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Detection of trace sub-micron (nano) plastics in water samples using pyrolysis-Gas chromatography time of flight mass spectrometry (PY-GCToF).

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HIGHLIGHTS

- Pyrolysis - Gas chromatography time of flight mass spectrometry (Py-GCToF) presented as a standard methodology for identification and semi-quantification of micro and nanoplastics.
- Fast sample preparation and obtainment of repeatable results even in real environmental aqueous samples.
- Use of PTFE membranes as a sample support; an affordable, common and broadly applied material in the industry.

Abstract

The identification and quantification of micro and nanoplastics (MPs and NPs respectively) requires the development of standardised analytical methods. Thermal analysis methods are generally not considered a method of choice for MPs analysis, especially in aqueous samples due to limited sample size introduction to the instrument, decreasing the detection levels. In this article, pyrolysis - Gas chromatography time of flight mass spectrometry (Py-GCToF) is used as a method of choice for detection of MPs and NPs due to its unprecedented detection capabilities, in combination with PTFE membranes as sample support, allow for smaller particle sizes (>0.1μm) in water samples to be identified. The utilisation of these widely used membranes and the identification of several and specific (marker) ions for the three plastics in study (polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC)), allows for the extraction of individual plastics from complex

signals at trace levels. The method was validated against a number of standards, containing known quantities of MPs. Detection levels were then determined for PVC and PS and were found to be below <50 μg/ L, with repeatable data showing good precision (%RSD <20 %). Further verification of this new method was achieved by the analysis of a complex sample, sourced from a river. The results were positive for the presence of PS with a semi-quantifiable result of 241.8 μg/L. Therefore PY-GCToF seems to be a fit for purpose method for the identification of MPs and NPs from complex mixtures and matrices which have been deposited on PTFE membranes.

Key words

Microplastics

Nanoplastics

Pyrolysis

Time of Flight Mass Spectrometry

Micropollutants

Water Quality

1. Introduction

Despite their vast applications, plastics are an environmental sensitive issue due to their resistance to biological, and chemical degradation, which entail the accumulation of these materials in the environment (da Costa et al., 2016). Plastic debris is fragmented into smaller particles by action of natural ultraviolet radiation and other physical forces (Andrady A. L., 2015). Microplastics (MPs) are considered synthetic materials below 5 mm (Arthur et al., 2009) whereby, the nanoscale is defined between 1nm to 100 nm (Klaine et al., 2008).

The environmental fate of these micropollutants is a cause of concern. MPs and have been identified in samples of air (Gasperi et al., 2018), soil (Rillig et al., 2017), found in a number of water bodies (Horton et al., 2017), and a recent study have found MPs in rain,

describing them as a new atmospheric pollutant (Wetherbee et al., 2019). It is postulated that NPs are small enough to cross the cell wall and can interact with the cell functions, causing increased cytotoxicity associated with oxidative stress (Barboza et al. (2018), de Souza Machado et al. (2018) and Schirinzi et al. (2017)). Tamara Galloway, (2015), expounded the different risks and hazard levels associated with plastics and potential dangerous additives. According to the study, leaching of toxic chemicals, from the core of the particle to the surface, could result in a long-term source of chemicals in body fluids and tissues.

A standardised method to quantify MPs is yet to be published. It is also challenging to detect micropollutants smaller than 50-100 µm with current methods. According to Lehner et al. (2019), there is still no methodology reliable enough to detect NPs in complex environmental samples such as surface, ground, potable and raw waters. Common analytical techniques currently employed are FTIR, Ramman, Light microscopy and SEM. Although practically useful for qualitative work, they do have associated limitations, such as identification amongst complex mixtures (Lusher et al. (2017), Song et al. (2015)), and detection levels of these instruments are limited by resolution. This makes it difficult to identify small particle sizes (<50 µm) and also to quantify low level material (Lusher et al. (2017), Song et al. (2015)).

To overcome these limitations of resolution, this article focuses on the identification of MPs and NPs by analysis of their thermal degradation products using chromatographic and mass spectrometry, a highly sensitive technique commonly used to analyse complex samples and has been used in characterisation of synthetic polymers and copolymers (Silva et al. (2018), Fries et al. (2013), Hermabessiere et al. (2018), Ter Halle et al. (2017)).

The following method employs a commonly used PTFE membranes (pore sizes 0.45 and 0.1 µm) to deposit MPs and NPs from aqueous samples working in the limit range of nanoscale. After processing, these filters are analysed using Gas chromatography coupled to time-of-flight (ToF) mass spectrometry (see figure 1). The verification of MP and NP type is based purely on the identification of the evolved degradation products. The

plastics studied in this article are known to breakdown at temperatures below 500 °C (McNeill et al. (1995), Bockhorn et al. (1999), Faravelli et al. (2001)). The degradation products produced from the different plastics may be used as potential markers to identify a variety of plastics in a complex mixture or identify plastics from samples containing potentially difficult matrices, such as untreated water samples. The choice of membrane was also decided based on PTFEs' higher thermal resistivity than common plastics, as it begins to degrade (>550°) (Odochian et al., 2014) and is less likely to contribute to background interference.

The advantage of using a ToF (pulsed) analyser over more common scanning mass spectrometers (quadrupole analysers), is its superior acquisition speed and mass resolution (El-Aneed et al. (2009), Choi et al. (2003)), which is anticipated to bring sensitivity down to sub part per billion levels. In case of our system, this ToF operates in nominal mass units (not accurate mass) similar to the quadrupole but offers substantial sensitivity due to the fact that all ions in a ToF are transmitted to the detector. This has an added advantage of providing full scan (FS) data for structural interpretation. In quadrupole analysers sensitivity is reduced in FS operation, due to division of acquisition time across the full mass range (duty cycle) (Hoffmann and Stroobant, 2007). However, sensitivity can be boosted in scanning mass spectrometers by utilising single ion monitoring acquisition mode or combination of the two, but this comes at a compromise between sensitivity and acquiring full spectral data (Hoffmann and Stroobant, 2007). The combination of gas chromatography and mass selectivity of the mass analysers allows to match markers from reference materials to unknowns based on two parameters; retention time and spectral matching to further aid in plastic type identification. Together with unprecedented low-level detection capability of the instrument and small pore sizes of the membranes, it is anticipated the detection of MPs and NPs down to 0.45 and 0.1 µm in cross sectional size at part per billion level.
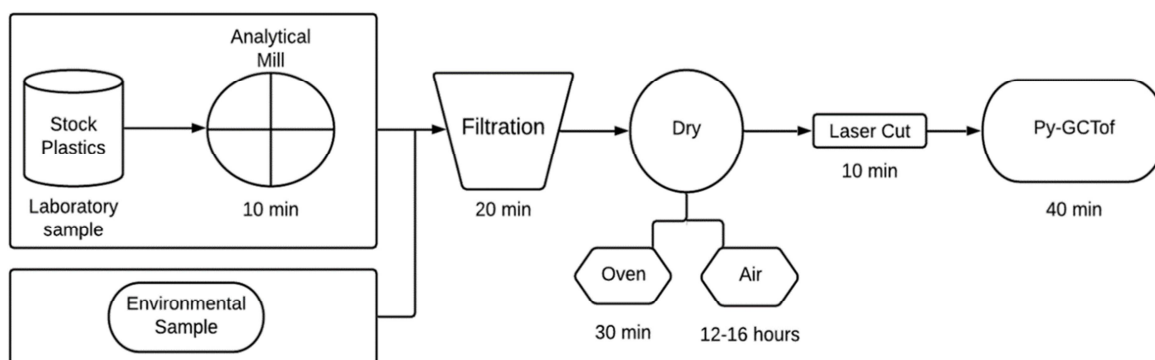
Figure 1. Illustrating the novel standard methodology proposed in this study for the identification of micro and nanoplastics using both laboratory and real environmental samples.

