacity for automation of up to 100 samples or up to 199 samples on an ULTRA-xr Pro™.
- **Representativeness:** TD accommodates a range of sample sizes, allowing meaningful results to be obtained from relatively inhomogeneous samples.
- **Versatility:** TD allows both composition analysis and characterisation of surface contaminants.
- **Sensitivity:** TD enables analysis of trace-level compounds such as contaminants adsorbed to the surface. (The profile for adsorbed VOCs can indicate the source of a sample, the length of time it has been in the environment and/or the type of environment it has been exposed to.)
- **Productivity:** Direct desorption of subsequent samples can begin while GC analysis of the previous sample is ongoing.
- **Enhanced analytical quality control:** in compliance with best laboratory practice, Markes' automated TD systems allow a gas-phase internal standard to be added to the sampling end of the focusing trap just before the start of sample desorption.

Specifically regarding microplastics, direct desorption enables sample sizes from low microgram to mg levels to be analysed at a variety of temperatures and split flows for different purposes of microplastic analysis. In this application note, filtrates from salt samples were analysed.

Sample preparation and analysis

A small aliquot of each salt was weighed onto a quartz microfibre filter, which was then placed inside glass filtration equipment (Figure 2). Microplastic-free Milli-Q™ water was used to dissolve the salt, and the solution passed through the filter to leave the filtrate on the surface. The filtrate was then washed with solvents to remove sample matrix (see Application Note 150 for details[4]).

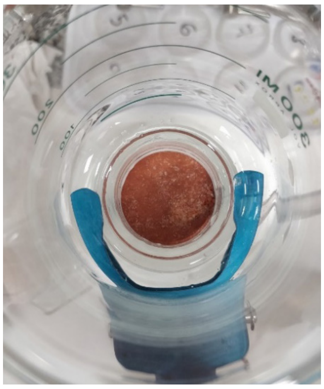

Figure 2: Salt sample dissolved onto filter paper in glass filtration equipment.

For other types of samples, the exact protocol required for extraction of microplastic residues will vary depending on the matrix under study and so this part of the sample preparation process can be modified depending on the type of sample under investigation.

The filters were then dried and transferred to empty TD tubes and either sealed with long term caps ready for storage, or fitted with DiffLok™ caps (Figure 3) for processing by automated TD–GC–MS. After the analytical sequence, the filters were removed from the tubes for disposal so that the tubes could be re-used. Analysis was carried out using the 100-tube TD100-xr thermal desorber (Figure 4) coupled to a GC–MS.

TD:

Instrument:	TD100-xr (Markes International)
Tubes:	Empty stainless-steel tubes (C0-AXXX-0000)
Flow path:	200°C
Pre-purge:	1 min at 20 mL/min
Tube desorption:	320°C (12 min)
Trap flow:	20 mL/min
Split flow:	60 mL/min
Trap purge:	1.0 min at 50 mL/min
Focusing trap:	'Air toxics' (part no. U-T15ATA-2S)

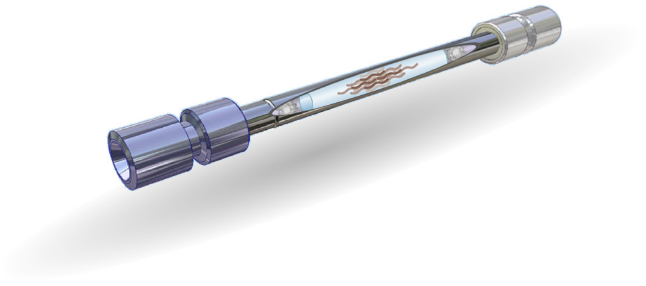

Figure 3: Schematic of a sample tube containing a sample for direct desorption capped with DiffLok™ caps. The caps allow gas to flow when pressure is applied and prevent contaminant ingress and analyte loss before and after automated TD–GC–MS analysis.

Figure 4: Markes' TD100-xr automated thermal desorber (100-tube capacity).

