Non-target screening with high resolution mass spectrometry: Ready for real-world applications?

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Non-target is becoming popular in science

Search for publications in web of science (May 23, 2017)

Nontarget or non-target* & mass spectrom*
Chance to explore the iceberg of chemicals?

- **Targets**: Confirmation with reference standards
- **Suspects**: Expected compounds which exact mass can be screened
- **Non-targets**: All remaining components with no prior information

Definitions from Schymanski et al., 2015, ABC
Content

General workflow

Three application examples

- Daily monitoring of river Rhine
- Evaluation of wastewater treatment technologies
- Assessment of (political) mitigation measures using sediment archives

Conclusions
Non-target screening workflow

Experiments → Treatment → Monitoring

- Sampling: Space & Time
- Analysis: Sample enrichment, Chromatography (GC, LC), Appropriate ionization, HR-tandem MS
- Data Pre-processing: Detect Peaks, Subtract Blind, Replicates & Grouping, Build Profiles, Series
- Prioritization: Isotopes, Mass Defect, Time/Spatial Trends, PCA/Clustering, Processes, Effects
- Identification: Database, Suspect Search, Assign Formula, Fragments, Analytical (RT, Ionization,...) & Meta Information

Commercial/Open Software

Databases → In Silico Prediction

Sample Archive → Digital Archive

Pollution Overview → Pattern → Structures

Hollender et al, submitted
Core needs – HRMS instruments & databases

- high mass accuracy (< 5 ppm)
- high mass resolution (0.001 Da)
- high sensitivity in fullscan mode
- high stability over time

Resolution: \[ R = \frac{m}{\Delta m} \]

- Spectra libraries
- Compound databases
- Computational tools

\[ \Delta m = \text{mass difference full width at half maximum (FWHM)} \]
Prioritization depends on (research) question

<table>
<thead>
<tr>
<th>Data-driven</th>
<th>Experiment-driven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency, abundance of masses</td>
<td>Persistence, elimination/formation over process</td>
</tr>
<tr>
<td>Component with <strong>characteristic isotope</strong> pattern (C, Cl, Br, N, O, S)</td>
<td>Reaction-based search of <strong>TPs</strong> to link masses <strong>before and after treatment</strong></td>
</tr>
<tr>
<td>Part of <strong>homologue series</strong> (mass difference, Kendrick mass defect)</td>
<td><strong>Biological, electrochemical, oxidative transformation products (TPs) formation</strong></td>
</tr>
<tr>
<td><strong>Suspect</strong> screening</td>
<td><strong>Reaction</strong> with <strong>isotopically-labelled</strong> reagents</td>
</tr>
<tr>
<td>Specific functional groups (<strong>MS/MS</strong>, neutral loss)</td>
<td><strong>Effect.directed selection</strong> of masses in toxic fractions</td>
</tr>
<tr>
<td><strong>Temporal</strong> or <strong>spatial profile</strong> over several samples</td>
<td></td>
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</tbody>
</table>
Daily screening at the International Rhine monitoring station – River Rhine

http://www.eawag.ch/en/department/uchem/software/
**Analytical strategy**

SPE followed by LC-ESI-HRMS (LTQ-Orbitrap)
High resolved full scan spectra & MSMS fragmentation spectra

**Target analysis**

- Priority substances
  - **x** calibration
  - **x** exact quantification
  - **x** manual data processing

- Site specific substances
  - **x** calibration
  - **x** semi-quant. quantification
  - **x** automated processing

**Non-target analysis**

- Remaining masses
  - **∞** calibration
  - **∞** semi-quant.
  - **∞** automated processing

**Time profiles**

**Statistical analysis**

**Changes / trends**

**Prioritization**

Ruff et al., Aqua & Gas 2013, 5: 16-25
Prioritization using time profiles

**Identification**
- Molecular formula assignment,
- Database search
- Prioritization of hits with information on industrial production
- Confirmation with reference standard

Sample intensity
Blank intensity
mean m/z = 223.154
mean RT = 4.9 min

Tetraglyme

Ruff et al., Water Research 2015
Real-time monitoring of the Rhine River

Cumulative loads over three years
Real-time monitoring of the Rhine River

Cumulative loads over three years

![Graph showing cumulative loads over three years with peaks labeled as 0.6 t, 0.1 t, 0.4 t, and 0.2 t. The graph also includes chemical structures for PPA and TCP, with a production break indicated.]
Evaluation of wastewater treatment technologies

Complex wastewater matrix with contaminants
Wastewater treatment with ozonation

Full scale Wastewater treatment plant (WWTP) in Dübendorf, Switzerland

Conventional Activated Sludge → Secondary Clarifier → Ozone addition 2, 3, 4, 5 mg/L O₃ → Post treatment: sandfiltration

Influent, before bio → After bio → After ozone → After post

24-hr flow-proportional composites (3 consecutive days)
**Workflow – Suspect & non-target screening**

**Data Acquisition**
- Online solid-phase extraction
- LC-Orbitrap MS/MS (Atlantis; +/- ESI R= 70,000 – 140,000)

**Data Processing**
- Peak detection
  - enviPick package
- Componentization
  - nontarget package
- Profile building
  - enviMass package
- Blind subtraction
  - R script

**Data Analysis**
- Multivariate Statistics – PCA, HCA
- Linking Parent/TP
  - R script
- Suspect Screening
  - R script
- Structure elucidation
  - MetFrag, MassBank, STOFF-IDENT

Workflow modified from: Schollée et al. 2015, Anal. Chem
Using multivariate statistics to explore transformation product formation

Principal component analysis of Non-target Peaks

Schollée et al., 2015, Anal. Chem.
PCA of non-target peaks & linkage of peaks

Loading Plot for Nontarget scaled peaks

- hydroxylation
- demethylation
- deethylation
- dehydrogenation
- hydrogenation
- dehydration
- dechlorination
- deconjugation

5872 linkages
Example of a linkage related to a hydroxylation

Parent exact mass: 337.2221 $\text{C}_{16}\text{H}_{32}\text{O}_7$

TP exact mass: 353.2170 $\text{C}_{16}\text{H}_{32}\text{O}_8$

MS/MS Similarity score: 0.73
Hierarchical clustering of profiles along treatment chain

N = 10818

High

Low

Before bio After bio After ozone After post

N = 586
Lake sediments as archive of pollution

- Pharmaceuticals
- Personal care products
- Veterinary medicines
- Pesticides
- Industrial chemicals
Analytical procedure

Lake sediment cores

~1990s

Freeze-drying & homogenization

Pressurized liquid extraction & purification using liquid-liquid extraction (Quechers)

RP chromatography (Xbridge)
ESI +/-
Orbitrap-HRMS/MS


- 180 Targets
- 840 Suspects
- Non-targets
Non-target screening of m/z 450.9619 (ESI-)
with negative mass defect & clear isotopic pattern

Chlorine adduct

Exact mass (m/z) 414.9846

MS & MS/MS

MOlGEN: C, H, O, N, Cl, F

C₁₅H₈Cl₂F₆N₂O

Top score of 0.96

Number of references

5 Structures

Score: 1
References: 13

Score: 1
References: 1

Score: 0.802
References: 8

Score: 0
References: 5

Score: 0
References: 20

Flucofuron

http://molgen.de/
http://msbi.ipb-halle.de/MetFrag/

Characteristic trends using hierarchical clustering – Lake Lugano

Flucofuron
Features: 849

Features: 651
Characteristic trends using hierarchical clustering – Lake Lugano

Upgrade of wastewater treatment

Ban of other antifouling paints

Trichlocarban (TCC)/Dichlorocarbanilide (DCC) Features: 651

Flucofuron Features: 849

Terbutryn /Promethryn Features: 433

Irgarol Features: 653
Is non-target screening ready for real-world applications?

Yes almost, but…

- excellent instrumentation, as well as proper data analysis tools and expert knowledge is needed
- Prioritization is mandatory to master the number of features
- Currently more characterization than identification
- Not every peak can be identified with mass spectrometry

Future challenges

- application to other ionization techniques (e.g., APPI, APCI)
- Inclusion of other information into identification workflow (e.g., ion mobility, meta information)
- Implementation in practice labs
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Thank you for your attention

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Achievements on the Rhine

- 300 relevant compounds included in target screening
- Captured load increased from 7 to 100 t per year (+ ca. 56 t complexing agents)
- Non-target analysis using enviMass has triggered several Rhine alarms

Daily trend monitoring and spill detection with enviMass since 2012
Temporal pollution pattern

Time series of PCPs in sediment cores from Lake Greifensee

Upgrade wastewater treatment

Triclosan / Triclocarban

Tonalide

ng/g_{dw}

Chiaia-Hernandez et al. ES&T 2013, 47(2) pp. 976-986
Temporal pollution pattern

Time series of the pesticide irgarol in sediment cores from Lake Greifensee

Ban of other antifouling paints

Validation with Target Compounds
Screening of transformation products

Triclocarban (TCC)
Biocide
\[ \text{Log} \, K_{ow} = 4.71 \pm 0.46 \]

Dichlorocarbanilide (DCC)
Transformation product
\[ \text{Log} \, K_{ow} = 4.07 \pm 0.29 \]

Temporal resolution of TCC and DCC based on peak areas
Characterisation of non-target features

Sand Filter Post-treatment

Assigned Trend
- Removed in biological treatment
- Biological TP, removed in ozone
- Biological TP, not removed in ozone
- Ozone TP, removed in post-treatment
- Ozone TP, not removed in post-treatment
- TP formed in post-treatment
- Persistent in bio, removed in ozone
- Persistent
- Other

Trend
1
2
3
4
5
6
7
8
9

m/z
200
400
600
800

(a)
Transformation Products of Homologue series ($\Delta C_2H_4O$)
Non-Target Screening von intensiven Peaks mit negativen Massendefekt und Chlorisotopenpattern

**Exact mass (m/z): 402.8427**

- **Molgen** + **Metfrag**
- **MS + MS/MS**

**Molecular Formulas:** 6

- $C_{13}H_6Cl_6O_2$
- $C_{12}H_6BrClN_2OS_3$
- $C_{11}H_5Cl_5N_2O_2S$
- $C_{12}H_6ClS_3Sb$
- $C_{12}H_6Cl_5O_3P$
- $C_8H_4Br_F_6O_2$

- **Isotopen pattern match**

**Structures:** 11

- $C_{13}H_6Cl_6O_2$

**Anzahl Referenzen:** 11

**logKow Vorhersage – Retentionszeit match**

Hexachlorophene

http://msbi.ipb-halle.de/MetFrag/

PCA of Nontarget Peaks before/after ozonation

Example of 3 mg/L Ozone

Scores Plot for Nontarget scaled peaks

After bio
After ozone

Loading Plot for Nontarget scaled peaks
Loads along the river Rhine: example lamotrigin

Hollender et al., Chimia (2015) 68: 793–798