QUALITATIVE SCREENING FOR EMERGING CONTAMINANTS AND THEIR METABOLITES/TRANSFORMATION PRODUCTS IN SEWAGE SLUDGE OF ATHENS BY UHPLC-QTOF MS

Viola Borova
Ph.D Researcher

Acknowledgements: Anna Bletsou and Nikolaos Thomaidis

University Of Athens
Department of Chemistry
Overview

- Emerging contaminants, Issue of concern
- Their occurrence in the environment

Introduction

Analytical methodology
Method Validation

Application in real sewage sludge samples

Conclusions
Definitions

ECs: Pharmaceuticals, Illicit drugs, Personal care products, Endocrine disruptive compounds (EDCs), Flame retardants, Food additives, Disinfection by-products, Pesticides, PLUS metabolites & TPs

They Come from “You”

What’s in wastewater?

- human feces and urine
- food from sinks
- soaps and other cleaning agents
- runoff from streets and lawns
- industrial discharges
So what's the problem with sewage sludge?

ECs remain in the sewage sludge (SS) generated
Efforts on improving water quality led to an increased sewage loads
Sorption processes are complex and difficult to predict
Additional route of entry of organic pollutants to the environment, Toxicity, Plant growth (amendment)

*Sewage Sludge* - any solid, semisolid, or liquid residue removed during the treatment of municipal waste water or domestic sewage

In Europe is estimated 90 g *d.w.* per person per day

- Land application: 57%
- Incineration: 20%
- Landfilling: 13%
- Sea disposal: 10%
Issue of concern

- Detailed studies on the presence of ECs and their metabolites and TPs in sewage sludge are necessary in order to have the whole picture of the distribution of these emerging pollutants in the environment and to perform a reliable risk assessment.

Imperative need for...

Capable of monitoring a large variety of compounds, belonging to different group of compounds, with different characteristics with one analytical procedure in one single run.
Analytical Methodology
~ Workflow for Screening of ECs in sewage sludge

Sample Preparation
Extraction from sewage sludge

UHPLC-QTOF-MS
(+), (-) ESI / bbCID mode
High sensitivity & resolution
Accurate mass data

Processing: Target Analysis
In house database (2327 compounds)

Report
Identification, Confirmation

✓ Unlimited number of analytes monitored
✓ No compound-specific method development
✓ Target and non-target approach

maXis Impact
Ultra High Resolution
Time-Of-Flight Mass Spectrometer
UHR-TOF-MS

BRUKER
Analytical Methodology
~ Sample preparation*

1. Samples were collected after sewage sludge dewatering. Then, they were freeze-dried and stored in the dark at -20 °C until analysis.
2. Finely homogenization in a mortar.
3. Weigh 0.1 gr of dried sludge.
4. Internal deuterated standards of the compounds were added to all samples.
5. Addition of 2 mL mixture solution.
6. Solution: MeOH : Milli Q water (pH 2.5, FA 0.5% and 0.1% EDTA), 50:50 v/v.
7. 15 min in ultrasonic at 50°C.
8. Centrifugation 4000 rounds for 10 min.
9. The supernatant collected in glass tube.
10. Steps 5, 7, 8, 9 repeated two more times.
11. Total collected 6 mL.
12. The extracts were evaporated to dryness under constant steam of nitrogen, N₂ (g) at 40°C.
13. Reconstitution in 500 μL of 25% MeOH and 75% ultra purified water with 0.05% v/v formic acid.
14. 1-2 min vortex stirring.
15. Final filtering step of the extract on a 0.2 mm syringe filter.

Analytical Methodology
~ UHPLC-QTOF-MS

Mobile phase:
H₂O:MeOH (gradient)
- both 0.01% HCOOH & 5 mM NH₄HCO₂ (ESI+)
-5 mM CH₃COONH₄ (ESI-)
Flow rate: gradient

Column
AcclaimTM RSLC 120 C18 (2.1 × 100 mm, 2.2 µm)
Injection volume: 5 µL

Pre-column
VanGuard (Waters): Acquity UPLC BEH C18 1.7 µm, 2.1 × 5 mm

UHPLC
Dionex UltiMate 3000 RSLC (Thermo Fisher Sci.)

QTOF MAXIS IMPACT
(Bruker Daltonics)
Range: m/z 50-1000
Scan: 2 Hz

(+), (-) ESI bbCID mode
Low CE (4 eV) (pass all) → MS spectra
High CE (25 eV) (fragment all) → MS/MS spectra
Analytical Methodology
~ Method development

In-house database: 2327 compounds

- 2224 compounds for (+) ESI
- 580 compounds for (-) ESI

- > 700 pesticides
- > 200 pharmaceuticals, illicit, DoA
- ~ 300 steroids & doping compounds
- ~ 100 compounds like industrial chemicals, food additives, dies and natural occurring compounds (aminoacids)
- ~ 300 metabolites & TPs
Analytical Methodology
~ Method development

The in-house database is a list of compounds for identification.

Retention times for the matched UHPLC method.

Adduct information.

Isomer information.

Fragment ions on MS data level.

Isotopic confirmation.

Qualifier ions for confirmation in broad band MS/MS mode.

<table>
<thead>
<tr>
<th>m/z</th>
<th>RT</th>
<th>sum formula</th>
<th>name</th>
<th>CAS</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>158</td>
<td>1.370.835.159.5.15</td>
<td>C8H11NO+</td>
<td>Aminocarb (Metal) Fragn 137</td>
<td>(2032-59-9)</td>
<td>1.521.072</td>
<td>1.370.836</td>
<td></td>
<td></td>
</tr>
<tr>
<td>159</td>
<td>1.521.065.959.5.15</td>
<td>C8H11NO+</td>
<td>Aminocarb (Metal) Fragn 152</td>
<td>(2032-59-9)</td>
<td>1.521.072</td>
<td>1.370.836</td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>233.128.454.98</td>
<td>C13H16N2O2</td>
<td>Aminogluthetimide</td>
<td>(125-84-8)</td>
<td>146.096.426</td>
<td>94.053.126</td>
<td>18.810.699</td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>232.144.435.21</td>
<td>C13H17NO3</td>
<td>Aminopyrazone. Amispyra</td>
<td>(58-15-1)</td>
<td>56.049.476</td>
<td>97.076.025</td>
<td>111.091.675</td>
<td></td>
</tr>
<tr>
<td>162</td>
<td>328.184.198.546</td>
<td>C13H2NO3S</td>
<td>Aminoprazine</td>
<td>(58-37-7)</td>
<td>210.052.847</td>
<td>238.068.497</td>
<td>58.005.126</td>
<td></td>
</tr>
<tr>
<td>163</td>
<td>1.630.865.890.8.67</td>
<td>C8H10N2O</td>
<td>Aminorex Isomer 1</td>
<td>(1220-50-3)</td>
<td>1.200.808</td>
<td>1.030.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>120.081.776.8.67</td>
<td>C8H10N+</td>
<td>Aminorex Isomer 1 Fragn 120</td>
<td>(1220-50-3)</td>
<td>1.200.808</td>
<td>1.030.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>1.630.865.890.8.67</td>
<td>C8H10N2O</td>
<td>Aminorex Isomer 2</td>
<td>(1220-50-3)</td>
<td>1.200.808</td>
<td>1.030.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>166</td>
<td>120.081.776.8.67</td>
<td>C8H10N+</td>
<td>Aminorex Isomer 2 Fragn 120</td>
<td>(1220-50-3)</td>
<td>1.200.808</td>
<td>1.030.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>167</td>
<td>64.603.097.124</td>
<td>C12H9N1O3S2</td>
<td>Amiodarone</td>
<td>(1951-25-9)</td>
<td>73.088.601</td>
<td>86.069.426</td>
<td>100.112.076</td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>2.941.964.742.13.3</td>
<td>C19H23N3</td>
<td>Amitraz</td>
<td>(3009-61-1)</td>
<td>163.122.975</td>
<td>122.096.426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>163.122.975.13.3</td>
<td>C10H15N2O+</td>
<td>Amitraz Fragn 163</td>
<td>(2032-59-9)</td>
<td>163.122.975</td>
<td>122.096.426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>278.190.326.8.23</td>
<td>C20H23N1</td>
<td>Amitriptyline</td>
<td>(50-48-6)</td>
<td>57.049.725</td>
<td>58.039.974</td>
<td>68.024.523</td>
<td></td>
</tr>
<tr>
<td>171</td>
<td>850.308.722.144</td>
<td>C2H3N4</td>
<td>Amitrole</td>
<td>(51-67-7)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>172</td>
<td>409.152.478.8.36</td>
<td>C20H25N2O2SC1</td>
<td>Amloidipine</td>
<td>(88150-42-9)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>173</td>
<td>447.108.356.8.36</td>
<td>C20H25N2O2SC1 tighter+</td>
<td>Amloidipine (K)</td>
<td>(88150-42-9)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>174</td>
<td>43.113.442.8.36</td>
<td>C20H25N2O2SC1 tighter+</td>
<td>Amloidipine (Na)</td>
<td>(88150-42-9)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>175</td>
<td>238.062.933.8.36</td>
<td>C12H13CON2O2*+</td>
<td>Amloidipine Fragn 238</td>
<td>(88150-42-9)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>176</td>
<td>294.089.148.8.36</td>
<td>C15H17NO3*+</td>
<td>Amloidipine Fragn 294</td>
<td>(88150-42-9)</td>
<td>238.062.933</td>
<td>294.089.148</td>
<td>334.084.062</td>
<td></td>
</tr>
<tr>
<td>177</td>
<td>318.275.143.12.8</td>
<td>C21H9NO</td>
<td>Amorolfin</td>
<td>(78613-35-1)</td>
<td>130.122.641</td>
<td>161.132.477</td>
<td>11.610.699</td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>3.141.054.668.7.66</td>
<td>C17H16ON8</td>
<td>Amoxepine</td>
<td>(14028-44-4)</td>
<td>271.063.367</td>
<td>70.065.126</td>
<td>245.047.017</td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>136.112.076.4.15</td>
<td>C9H13N</td>
<td>Amphetamine</td>
<td>(300-62-9)</td>
<td>91.054.227</td>
<td>65.038.577</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>119.085.527.4.15</td>
<td>C9H11N+</td>
<td>Amphetamine Fragn 119</td>
<td>(300-62-9)</td>
<td>91.054.227</td>
<td>65.038.577</td>
<td></td>
<td></td>
</tr>
<tr>
<td>181</td>
<td>91.054.227.4.15</td>
<td>C9H7N+</td>
<td>Amphetamine Fragn 91</td>
<td>(300-62-9)</td>
<td>91.054.227</td>
<td>65.038.577</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analytical Methodology
~ Validation data set

I. Dataset
114 compounds: 106 in (+) ESI, 8 in (-) ESI, 5% of the compounds in the database

II. Optimization of the evaluation method (TargetScreening)

<table>
<thead>
<tr>
<th>Find</th>
<th>Area</th>
<th>1000 (+)/ 600 (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity</td>
<td>250(+)/ 150 (-)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scoring</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>ret. Time (min)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>accuracy (ppm)</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>mSigma threshold</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>
Analytical Methodology
~ Validation data set

Chromatogram for 106 in (+) ESI in a spiked sample (500 ng/g)
Analytical Methodology

~ Validation data set

Validation Parameters

- **Calibration curves** of standard solution in solvent and in spiked samples were built (6 levels of concentration)

- **Repeatability, Recoveries** (in two levels of concentrations) and Matrix Effect

- The screening detection limit (SDL) and the limit of identification (LOI): estimate the threshold concentration at which detection and identification become reliable, respectively.

  - **SDL**: the lowest concentration level tested for which a compound was detected in all samples; \((t_R + \text{precursor ion})\)

  - **LOI**: the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples; \((t_R + \text{precursor ion} + \text{fragment ion})\)

A CRM 145R, sewage sludge from European Commission, was used for validation.
Analytical Methodology
~ Validation data set

Recoveries, high level (500ng/g)

RSD% (n=6) : 0.4 – 23.0 %

Recoveries, low level (50ng/g)

RSD% (n=6) : 0.7 – 26.8 %

- > 30%
- 30% - 50%
- 50% - 70%
- 70% - 90%
- 90% - 120%

- > 30%
- 30% - 50%
- 50% - 70%
- 70% - 90%
- 90% - 120%
Analytical Methodology
~ Validation data set

Matrix Effect

<table>
<thead>
<tr>
<th>Suppression</th>
<th>Freq. of the compounds, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% - 80%</td>
<td>40.0</td>
</tr>
<tr>
<td>80% - 60%</td>
<td>50.0</td>
</tr>
<tr>
<td>60% - 40%</td>
<td>20.0</td>
</tr>
<tr>
<td>40% - 20%</td>
<td>10.0</td>
</tr>
<tr>
<td>&lt;20%</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Sewage sludge
Analytical Methodology
~ Validation data set

Screening Detection Limits (SDLs)

- 2.5 ng/g d.w: 26%
- 5.0 ng/g d.w: 25%
- 10 ng/g - 25 ng/g d.w: 28%
- 100 ng/g - 250 ng/g d.w: 14%
- 500 ng/g d.w: 7%
- 1000 ng/g - 2500 ng/g d.w: 2%

Limit of identification (LOI)

- 2.5 ng/g d.w: 21%
- 5.0 ng/g d.w: 25%
- 10 ng/g - 25 ng/g d.w: 26%
- 100 ng/g - 250 ng/g d.w: 11%
- 50 ng/g d.w: 10%
- 500 ng/g d.w: 5%
- 1000 ng/g - 2500 ng/g d.w: 2%
Application in real sewage sludge samples from WWTP of Athens

Location: WWTP of Athens, Greece
Period: 1 day in March 2014 & 1 day in March 2015
Samples: After sewage sludge dewatering

Results

March 2014
- 109 in (+) ESI
- 29 in (-) ESI

March 2015
- 112 in (+) ESI
- 25 in (-) ESI

Common in both years
- 66 in (+) ESI
- 16 in (-) ESI
Application in real sewage sludge samples from WWTP of Athens

(+) ESI

Area

(+ ESI)

Area

(+ ESI)

Area

(+ ESI)
Application in real sewage sludge samples from WWTP of Athens

(-) ESI

<table>
<thead>
<tr>
<th>Compound</th>
<th>Area 2015</th>
<th>Area 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ursodeoxycholic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niflumic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Nonylphenol (4-NP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-tert-Octylphenol (4-OT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

- In-house database with information for 2327 compounds was applied in sewage sludge samples.
- Generic solid liquid extraction of a wide range of compounds.
- Validation of the target screening method.
- Comparison of the results for 2 consecutive years.
- Screening and Identification of the analytes (antihypertensives, antidepressants, pesticides etc.)
Acknowledgements:
This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEIA 624 (TREMEPOL project).

Any Questions???

E-mail Address: ntho@chem.uoa.gr
vborova@chem.uoa.gr