Using a GC based effect-directed approach for the analysis of AhR active contaminants on plastic litter

MTM Research Centre
Man Technology
Environment

Christine Schönlau[1], Jeroen Kool[2], Maria Larsson[1], Magnus Engwall[1] and Anna Kärrman[1]

[1]Man Technology Environment (MTM) Research Centre, Örebro University, 701 82 Örebro, Sweden,

[2]Division of BioAnalytical Chemistry, VU University Amsterdam, De Boelelaan 1108, 1081 HZ, Amsterdam, The Netherlands

Background

Why effect-directed analysis???

- Contamination in environment often by multitude of chemicals → **complex samples**

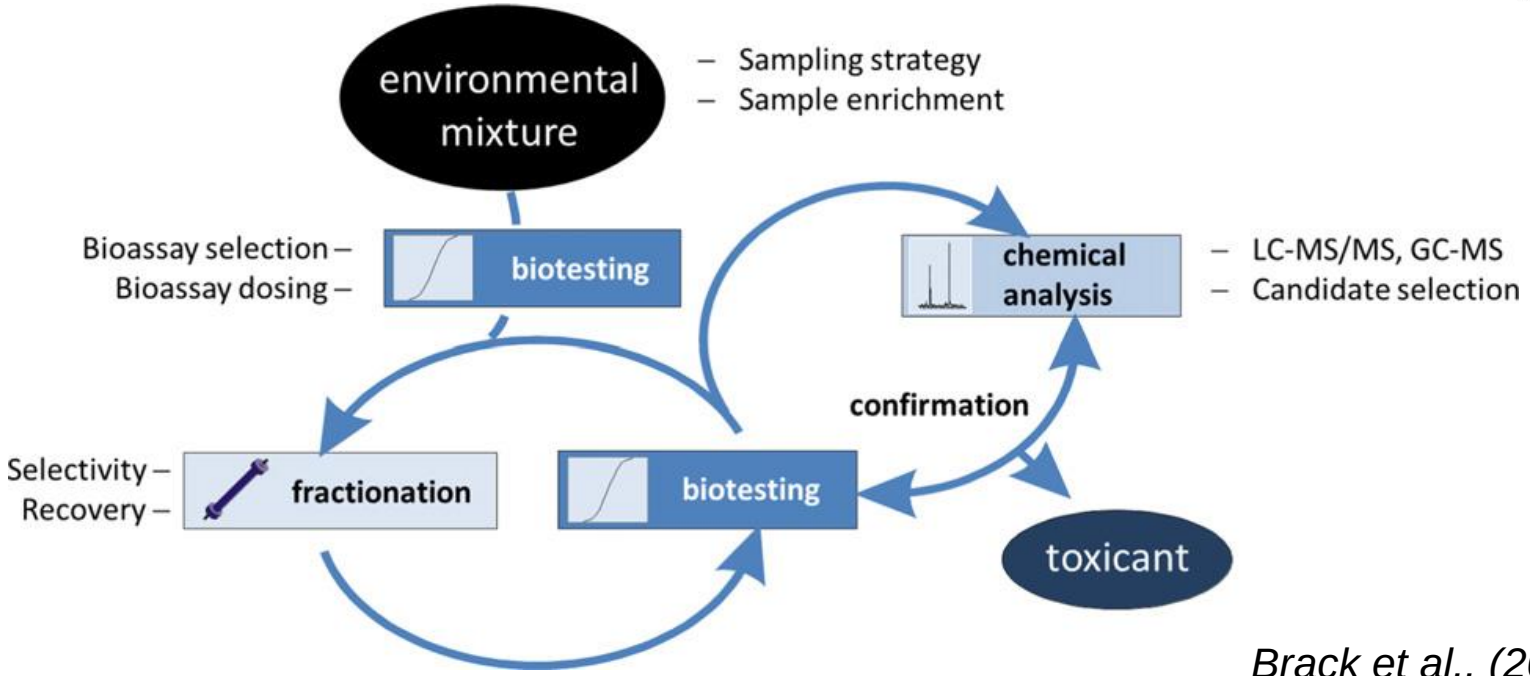
- Risk assessment: Traditionally chemical target analysis of contaminants, e.g. priority pollutants → modelling of toxicity based on individual compound toxicity data

- Key contaminants unknown?!!

- Basic idea: sequentially **reduce complexity** of an environmental sample by removing nontoxic components

  → enable chemical identification of causative agents

Traditional EDA approach



Brack et al., (2016)

- How much of measured effects can be explained by identified compounds?
- How much is not explained by them?

Background of the project

- Plastic pollution – chemical risk for marine ecosystems

➢ World plastic producti… …Europe, 2016)

➢ More …

➢ Absor… …ves and monol…

Aim

Assessment of potential toxicological effects of marine plastic and identification of causative agents.