2. Materials and methods

2.1. Standard reference materials

Powdered PVC (high molecular weight and bulk density of 1.4 g/ml) and PP (pellets and bulk density of 0.9 g/ml) were purchased from Sigma-Aldrich UK. PS, on granules of 900 μm and a bulk density of 0.62 g/ml, was purchased from Goodfellow Cambridge Limited. Fluoropore® Polytetrafluoroethylene (PTFE) membranes (Merck Group UK) of 0.45 μm and 0.1 μm pore size were purchased for filtration purposes. Helium and nitrogen gases were purchased from BOC Gas & Gear (Port Talbot, UK).

2.2 Instrumentation

The plastic reference material was ground to less than 100 μm using an IKA® England LTD M20 Universal Mill to generate material considered to be in the micro to nano size range. Filtration experiment was then carried out using a glass vacuum filtration funnel and receiving flask with samples drawn through under negative pressure using Sparmax® vacuum pump (The Airbrush Company Ltd, UK). The filtrated membranes were cut using a TROTEC Speedy 300™ laser cutter (Trotec Laser UK). Light microscopy of the membranes was used to determine coverage and particle size, these were done using Zeiss Primotech microscope (Carl Zeiss Ltd., Cambridge, UK) at 5 x and 10 x magnification.

Thermo-gravimetric analysis of the raw materials (plastics and membranes) was performed on a simultaneous thermal analysis (STA) 6000 by Perkin-Elmer 6000 (Llantrisant UK) this was used to determine the mass losses over a temperature range of 30 -700 °C.

Pyrolysis of the filtrate membranes was achieved using a CDS Pyroprobe® 5150 from Analytix Ltd (Boldon, UK). The pyrolysis products was then analysed using gas chromatography time of flight mass spectrometer (GC-ToF) that consisted of an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) and a Pegasus BT mass analyser (LECO Corporation, Saint Joseph, MI).

2.3 Microplastic preparation

In order to obtain particle sizes below 100 μm in cross sectional area, the raw plastics (PVC, PP and PS) were mechanically ground using an analytical mill. Preliminary experiments by Steyaert et al. (2012) demonstrated that milling was effective at generating consistent pulverised mixtures with particles smaller than 1 mm. The frequency of rotation used for this investigation was 20,000 rpm and plastics were milled for 10 min. The grinding chamber was loaded with 15 g of each plastic. To avoid the overheating of the unit and the agglomeration of the plastic particles, the mill was stopped for at least one minute after a minute of grinding. Light microscopy results show MPs were evenly distributed across the filter and all appear to below 100 μm in size as expected (see figure 2).
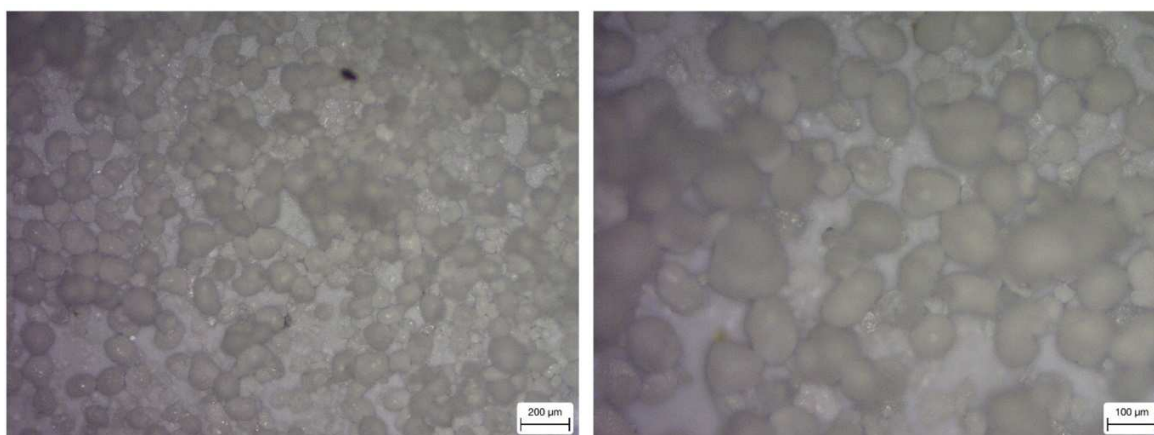
Figure 2. Illustrating light microscopy images of MPs generated by milling then filtered on to PTFE membrane. Left image is 5 x magnification and image on right is 10 x magnification.

2.4 Microplastic filtration and sample preparation

For membrane samples containing only one of the plastics, 1 g of ground of the material was added 500 ml of deionised water and stirred to ensure homogeneity of the solution and that sufficient material was deposited on the membrane. The same procedure was followed to prepared mixed samples of each plastic, reducing the quantity of material used to 0.25 g of each ground plastic.

A vacuum pump connected to the side arm of the receiver flask created a negative pressure of 675 mmHg which was used to draw through the sample (water containing MPs). The 0.45 and 0.1 μm pore size filtered membranes were dried in an isolated fume cupboard at room temperature under constant air flow to aid evaporation.

Afterwards, several 10 mm x 1 mm shards of the membranes were cut systematically to give representative coverage of the filter. This process ensured high precision and repeatability of the filter cutting process. All samples were placed into small glass bottles with a metal and natural rubber lid to keep them in the same conditions.

2.5 NP and MP detection

2.5.1 STA analysis

Simultaneous thermal analysis (STA) of all plastics in study and PTFE membranes were carried out utilizing nitrogen as the purge gas at a flow rate of 20 ml/min. The samples were heated up to a temperature of 700 °C at a heating rate of 15 °C/min.

2.5.2 Pyrolysis GC-ToF

Samples were pyrolysed using the CDS Pyroprobe. The samples were introduced into quartz tubes and heated from 30 °C to 500 °C at a rate of 20 °C/ms with a hold at 500 °C for 20 seconds. A nitrogen purge at 20 ml/minute around the probe ensures the sample is

maintained in a pyrolytic environment. Pyrolysis products were captured using a Tenax®
trap utilised for sorption of broad range of compounds (C6-C40). This was initially held at
50 °C. Prior injection, the trap was heated to 300 °C to desorb the captured analytes. The
transfer lines connecting the pyroprobe unit to the GC were held at 300 °C to avoid
analyte condensation. The trapped analytes are then exchanged into a gas flow of ultra-
pure helium before being transferred to the GC unit.

To ensure good chromatography and analyte separation a 30 m, a non-polar column with
a 5 % diphenyl dimethyl polysiloxane phase was purchased from Restek Ltd (Worthing,
UK) with a column id 250 µm and a film thickness of 0.25 µm. The column flow was
established at 1.2 mL minute (ultra-pure helium) and GC oven ramp of 40 to 300 °C at 10
°C/min. The inlet conditions were set at 250 °C with the split vent open at ratio of 75:1 to
prevent column overloading. The transfer line connecting the GC to the mass analyser
was heated at 250 °C to minimise peak broadening. In the mass analyser the source
(electron ionisation) operated at standardised 70 eV for reproducible spectra and source
kept hot at 250 °C to limit source contamination. The MS acquisition parameters were set
at 15 spectra/s with an extraction frequency of 15 kHz, collecting spectra between 50-500
mz. The data software package used to interpret the mass spectral information was
ChromaTOF (LECO Corporation, Saint Joseph, MI).

