NON-TARGET SCREENING OF ENVIRONMENTAL SAMPLES BY LOW AND HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROMETRY (TOF-MS)

P. ROSTKOWSKI[1], P. HAGLUND[2], C. DYE[1]AND M. SCHLABACH[1]

[1]Norwegian Institute of Air Research (NILU), PO Box 100, 2027 Kjeller, Norway
[2]University of Umeå, SE-901 87 Umeå, Sweden
E-mail: Pawel.Rostkowski@nilu.no

ABSTRACT

The already substantial number of different organic chemical substances emitted to, and circulating in the environment is increasing. Due both to this growing number of chemicals, and the accumulating knowledge of their potential negative environmental and health effects, the number of emerging contaminants in Europe and elsewhere is also increasing. In regular environmental analysis, a targeted approach is generally used, i.e. the analytes of interest are selected before making measurements. However, the problem with targeted methods is that chemicals, which are not initially anticipated, are not detected regardless of how high their concentration might be. Thus the non-target approaches are needed to identify the unknowns and to reveal a more complete profile of contaminants. In this study we utilized modern techniques, such as high and low resolution time of flight mass spectrometry (TOF-MS) combined with either ultra-high-performance liquid chromatography (UPLC), gas chromatography (GC) or multidimensional gas chromatography (GCxGC) to analyze wastewater, sludge, sediment and biota samples. This approach proved to be useful, a number of anthropogenic compounds have been tentatively identified and included: pharmaceuticals and personal care products, plasticizers and flame retardants, polymer additives, and other well-known persistent organic pollutants. Additionally, full-scan acquisition allows retrospective analysis for emerging contaminants years after the data has been acquired.

Keywords: non-target screening, time of flight mass spectrometry, environmental contaminants

1 Introduction

Analysis of complex mixtures in environmental samples is an extremely difficult task. Since sample matrices in most cases are complex, traditionally ultra-trace analytical methods were specifically developed for a certain group of substances. This traditional *targeted* approach gives good sensitivity and reliable identification and quantification of the target compounds, but has a significant drawback, as it always will miss all compounds, which were not selected at the start of the analyses. In addition, ecotoxicological studies have shown in many cases that the concentrations of the known compounds are not high enough to explain some of the toxic potentials of samples (Johnson et al. 2007, Hill et al. 2010, Rostkowski et al. 2011). To fill this knowledge gap *non-target* screening methods are a very important tool for environmental chemistry.
During the last decade mass spectrometer (MS) based on time-of-flight technology (TOF-MS) has become more affordable, stabile, and useful for environmental trace analysis. TOF-MS acquire full mass spectra with a much better sensitivity than a standard quadrupole MS and make it a versatile tool for both target and non-target analysis of environmental contaminants. Combined with gas or liquid chromatography (GC-MS or LC-MS) it is possible to separate and detect a very broad range of chemical compounds (Figure 1) in only one or a few single runs.

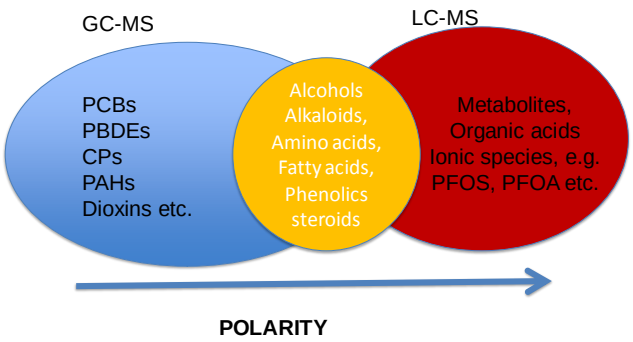

Figure 1: Typical application range for GC-MS and LC-MS

In this pilot study, designed to test the possibilities, strengths and weaknesses of the non-target screening approach, we applied state of the art techniques, GCxGC-LRTOFMS, GC-HR-TOFMS and LC-HRTOFMS to a limited selection of samples often used for environmental contamination studies. For the necessary data mining, the raw data from instrumental analysis were treated with advanced software tools tailored to filter out as most as possible of relevant information.