Focusing trap low: -15°C
Focusing trap high: 300°C (3 min)
Trap heat rate: MAX
Outlet split: 30 mL/min

GC:
Column: DB-624™ 30 m × 0.25 mm × 1.4 µm
Carrier gas: Helium, constant flow
Column flow: 1.2 mL/min
Oven: 40°C (2 min), 10°C/min to 60°C, 30°C/min to 250°C (10 min)

MS:
Source: 230°C
Transfer line: 230°C
Quadrupole: 150°C
Scan range: m/z 34–350

Samples

Step 1: Marker compounds and calibration

When the TD–GC–MS methodology was finalised, the polymer standards were directly desorbed and analysed to check the specificity and validity of the marker compounds for the following four polymers:

- Polyethylene terephthalate (PET)
- Polyvinyl chloride (PVC)
- Nylon-6
- Polystyrene (PS)

The polymers were chosen because they are abundant in surface water samples,[5] so are also expected to be found in salt.

The polymers were sourced in pellet form (Sigma Aldrich) and ground into powder using a ceramic ball mill. The powders were weighed onto 0.3-µm quartz microfibre 47-mm filter papers (QF1-047 CHM Lab) using a metal spatula and microbalance before the filters were placed inside empty stainless-steel tubes. The tubes were then capped with DiffLok™ caps ready for analysis.

Step 2: Salt samples

20g of each salt sample were weighed and placed on a quartz microfibre filter. Each sample was dissolved using Milli-Q ultra-pure water and then washed in multiple stages with solvents, including hydrogen peroxide, acetone and methanol, with water washes in between. This procedure was optimised to break down external contamination from the salt, before the filters were dried in an oven for 30 minutes at 100°C.

The dried filters were folded and placed inside empty stainless-steel TD tubes, which were then capped before analysis (Figure 5). Sample preparation takes less than an hour, can be applied to multiple filters at the same time and is used for a wide range of sample types with optimised solvent choices.

Salt samples from five different sources were analysed:

- Laboratory-grade sodium chloride
- Table salt (brand A)
- Table salt (brand B)
- Himalayan rock salt
- Celtic Sea salt

Results and discussion

Step 1: Confirming the marker compounds for polymer identification and quantitation

Each of the four powdered polymer standards thermally degraded during analysis to form characteristic compounds that were significant and specific enough to be used as markers to identify and quantify the presence of the four plastics (Figure 5). The thermal degradation processes are well-known in each case.[6,7]