In order to limit chromatographic data, marker ions for the characteristic compounds
generated by PS, PP, PVC, will be used for ease of data interpretation (see table 1.). For PS
and PVC, light alky aromatics and polyaromatics will be predominate, such as toluene,
styrene and naphthalene (McNeill et al., 1995). These spectra are well defined and
typically their fragmentation patterns produce high abundance fragment or precursor ions
such as 91 *mz* for toluene, 104 *mz*, for styrene and 128 *mz* for naphthalene (Watson and
Sparkman, 2008) For PP, the typical products generated are aliphatic (Bockhorn et al.,
1999) and fragmentation of these compounds generate very similar spectra predominant
with low mass fragment ions (Stevensons rule for sigma cleavage) (Watson and Sparkman,
2008). These low fragmentation ions are fairly common with many other compounds. For
this reason, a higher molecular weight fragment ion will be chosen at a compromise

(although less abundant) such as 97, 111 or 125 *mz,* as higher masses are deemed less common and more distinguishing. Retention time will also be crucial distinguishing feature for characterisation of PP by overlaying samples with a reference material

2.5.3 Semi-quantification of PVC and PS breakdown products

A sample containing a mixture of PS and PVC was filtered and prepared as in 2.4 but on this occasion each cut filter was spiked with 1 ng of chlorobenzene d5 internal standard prior pyrolysis and run in triplicate. This was used for semi quantification of the emerging plastic break down products for PVC and PS, which were typically aromatic. Semi-quantification was based on the summation of the area count of the individual marker ions of PS and PVC, compared to the area count of the spiked internal standard (equation 1). In addition to this, three blank PTFE samples were also run and spiked with 1 ng of chlorobenzene d5 for determination of analytical limit of detection (LOD) of PS and PVC. The LOD for each plastic was calculated by the 3 x standard deviation of the marker ions in the PTFE filter blank.

$$\frac{\sum Area\ count\ of\ mircoplastic\ marker\ ions}{Area\ count\ of\ \text{chlorobenzene d5}} \times Mass\ of\ chlorobenzene\ d5 \quad \text{Equation 1.}$$

3. Results

3.1 Verification of thermal degradation

In order to corroborate the data shown in the literature (Matsuzawa et al. (2004), Yu et al. (2016)), thermo-gravimetric analysis was performed on the raw plastic material and PTFE membranes using a Perkin-Elmer STA6000 apparatus. The outcome can be observed in figure 3.

Results for PVC, in line with Yu et al. (2016), show the expected curve in two phases, with the first one associated to a mass loss around 65 % at temperatures between 250-350 °C and the second one presenting a mass loss of around 95 % of the material at

temperatures of 500 °C with a total decomposition at temperatures of 525 °C. For PP, the curve obtained demonstrates how this material starts decomposing at temperatures of 300 °C, with a drastic mass loss in the interval of 400-475 °C where the total amount of PP is completely disintegrated. PS showed an even earlier degradation than PP, started breaking down at 375 °C and degrading completely before reaching 450 °C. A small percentage of mass, in the order of 3 %, was lost from PTFE membranes before reaching the temperature of 500 °C. Once this threshold was exceeded, this material started decomposing and was completely consumed at the temperature of 650 °C. Therefore, a temperature of 500 °C was deemed appropriate for analysis of MPs. The increased mass transfer of the MP decomposition products to the column, will overall enhance the signal of plastic markers to that of the background noise contributed by the PTFE filter.
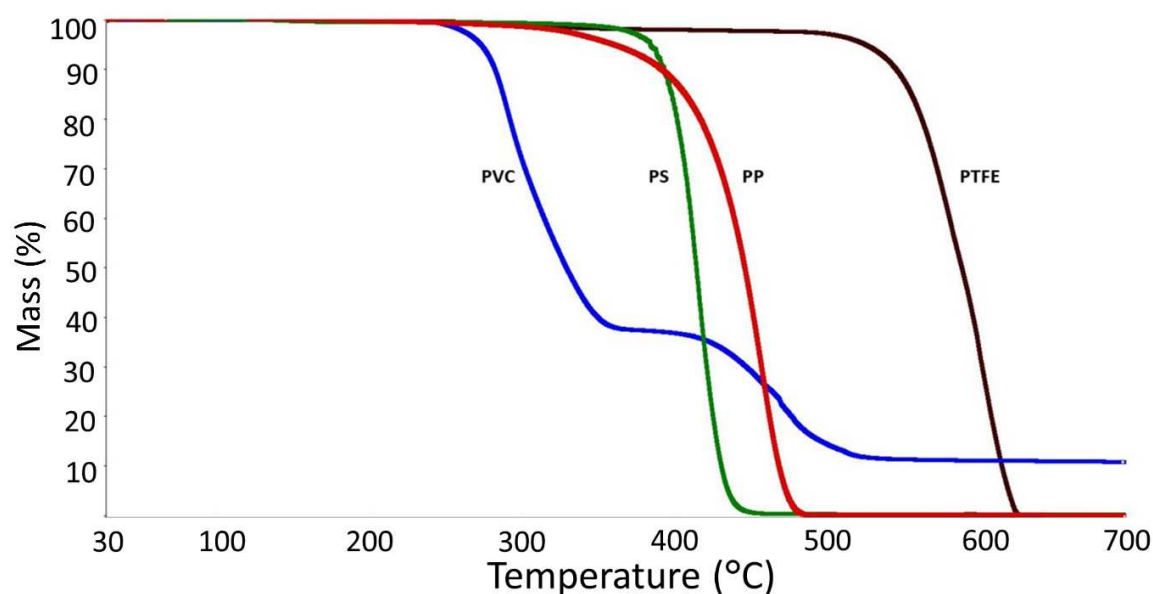

Figure 3. A thermo-gravimetric plot for different polymers; PVC (blue curve), PS (green curve), PP (red), and PTFE (black). PTFE degrades at higher temperature compared to the other plastics.

3.2 Detection of plastic markers.

Samples of raw PTFE membranes (process blanks) and PTFE membranes containing MPs (from filtration of water samples spiked with MPs) were prepared before being analysed using the Py-GCToF. The samples contained PVC, PS and/or PP. The markers ions for these

