



# Microplastics Identification and Quantification by Pyrolysis-GCMS



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**Technical Marketing Manager**  
**SEA/INDIA**

# Frontier Laboratories, Japan

- **Japanese Organization, located in Koriyama, Fukushima prefecture**
- **Entering 32<sup>nd</sup> year of our journey**
- **Global Leader in Analytical Pyrolysis Technology**
- **Manufacturer of Pyrolyzer and peripheral devices & UA-Columns**

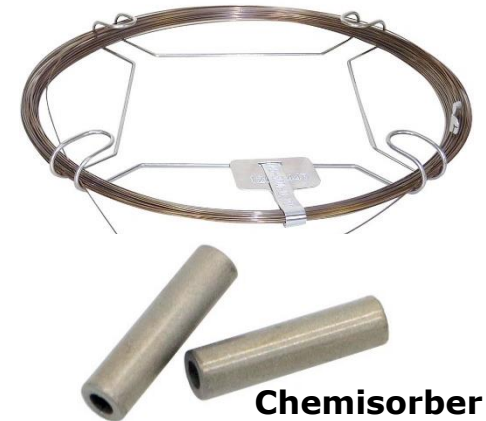
**Pyrolyzer**



**Micro Reactor**



**UA-Columns**



# Frontier Laboratories, Japan

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Pyrolyzer



- **Chemical Analysis of Polymeric materials for polymers & additives determination**

Micro Reactor



- **Investigation of Waste-Energy studies**
- **Rapid Catalyst Screening**
- **Catalytic Pyrolysis & Co-pyrolysis**

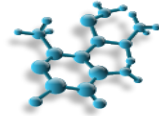
Chemisorber



- **Smart device for offline preconcentration of VVOC, VOC & SVOC [polar and semipolar]**
- **Food and Environmental Applications**

# Application areas of PY-GC/MS system

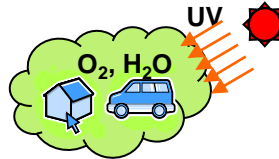
1: Characterization of polymers



2: Quality control



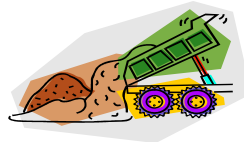
3: Degradation/life evaluation of polymeric materials



4: Recycling of polymeric materials, biomass utilization



5: Organic geochemistry and soil chemistry



6: Clinical science, pathology



7: Biochemistry, microbiology



8: Coal liquefaction, energy conservation



9: Forensic science



10: Wood science, pulp industry



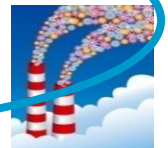
11: Tobacco smoke, toxicology



12: Extraterrestrial science



13: Environmental science



# Development of Py-GC/MS System for Microplastics Analysis



# What will you learn from this presentation?

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- What are Microplastics?
- Where do we find them? What are the sources?
- Why do we worry about microplastics?
- What are our current knowledge on MP distribution?
- Where are we heading into?
- What is the state of art for MP analysis?

# What are microplastics?

L. Van Cauwenberghe,\* L. Devriese, F. Galgani, J. Robbins, C. R. Janssen

*Marine Environmental Research* 111 (2015) 5-17.

Microplastics in sediments: A review of techniques, occurrence and effects

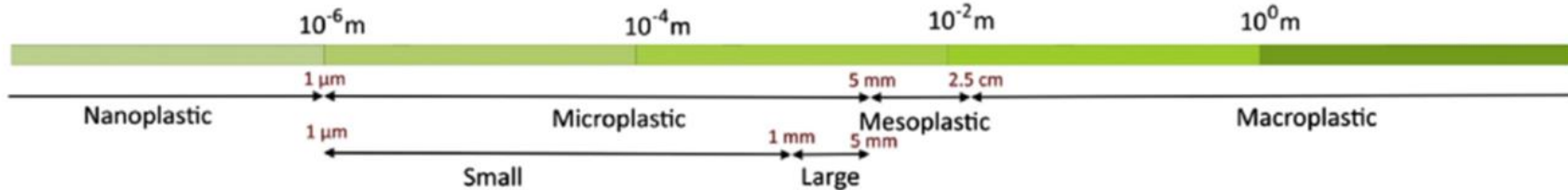


Fig. 2. Size matters. Suggestion of plastic debris nomenclature based on size, as proposed by the European MSFD technical subgroup on Marine Litter (MSFD GES Technical Subgroup on Marine Litter, 2013). The overall term “microplastic” is composed of small microplastics (SMPs, smaller than 1 mm) and large microplastics (LMPs, 1-5 mm), to differentiate between two commonly used definitions of microplastics.

**Microplastics : 1 µm ~ 5 mm**  
**Nanoplastics : 1 nm ~ 1000 nm (1 µm)**

**350 µm : sieve/net (333 µm in EU & US)**  
**10 µm : µ-FTIR spatial resolution**  
**1 µm : µ-Raman (& optical microscope) spatial resolution**

J. P. da Costa *et al.*, *Sci. Total Environ.*, **566–567**, 15–26 (2016). (Nano)plastics in the environment – Sources, fates and effects

Fig. 1. A size-based definition of plastics as proposed by different authors.

## Microplastics: (1 µm - 5 mm)

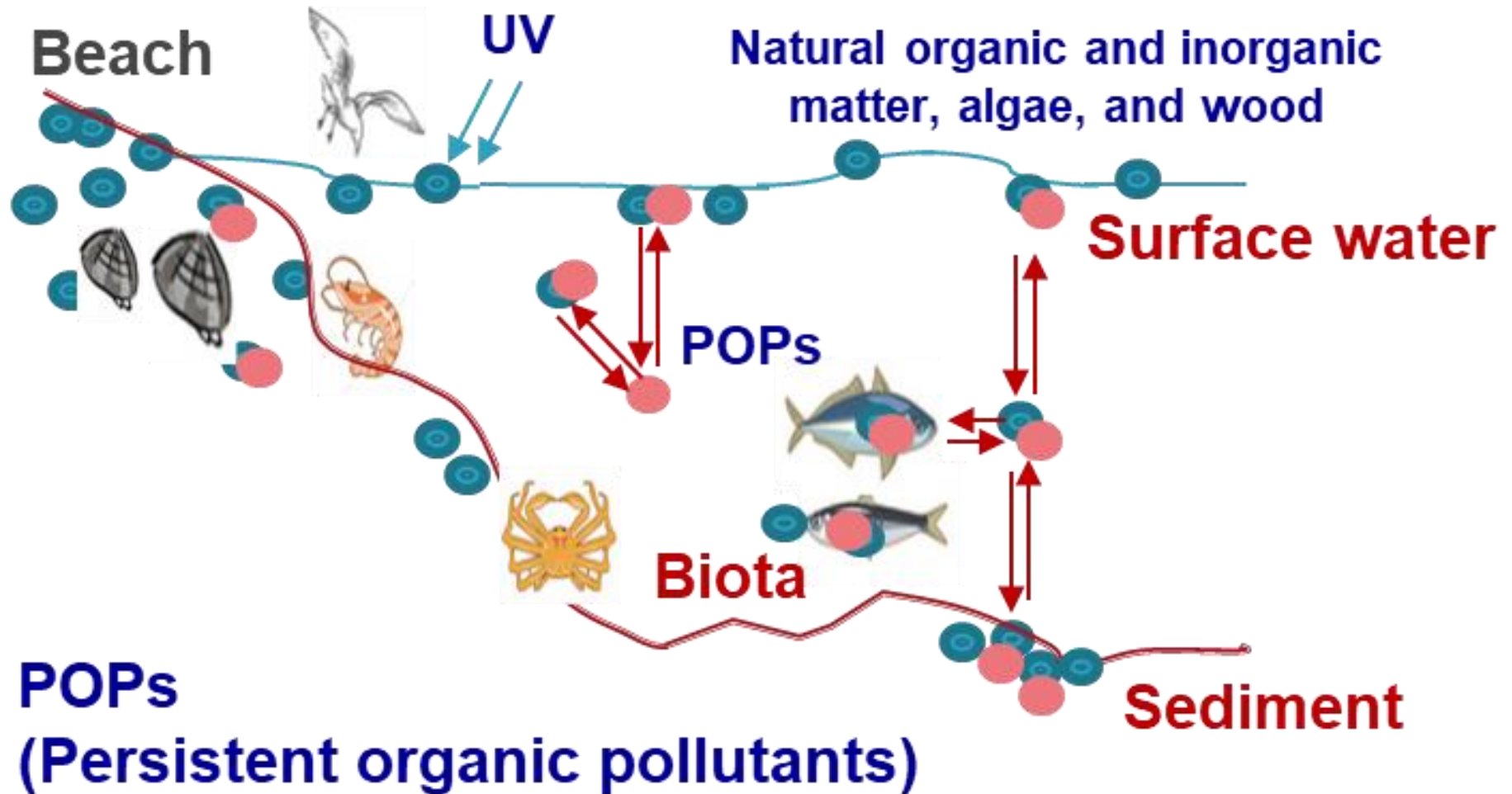
- Primary - pellets and fibers
- Secondary - degraded pieces
- Damage to wildlife by ingestion

## Nano-plastics: (< 1 µm)

- Impact on marine life by accidental ingestion
- Health effects by the concentration of POPs through the food chain

Nanoplastics   Microplastics   Mesoplastics   Macroplastics

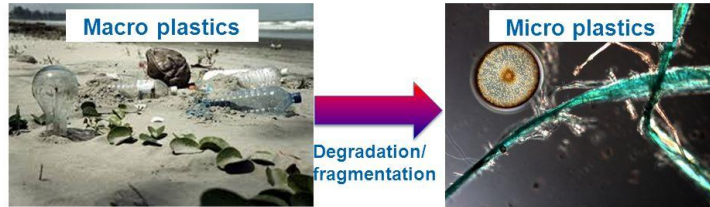
# Where do we find them?





# Where do we find them?

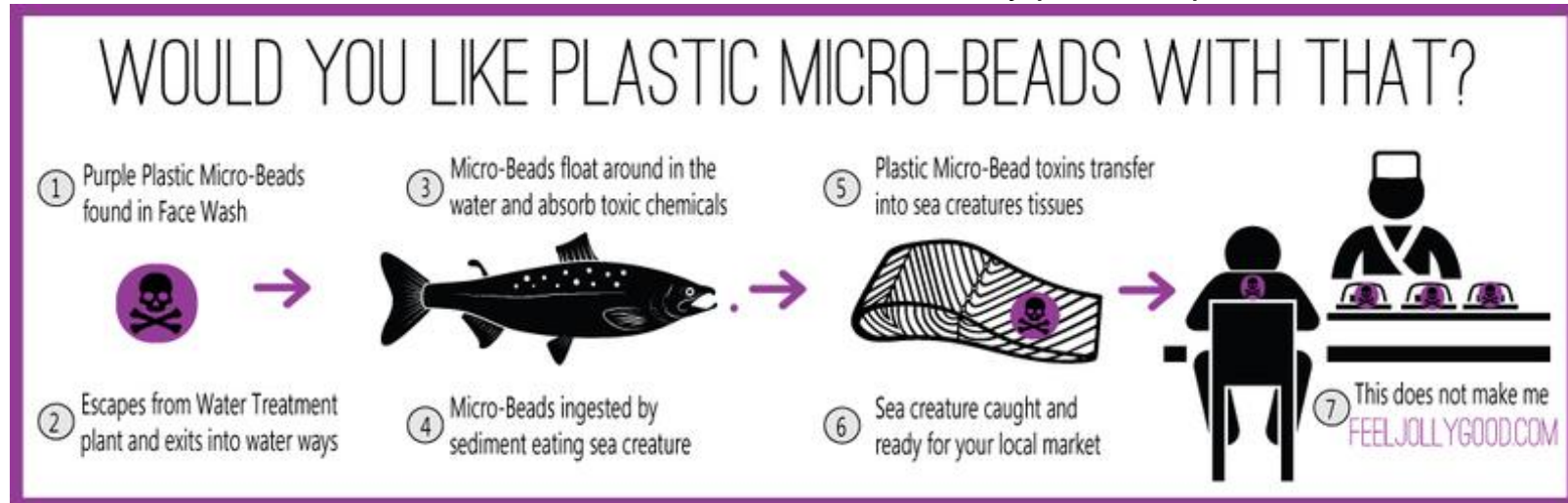
Contamination of marine and coastal environment by plastic waste emerging problem worldwide



- recent studies (US, UK, BE, NL, MICRO, CLEANSEA):
- micro plastics accumulate on beaches, water column, sea floor
  - real distribution not clear, different sources
  - more close to densely populated areas and areas receiving sewage



- Microparticles including microplastics have been shown to pass from the intestines into the blood and potentially into other organs.
- One study found that plastic fibers were present in 87% of the human lungs studied. The researchers proposed this may be due to microplastics present in the air.
- The World Health Organization (WHO) has announced a review into the potential risks of plastic in **drinking water** after a new analysis of some of the world's most popular **bottled water** brands found that more than 90% contained tiny pieces of plastic



# Where do we find them?



Chemosphere

Volume 303, Part 1, September 2022, 134918



Contents lists available at ScienceDirect

Environment International

journal homepage: [www.elsevier.com/locate/envint](http://www.elsevier.com/locate/envint)



## Investigation of microplastic contamination in blood cockles and green mussels from selected aquaculture farms and markets in Thailand

Anh Tuan Ta<sup>a</sup>, Piyathida Pupuang<sup>a</sup>, Sandhya Babel<sup>a,\*,</sup>, Li Pang Wang<sup>b,\*,</sup>

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<https://doi.org/10.1016/j.chemosphere.2022.134918>

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### Highlights

- MPs were found in bivalves collected from selected farm and markets in Thailand.
- MPs in blood cockles from Khlong Dan market was  $11 \pm 5$  particles/individual.
- MPs in blood cockles from BangBo aquaculture farm was  $6 \pm 1$  particles/individual.
- Most MPs were PP, PE with size of 0.05–0.3 mm and shapes of fragments, fibers.
- Number of MPs in bivalve from markets is much higher than in aquaculture farms.

Full length article

## Discovery and quantification of plastic particle pollution in human blood

Heather A. Leslie<sup>a</sup>, Martin J.M. van Velzen<sup>a</sup>, Sicco H. Brandsma<sup>a</sup>, A. Dick Vethaak<sup>a,b</sup>, Juan J. Garcia-Vallejo<sup>c</sup>, Marja H. Lamoree<sup>a,\*</sup>

<sup>a</sup> Dept. of Environment and Health, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1105, 1081 HZ Amsterdam, the Netherlands

<sup>b</sup> Deltares, Delft, the Netherlands

<sup>c</sup> Cancer Center Amsterdam and Amsterdam Infection and Immunity, Amsterdam University Medical Center (VUmc location), De Boelelaan 1105, 1081 HZ Amsterdam, the Netherlands

### ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:  
Nanoplastics



microplastics

### ABSTRACT

Plastic particles are ubiquitous pollutants in the living environment and food chain but no study to date has reported on the internal exposure of plastic particles in human blood. This study's goal was to develop a robust and sensitive sampling and analytical method with double shot pyrolysis - gas chromatography/mass spectrometry and analyze the presence of plastic particles >200 nm in human whole blood from 20 health-care workers.