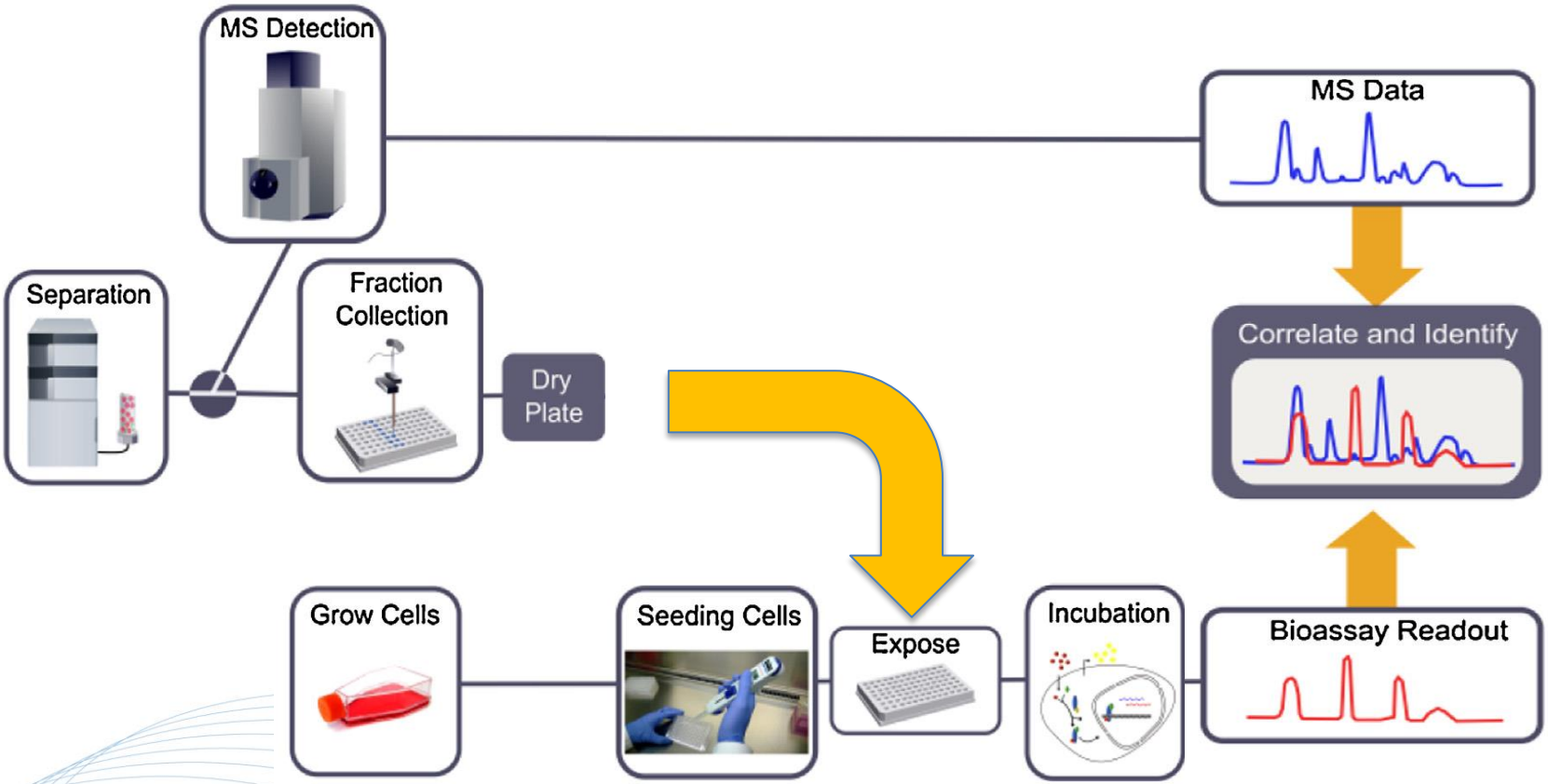
Fractionation in traditional EDA

- Liquid chromatography preferably used
- For analyzing the nonpolar POPs → normal phase LC
- Usually GC used for nonpolar substances
- Fractionation after GC analysis requires complex setup

- Drawback: Very **time-consuming**

Principle of simultaneous analysis

- Aim: rapid activity-directed screening of compounds by parallel coupling of liquid or gas chromatography with a reporter gene assay and mass spectrometry