MPs were identified by overlaying the results of the filtered sample membranes with the PTFE process blanks. Further confirmation of the markers was done be comparison against raw plastics run under the same pyrolysis conditions. By comparing the blank (PTFE filter only) with the respected samples of MPs, it is possible to clearly identify the components of the individual plastics from the PTFE filter (see figure 4).
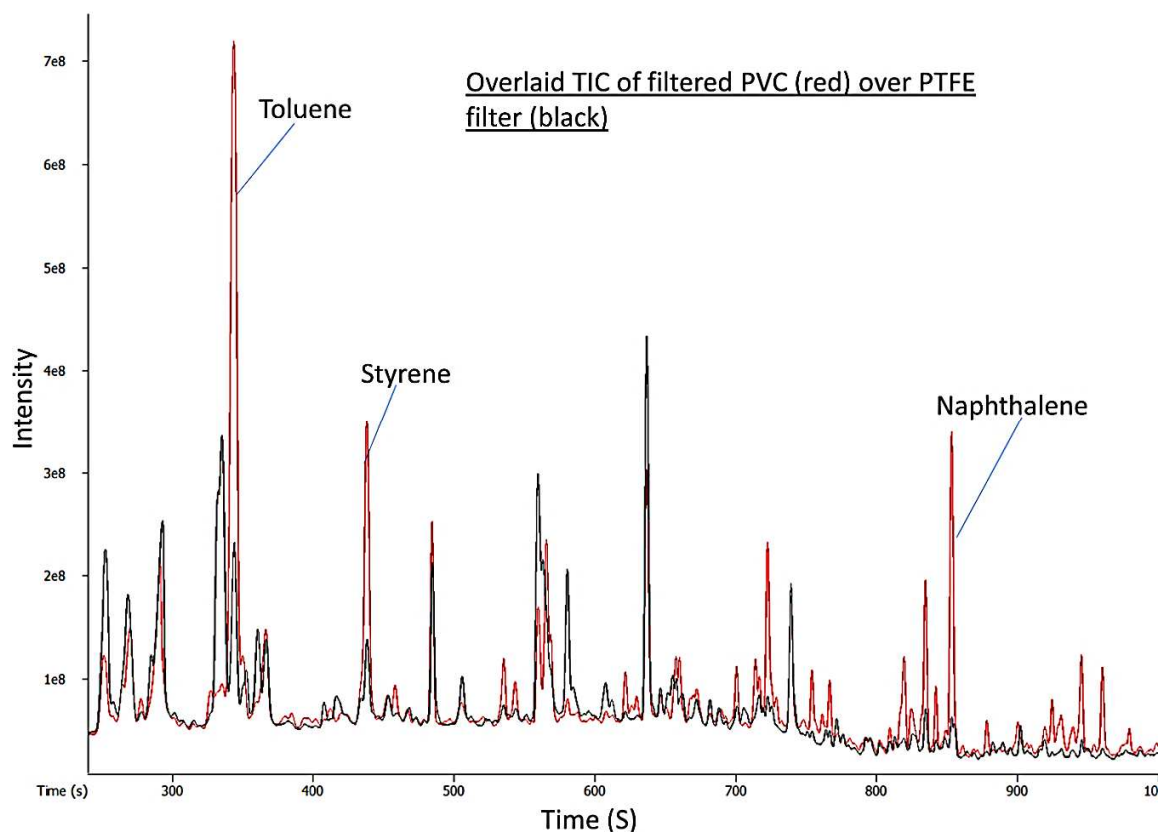

Figure 4. Shows overlaid total ion chromatogram (TIC) of PCV deposited by filtration compared with PTFE filter blank. Clearly there are identifiable differences, coming from the PVC such as toluene, styrene and naphthalene.

3.2.1 PVC thermal decomposition

The thermal breakdown of PVC at 500 °C was investigated by McNeill et al., (1995), Bockhorn et al. (1999), Faravelli et al. (2001))., and showed that over 50 % of the material is lost as HCl gas and the remaining organic product (tar), light aromatic compounds such as benzene and toluene make up the majority of the liquid (80 %) (McNeill et al., 1995).

Benzene and HCl emerge at temperatures as low as 200 °C, and it is not until higher temperatures that the emergence of the other alky aromatics and polyaromatic hydrocarbons emerge (above 300 °C) (McNeill et al., 1995). Toluene being the most abundant of the aromatic compounds during the second loss and the presence of alky aromatic species (styrene, xylene) and poly-aromatic compounds (indene, naphthalene) are present but at lesser amounts (McNeill et al., 1995). The same authors proposed that the main driving force for PVC thermal degradation occurs as a result dechlorination via random scission of secondary chlorine atoms, to form initiation sites (see figure 5 (3)). Benzene and alkyl benzenes are then formed through intramolecular cyclisation of the chain ends (McNeill et al., 1995) . The inner part of PVC is cross-linked via Diels-Alder condensation reaction of double bonds belonging to other chains, at higher temperatures these structures degrade to produce aromatic hydrocarbons through cyclisation of free ends (McNeill et al., 1995).
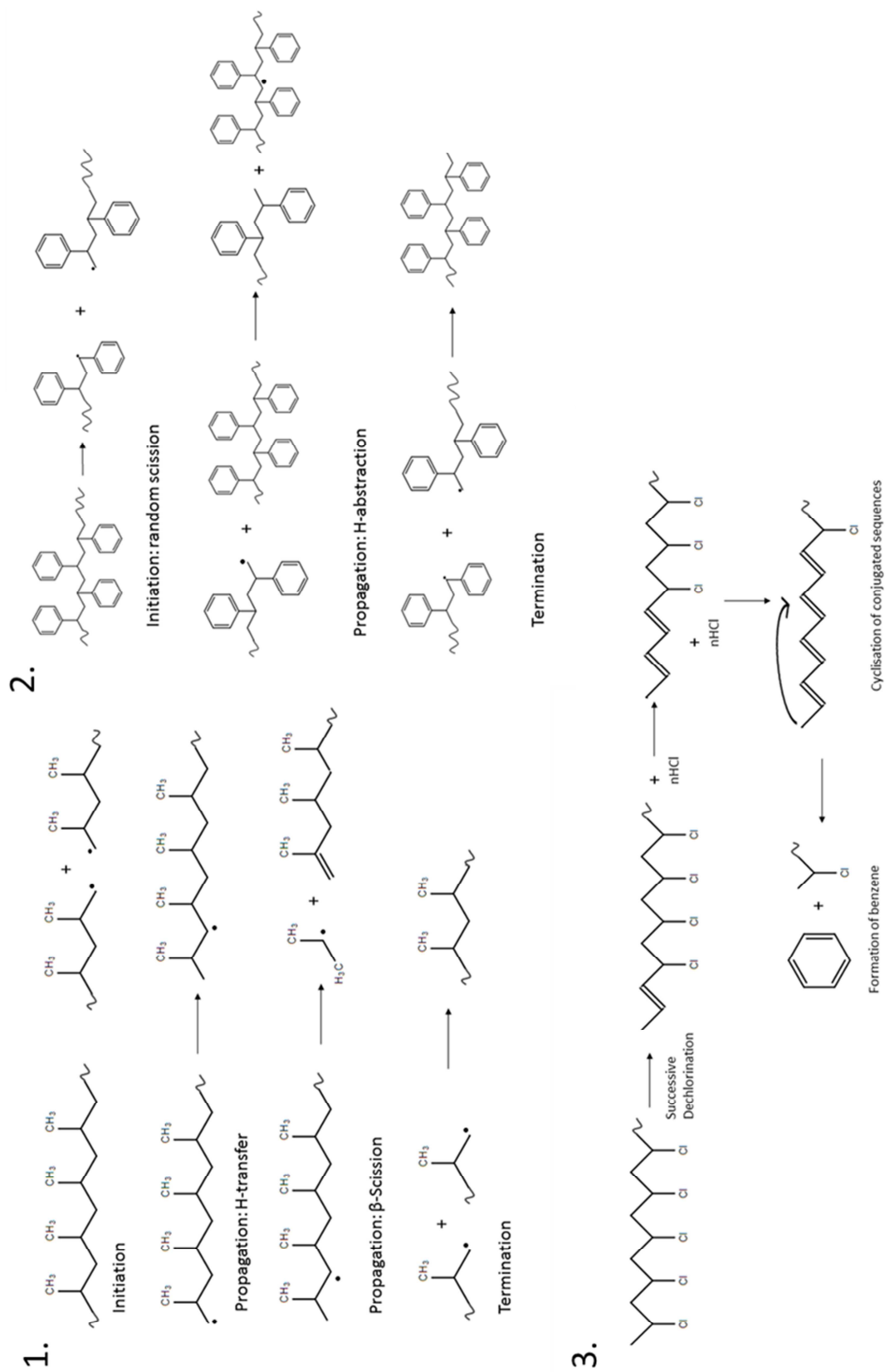
Figure 5. Shows the simplified mechanism for thermal decomposition of PP (1), PS (2), PVC (3), and degradation is initiated through formation of primary radicals.