Article

## Quantification of Microplastics by Pyrolysis Coupled with Gas Chromatography and Mass Spectrometry in Sediments: Challenges and Implications

Nadia Bouzid<sup>1,2</sup>, Christelle Anquetil<sup>2</sup>, Rachid Dris<sup>1,\*</sup>, Johnny Gasperi<sup>3</sup>, Bruno Tassin<sup>1</sup> and Sylvie Derenne<sup>2</sup>

<sup>1</sup> Leesu, Ecole des Ponts, Université Paris Est Creteil, F-94010 Creteil, France; nadia.bouzid@enpc.fr (N.B.); bruno.tassin@enpc.fr (B.T.)

<sup>2</sup> UMR METIS, Sorbonne Université, F-75005 Paris, France; christelle.anquetil@sorbonne-universite.fr (C.A.); sylvie.derenne@sorbonne-universite.fr (S.D.)

<sup>3</sup> LEE, Université Gustave Eiffel, F-44344 Bouguenais, France; johnny.gasperi@univ-eiffel.fr

\* Correspondence: rachid.dris@u-pec.fr

# Where are they coming from?

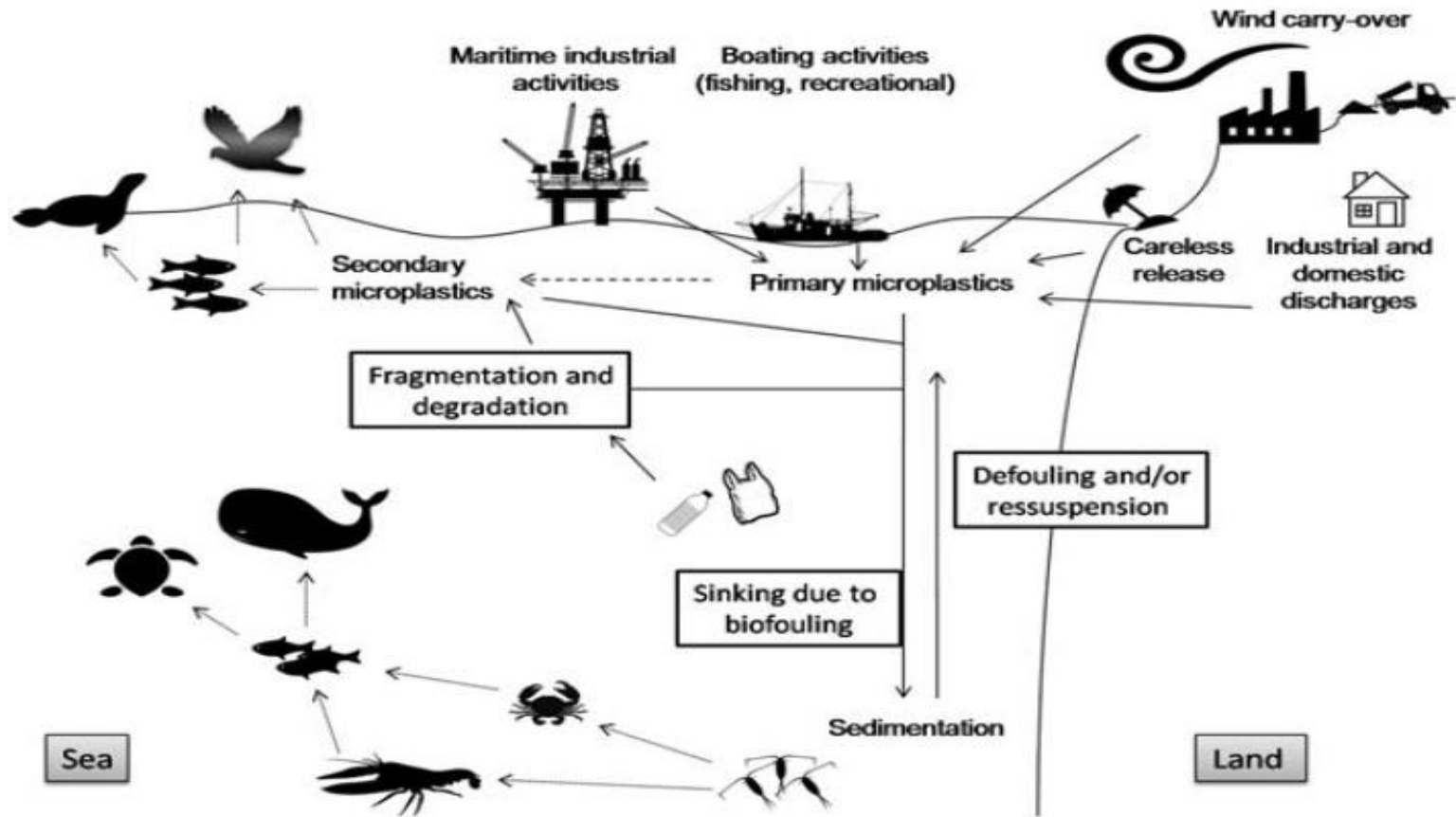


FIGURE 4 Potential fate, pathways and biological interactions of microplastics in the aquatic environment. Adapted from W.C. Li, H.F. Tse, L. Fok, *Plastic waste in the marine environment: a review of sources, occurrence and effects*, *Sci. Total Environ.* 566–567 (2016) 333–349.

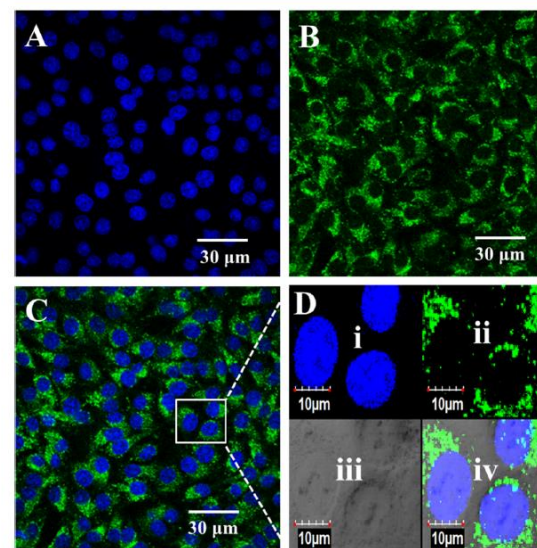
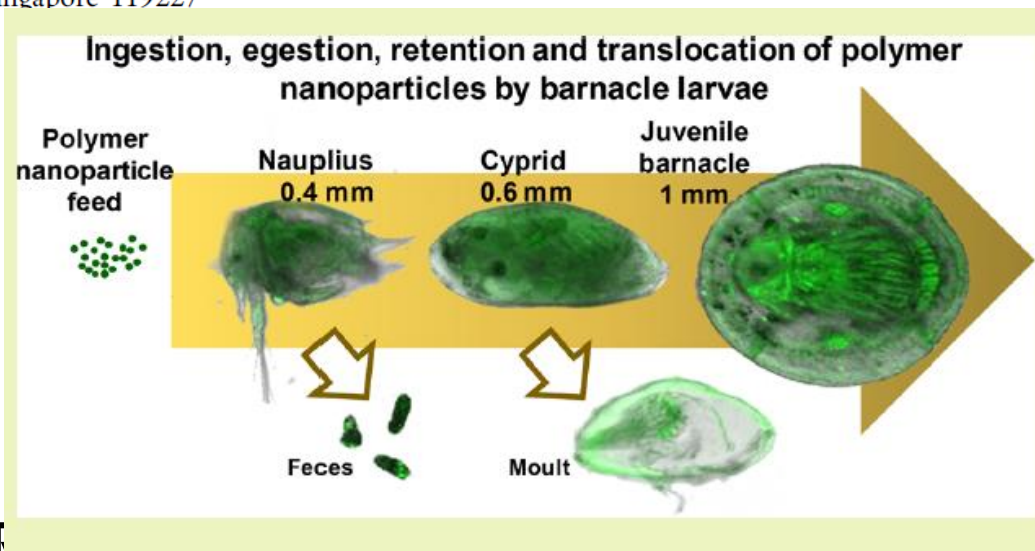
# Potential effects of MP in organism

## Fate of Nanoplastics in Marine Larvae: A Case Study Using Barnacles, *Amphibalanus amphitrite*

Samarth Bhargava,<sup>†</sup> Serina Siew Chen Lee,<sup>‡</sup> Lynette Shu Min Ying,<sup>‡</sup> Mei Lin Neo,<sup>‡</sup>  
Serena Lay-Ming Teo,<sup>\*,‡</sup> and Suresh Valiyaveetil<sup>\*,†, ID</sup>

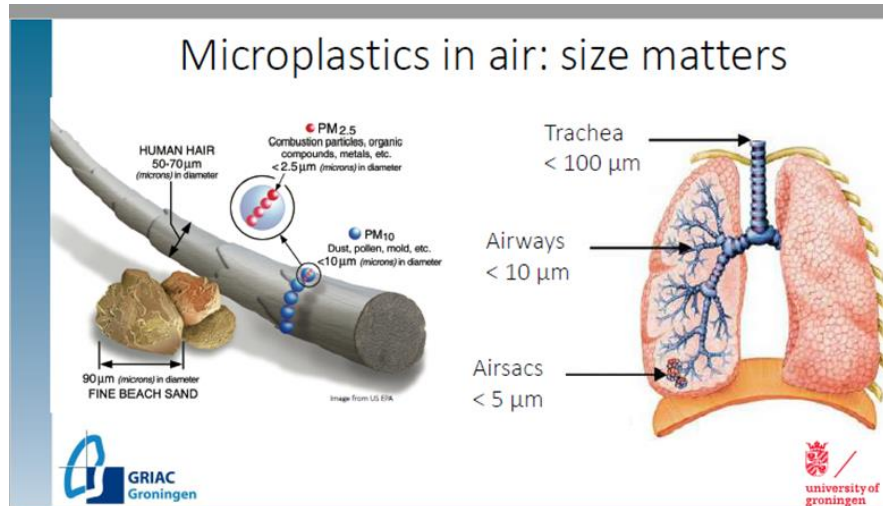
<sup>†</sup>Materials Research Laboratory, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543

<sup>‡</sup>St. John's Island National Marine Laboratory, Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, Singapore 119227



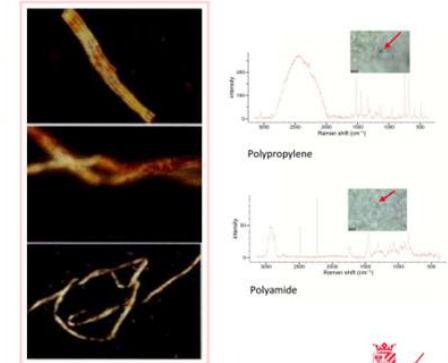
# Microplastics effects in lung tissue

Prof. Barbro Melgert, Netherlands

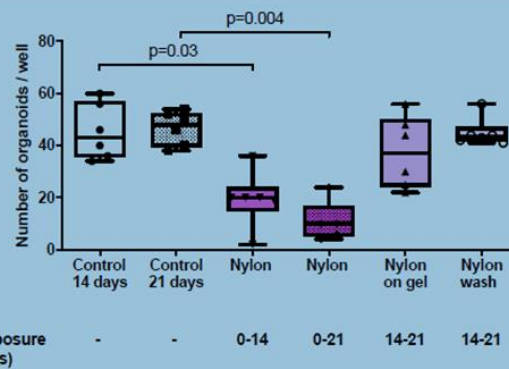


## Microplastics in lung tissue

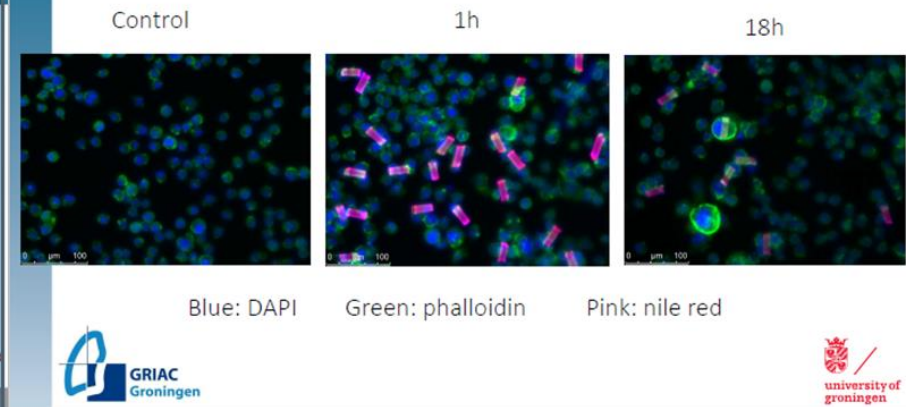
- Fibers in patients with lung cancer
- Natural and synthetic fibers
- Particles in normal alveolar lung tissue
- Distributed throughout lungs



## Nylon particularly affects developing organoids



## Uptake of nylon microfibers by macrophages



# Microplastics Distribution Data

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**Microplastics presence in the environment is reported globally  
But without any consistency.....**

- **Differences in reporting format**
- **Differences in reporting sizes**
- **Differences in Sampling method and sample preparation**
- **Differences in Analytical Techniques**
- **Insufficient evidence on data quality**



International Symposium on  
**Environmental Microplastics**  
10-12 November 2021 (2-7 PM GMT+8:00)

## Program Agenda

10 Nov 2021 (Day1) – Microplastics Analysis – Challenges & New Developments

11 Nov 2021 (Day 2) – Environmental Microplastics – Monitoring and Assessments

12 Nov 2021 (Day 3) – Toxicological effects of Microplastics on human cells and other living organisms



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## Outcome of Microplastics 2021 Symposium

- ❑ The impact of microplastics in the environment is not fully understood and data are insufficient for regulators/policy makers to develop any policy or regulation on this. However, guidelines like policy from OECD and other federal agencies are an indications that they are also closely watching the progress and developments.
- ❑ More of research is required to fill the gaps. At the same time, harmonization and standardization are required to have wholistic approach and fruitful comparison of data distribution around the world.
- ❑ More collaboration among different research groups/researchers should be encouraged. Panel members were encouraged to make of use this community forum to develop collaborative work on mutual interests and common research topics.
- ❑ Lack of reference materials were high lighted. Inter comparison exercises are suggested. We will explore both with few organizations support.