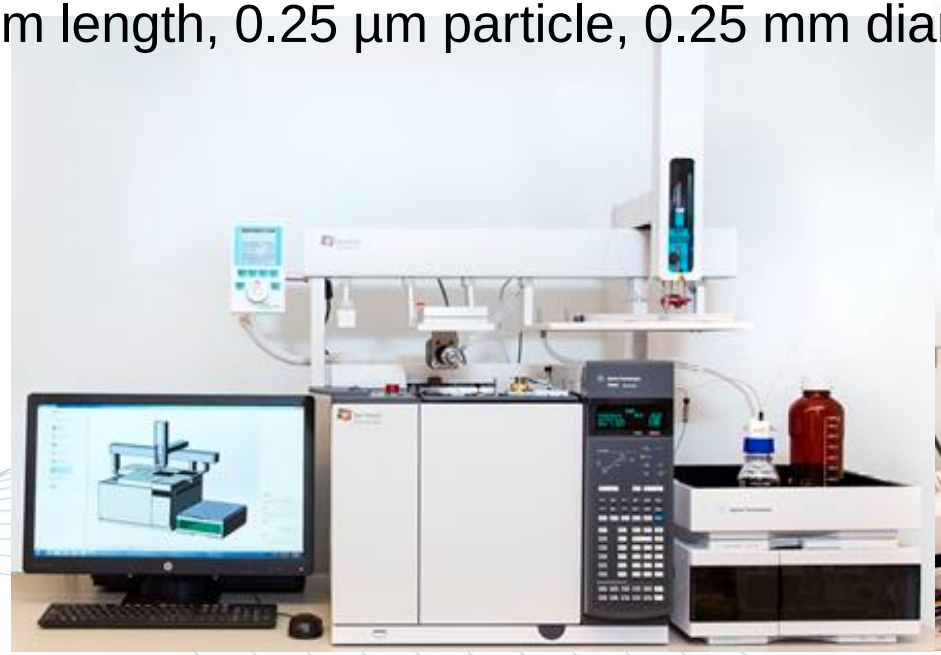
Modified after Jonker et al., (2015)

GC fractionation

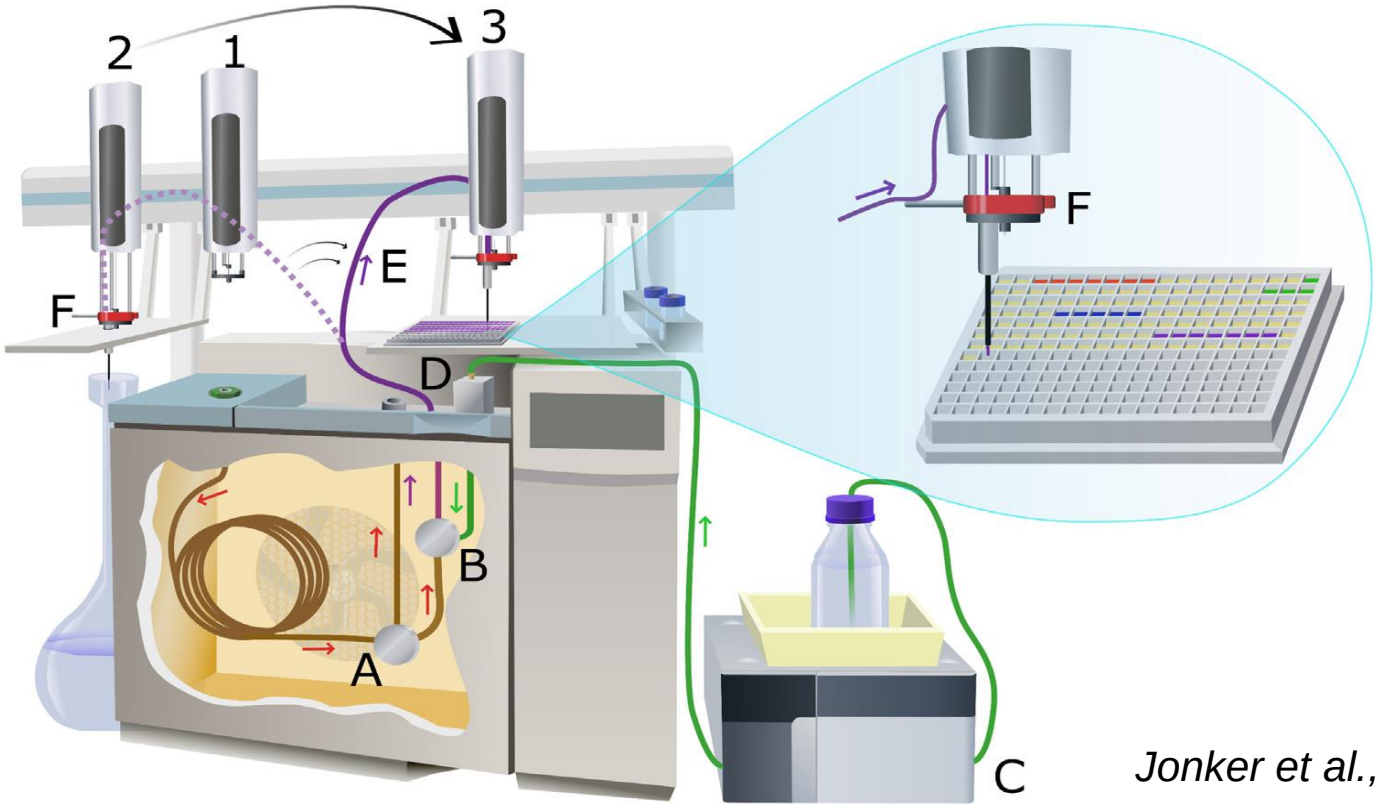
- Gas chromatograph: Agilent 7820A GC-MS with PAL robotic system modified by Da Vinci Laboratory Systems (Rotterdam, Netherlands)