For filtered PVC, the main compounds identified were toluene, alkyl aromatics and polyaromatic hydrocarbons which are typical with PVC thermal degradation (McNeill et al., 1995) (see figure 6). The only chlorinated product identified was chlorobenzene and this was of low abundance, which is typical of PVC thermal decomposition (Sin et al., 2012),. The increase in pyrolysis temperature then showed the emergence of aromatic and poly aromatic compounds, with their formation likely due to rearrangements of the scission products of the polymer back bone.
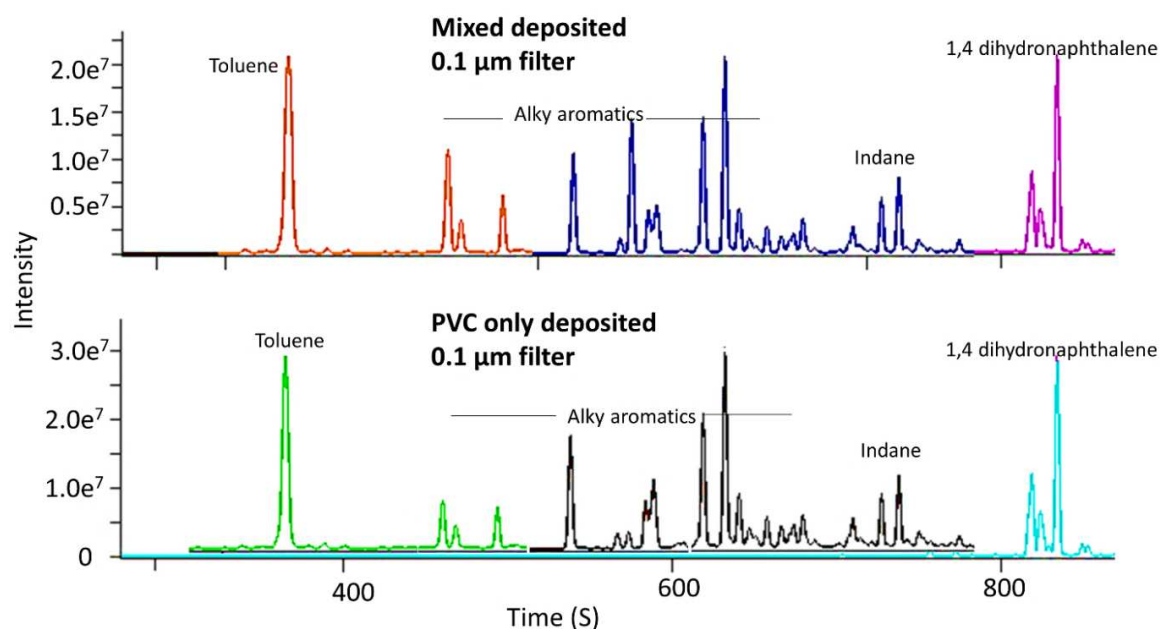

Figure 6. *Comparison of extracted ions (91, 117, 130 m/z) for PVC from sample deposited with only PVC (bottom) particles and a sample deposited with a mix of plastics particles (PP,PS,PVC), (above) deposited on 0.1 μm.*

3.2.2 Polypropylene thermal decomposition.

For PP, a repeating pattern of branched alkenes (see figure 7) is prominent throughout the chromatogram, this is likely from random thermal scission of the polymer back bone, which reveals a homologous pattern (Bockhorn et al., 1999). For PP, Bockhorn et al. (1999)

showed that above 400 °C, PP degrades into a large number of aliphatic compounds which were mainly branched and unsaturated hydrocarbons ranging from C11 to C31. Typical compounds identified were 2,4,6-trimethyl-1-nonene and 2,4,6,8-tetramethyl-1-decene. The proposed mechanism is a radical chain mechanism, initiated by random scission of the polymer into primary radicals (Figure 5 (1)). Propagation reactions progress with either β scission of the primary radicals to produce secondary radicals and ethane, or through cyclic intermolecular hydrogen transfer of the primary radicals. Intramolecular hydrogen transfer advances with another β scission generating predominantly alkenes. At higher temperatures, more scissions lead to more primary radicals that can react with each other to form alkanes (termination reaction) (Bockhorn et al., 1999).
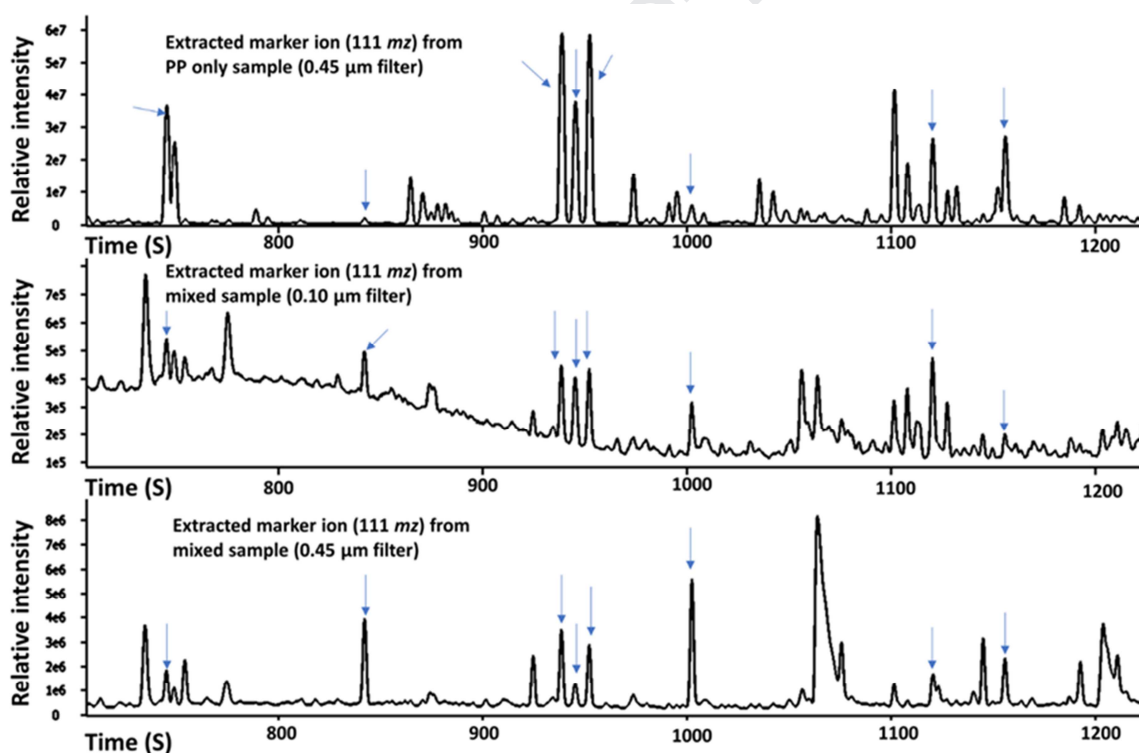

Figure 7. Comparison of PP ions from sample filtered with only PP (top), with sample containing a mix of plastics (PP, PS, PVC), filtered using 0.10 μm filter (middle) and sample containing a mix of plastics filtered using 0.45 μm (bottom). The chromatogram from the filter containing mixed plastic aligns neatly with the filter containing only PP. Retention, is

therefore a good verification parameter for PP. Although peak intensity varies, this is directly linked to the amount of material deposited on the filter (PP deposited only having the greatest intensity as more PP is present).