# Where are we heading to????

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- **Establishing some harmonization in sampling and analysis**
- **Developing some standards for sampling and analysis**
- **Collecting Baseline data for a period of time**
- **Improve our understanding on MP impacts on human and environment**

# Steps taken by International agencies

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- UN environment agency urges ban of microplastics in cosmetics and personal care products
  - The number of scientific investigations has increased, along with public interest and pressure on decision-makers to respond
- The European Commission would put forward in 2022 a legislative proposal to reduce the release of microplastics in the environment and to restrict the addition of microplastics to products
- **WHO** calls for more research into microplastics and a crackdown on plastic pollution. Their studies revealed that particles above 130 micrometers are not likely to be absorbed by human body but smaller particles including nano size may affect but data is limited.
- **ISO/DIS 24187**- Principles for the analysis of plastics and microplastics present in the environment
- **ASTM D8332-20**: Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers
- **ASTM WK67788**: New Test Method for Identification of Polymer Type and Quantity (Mass) Measurement of Microplastic Particles and Fibers in Waters with High-to-Low Suspended Solids Using Pyrolysis-Gas Chromatography/Mass Spectrometry: Py-GC/MS

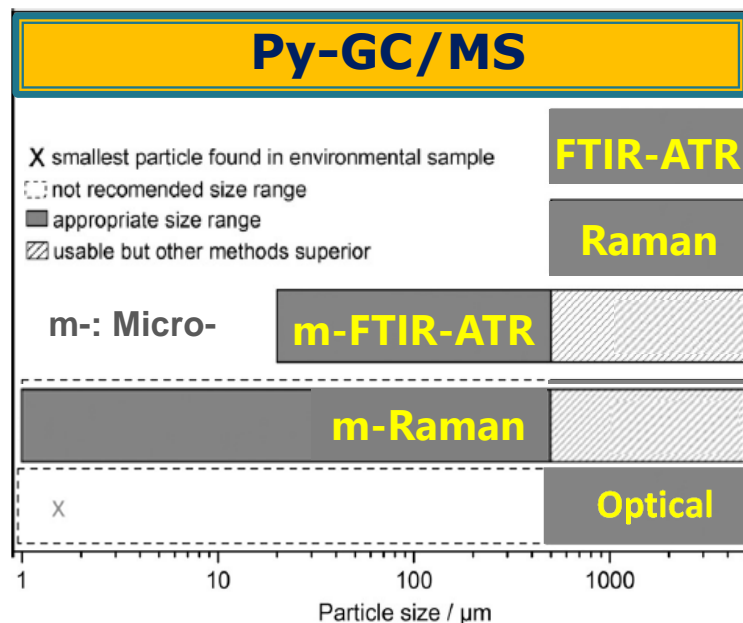
# State of art for MPs analysis

Dimensional characteristics (1  $\mu\text{m}$  ~ 5 mm)  
Surface area, shape, size, dispersion (value), diameter, color, etc.



C. Wu, K. Zhang et al.  
*Freshwater Microplastics*,  
p.85-99 (2018).

## Comparison of analytical methods



\*Based on N. P. Ivleva, A. C. Wiesheu, R. Niessner, *Angew. Chem. Int. Ed.* 2017, 56, 1720–1739.

## Micro-FTIR-ATR, micro-Raman

### Features:

- Non-destructive
- Widely dependent of particle/fiber mass

### Challenges:

- Extensive sample preparation
- Longer analysis time (2 - 8 hr)
- Limitation by particle size (FTIR-ATR)
- Quantitative analysis (number of particles/m<sup>2</sup>)

\*\*Based on A. K ppler,\* M. Fischer,\* and others, *Anal. Bioanal. Chem.*, 410 (2018) 5313–5327.

## Pyrolysis (Py)-GC/MS

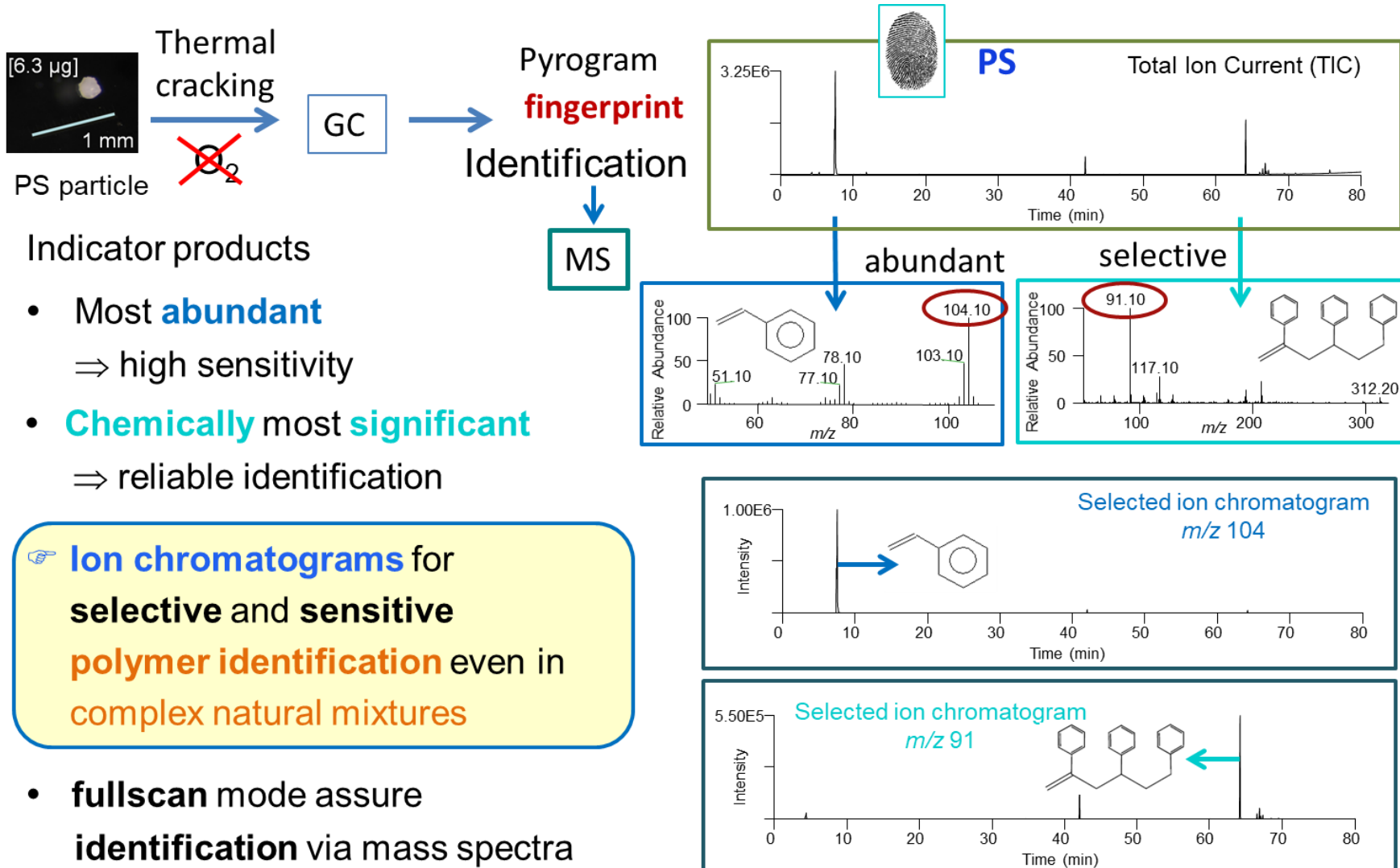
### Features:

- Widely independent of shape and size
- Inorganic contaminations do not disturb
- Shorter analysis time (60 min to 3 hrs)
- Software development for efficient qualitative/quantitative analysis is needed

### Challenges:

- Destructive analysis
- Standard method (ASTM etc. ) in progress

# PY-GCMS – Analytical Principle



Indicator products

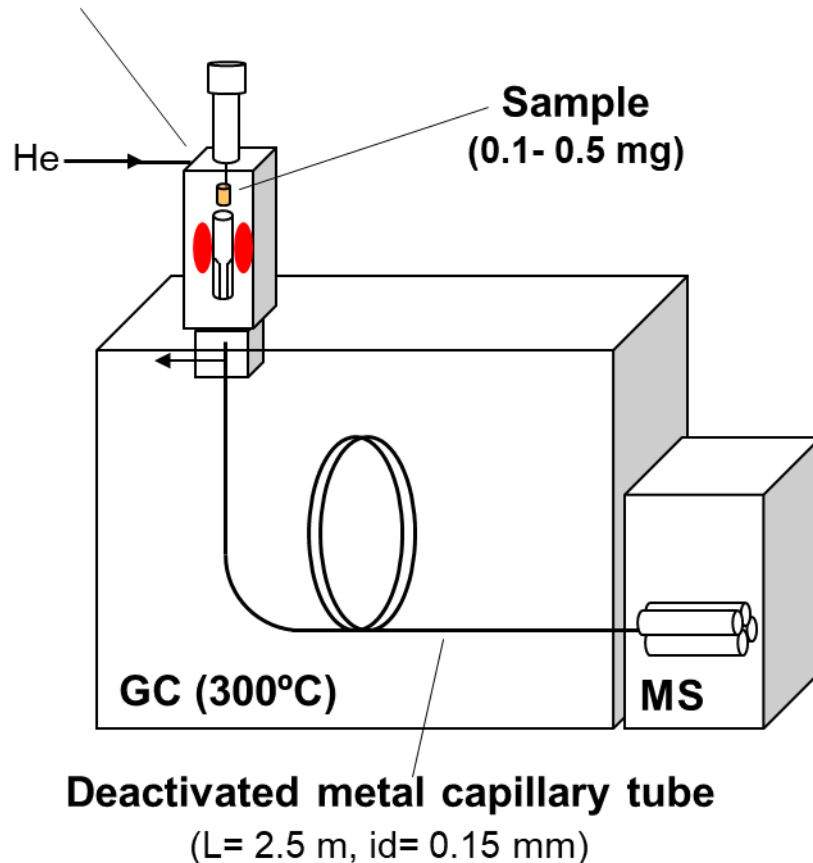
- Most **abundant**  
⇒ high sensitivity
- **Chemically** most **significant**  
⇒ reliable identification

👉 **Ion chromatograms** for **selective** and **sensitive** **polymer identification** even in **complex natural mixtures**

- **fullscan** mode assure **identification** via mass spectra

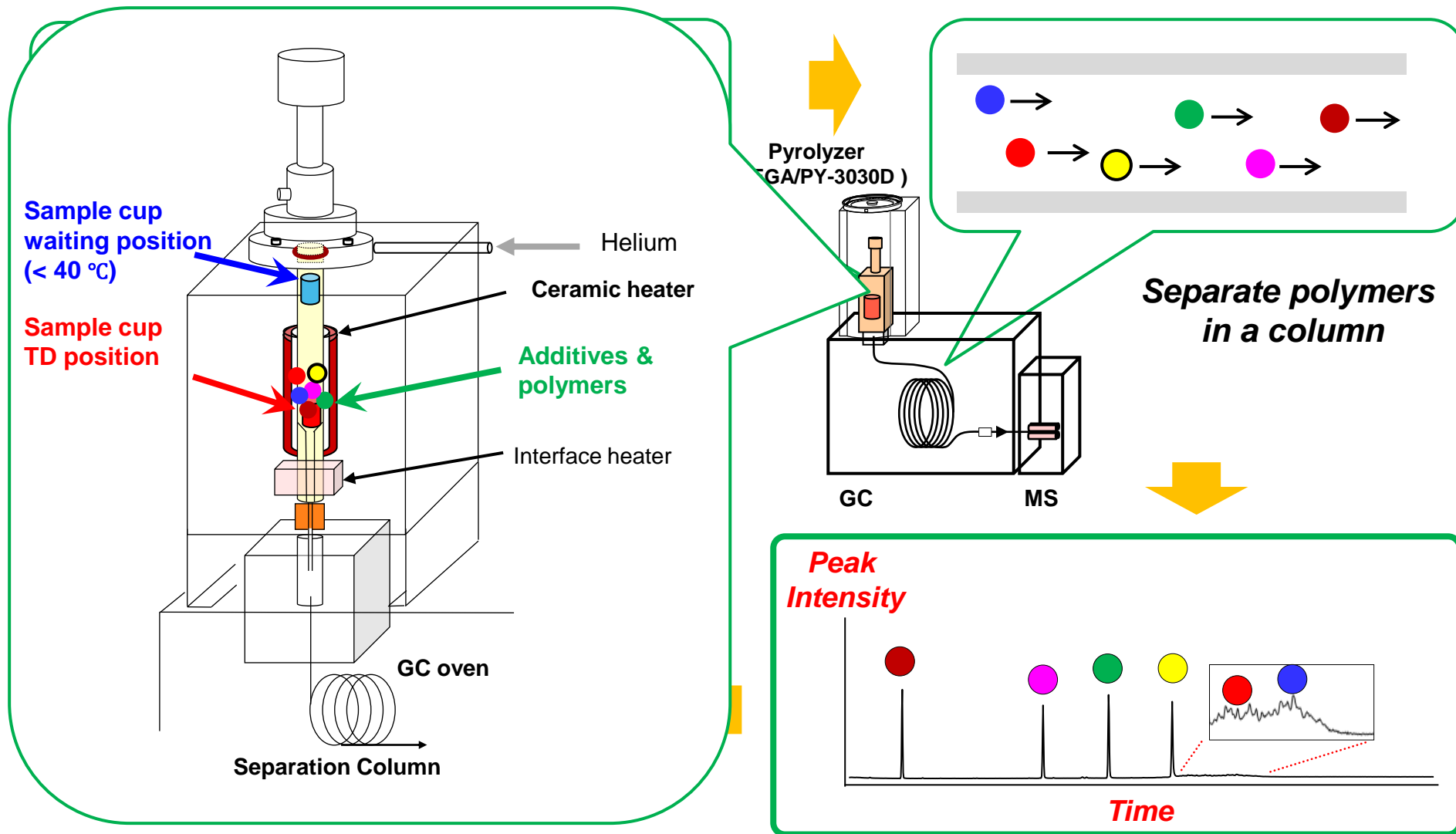
# Analytical Pyrolysis Introduction

Pyrolyzer with heating furnace  
100 - 700°C (20°C/min)



- **Analytical Pyrolysis is a technique in which the sample is thermally treated in a controlled environment. Evolved/ released compounds are introduced to GCMS for detailed analysis of volatiles, semi-volatiles and non-volatiles compounds**
- **A simple sample introduction system for GCMS applications where solid or liquid can be introduced without any detailed sample preparation.**