- PAL system programmed to pick-up and move a smart grip unit over a 96 or 384-well plate for fraction collection

- Agilent HP-5MS column (5% Phenyl-methylpolysiloxane), 30m length, 0.25 µm particle, 0.25 mm diameter



DVLS GC Fractionator



Jonker et al., (2016)

A) Y-piece for post column split
B) Inverted y-piece
C) LC pump
D) Heating element
E) Transport capillary
F) Smart grip unit

Samples

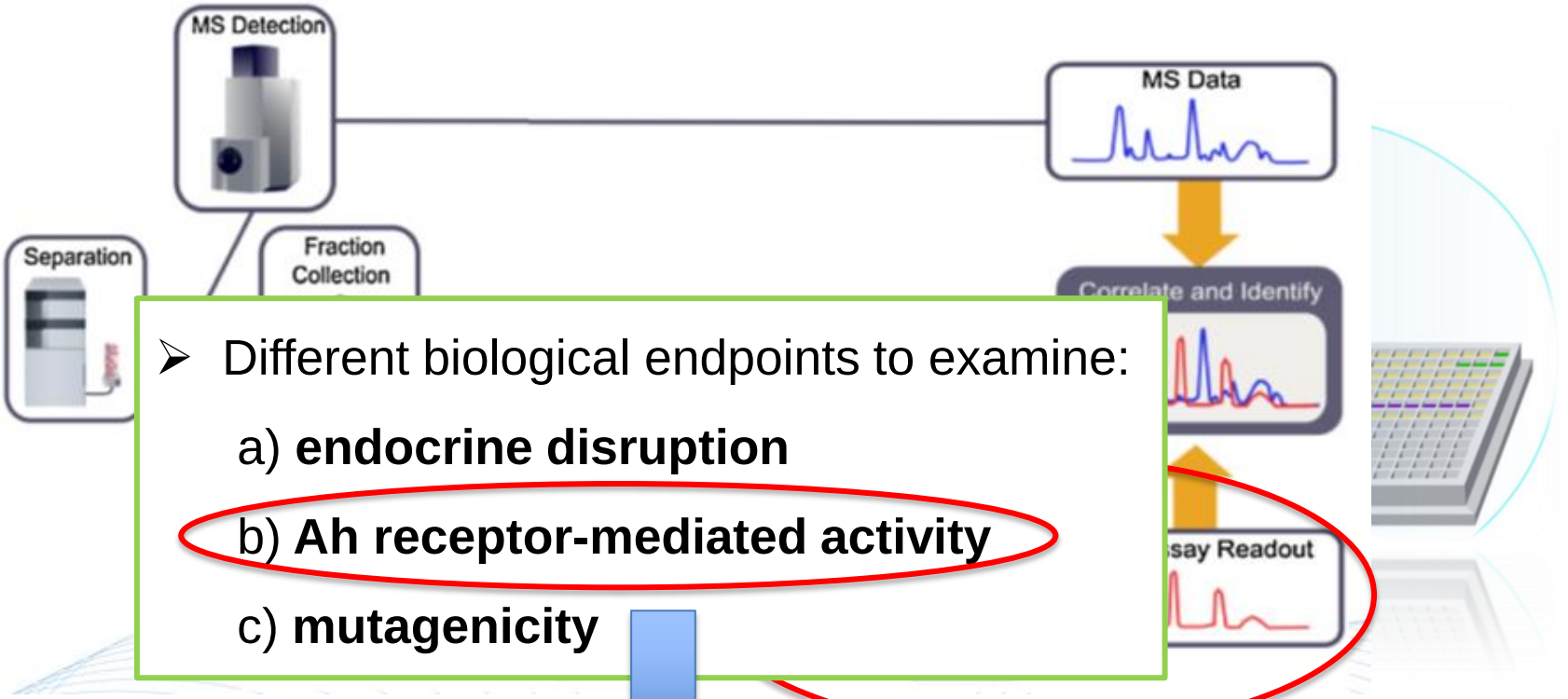
- 2 g of **beached plastic**
- Polyethylene (**PE**) and polypropylene (**PP**)

- Total of 86 compounds

Compound	Concentration in ng/g plastic	Concentration in 0.5 ml extract [ng]
PAHs	2000	333.33
Pesticides	20	3.33
PBDEs	7	1.16
PCDD/DFs	5 - 25	0.83 – 4.16
Planar PCBs	2 - 20	0.16 – 1.66

Testing of DVLS Fractionator

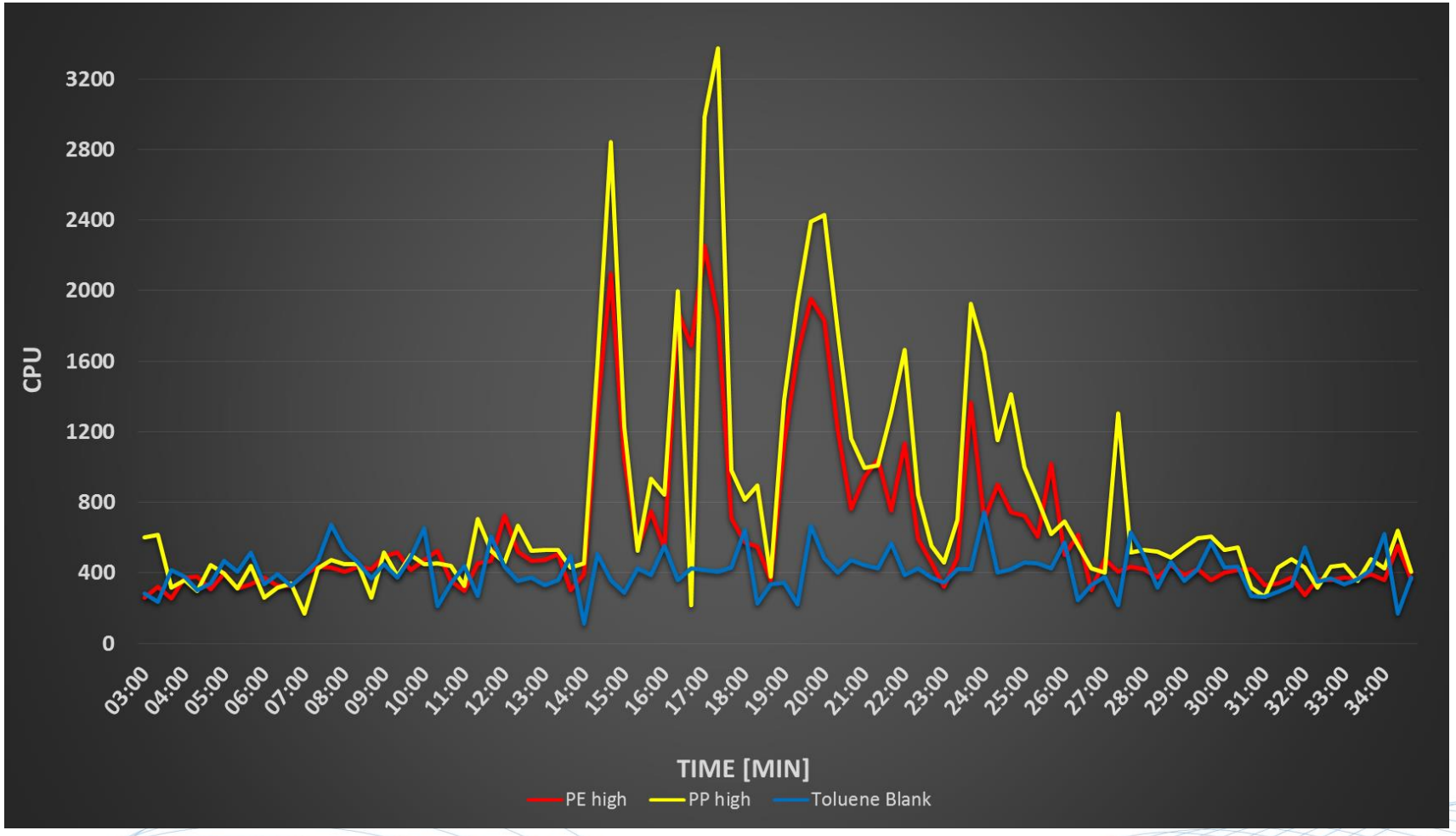
- Injection of 2µl of extract



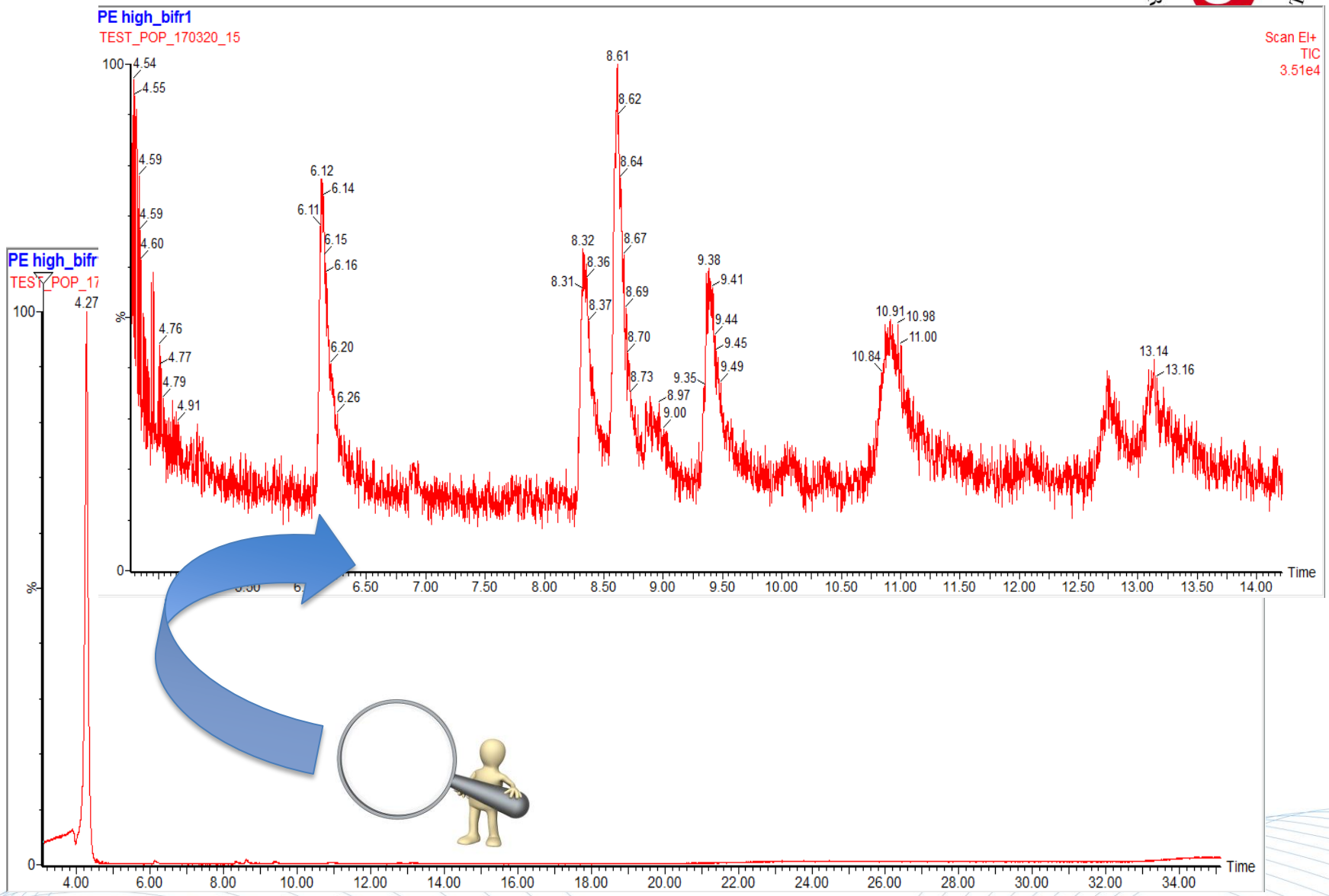
- Different biological endpoints to examine:
  - a) **endocrine disruption**
  - b) **Ah receptor-mediated activity**
  - c) **mutagenicity**

H4IIE-luc assay

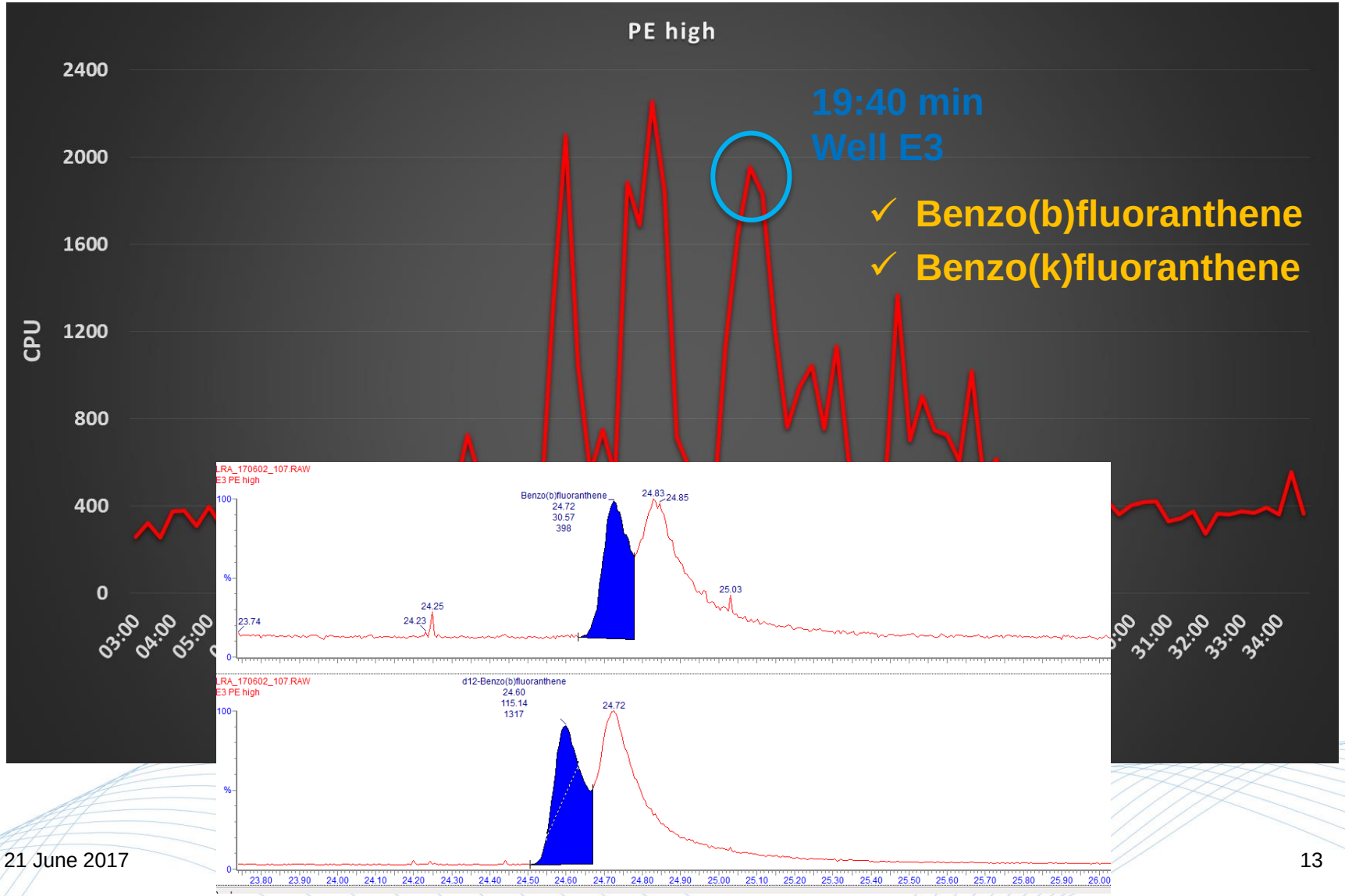
Bioassay chromatogram



MS chromatogram

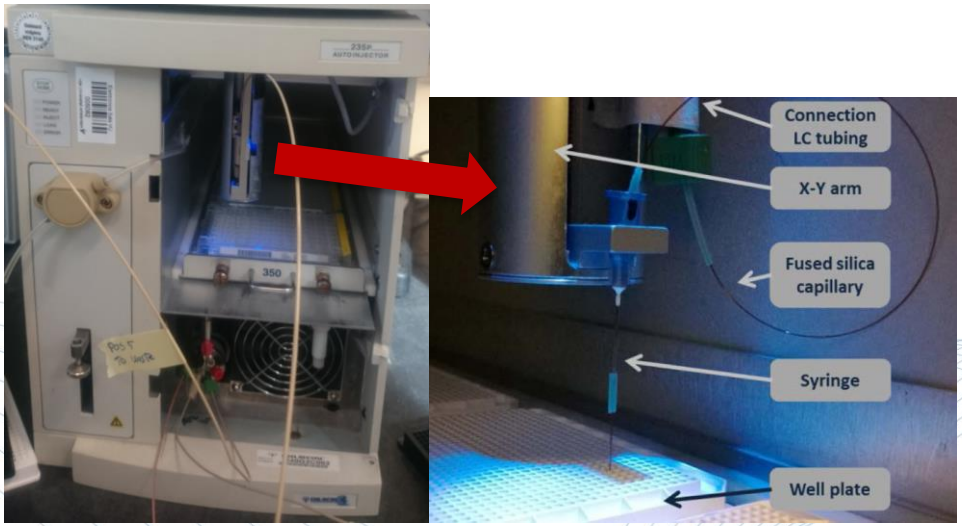


Correlating bioassay and chemical results

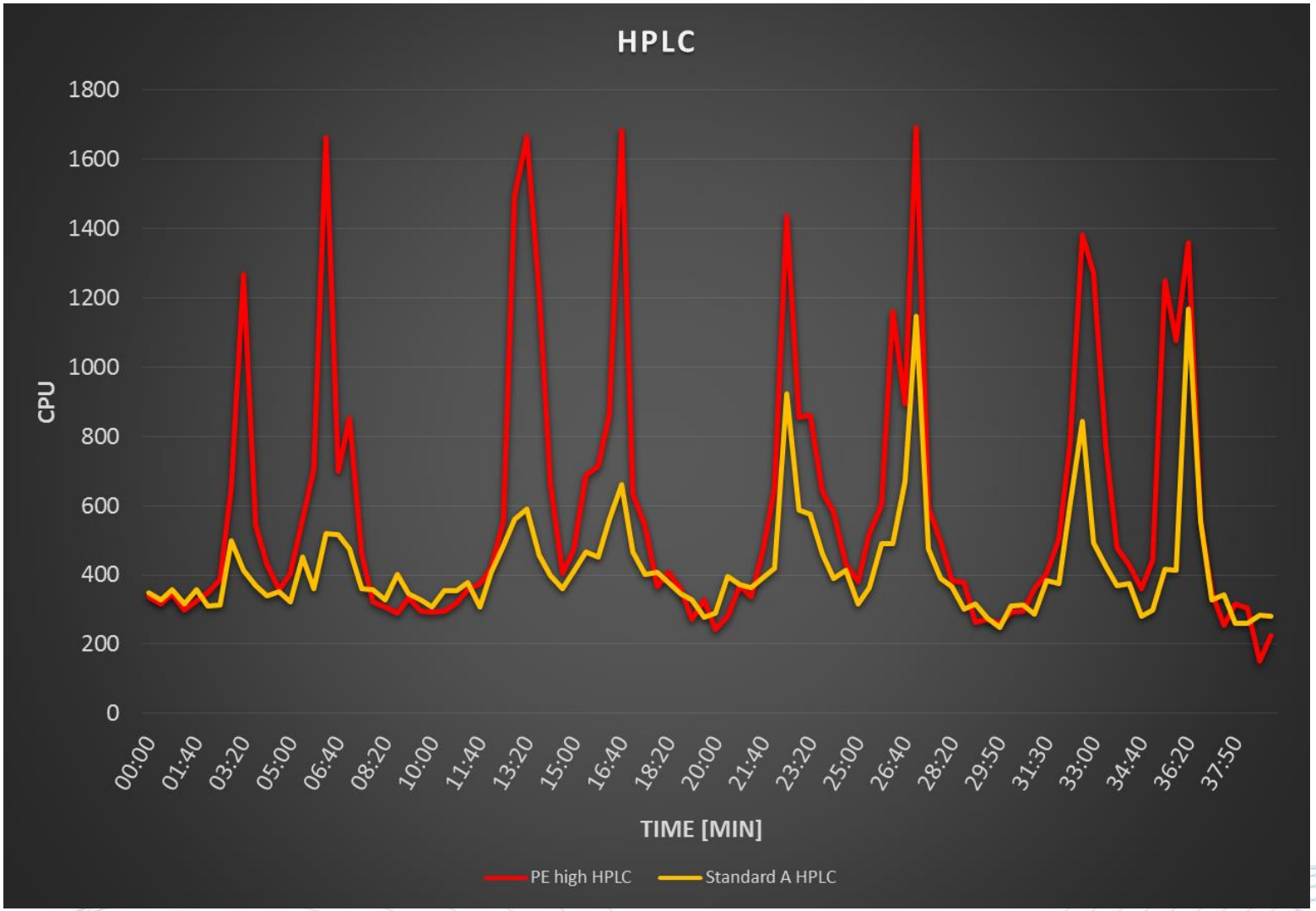


LC fractionation

- Liquid Chromatography separation on a **Shimadzu UFLC** system with a photodiode array detector
- MS system: **maXis™ HD** Ultra high resolution QTOF
- A Gilson 235p autosampler was modified for fraction collection in 96 or 384 well plates



Bioassay chromatogram



Conclusions and Outlook

GC fractionation was successful in detecting bioactive substances in a fortified plastic extract

Bioassay demonstrated high sensitivity

- Analyze marine plastic extracts with fractionation system to identify causative agents
- Use HRMS
- Apply a battery of different bioassays

Acknowledgement

Special thanks to: **Jeroen Kool**
Maria Larsson
Anna Kärrman

formas
The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning

Da Vinci LABORATORY SOLUTIONS