3.2.3 Polystyrene Thermal decomposition.

Pyrolysis of PS, revealed a rather complex array of compounds. As with PP, the thermal degradation follows a typical radical chain initiated mechanism, where initiation, propagation and termination reaction are the main mechanism (Faravelli et al., 2001). For PS, initiation can take place through either random scission of the C-C backbone, forming a primary radical ($^\bullet$carbon adjacent to the benzyl group) and a benzyl radical ($^\bullet$carbon directly bonded to the benzene functional group) and through chain-end scission (See figure 5 (2)). Chain-end scission forms a benzyl radical as before, and a stabilized allyl benzyl radical (Faravelli et al., 2001). Propagation then consists of H-abstractions and β scissions. There are two types of H-abstractions, intermolecular, in which radicals abstract H from other molecules and intramolecular abstractions, whereby primary radicals from random scission and chain end scission react to form another molecule and radical (Faravelli et al., 2001). Termination proceeds under second-order kinetics as either recombination or disproportionation reactions of radicals to form molecules (Faravelli et al., 2001). In practice the thermal degradation of PS predominantly generates the monomer styrene (60-80 %) and to a lesser degree, aromatics, light hydrocarbons, alkyl aromatics and dimers and trimers (all below <10 %) (Faravelli et al., 2001). Therefore, not surprisingly, styrene being the most predominant due to the polymer being comprised of polymerized styrene monomers. However, a similarity between PS and PVC is the fact that they both contain toluene and styrene, but a distinguishing feature of PS, is the peak ion ratio of styrene to toluene is around >40 :1 and PVC has a peak ion ratio of toluene to styrene of 100 :1 with toluene peak at significant abundance (see figure 8) . Notably with PS is the increase in single ring aromatic compounds such as 1,2,3-trimethyl-benzene and 3-butenyl-benzene, each as a result of scission the PS polymer and rearrangements of side groups. Towards the later end of the chromatograms, PS degradation compounds have

increased molecular mass and it is apparent these are larger units of the polymer such as stilbene, which appears to be a dimer of styrene.

A number of markers (ions) were identified for PVC, PS and PP and are listed in table 1. These plastics markers were identifiable on 0.45 µm and 0.1 µm filters, putting the detection of cross-sectional area to as low as a 100 nm.
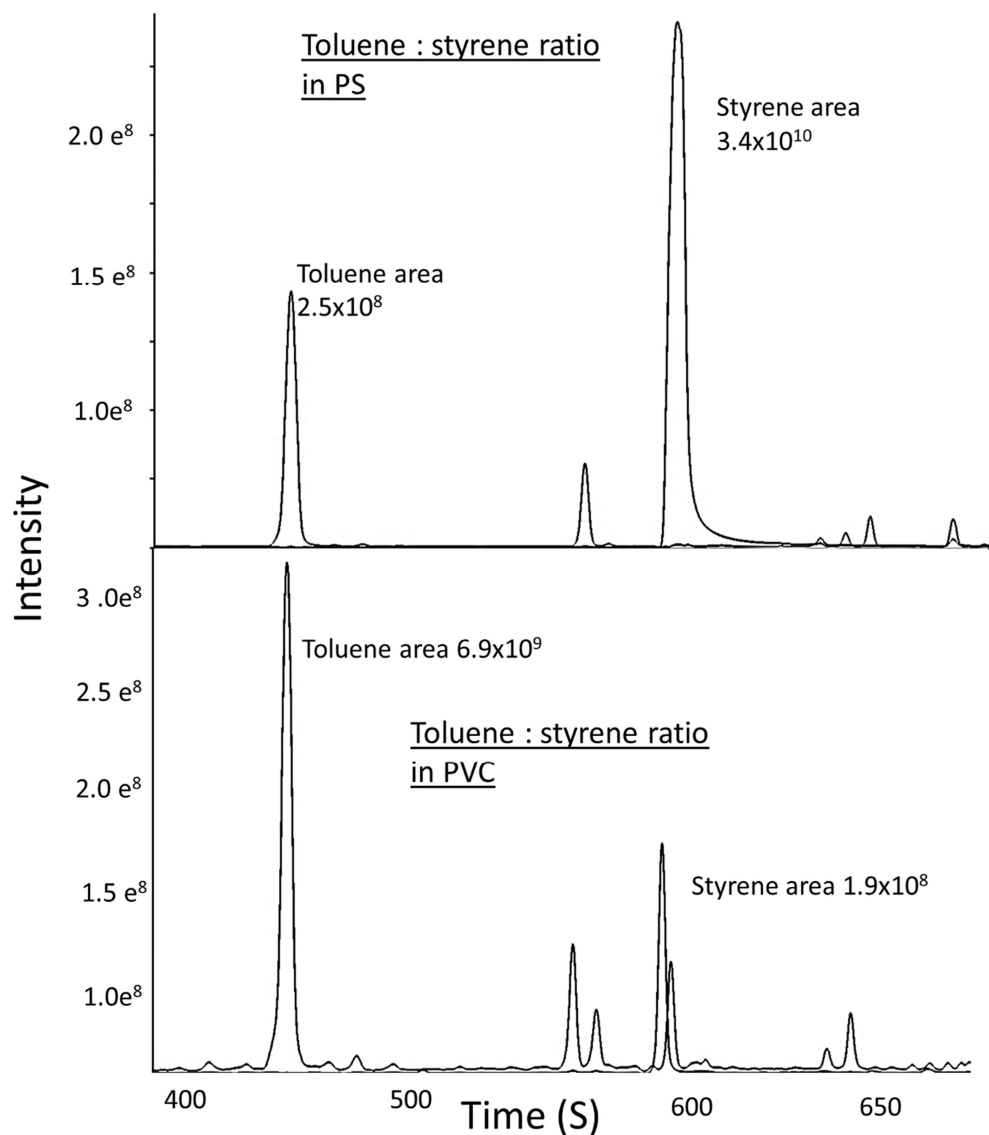

Figure 8. Shows the ions ratio of 104 mz (styrene): 91 mz (toluene), in PS (top) and PVC (bottom). PS contributes to significantly more towards styrene compared to PVC, whose main contribution is toluene.
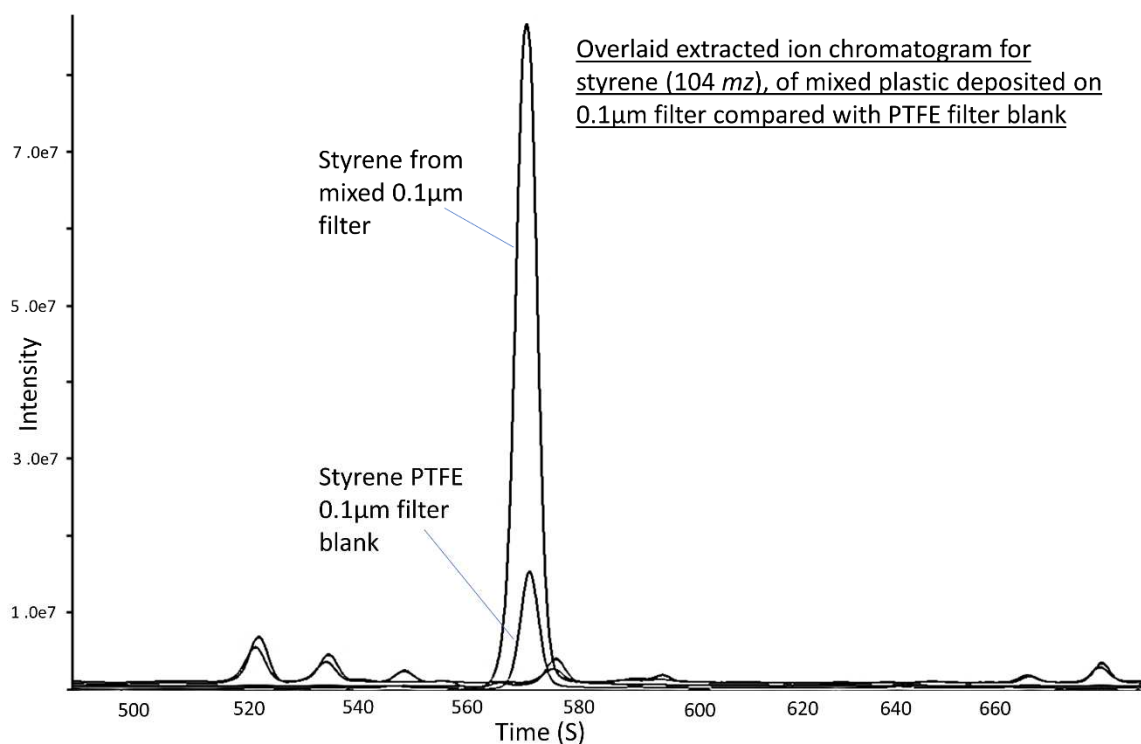
Figure 9. Shows the detection of styrene from plastic mixture deposited on 0.1 µm PTFE filter. Styrene peak (104 mz) is significantly greater in abundance compared to the process PTFE filter blank.