2. Materials and methods

2.1. Sample collection

In order to fulfill the testing objective of the study a broad range of samples from different areas of Norway was chosen: ambient air, sewage water (influent and effluent), sludge, sediment, and different biota samples (prawns, cod liver, eggs). All the samples were prepared in duplicate, one to be extracted for LC-TOF analyses and another for GCxGC and GC-HR-TOF analyses.

2.2. Extraction

Wastewater influent and effluent samples (75 and 750 ml, respectively) were extracted with Waters Oasis HLB SPE cartridge in parallel with dichloromethane as an elution solvent of the samples used in GCxGC-TOF and GC-HR-TOF analyzed and with methanol/acetonitrile (50:50) for the purpose of LC-HR-TOF analyses. In order to avoid clogging the SPE cartridges with particles influent samples were passed through a glass fiber filter prior to extraction. All filters containing particles were extracted in the ultrasonic bath with solvents chosen for different analytical techniques. Sediment and sludge samples prior to extraction were mixed with activated copper powder to remove elemental sulphur and then approximately 5 g was extracted using ultrasonic bath with dichloromethane (for gas chromatography mass spectrometry analyses) and with acetonitrile:methanol for LCMS analyses. In order to remove water biota samples were treated with anhydrous sodium sulphate and extracted in the ultrasonic baths with either dichloromethane or methanol:acetonitrile (50:50). The samples with heavy matrix, i.e. sludge, sediment and biota samples were subjected to filtration, dichloromethane extraction and non-discriminating gel permeation chromatography (GPC) clean up. Sediment and sludge samples were also treated with cupper powder to remove elemental sulfur. All samples were then concentrated to approx. 100µL and analyzed by both techniques. The total ion chromatograms revealed a relatively high “background” of lipids in several “heavy matrix” samples and these samples were therefore filtered through silica using acetone: hexane (1.1, v/v), and were reanalyzed.

2.3. Instrumental analysis

The GCxGC-MS analyses were performed using a Leco 4D equipped with a HP6890 GC and a 30m x 0.25mm x 0.25µm SGE BPX-50 (50% phenyl-methylsilicone) and a 2m x 0.15mm x 0.15µm Varian VF-1ms (100% methylsilicone) column. Helium was used as

carrier gas at a constant flow of 1 ml/min and the GCxGC modulator was operated at a modulation frequency of 7s. The main GC oven temperature program was 60°C (1 min) – 5 C°/min – 300°C (2 min) and the second oven was ramped at +20°C bias. One microliter aliquots was injected in the split-less mode and EI mass spectra (70 eV) was collected at 100 Hz over the mass range 35-700 Dalton. The GC-HRMS analyses were performed using a Leco GC-HRT equipped with a HP7890 GC and a 30m x 0.25mm x 0.25µm J&W DB5MS-UI (5% phenyl-methylsilicone). Helium was used as a carrier gas at a constant flow of 1 ml/min. The GC oven temperature program was 60°C (1 min) – 5 C°/min – 300°C (2 min). One microliter aliquots was injected in the split-less mode and EI mass spectra (70 eV) was collected in the high-resolution mode (>25000 resolution) over the mass range 35-700 Dalton. LC-HR-TOF analyses were performed with Agilent 1290 Infinity UHPLC coupled with Agilent 6530 QTOFMS with Agilent JetStream ESI source operated in positive and negative modes. Samples were separated using a reverse phase Waters Acquity UPLC HSS T3 column (100Å, 1.8µm, 2.1 mm x 100mm). Mobile phases A and B were water with 0.1% formic acid, acetonitrile with 0.1% formic acid (positive mode) and water with 0.1% ammonium acetate and methanol with 0.1% ammonium acetate (negative mode). Separation was achieved using a flow rate of 0.4 ml/min with the following gradient: 90:10 to 78:22 in 3.5 min, 50:50 at 20min and 0:100 at 30min for 10 min.

2.4. Data treatment

The automatic GCxGC peak detection and deconvolution routine was used with a signal-to-noise ratio of 20 and the spectra was compared to the NIST 2011 library. Peaks with "similarity" >700 (70% match) was manually evaluated. Candidates that did not hold for this inspection were discharged. Similarly, peaks that also were detected in the blanks were eliminated. The remaining components were semi-quantified vs. the internal standard (D10-phenanthrene) using MS Excel and were annotated. CAS number was presented for components with "unambiguous" spectra. For the remaining, a chemical class was assigned. The GC-HRT data was very complex and it as clear that the chromatographic resolution was not sufficient for this type of complex matrices. Consequently, the automatic peak detection and deconvolution routine produced fewer tentative structures than GCxGC-MS and mostly for high abundance components. It was however useful for confirmation/discrimination of GCxGC candidates. In addition, the isotope filter option of the software proved useful to automatically detect halogenated (chlorinated and brominated) compounds in the samples. Raw LC/MS data was analyzed with Agilent Mass Hunter Qual software. In the first step molecular feature extraction module (MFE) was used to find peaks in the total ion chromatogram. The software removed the chemical background from the three dimensional LC/MS dataset, found the true ion signals, grouped the chemically related ion signals (isotopes, adducts and dimmers). This resulted in a compound table with associated chromatograms and pure spectra with each compound with a quality score calculated. As a compromise, to avoid extracting too much of background noise only peaks with more than 50-500 counts (sample dependent) and quality score over 60% were extracted. To take advantage of mass accuracy of the data the results of this data processing were further used to derive molecular formulas of compounds extracted by the MFE feature. Besides accurate mass isotope ratios and isotope mass values were used to logically narrow the list of possible formulas. Following elements were selected as acceptable in this procedure: C,H,O,N,S,Cl,Br, P with a minimum overall score per charge carrier set to 35 and a mass error window defined to 5ppm. For each compound, a probability score was calculated that is based on how well the isotope abundance ratios for the candidate molecular formulas match those from the experimental data. This resulted in a shorter list of ranked candidate molecular formulas, with the top score (highest score = 100) being more likely to be correct. In the last step the formulas with overall score of 80 % and up were compared with Agilent databases of contaminants and a public database Chemspider. It

allowed for a tentative identification (based on the possibility of the compound to be likely present in the tested sample) of some structures and for a provision of elemental formulas of compounds with too many candidates in these libraries. Overall relative score was calculated based on scores from different steps in the structure elucidation procedure with the assumption that lack of the candidate or too many candidates in databases were not decreasing overall score of compounds with only elemental composition available.

3. Results

Based on the presented approach a number of different environmental contaminants have been tentatively identified (Table 1).

SampleID	1	2	3	4	5	7	6	8	9	10	11	12	13
Sample type	Air (remote)	Air (urban)	STP	STP	STP	STP	STP	Sediment	Biota	Biota	Biota	Biota	Biota
			sludge	sludge	Influent	Influent particles	Effluent		Prawns	Cod liver	Egg (Common eider)	Egg (Common shag)	Egg (Herring gull)
Phthalates	9	8	8	8	7	8	8	17	1	1	0	0	1
OPs	4	7	4	7	7	4	9	5	3	2	2	4	7
Poly/add	12	15	13	21	34	15	28	15	9	6	8	3	5
PPCP	26	18	39	25	33	15	242	25	39	54	33	4	34
Pesticides	4	5	5	4	2	2	33	1	5	1	6	2	12
PACs	1	9	50	94	9	3	4	96	45	0	4	2	2
Halogens	0	0	6	6	0	1	0	12	0	14	2	10	29
PFCs	0	0	0	0	3	0	0	0	1	0	0	0	0
Oxy-compounds	0	0	0	12	9	0	4	7	0	0	0	0	0
N-compounds	0	0	2	7	1	0	2	0	0	0	0	0	0
Unidentified	252	212	265	82	271	291	209	237	305	63	216	230	286

Besides the long list of already known and monitored compounds, some of the compounds identified in this study were not detected earlier in these types of samples or reported only occasionally and included adipates, antioxidants, benzothiazoles and benzotriazoles.

4. Conclusions

Non-targeted screening with time of flight mass spectrometry is a useful tool for identification of environmental contaminants. Further work is needed to reduce number of not identified compounds detected in this study.

References:

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