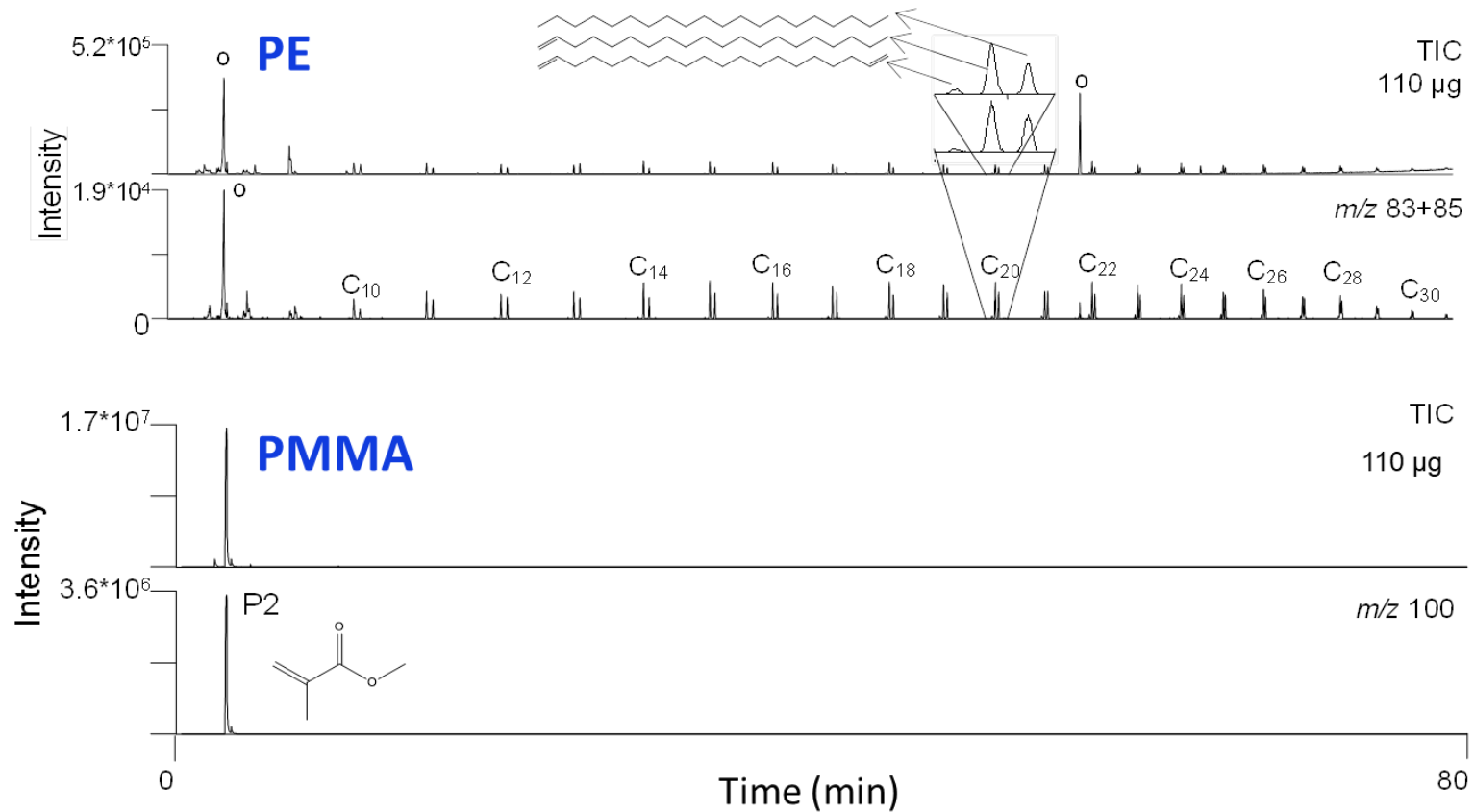
# PYGCMS working principles



Detection of peaks

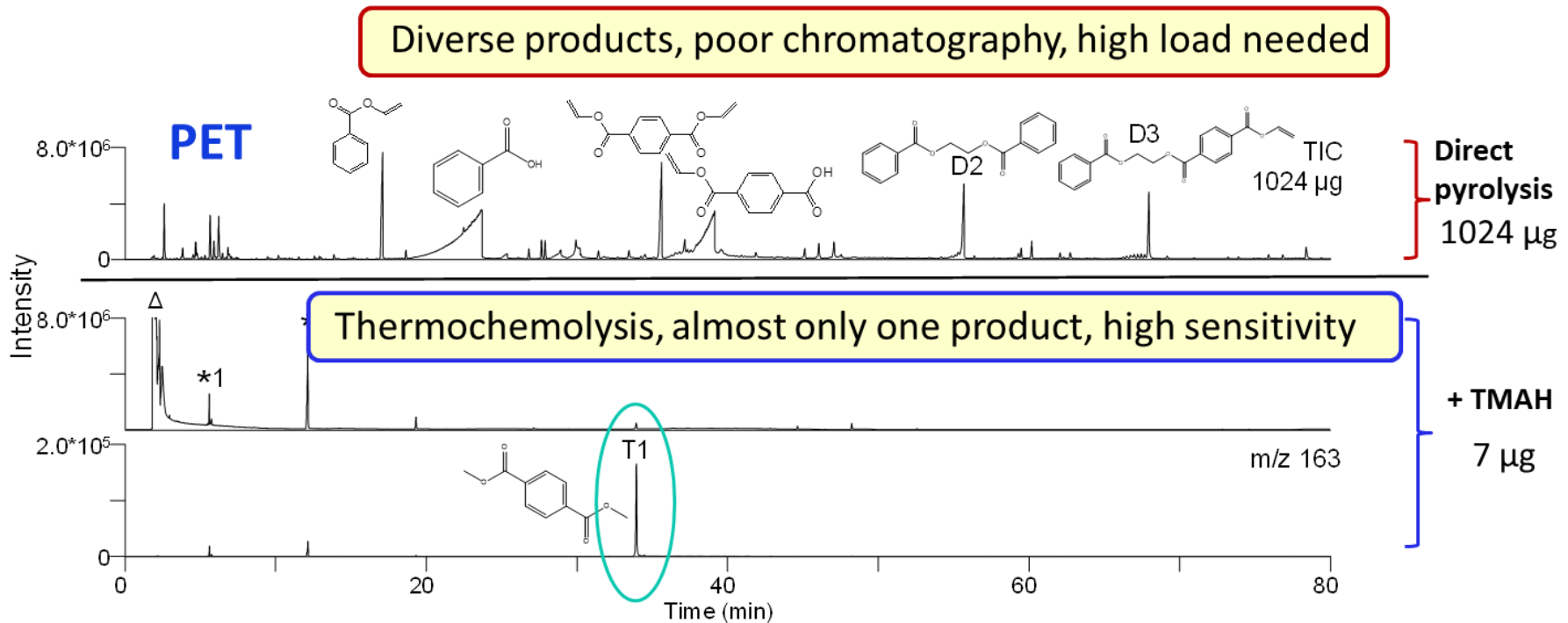
# PY-GCMS – Analytical Principle

## Different pyrolytic behavior of polymers



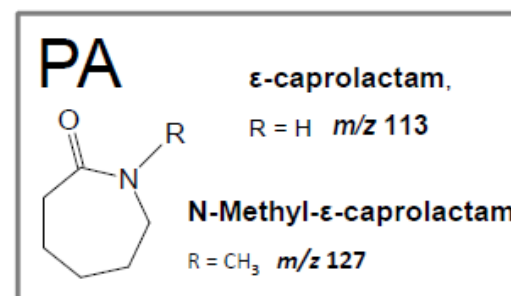
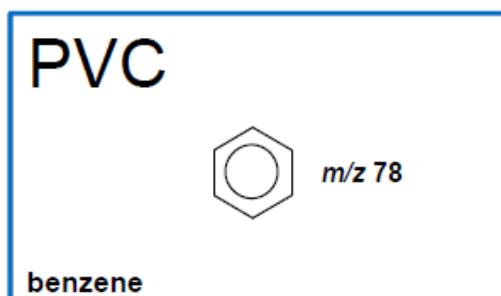
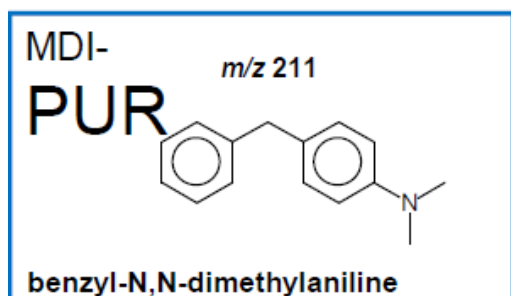
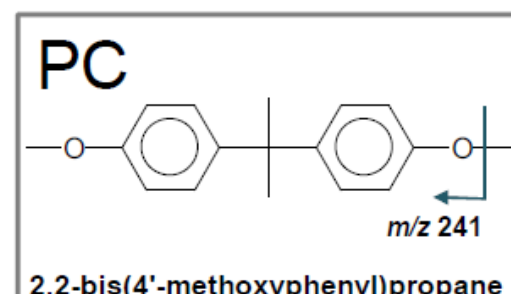
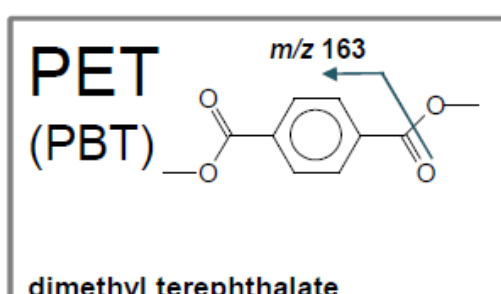
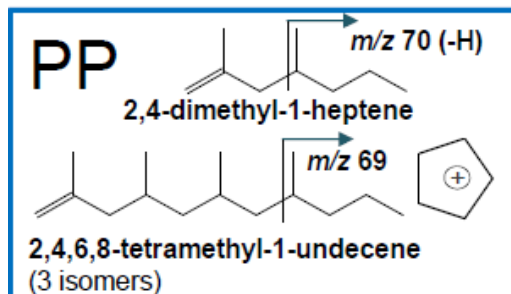
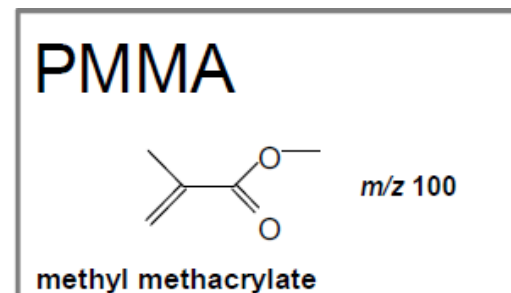
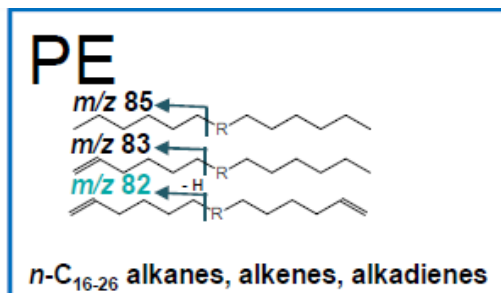
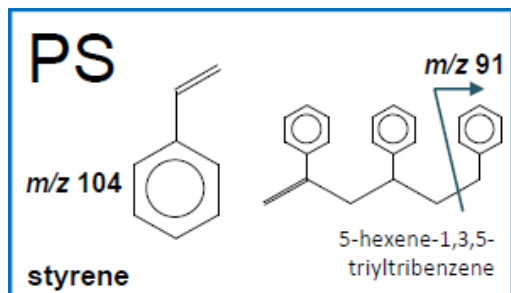
# PY-GCMS – Analytical Principle

- 👉 On-line derivatization with tetramethylammonium hydroxide (TMAH)
- 👉 Thermally assisted hydrolysis and methylation (e.g. Challinor, 2001)





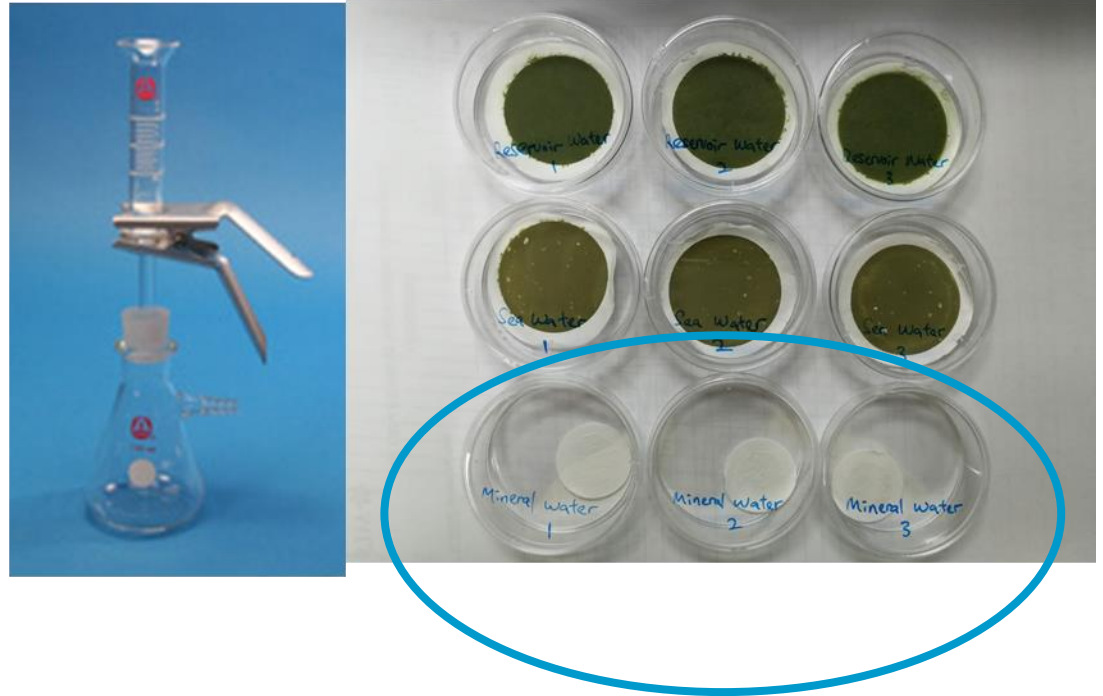
# PY-GC/MS MP quantification: Marker Ions



# Microplastics analysis in Water samples

## Sample Preparation

- **Glass fibre Filter**
- **Use small size (25mm)**
- **Prebake filter @ 700 oC**
- **Filter 2 Lit sample**
- **Analyse the filter directly**



**For Large volume filtration**

# Microplastics: Preconcentration (Water)

North Sea surface water (1.5 m bsl.), 220 L

Dibke et al.  
in prep.