Table 1. A table listing the compounds and marker ions for the different plastics, PVC, PP and PS.

ample	Compound identified	Retention time S^{-1}	*mz* marker ion.
	Toluene	437	**91**
	Ethyl benzene	534	91
	Styrene	565	104
PVC	**Indane**	714	**117**
	Indene	722	116
	1,4 dihydronaphthalene	834	**130**
	Naphthalene	852	**128**
	1-Methyl-naphthalene	945-960	**142**
	Biphenyl	1009	154
	7-Methyl-decene	706	**111**, 97
	3- Dodecene	709	**111**, 97
	2,4-Dimethyl-1-decene	711	**111**, 97
	5-Methyl-undecene	745	**111**, 97

PP	7-Methyl-1-undecene	788	**111**, 97
	7-Methyl-2-decene	864	**111**, 97
	1-Tridecene	924	**111**, 97
	2,6,8-Trimethyl-octene	1101	**111**, 97
	2,4,6-Trimethyl-nonene	1120	**111**, 97
	2,4,6,8-tetramethyl-1-decene	1244	**111**, 97
PS	Toluene	441	91
	Styrene	561	**104**
	1-Ethyl-2-methyl benzene	621	**117, 105**
	1,2,3-Trimethyl-benzene	656	120, **105**
	2-Propenyl-benzene	697	**117, 91**
	3-Butenyl-benzene	716	132, **91**
	3-Phenyl-1-propyne	722	115
	1-Methylenepropyl-benzene	729	132, 117 ,91
	Bibenzyl	1112	182, 104, 91
	1,1'-(1-Methyl-1,2-ethanediyl)bis-Benzene	1138	105, 91
	Stilbene	1239	**180**, 165
	1,2-Dihydro-3-phenylnaphthalene	1311	191, 128, 91
	2,5-Diphenyl-1,5-hexadiene	1351	143, 130, 104, 91

3.3 Identification of microplastics in complex mixture.

Using ion filters for PVC (91, 117, 128, 130, 142), PP (111, 97) and PS (91, 104, 105, 117, 180) on the software, it was possible to differentiate individual MP types from a mixture that had been filtered on to 0.45 and 0.1 µm filter. PVC, PP and PS markers were all extractable from the chromatogram and each plastic identifiable. This was confirmed by comparison samples of the raw plastics that were previously run. Figure 6 shows an example of PVC ions (91 *mz*) identified from a mixture of plastics deposited on 0.1 µm filter, while figure 7 shows markers identified for PP in the mixture deposited on 0.45 µm filter and compared with raw PP for confirmation.

3.4 Semi-quantification of PVC and PS breakdown products

The samples prepared in 2.5.3 for semi quantification were run in triplicate from randomized positions on the filter and processed accordingly for the marker ions for PVC and PS to determine analytical limit of detection (LOD) and precision of the method. The analytical LOD for PVC and PS marker ions was determined by assessing standard

deviation of multiple blank samples (filter only and internal standard) and multiplying by a factor of three, to give a value of 48.6 and 42.7 ng (per 10:1 mm) respectively. The sample LOD value for PVC and PS can then be back calculated for 1 L sample, by utilising all the known parameters of the filtration process, such the diameter of the filter (40 mm) and sample volume filtered (500 ml filtered) are known, which puts the LOD at value at 48.9 and 42.9 µg/L respectively (please see equation 2 below for further clarification). Using this methodology, semi-quantification of PVC and PS from the mixed sample on the 0.45 µm filter determined an average mass for PVC markers 282 µg/L with a % RSD of 19.72 % and 299.6 µg/L for PS with an RSD of 4.75 %. The low % RSD for both MPs suggest the analytical method is repeatable and that the MPs form a homogenic layer on the PTFE filter which is in line with the microscopy images (figure 2).

$$C = \frac{\left(\left(\frac{AF}{AS}\right) \times m\right)}{v}$$

Equation 2.

C is concentration of nano plastic material per volume of sample (ng/mL ~ µg/L)

AF is the area of the filter (mm^2)

AS is area of sample shard

M is mass calculated by semi-quantitation

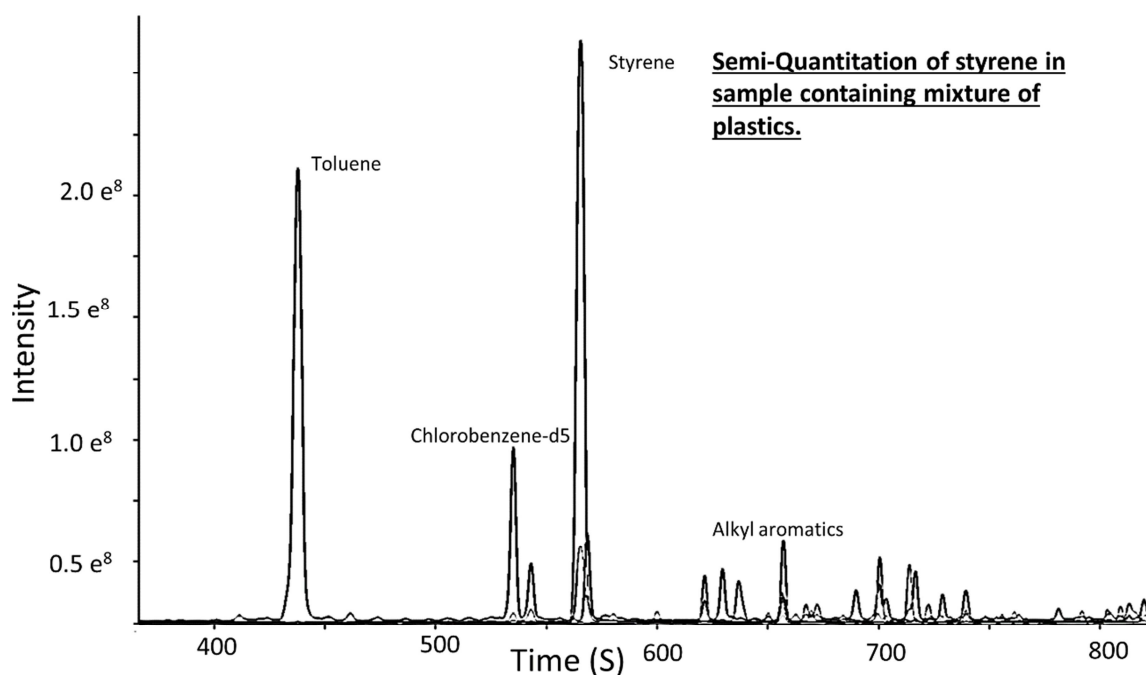V is volume of sample used in mL

Figure 10. *Example of semi-quantification of polystyrene from a sample containing a mixture of plastics. It is assumed that majority of toluene emerges from PVC and therefore ignored from styrene calculation, whist the main product from polystyrene degradation is styrene.*