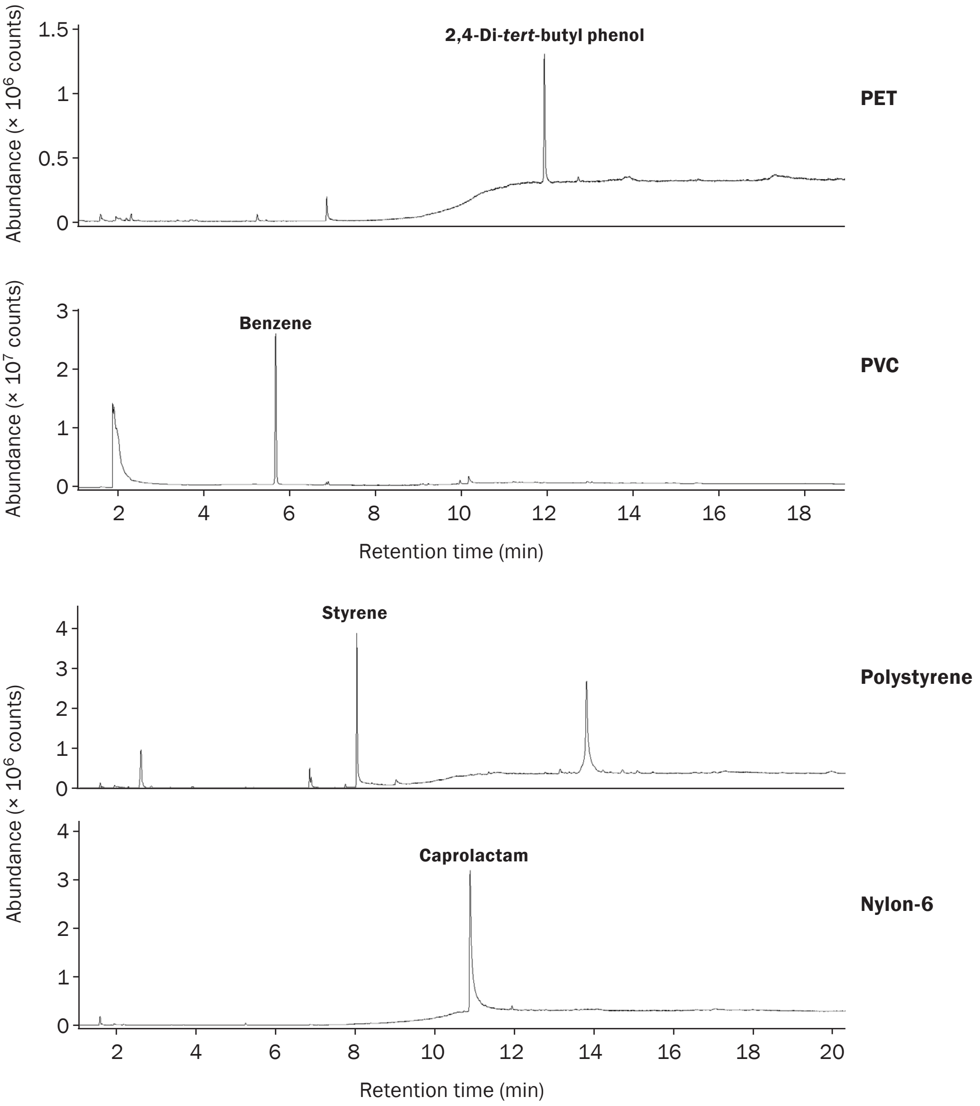

Figure 5: Identification of marker compounds for the four polymer standards displayed.

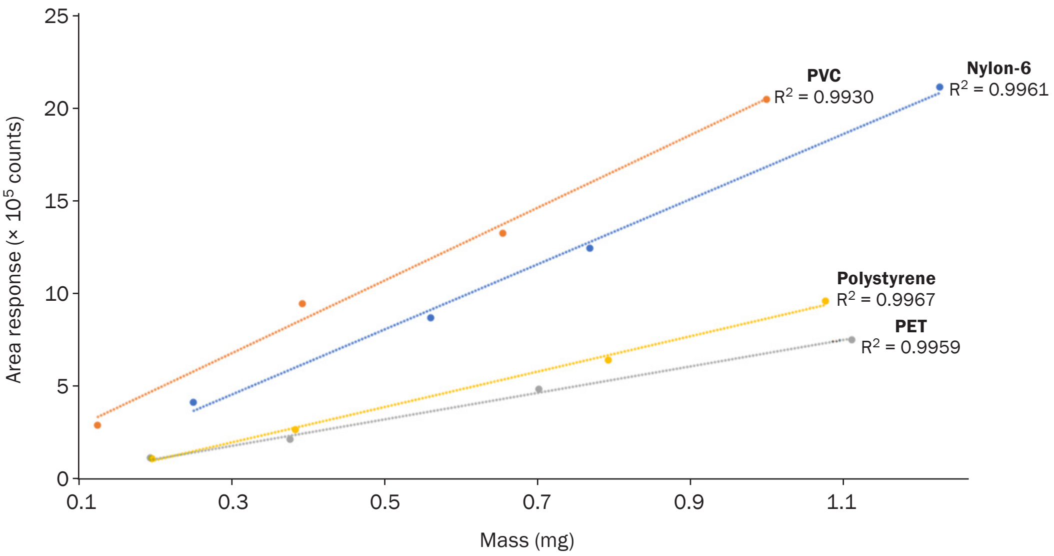

Figure 6: Linearity plots of polymers (0.1 to 1.2 mg) using quantitative marker compounds.

Polymer	Marker compound	Retention time	Linearity	Degradation process
Nylon-6	Caprolactam	10.9	0.9961	Depolymerisation of caprolactam
PET	2,4-DTBP	11.9	0.9959	PET glass transition[8]
Polystyrene	Styrene	8.05	0.9967	Depolymerisation of styrene
PVC	Benzene	5.67	0.9930	Dehydro-chlorination process of PVC

Table 1: Retention time and linearity for each polymer's marker compound and the degradation process.

The value of each marker compound for quantifying the mass of each plastic present was then evaluated by varying the mass of plastic desorbed and checking the linearity of the marker compound response. The results obtained for each polymer marker under these method conditions are illustrated in Figure 6 and presented in Table 1.

Step 2: Applying the method to characterise microplastics present in the salt samples

Five different salt samples and associated blanks were analysed using the method described for comparison.

The resulting chromatograms for three samples – the Celtic Sea salt, Himalayan rock salt and brand A table salt – are shown in Figure 7. The Celtic Sea salt has a busy profile with many peaks at high concentrations. This is due to the increasing amount of pollution in oceans, particularly in areas such as the Celtic Sea, which is close to the English Channel – one of the world's busiest shipping lanes.

The Himalayan rock salt displays a cleaner profile, with fewer peaks at lower concentrations relative to the Celtic Sea salt. Despite being deposited ~150 million years ago, this salt still exhibits some traces of microplastic contamination. During excavation, production, packaging and transport, the Himalayan rock salt is exposed to today's environment and therefore the contamination of microplastics, hence the presence of different polymers within this sample. It is important to point out that mixtures of different marker compounds were observed in most samples analysed. These were easily identified using the method described even within these complex matrixes.

Of all the samples, branded table salt A exhibited the fewest peaks at the lowest concentrations, suggesting that the process of reversed osmosis used in the production of this table salt removes most of the microplastic contamination from the four targeted polymers.

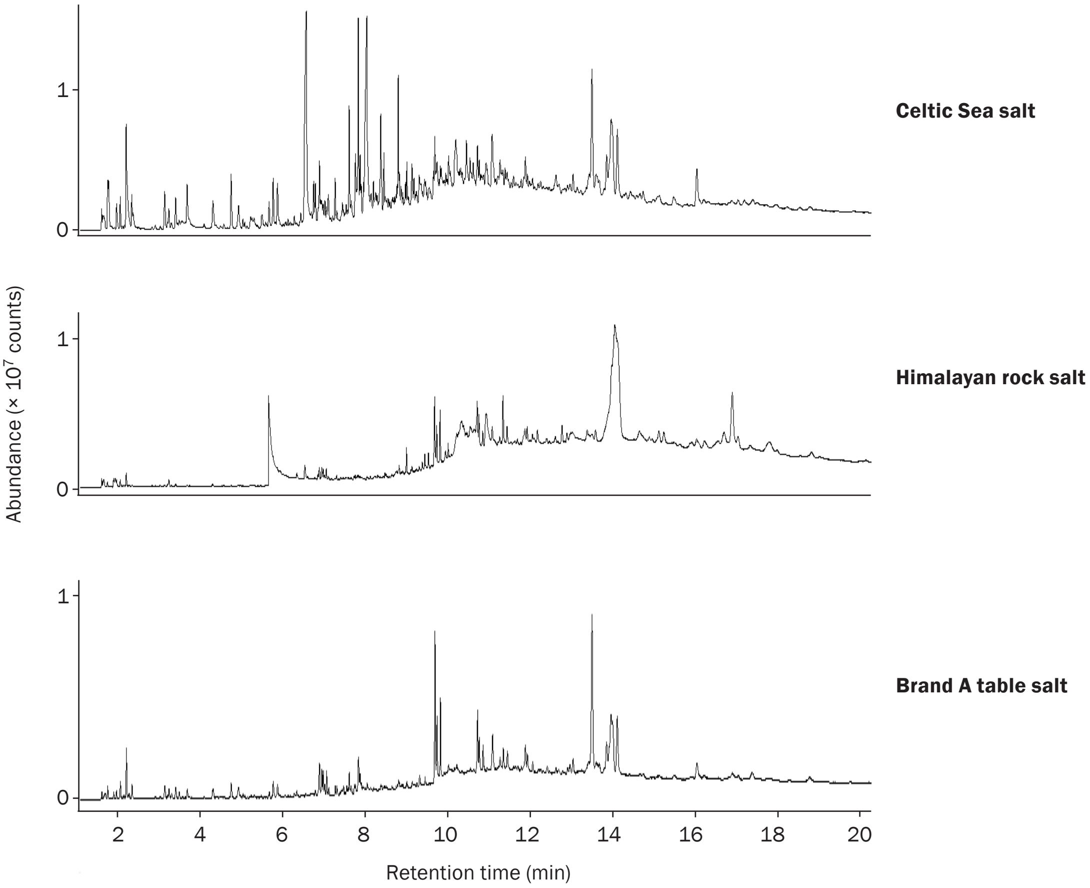

Figure 7: Total ion chromatograms for Celtic Sea salt (top), Himalayan rock salt (middle) and brand A table salt (bottom).

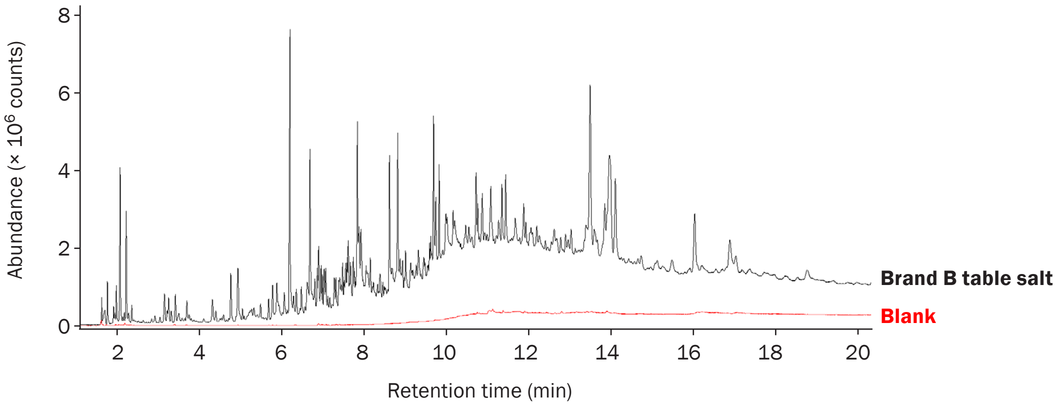

Figure 8: The chromatogram for the table salt sample (black) was compared with a laboratory-grade NaCl sample blank (red), demonstrating that the analytical system and sample preparation process are free from microplastics as well as other forms of contamination.

Further, a table salt chromatogram was compared with that of a blank run with laboratory-grade NaCl (Figure 8). Note that the same filter as that used for sample preparation was used for the blank run. The filter underwent the same extraction and preparation processes (water, solvent, drying, *etc.*). No significant artefacts from sample preparation or analysis can be seen in the chromatogram for the blank run.

Blanks are a vital part of method development; in this study, system blanks, filter blanks and microplastic-free water blanks showed that the workflow was free from contamination that could lead to false positive results, as shown in Table 2. In addition, the simple sample preparation steps and direct analysis of the filter reduced the possibility of contamination from additional sources.

Two spiked samples were also analysed for confirmation of the identified markers. These comprised laboratory-grade NaCl spiked with (a) 0.3 mg of nylon-6 or (b) a mixture of unknown quantities of PET, PVC and polystyrene. The results led to the correct identification of the polymers present in the mixture and quantified the spiked nylon-6 salt sample, confirming the robustness of the method.

Salt type	PET mg/g	PVC mg/g	Polystyrene mg/g	Nylon-6 mg/g
NaCl internal blank	0.000	0.000	0.000	0.000
QC 1 (NaCl spiked with 0.3 mg of Nylon-6)	0.000	0.000	0.000	0.330
QC 2 (NaCl with polymer mix)	0.0236	0.194	0.184	0.000
Himalayan rock salt	0.00775	0.000165	0.0207	0.000
Celtic Sea salt	0.0159	0.00102	0.00463	0.000
Table salt (brand A)	0.00257	0.000265	0.00298	0.000
Table salt (brand B)	0.00173	0.00012	0.00152	0.000

Table 2: Results for various salt samples showing quantified values of each polymer per 20g sample size.

The quantities of each polymer found in the 20-g salt samples, blanks and spiked NaCl samples are shown in Table 2.

Conclusions

TD–GC–MS was proven to be an excellent technique for identifying, characterising and quantifying microplastics from various salt samples. Marker compounds for quantitative analysis were identified for four polymers and linear calibration curves determined in each case. Microplastics were detected and identified in all the real-world salt samples analysed, which highlights the ubiquitous nature of microplastic contamination.

Future research in this area will focus on related aspects – for example exploring the wealth of additional forensic information hidden within the samples, such as surface-adsorbed VOCs. Here, TD–GC–MS will be used to apply lower desorption temperatures and split ratios, and to accommodate larger sample masses to facilitate trace analysis.

References

1. J.D. Peterson, S. Vyazovkin and C.A. Wight, Kinetics of the thermal and thermo-oxidative degradation of polystyrene, polyethylene and poly(propylene), *Macromolecular Chemistry and Physics*, 2001, https://onlinelibrary.wiley.com/doi/10.1002/1521-3935(20010301)202:6%3C775::AID-MACP775%3E3.0.CO;2-G.
2. Application Note 024: Overcoming the 'one-shot' limitation of thermal desorption by the re-collection of desorbed samples.
3. Instant Insight 006: How can I use re-collection to simplify method validation and development?

4. Application Note 150: Addressing the challenges of microplastic characterisation using thermal desorption.
5. G. Erni-Cassola, V. Zadjelovic, M.I. Gibson and J.A. Christie-Oleza, Distribution of plastic polymer types in the marine environment; A meta-analysis, *Journal of Hazardous Materials*, 2019, 369: 691–698, https://doi.org/10.1016/j.jhazmat.2019.02.067.
6. F. Tüdös, T. Kelent, T.T. Nagy and B.Turcsányi, Polymer-analogous reactions of polyenes in poly(vinyl chloride), *Macromolecular Microsymposia–XII and XIII*, Prague, 1973, 201–226, https://www.sciencedirect.com/science/article/pii/B9780408706391500153?via%3Dihub.
7. M.M. O'Mara, *Combustion of PVC in Pure & Applied Chemistry*, Pergamon Press, UK, 1977, vol. 49, pp. 649—660, https://old.iupac.org/publications/pac/1977/pdf/4905x0649.pdf.
8. C. Bach, X. Dauchy, I. Severin, J.-F. Munoz, S. Etienne and M.-C. Chagnon, Effect of temperature on the release of intentionally and non-intentionally added substances from polyethylene terephthalate (PET) bottles into water: Chemical analysis and potential toxicity, *Food Chemistry*, 2013, 139: 672–680, https://doi.org/10.1016/j.foodchem.2013.01.046.

Acknowledgements

This application note was developed in collaboration with Eurofins IPROMA (Madrid, Spain) and the data reproduced here were kindly provided by Antonio Rosado Sanz and Nuria Font Cardona.

Markes International gratefully acknowledges Dr David Megson and David Jones at Manchester Metropolitan University, UK, for their expert advice on the selection of marker compounds and mechanisms of production during thermal analysis

Trademarks

DiffLok™, TD100-xr™ and ULTRA-xr Pro™ are trademarks of Markes International.

DB-624™ is a trademark of Agilent Corporation.

Milli-Q® is a trademark of Merck KGAA.