Successive filtration 125  $\mu\text{m}$ , 20  $\mu\text{m}$ ; Fenton



> 20  $\mu\text{m}$

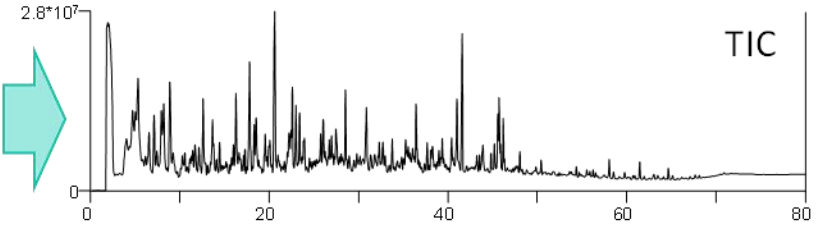
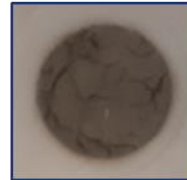


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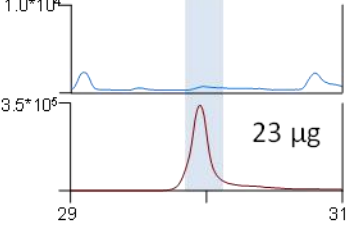
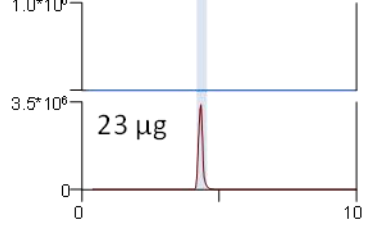
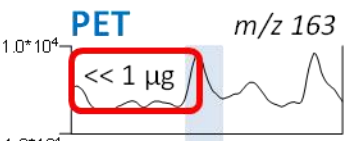
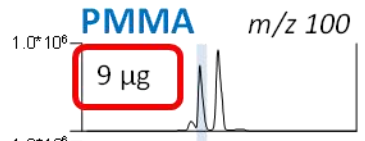
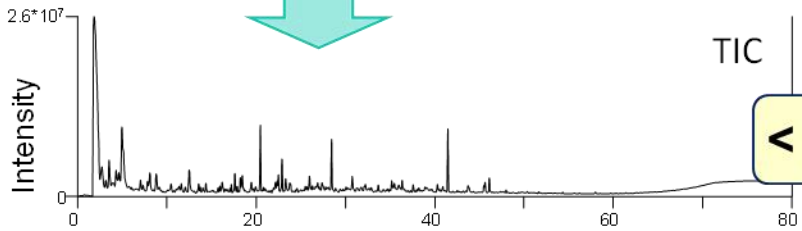
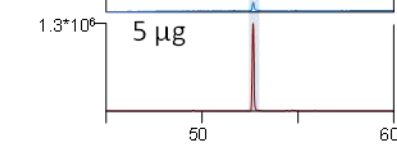
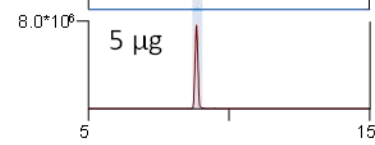
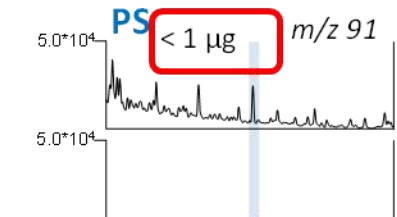
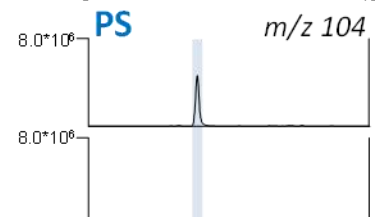
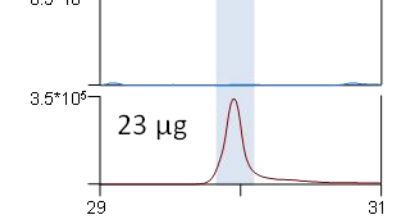
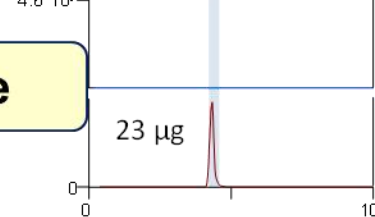
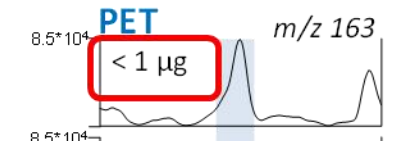
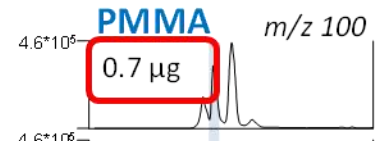


$\varnothing$  9 mm

> 125  $\mu\text{m}$



< ppb range



Sample  
Blank  
Standard

Time (min)

Time (min)

# Microplastics: Preconcentration (Sediment)

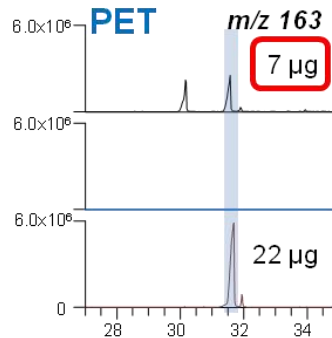
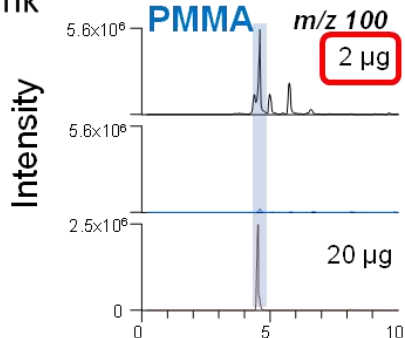
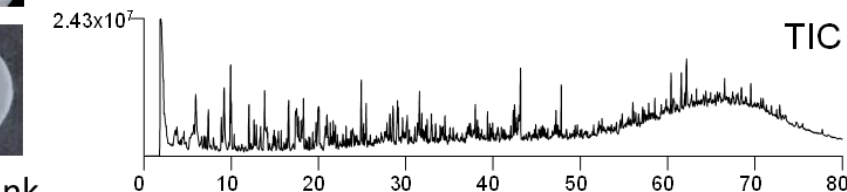
## Southern North Sea sediment 1 kg (wet weight)

Muddy, 1.29% TOC, 47% H<sub>2</sub>O

Density separation (NaBr); Fenton

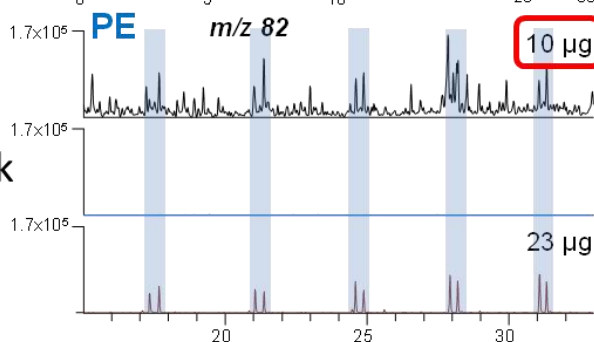


Proc. Blank



ppb range

Sample



Proc. Blank

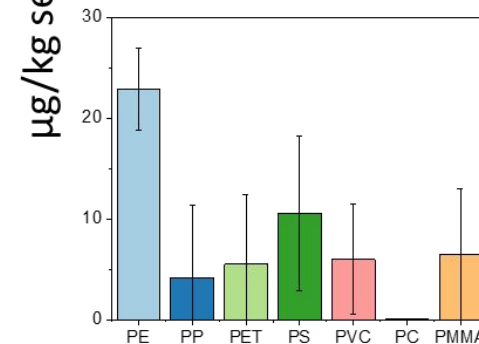
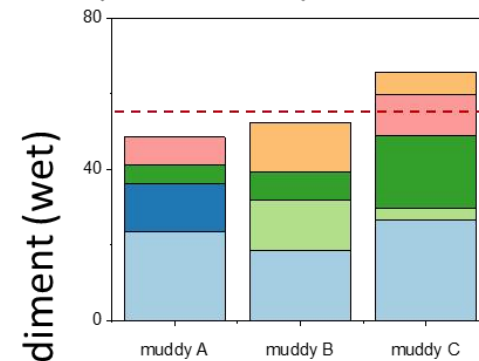
Standard

Time (min)

Fischer et al.



## Repeatability (n=3)



# Microplastics: Preconcentration (biota – fish)

**Sieve cascade** (stainless steel)

125 µm/ 64 mm / 40 µm/ 20 µm



Procedural blanks (secondary contamination)

## Density separation

NaBr solution ( $\rho \geq 1.46 \text{ g/cm}^3$ )

MPSS

Imhoff et al. 2012



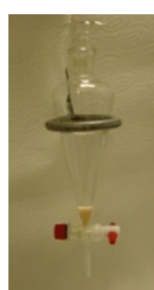
max. 6 kg

OPSS



max. 1 kg

Funnel



Small amounts

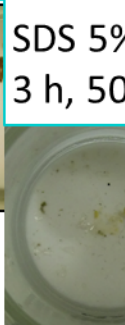
## Enzymatic-chemical-oxidative digestion

successive treatment with

5 stomachs



PE-washing



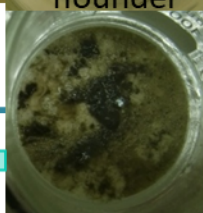
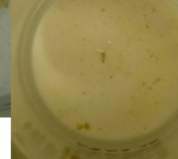
Glass crucible  
Ø 4 cm, 10 µm

SDS 5% Protease A-01

3 h, 50°C 24 h, 50°C

H<sub>2</sub>O<sub>2</sub> 30% (Chitinase 3 h, 50°C)

3 h, 50°C



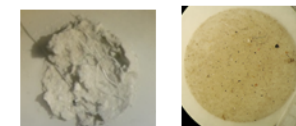
## Fentons-reagent



Fe (II)<sup>2+</sup> / H<sub>2</sub>O<sub>2</sub>

## Final filtration step

Anodisk- or GF-filter



Filter cakes

Ø 6-11 mm



Ø 4 mm

# Development of analytical method for MPs

---

**Develop an analytical method and analytical system for microplastics analysis using Py-GC/MS**



## **Challenges:**

1. Target plastic types
2. Secondary reactions among pyrolyzates (acid, alcohol, amine and unsaturated compounds)
3. Optimization of a separation column for various pyrolyzates
4. Qualification and quantification of plastics (Search software)
5. Matrices difficulties with environmental samples

# Development of analytical method for MPs

---

## **Our Solutions**

- 1. IQ Mill to homogenize the sample (filter/particles)**
- 2. Development of calibration standard**
- 3. UAMP Guard column**
- 4. Glass insert for improving peak shape**
- 5. MP Software for quick identification and quantification.**

# Microplastics Sample Preparation

**Water**

Proc. Blank



Ø 9 mm

> 125 µm



**Sediment**



**Biota**



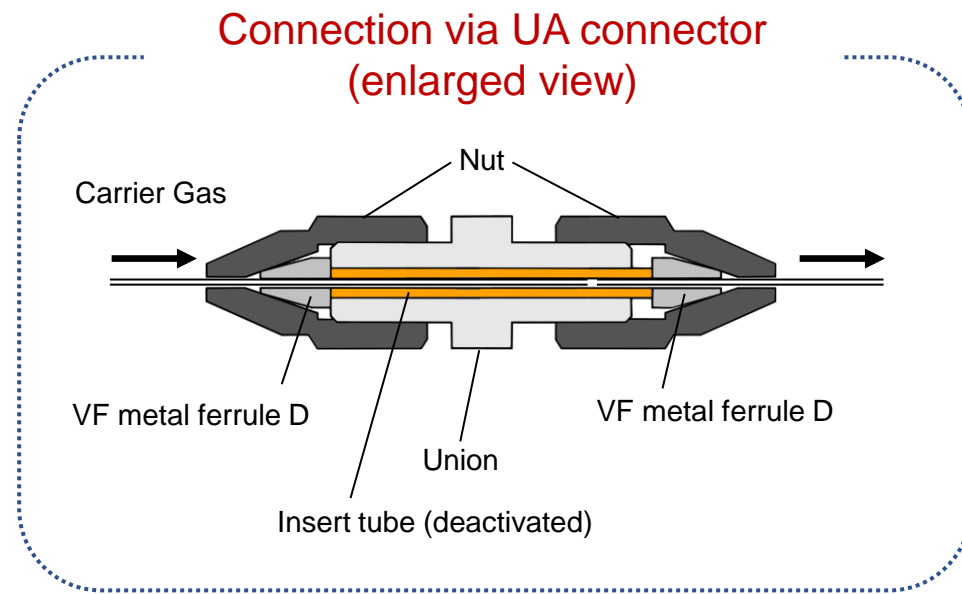
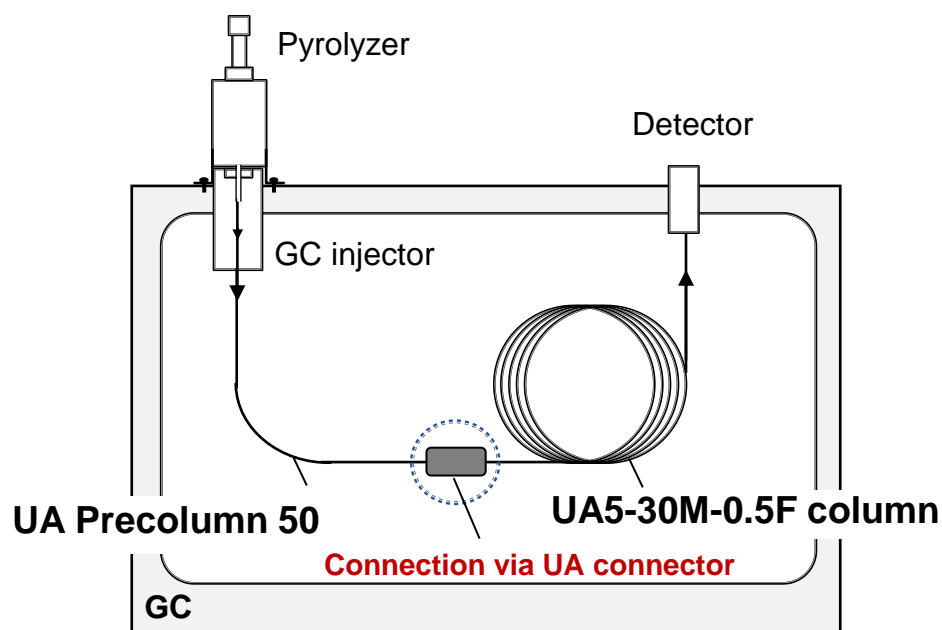
Ø 4 mm





# UAMP Column kit

This product consists of a separation column (UA5-30M-0.5F), a precolumn (UA Precolumn 50), and a connector (UA Connector). Using this product improves the accuracy of qualitative and quantitative analysis of plastics mixture samples using “F-Search MPs 2.0” software. Also, it reduces the contamination of the separation column, thus reducing the replacement frequency.



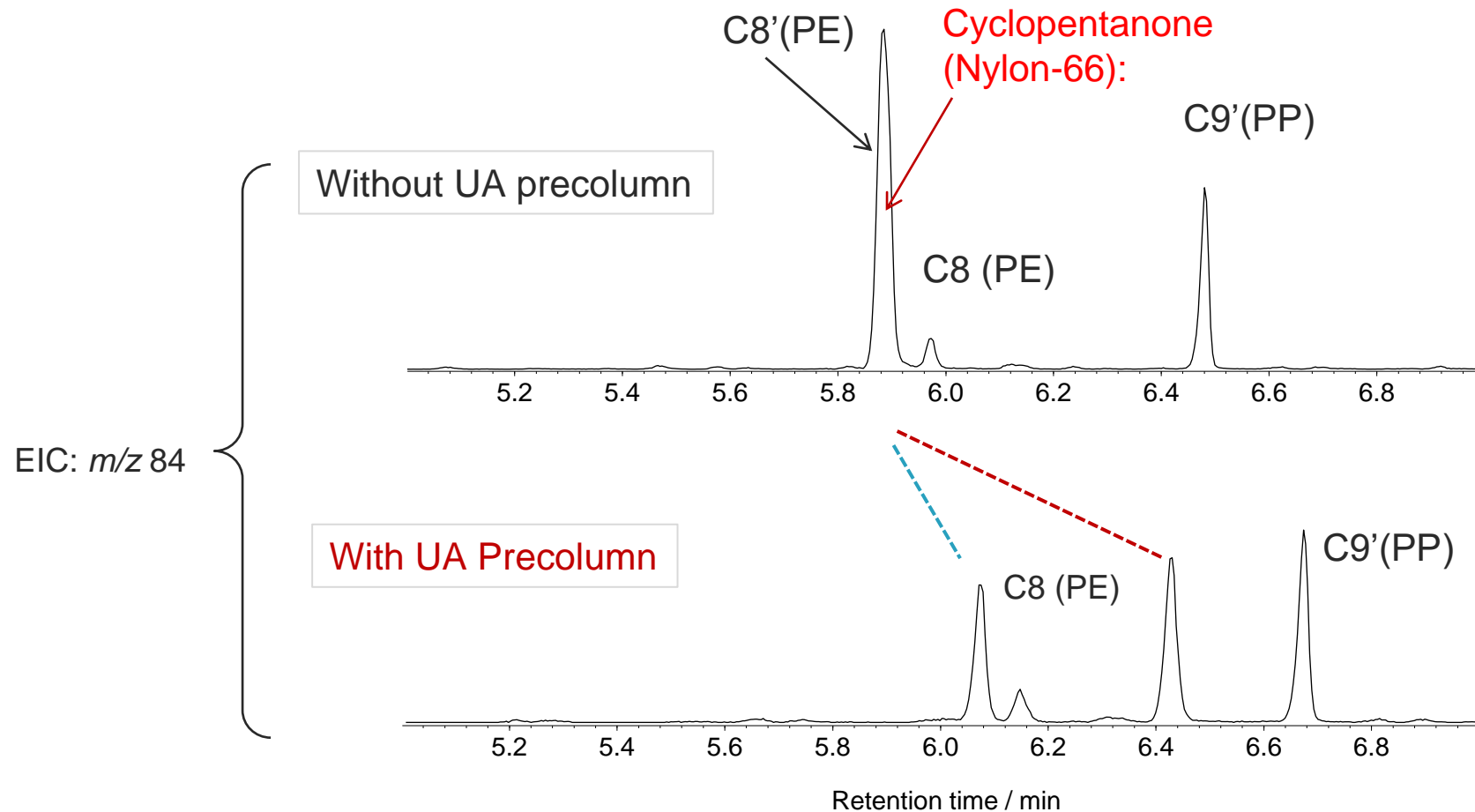
Product name	P/N	Contents
UAMP Column Kit	UAMP-K01	UA5-30M-0.5F (1ea.), UA Precolumn 50 (2 ea.), UA Connector (1 ea.), VF Metal Ferrule D (3 ea.), Wrench (6/8 mm, 2 ea.)

# Improvement of peak separation by UA precolumn

Oven: 40(2 min)-@20-280 °C, Column: Ultra ALLOY-5 (5% diphenyl-95% PDMS, 30 m, i.d. 0.25 mm, thickness 0.5 µm)

**Sample: PE: 320 µg, PP: 80 µg, Nylon-66: 18 µg**

Py at 600 °C

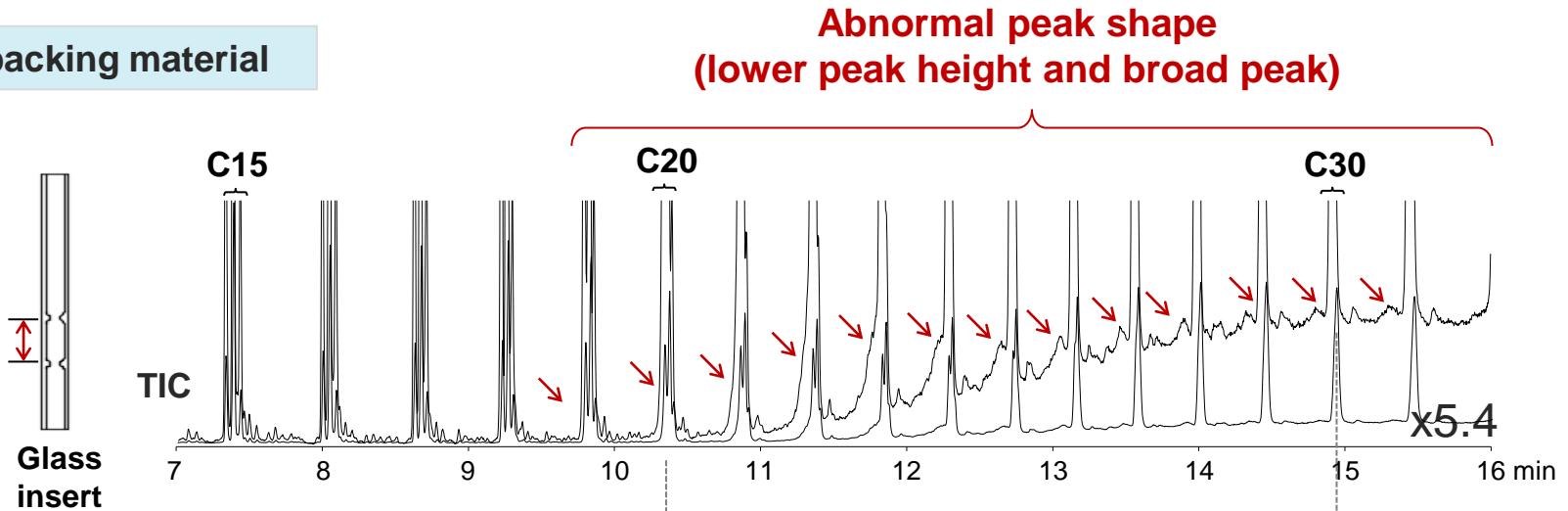


# Packed GC Glass insert

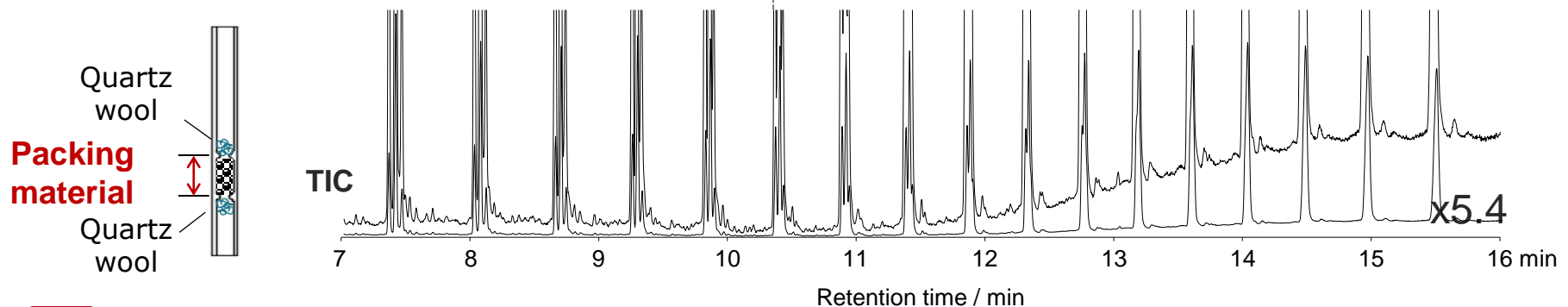
Py at 600 °C, Oven: 60-@20-320 °C, Column: Ultra ALLOY-5 (5% diphenyl-95% PDMS, 30 m, i.d. 0.25 mm, thickness 0.25 µm)

Sample: PE: 100 µg

Without packing material

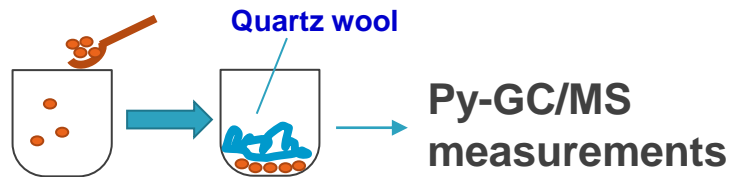


With packing material



# Development of Calibration standards

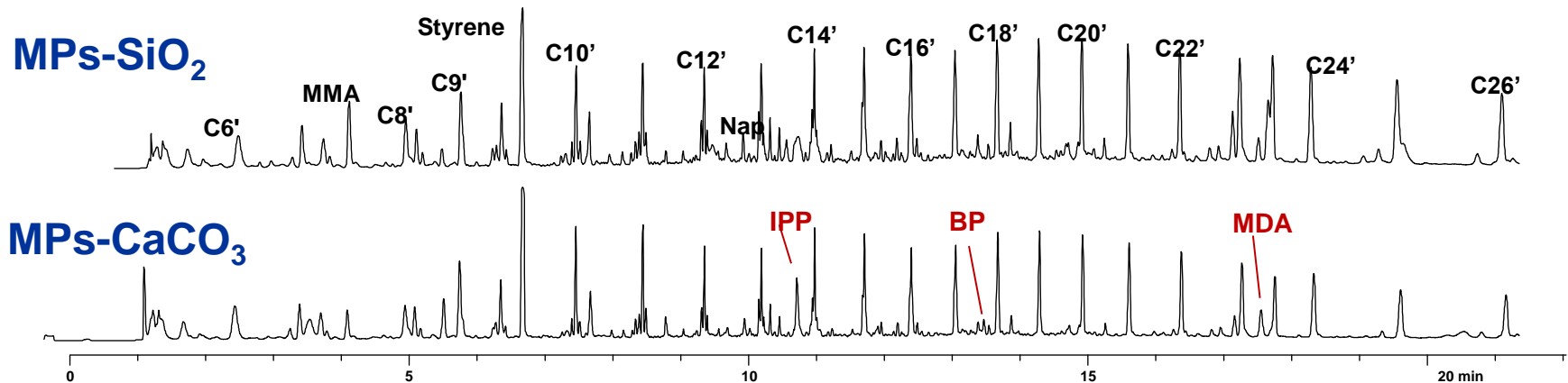
Put 4 mg of MP Calibration Standard into a sample cup



HR202i (A&D)  
< Min: 0.01 mg >



The amount of each polymer in the 4 mg of reference mixture (PE: 160  $\mu$ g, PP: 40  $\mu$ g, PS: 8  $\mu$ g, ABS: 16  $\mu$ g, SBR: 16  $\mu$ g, PMMA: 8  $\mu$ g, PC: 4  $\mu$ g, PVC: 40  $\mu$ g, PU: 4  $\mu$ g, PET: 16  $\mu$ g, N6: 5  $\mu$ g, N66: 18  $\mu$ g, diluent: 3.8 mg)

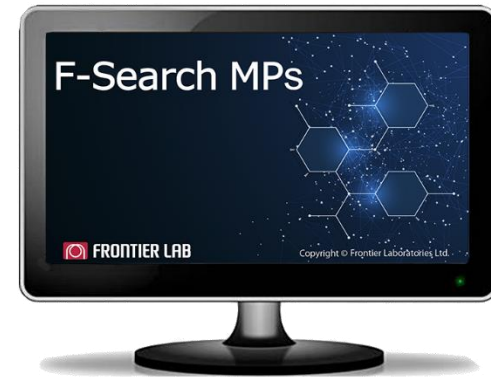


# Introduction F-Search MPs 2.0

---

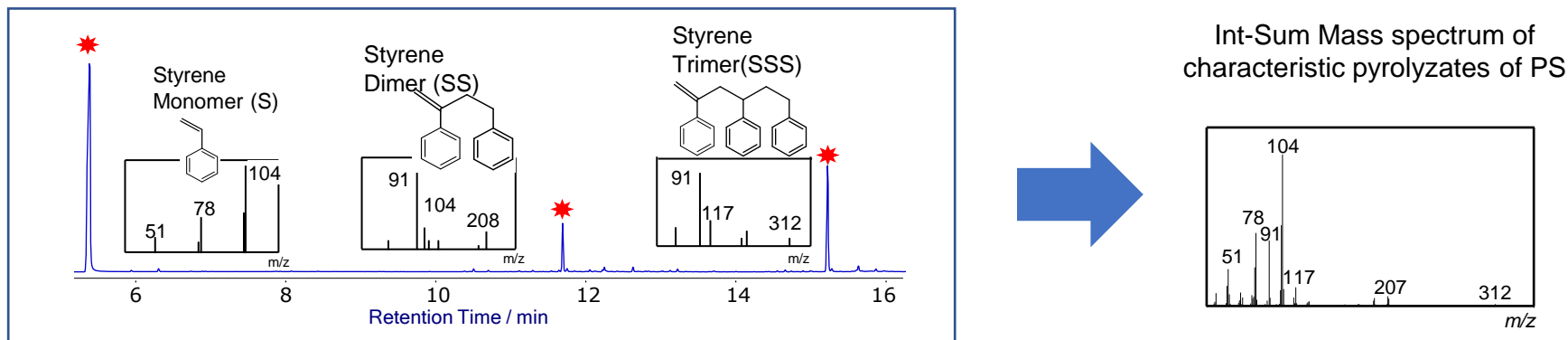
Recommended Py-GC/MS system configuration

- Microplastics Calibration Standard
- Sample prep procedure
- Select of MP Calibration Standard by targets
- Peaks used for quantitation - SiO<sub>2</sub>
- Peaks used for quantitation - CaCO<sub>3</sub>
- UAMP Column kit
- Improvement of peak separation by UA precolumn
- Consumable parts set for MPs
- Recommended Py-GC/MS system configuration (reiteration)

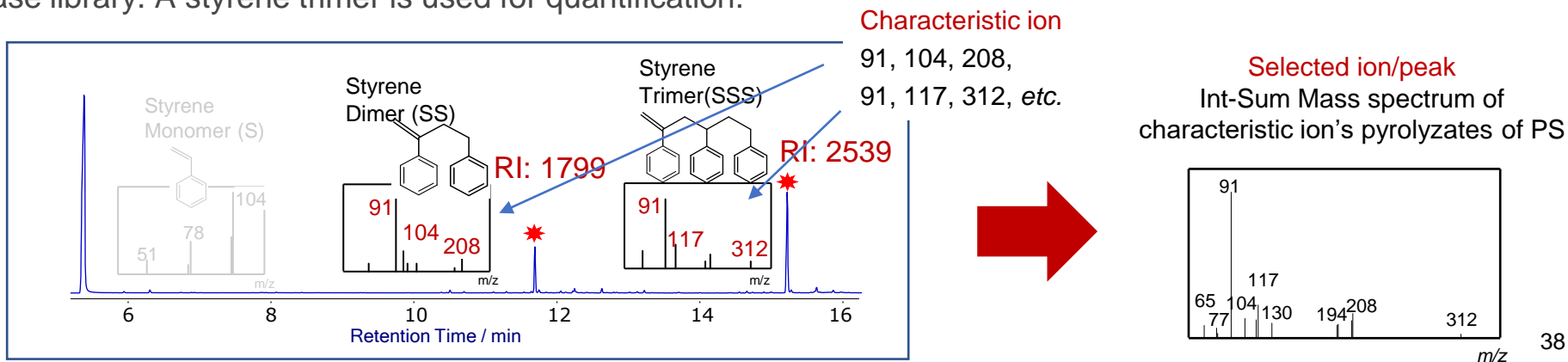


# Comparison of database and searching algorithm

**F-Search ver. 3.6** Styrene monomer, dimer, and trimer are selected as the characteristic pyrolyzates, and the mass spectrum obtained by Int-Sum for PS is registered in the database library. For qualification, detect peaks with a certain intensity, create Int-Sum mass spectrum for the sample, and search it on the database library.