3.5 Semi-Quantification of micro (nano)-plastics in river water.

A 200 mL sample of untreated river water taken from the river Tawe, South Wales at a location GPS location 51.7265391, -3.8386937 was filtered using a 0.45 μm PTFE membrane, before being dried, cut, spiked with internal standard and pyrolysed as before. The sample was run in triplicate and assessed for the marker ions for PS, PP and PVC. The results identified multiple marker ions for PS, but markers for PP and PVC were unobserved, once calculated, PS gave a semi-quantifiable result of 241.8 μg/L with a % RSD of 17.25 % for the repeated analysis. This value was above the LOD threshold of 48.9 μg/L for PS markers with multiple markers being identified, and it is likely that PS micro (nano)-plastics have been identified using a real sample matrix. The river sample was run as a proof of concept and the positive results suggest this method is appropriate for identifying low level of MPs and NPs above 0.45 μm in water samples containing a

complex matrix. As mentioned previously, MP's and NP's are associated with several potential risks for animals and human health due to their interaction with chemicals in the environment. The existence of these micropollutants in rivers, even at low concentrations, cannot be ignored and suitable methodology has be put forward to assess their wider environmental impact.
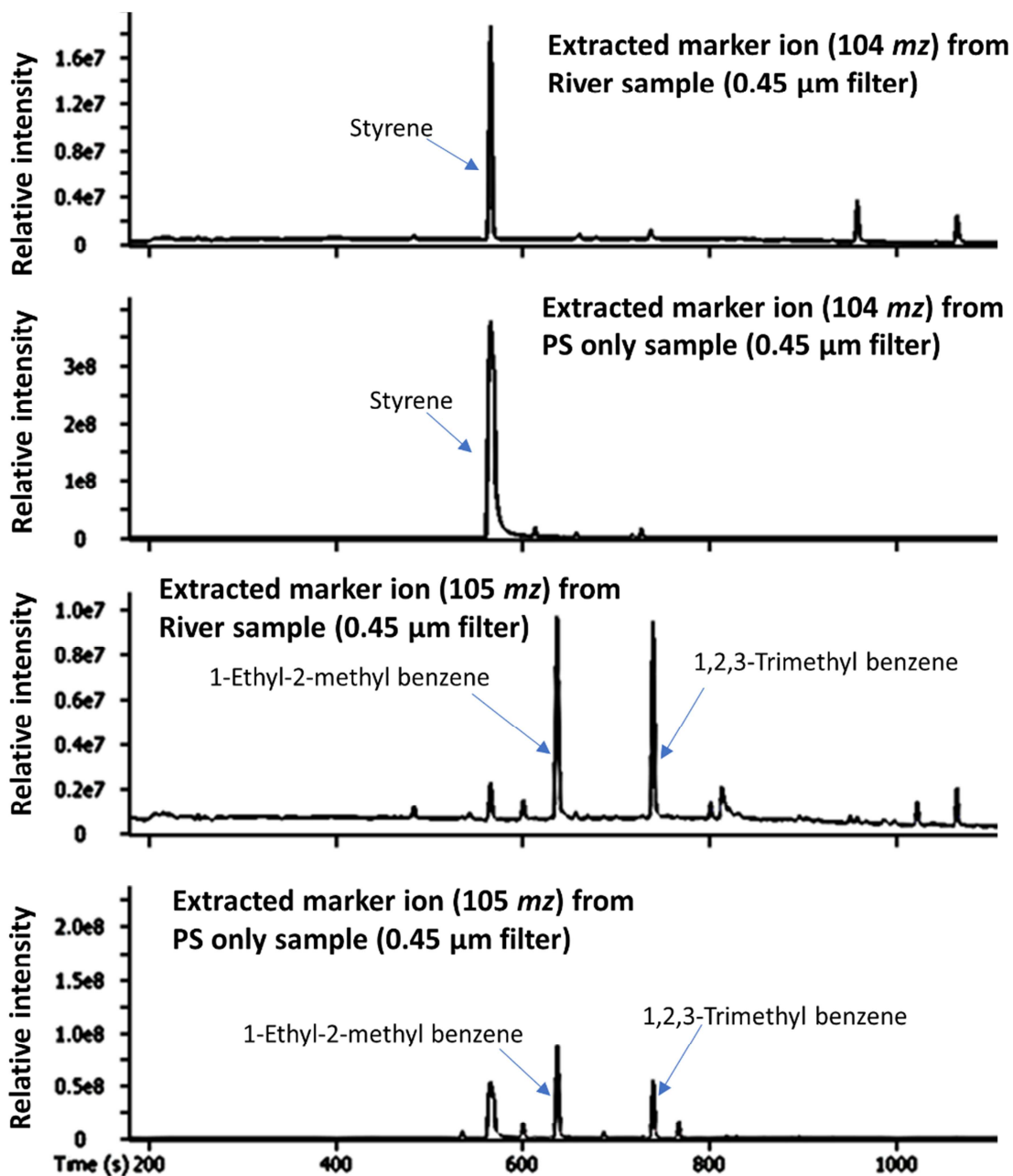
Figure 11. Is an example of the comparison of ion 104 and 105 mz from deposited PS on PTFE filter with filtered River Tawe sample. The ion 104 mz is associated with styrene and 105 mz for 1-ethyl-2-methyl benzene and 1,2,3-Trimethyl benzene, which are commonly found in polystyrene these have been identified in both samples.

Conclusion

The deposition and analysis of MPs and NPs on PTFE membranes, has proven to be a robust and reliable method for the identification of submicron particles, with a cross sectional area down to 0.1 μm extracted from aqueous samples. With the combined efforts of the superior sensitivity of Py-ToF, used as the analytical device in this investigation, MPs and NPs can be identified in complex aqueous samples at concentrations as low as 50 parts per billion. Although PTFE contributes to some of the ions (at 500 °C) present in the baseline, the MPs and NPs contribute to peaks with greater signal to noise ration and therefore are distinguishable. The utilisation of PTFE membranes is due to the characteristics of this material, being highly accessible and broadly used, inexpensive and considerably thermal resistant, which allows this technique to be easily applied without time-consuming procedures and with sample volumes under 500 ml. The membranes supporting the micropollutants can be prepared in under an hour. The use of specific differential markers ions for plastics allows the determination of PS, PP and PVC even when present in a mixture. By the introduction of a known compound as an internal standard it was now possible to quantify to the markers present on the filters. The summation of these values gave an indicator to the amount of MPs present on the filter. The repeatability of this analytical method was good with % RSD <20 % for the PVC and PS, which also indicates that the MPs have been laid down on the membranes homogenously. Finally this method is suitable for the analysis of complex matrix samples, and it was possible to identify PS on a membrane filtered with water obtained from a local river. The use of PTFE filter does have its limitations, the presence of background material emitted from the filter does increase the theoretical analytical detection level due to enhanced noise region, and this may also cause difficulty in analysis of plastics that may emit similar breakdown products as the filter. The applicability of this new approach in order to detect and quantify other type of plastics and eliminate the ion baseline associated with the use

of PTFE membranes is presently being carried out and results will be published in the near future.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: