# A multiresidue method to evaluate emerging micropollutants levels in waters

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## Context and objectives

One of the main issue of the Water Framework Directive (WFD, 2000/60/CE) is to reach good ecological and chemical status of waters bodies. The list of WFD priority substances has been amended in 2013 (2013/39/UE) and 2015 (2015/495/UE) by identifying new substances posing a significant risk to, or *via*, the aquatic environment. In fact, these new substances, so called "emerging contaminants", including numerous pharmaceuticals and pesticides, are frequently detected in rivers and wastewaters in concentrations from ng/L up to several µg/L. Hence, it is now of crucial importance to monitor wastewater treatment plant (WWTP) effluents since they are known one of the main vector of micropollutants to surface water.

➢ **Our challenge was to develop a reliable analytical method to quantify emerging contaminants at trace level in various types of waters.**

## **Target compounds:** typical indicators of urban domestic pollution

Indicator pesticides and pharmaceuticals were selected according to:

- their frequency of quantification in untreated wastewaters, secondary and tertiary wastewater effluents and in receiving rivers;
- their toxic effect in aquatic and terrestrial ecosystems;
- their large range of physico-chemical properties: octanol-water partition coefficient (log Kow), acid dissociation constant (pKa) and chemical classes.

↳ **49 selected substances**

including **4 priority substances** of the WFD and **5 watch-list substances** of the WFD daughter directive (2015).

| Use | Substances | Abbreviation | Log $K_{ow}$ | pKa | LOQ (ng/L) |
|---|---|---|---|---|---|
| Pesticides | Atrazine | ATZ | 2.61 | 1.6 | 0.1 |
| | Dimethoate | DIM | 0.77 | - | 0.1 |
| | Diuron | DIU | 2.68 | 13.6 | 0.2 |
| | Imidaclopride | IMI | 0.57 | - | 0.2 |
| | Isoproturon | IPU | 2.87 | - | 0.2 |
| | Mecoprop* | MCPP | 3.13 | 3.3 | N.D. |
| | Pirimicarb | PIRI | 1.70 | - | 0.4 |
| | Simazine | SMZ | 2.18 | 1.6 | 0.2 |
| Antibiotics | Azithromycin | AZI | 4.02 | 8.7 | N.D. |
| | Ciprofloxacin | CIPRO | 0.28 | 6.1 | N.D. |
| | Clarithromycin | CLARI | 3.16 | 9.0 | 0.2 |
| | Clindamycin | CLINDA | 2.16 | - | 0.2 |
| | Erythromycin | ERY | 3.06 | 8.9 | 0.2 |
| | Metronidazole | METRO | -0.02 | 2.4 | 0.1 |
| | Norfloxacin | NORFLO | 0.46 | 6.3 / 8.7 | 10 |
| | Ofloxacin | OFLO | -0.39 | 6.0 / 9.3 | 1 |
| | Sulfamethoxazole | SMX | 0.89 | 1.6 / 5.7 | 0.1 |
| | Trimethoprim | TRIM | 0.91 | 6.6 | 0.04 |
| Anti-depressants | Amitriptyline | AMI | 4.92 | 9.4 | 0.4 |
| | Fluoxetine | FLUOX | 4.5 | - | 1 |
| Betablokers | Acebutolol | ACE | 1.71 | 9.4 | 0.1 |
| | Atenolol | ATE | 0.16 | 9.6 | 0.4 |
| | Metoprolol | MET | 1.88 | - | 0.1 |
| | Propranolol | PROP | 3.48 | 9.4 | 0.1 |
| | Sotalol | SOT | 0.24 | - | 0.4 |

| Use | Substances | Abbreviation | Log $K_{ow}$ | pKa | LOQ (ng/L) |
|---|---|---|---|---|---|
| Anti-inflammatories | Acetaminophen | PARA | 0.46 | 9.4 | 1 |
| | Diclofenac | DICLO | 4.51 | 4.2 | 0.1 |
| | Ketoprofen | KETO | 3.12 | 4.5 | 0.2 |
| | Naproxen* | NAPROX | 3.18 | 4.2 | 0.1 |
| | Theophylline | THEO | -0.02 | 8.8 | 0.4 |
| HP | Bezafibrate* | BEZA | 4.25 | 3.3 | 0.02 |
| | Fenofibrate | FENO | 5.19 | - | 1 |
| | Fenofibric Acide | AC FENO | 4.00 | - | 0.02 |
| | Gemfibrozil* | GEMFI | 4.77 | 4.8 | 0.02 |
| AC | Cyclophosphamide | CYCLOP | 0.63 | - | 0.4 |
| AD | Metformine | METFOR | -0.50 | 12.4 | N.D. |
| AE | Carbamazepine | CARBA | 2.45 | 13.9 | 0.2 |
| BD | Salbutamol | SALBU | 0.64 | 10.3 | 0.2 |
| Anxiolytics | Alprazolam | APZ | 2.12 | - | 0.1 |
| | Diazepam | DIAZ | 2.82 | 3.4 | 0.2 |
| | Nordiazepam | NDZ | 2.93 | - | 0.1 |
| | Oxazepam | OXA | 2.24 | 1.5 / 10.9 | 0.2 |
| Diuretic | Furosemide* | FURO | 2.03 | 3.0 | 0.2 |
| Metabolites | 3,4-Dichloroaniline | DCA | 2.69 | - | 1 |
| | Acetylsulfamethoxazole | ACSMX | 1.21 | - | 0.1 |
| | Carbamazepine-EP | CARBA-EP | 1.26 | - | 0.04 |
| | Deisopropylatrazine | DIA | 1.50 | - | 0.1 |
| | Desethylatrazine | DEA | 1.51 | - | 0.2 |
| | Norfluoxetine | NFLUOX | 3.80 | - | 1 |

LOQ: limit of quantification; N.D.: not determined

HP: Hypolipemiants; AC: Anticarcinogen; AD: Antidiabetic; AE: Antiepileptic; BD: Bronchodilator
* : negative ionization (ESI -)

## **Materials and methods:** an efficient multiresidue method

250 mL of water sample

↓

**Sample filtration**: Glass filber filter 0.7 µm
**Sample acidification** to pH 5

↓

**Sample purification**: OASIS HLB ® 500 mg, 6 mL
Conditioning : 5 mL MeOH, 5 mL $H_2O$
Washing : 5 mL $H_2O$
Elution : 2*5 mL MeOH

↓

**Extract dilution** according to the matrix and expected concentrations

↓

**Analysis**: UHPLC-ESI(+/-)-QqQ-MS-MS
® Shimadzu Nexera 2 , API 4000 ® ABSciex
Column C18 BEH ® Waters (2.1 x 100 mm x 1.7 µm)

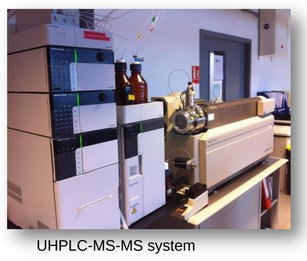
↓

**Identification/quantification**: MRM mode acquisition
Confirmation criteria are described in the EU council decision
Internal calibration
Use of 22 labelled molecular analogues as internal standards and surrogates

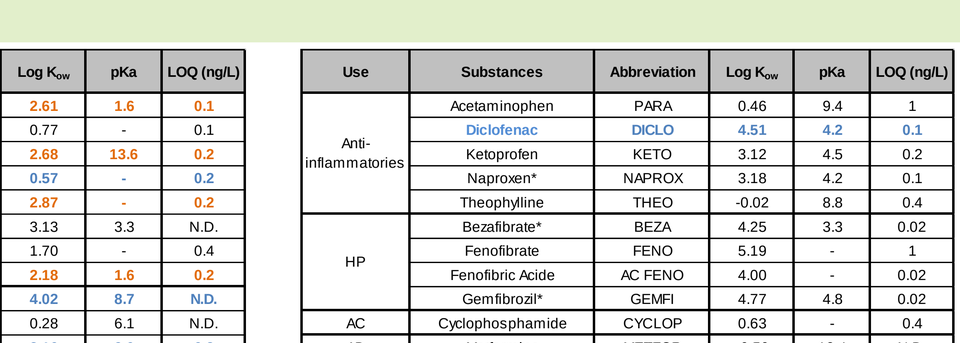
**Validation:**

- Linearity checked from LOQ values to 200 ng/L.
- Recoveries evaluated on a surface water spiked at 60 µg/L under reproducibility conditions (n=7):
  - Only qualitative analysis for NORFLO, CIPRO, METFOR, AZI, FENO.
  - Work in progress for negative ionization compounds.

water sample



UHPLC-MS-MS system

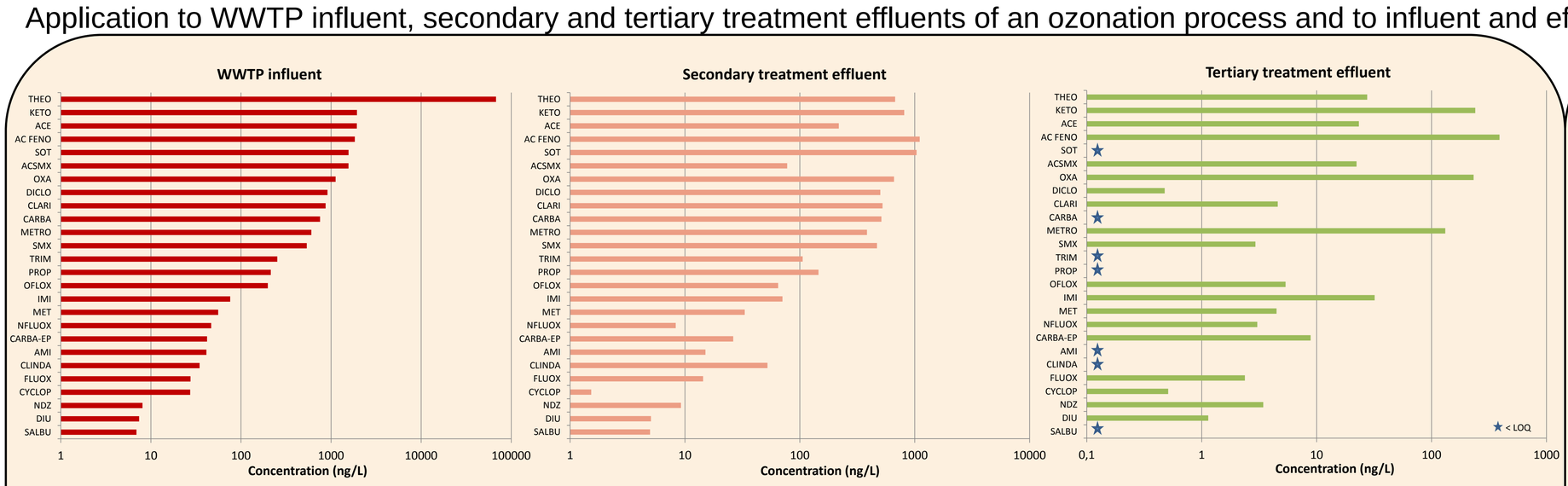



**Quality controls (QC):**

- Laboratory blanks (source water) to check non contamination.
- Spiked samples to check recoveries.
- QC standard solutions at 2 ng/L and 40 ng/L every 6 samples to check accuracy of the analysis and non deviation of instrumental measure. Acceptation criteria for QC standard solution: between 70 and 130 %.

## **Application to different types of wastewaters:** concentration levels

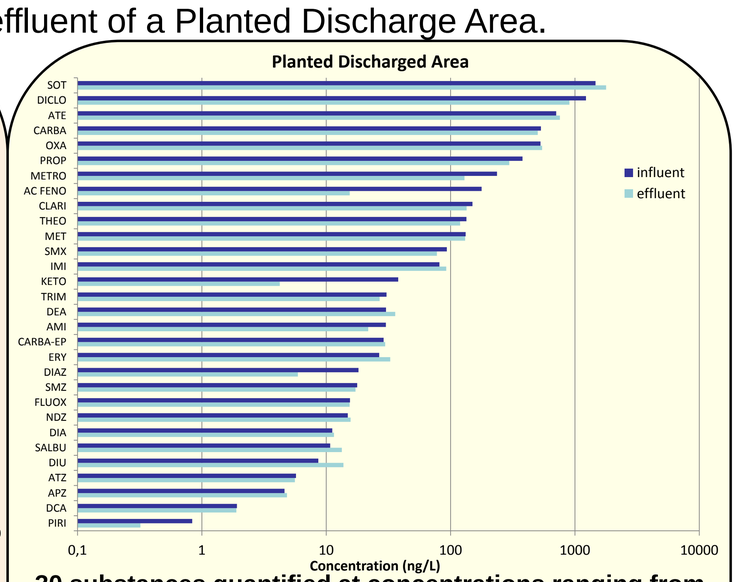
Application to WWTP influent, secondary and tertiary treatment effluents of an ozonation process and to influent and effluent of a Planted Discharge Area.




**26 substances quantified at concentrations ranging from 1.5 to 68 000 ng/L.**

**19 substances quantified at concentrations ranging from 0.5 to 390 ng/L.**

DCA, DIA, DEA, SMZ, ATZ, DIAZ, APZ and DIM were never quantified.
Analytical problems (matrix effects or QC out of specifications) for PIRI, PARA, IPU, ATE and ERY.




**30 substances quantified at concentrations ranging from 0.3 to 1 800 ng/L.**

IPU, CYCLOP and APZ were never quantified.
Analytical problems for NFLUOX, ACE, CLINDA, PARA, OFLO and DIM.

## Conclusions

- This study showed that our multiresidue analytical method is suitable for the quantification of 41 emerging micropollutants from ng/L to 100 µg/L in various water matrices.
- Further work is needed to better appreciate matrix effects on various aqueous matrices.
- This method will be adapted for biota matrices (*Gammarus fossarum*) and passive samplers extracts (Polar Organic Chemical Integrative Sampler).

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