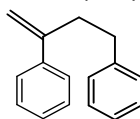
**F-Search MPs 2.0** Styrene monomer is not included as a characteristic pyrolyzate because it can be derived from other styrenic polymer, so the Int-Sum mass spectrum of characteristic ions of dimer and trimer is registered in the database. For qualification, these two peaks and characteristic ions are automatically detected from RI and search it on the database library. A styrene trimer is used for quantification.



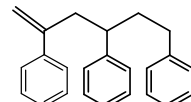
# Search algorithm of F-Search MPs 2.0

A: Registered polymer in database library (e.g. PS)

Styrene Dimer (SS)



Styrene Trimer (SSS)

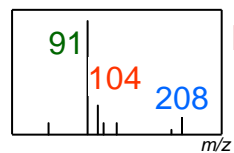


RI: 2488

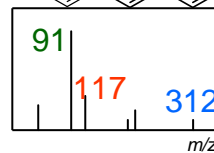
Int-Sum mass spectrum of SS and SSS

Database library

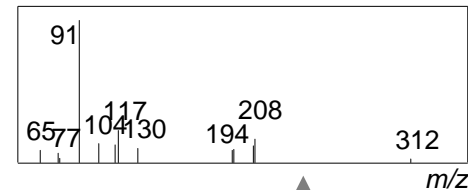
TICC



RI: 1749



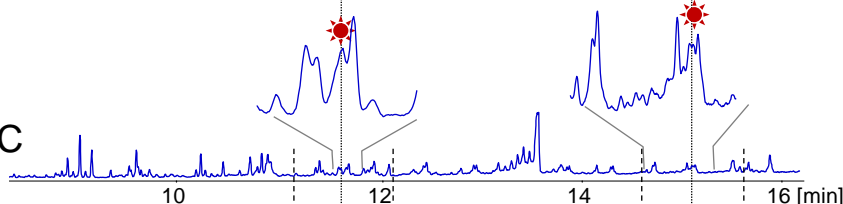
RI: 2488



10 12 14 16 [min]

B: Unknown sample (Search for PS)

TICC



Search range

EIC

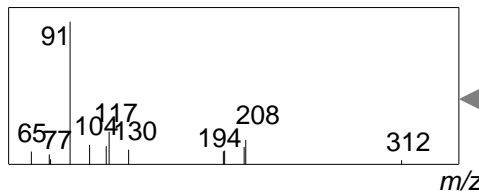
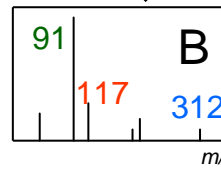
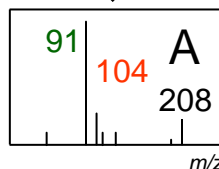
m/z: 91  
m/z: 104  
m/z: 208

m/z: 91  
m/z: 117  
m/z: 312

**Step 1 :**  
Detect EIC of characteristic ions of pyrolyzates

**Step 2:**  
Obtain mass spectrum based on Step 1

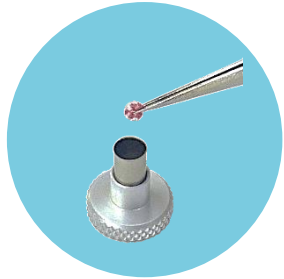
**Step 3:**  
Sum up mass spectrum of A and B



**Step 4:**

Calculate matching % of mass spectrum between unknown sample and registered polymer in database library

# Analytical procedure



**Sample  
Prep**



**Pyrolysis**

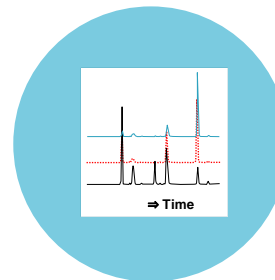


**Data  
analysis**



**Introduction**

**GC/MS**





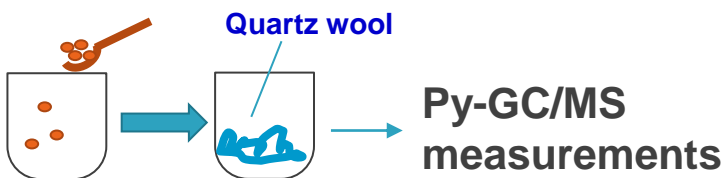
# Establishing Calibration Curve



Put Known weight of MP Calibration Standard into a sample cup



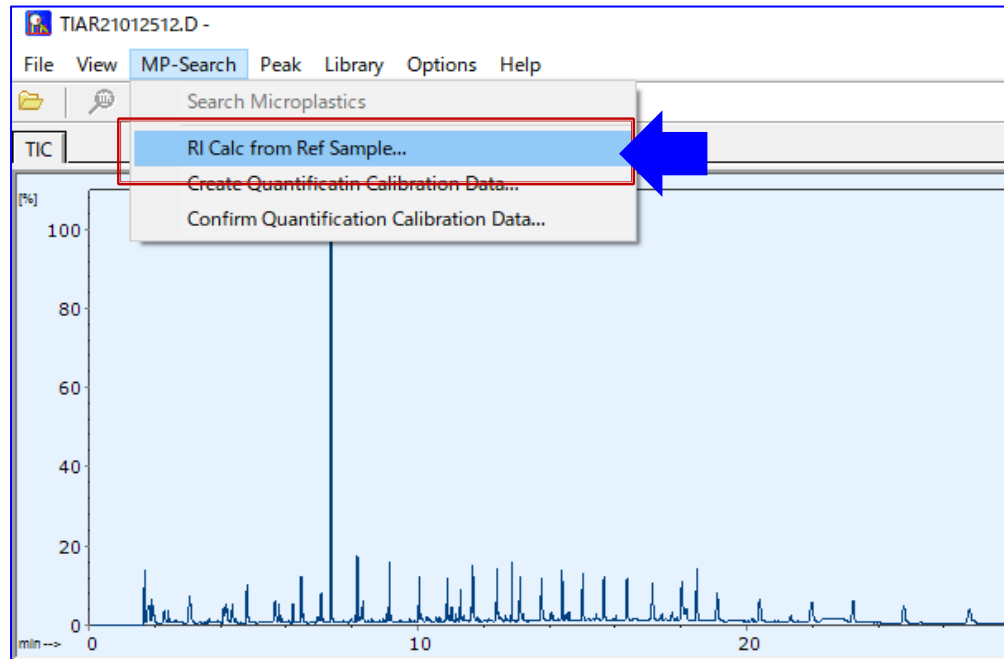
HR202i (A&D)  
< Min: 0.01 mg >



Polymer	4 mg std	2 mg std	1 mg std
	(concentration in $\mu\text{g}$ )		
PE	160	80	40
PP	40	20	10
PS	8	4	2
ABS	16	8	4
SBR	16	8	4
PMMA	8	4	2
PC	40	20	10
PVC	10	5	2.5
PET	16	8	4
N6	5	2.5	1.25
N66	18	9	4.5

# Step 1: Creating a plot of retention index (RI)

- RI is created based on the pyrolyzates of Polystyrene in MP Calibration Standard



Load a pyrogram data of MP Calibration Standard

The 'RI Calc From PS' dialog box contains the following data:

	Retention Index	Retention Time	
		Current	Detected
Toluene:	787	5.884	5.658 [min]
Styrene monomer:	917	7.332	7.359 [min]
Styrene dimer:	1799	13.498	14.508 [min]
Styrene trimer:	2539	17.543	21.344 [min]

Buttons at the bottom: Default RI, OK, Update, Cancel.

Auto-detection of RI

# Step 2: Creating a calibration file (3 of 4)

- Select "Add Rep. Files" and upload data files and input sample amount of each polymer.
- Then select Index level (different concentration) "2" and upload data files. You may use multiplier to automatically input sample amount of each polymer.

# Cal. Levels: 3 Apply Index Level: 1 Max number of files: 10

Add Rep. Files Delete Rep. Files

File name

Polymer	Amount [ug]
PE	160
PP	40
PS	8
ABS	16
SBR	16
PMMA	8
PC	4
PVC	40
PU	10
PET	16
N6	5
N66	18

Polymers with zero set cannot be quantified.

Next

Load data file and input sample amount

# Cal. Levels: 3 Apply Index Level: 2 Max number of files: 10

Add Rep. Files Delete Rep. Files

File name

Polymer	Amount [ug]
PE	80.0
PP	20.0
PS	4.00
ABS	8.00
SBR	8.00
PMMA	4.00
PC	2.00
PVC	20.0
PU	5.00
PET	8.00
N6	2.50
N66	9.00

Polymers with zero set cannot be quantified.

Next

Load data file of different conc.

# Cal. Levels: 3 Apply Index Level: 3 Max number of files: 10

Add Rep. Files Delete Rep. Files

File name

Polymer	Amount [ug]
PE	16.0
PP	4.00
PS	0.80
ABS	1.60
SBR	1.60
PMMA	0.80
PC	0.40
PVC	4.00
PU	1.00
PET	1.60
N6	0.50
N66	1.80

Polymers with zero set cannot be quantified.

Next Cancel

Load data file of different conc.

# Step 2: Creating a calibration file (4 of 4)

Confirm Quantification file - T1220614\_MP Calib\_JAS.qfl

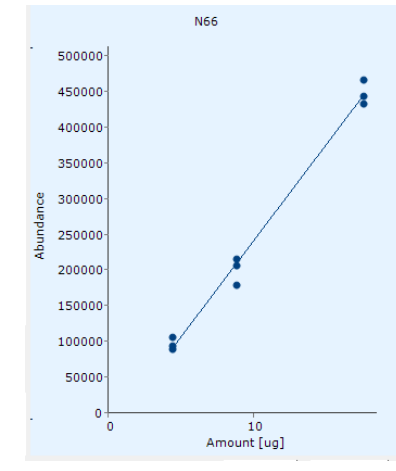
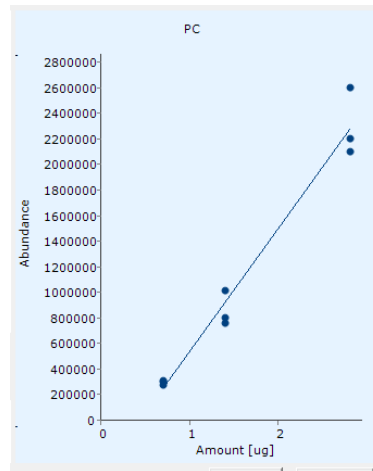
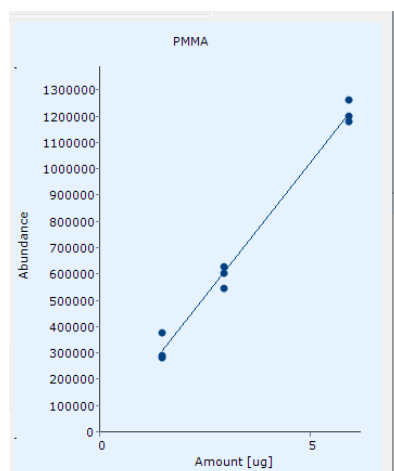
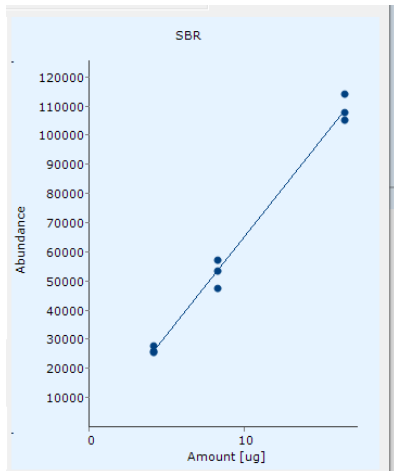
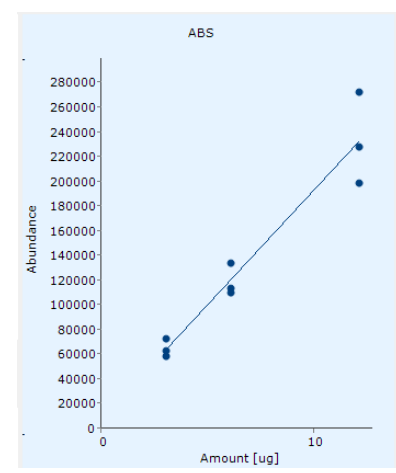
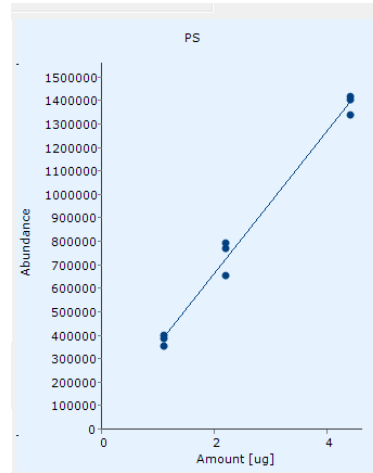
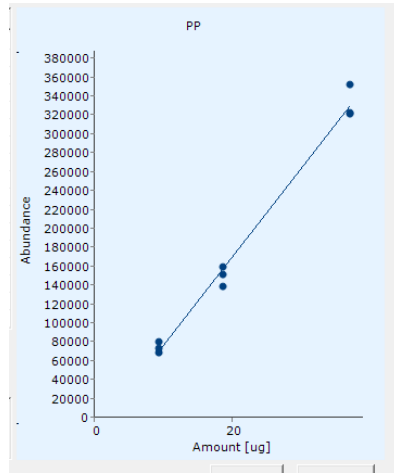
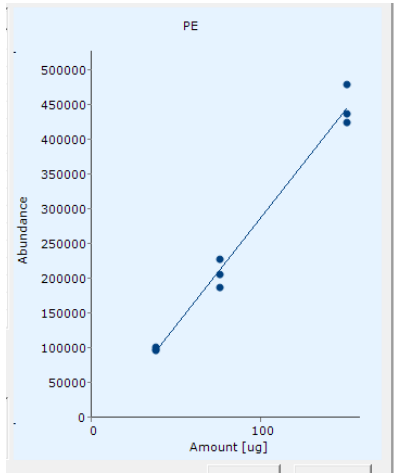
Index Level: 1 # Rep. File: 1/3 Calibration Curve: CF Type: Linear Chart: Area R<sup>2</sup> = 0.9865  
 Zero: Not Forced y = 3.1007E+03x - 2.1664E+04

Pol...	P...	m...	RT [min]	Area	Avr Area	Height	Avr Height	Amount [...]	T...	LOQ ...
PE	C...	82	17.884	96093	98492	21884	21639	37.7	B...	
PP	C...	1...	7.327	68696	74241	17250	17065	9.28	B...	
PS	S...	91	25.048	357739	382297	56004	59071	1.10	B...	
ABS	S...	1...	20.905	58229	64618	8352	9067	3.02	B...	
SBR	S...	1...	12.757	26134	26666	6676	6938	4.10	B...	
PMMA	M...	1...	6.057	284867	318547	60221	61560	1.48	B...	
PC	B...	2...	21.106	278797	300666	32716	34577	0.70	B...	
PVC	N...	1...	11.676	259928	251312	61340	59585	11.2	V...	
PET	B...	1...	11.087	87979	108534	16257	20530	3.58	B...	
N6	C...	1...	12.506	45813	53878	8495	9659	1.27	B...	
N66	C...	84	7.367	90002	97140	14455	15347	4.40	B...	

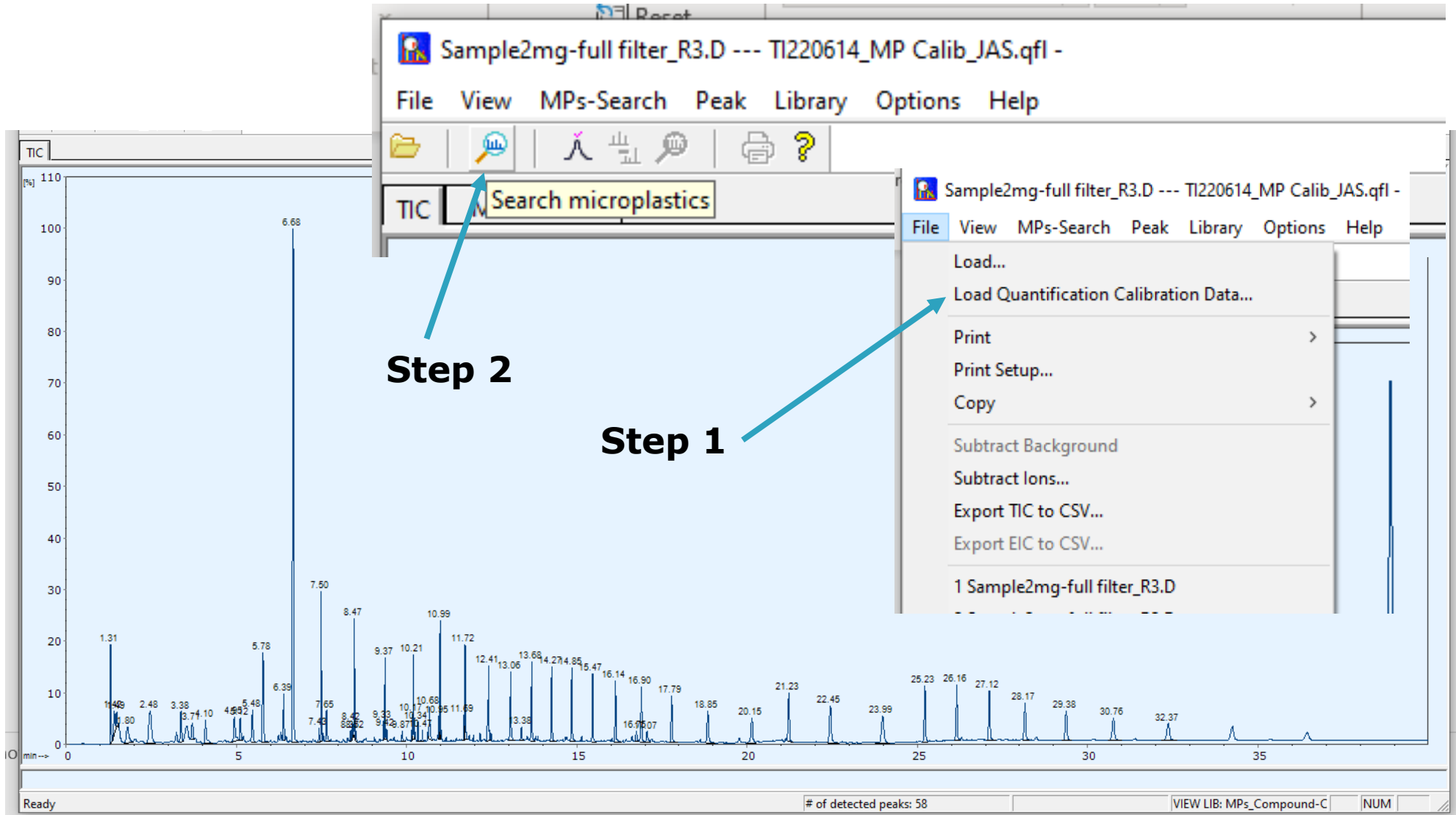
# Rep. File of ISTD

Save Copy Table

# Step 2: Creating a calibration file (4 of 4)



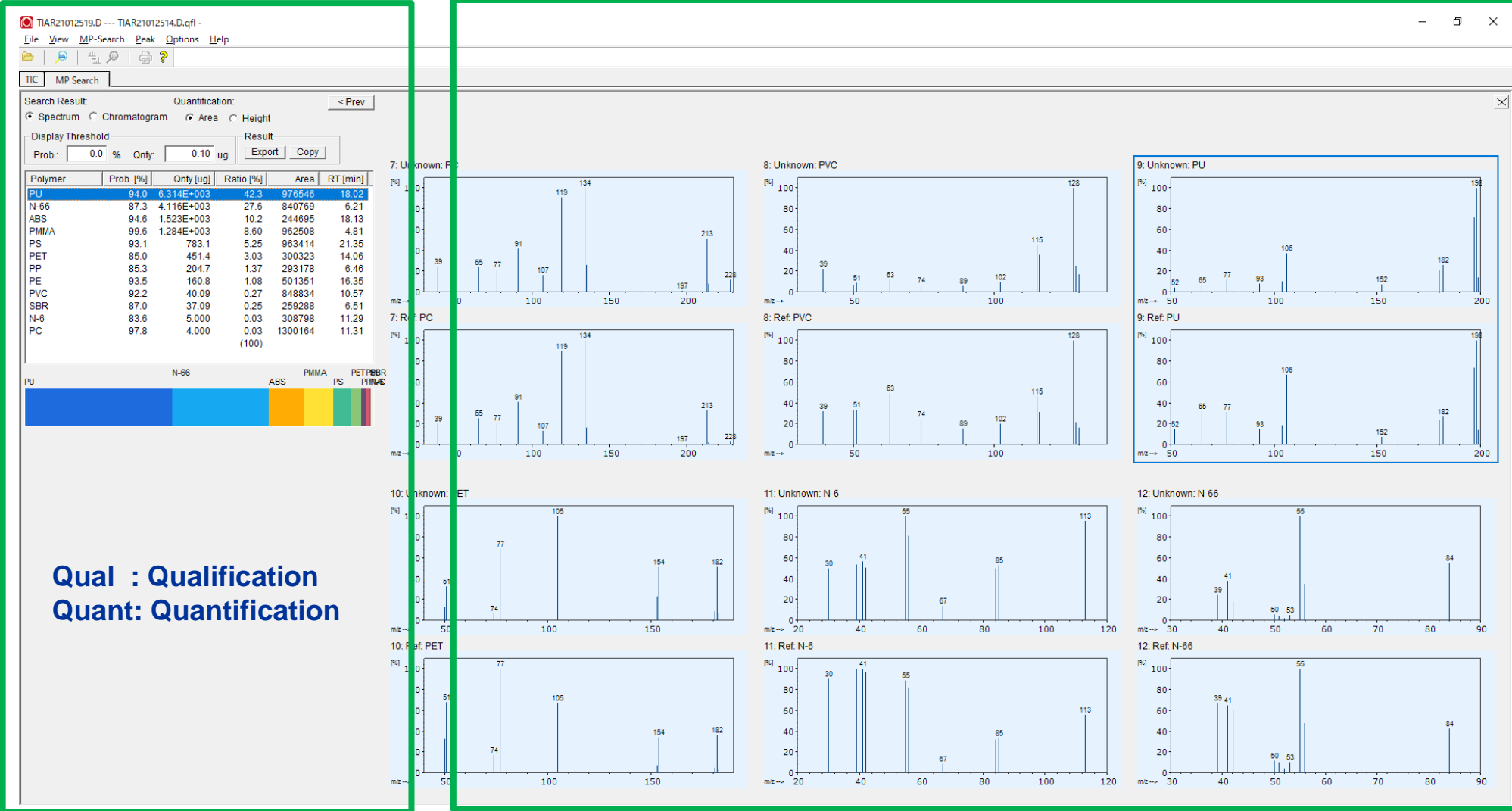
# Data interpretation of unknown sample



# Searching result (1/3) display overview

## Results of Qual/Quant\*

## Mass spectrum and peak shapes

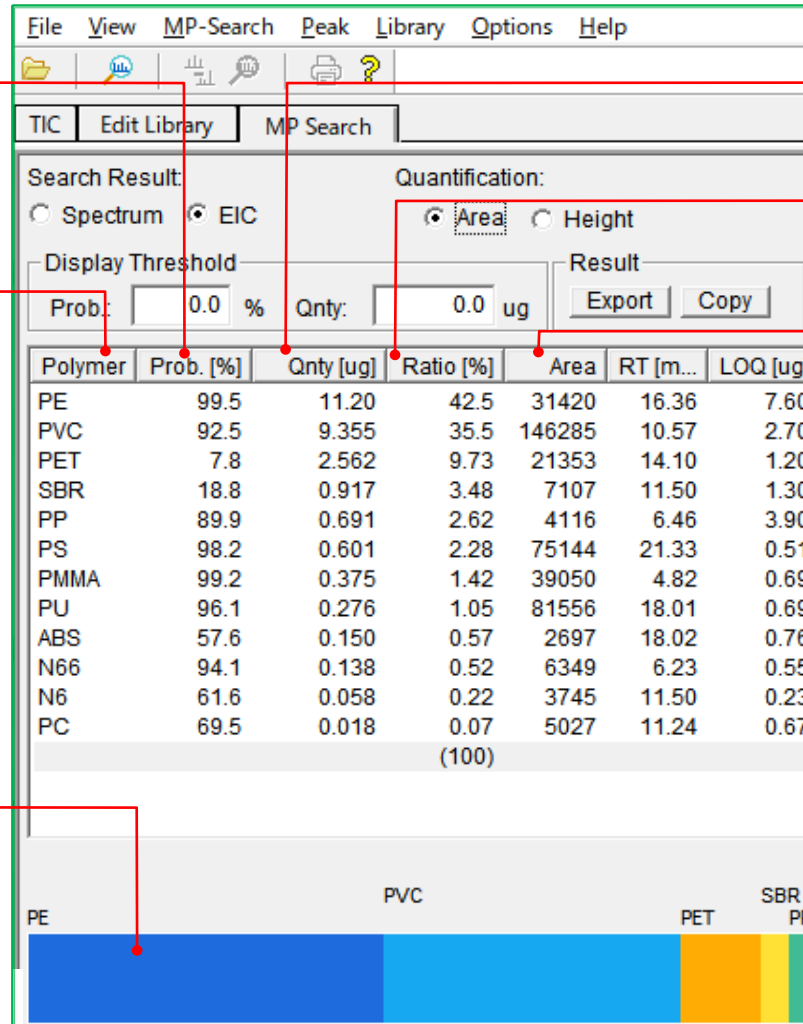


# Searching result (2/3) Qual. and Quant.

Library Match Quality (%)

Plastic Name

Bar graph of Relative Amount (%)



Quant Result ( $\mu\text{g}$ )

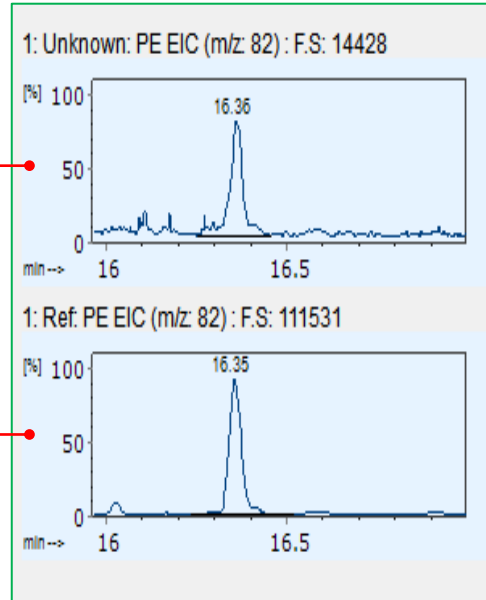
Relative Amount (%)

Peak area



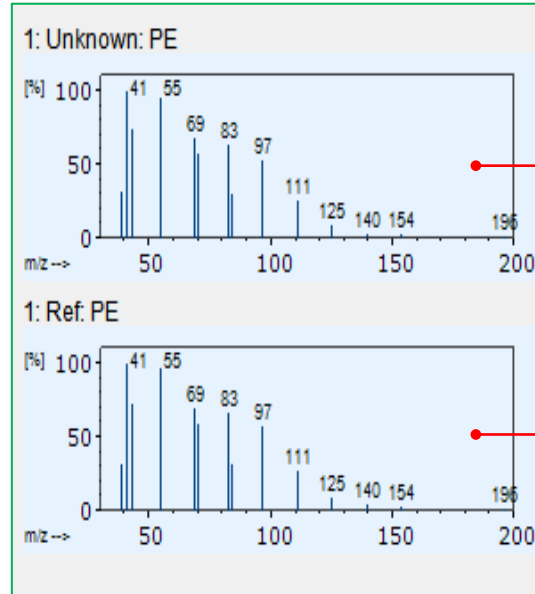
# Searching result (3/3) Mass spectrum/Peak shape

Marker peak of Sample



Marker peak of MPs Calibration Standard for calibration

Mass Spectrum of Sample peak



Mass Spectrum of MPs Calibration Standard for calibration

- Each polymer has its own marker peaks and ions

# Concluding Remarks

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- **Py-GCMS is a simple and fast technique for microplastics Analysis in Drinking Water and other environmental samples**
- **It is independent of size and shape of microplastics. It can analyze all sizes/shapes together**
- **It helps determining the mass concentration than particle numbers.**
- **For drinking Water, simply filter the water sample through 0.2-micron GF Quartz filter paper (10 mm or 25 mm dia). Pulverize the paper or 1/4<sup>th</sup> of the filter directly using Py-GCMS**
- **Use of Frontier Microplastics Software improves data interpretation for identification and quantification.**
- **LOQ for many of MPs are less than 1 µg**