Development of a quantitative screening method of organic pollutants by LC-Q-TOF in water and semi-quantitative approach

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Introduction

The quality of water (ground, surface and tap) is a major societal issue on national and European scale. Both public and private authorities are dedicated to upholding quality standards in accordance with regulatory mandates. The list of parameters subject to health monitoring may evolve with the identification of new substances. Therefore, it is essential to be able to provide answers regarding the identity and concentration of these substances to assess information on environmental pollution. This work introduces the development of a LC-Q-TOF method for targeted quantitative screening and suspect screening of environmental pollutants in water.

The aim was to maximize the identification of pollutants, including pesticides and their metabolites, pharmaceutical products and emerging contaminants, in two analyses. The lack of available analytical standards (due to non-commercial synthesis or high costs) raises concerns regarding the confidence levels associated with compound identification and semi-quantification. Therefore, initial semi-quantification trials, based on existing methods[1,2] were carried out on a proficiency test sample.

Materials and methods

Instrumentation:

- **Liquid chromatography:** ELUTE UHPLC (BRUKER)
- **Mass spectrometer:** Q-TOF Impact II (BRUKER)
- **Ion source:** VIP-HESI (BRUKER)

Acquisition method:

- m/z range 30 – 1200 m/z
- Data Independent Acquisition (DIA):
  - Full Scan spectra
  - Collision-Induced Dissociation

Chromatographic conditions:

- **Column:** intensity solo C18 (100 x 2.1 mm, 1.8 µm, Bruker)
- **Mobiles phases:**

Positive mode:

A) H_2O:MeOH (99:1 ; v:v), 0.01% formic acid, 5 mM ammonium formate

B) MeOH, 0.01% formic acid, 5 mM ammonium formate

Negative mode:

A) H_2O:MeOH (99:1 ; v:v), 5 mM ammonium acetate

B) MeOH, 5 mM ammonium acetate

Sample preparation: Filtration (0.7 µm)

Injection volume: 100 µL

Molecules and databases

Pesticides and micropollutants	Veterinary drugs and TPs	Toxic compounds
> **1000** compounds LC, MS and MS/MS information	> **200** compounds LC, MS and MS/MS information	> **1000** compounds LC, MS and MS/MS information
> **400** standards in QC sample	> **10** standards in QC sample	> **50** standards in QC sample
~ **200** metabolites		
Carbamates, chloroacetamides, triazines, triazoles, ureas,…	Benzimidazoles, coccidiostats, hormones, NSAIDs, sedatives,…	Alkaloids, betagonists, cannabinoids, corticoids, other sedatives,…

Quantitative method performances

455 molecules:

- Pesticides, metabolites
- Emerging pollutants
- Drugs

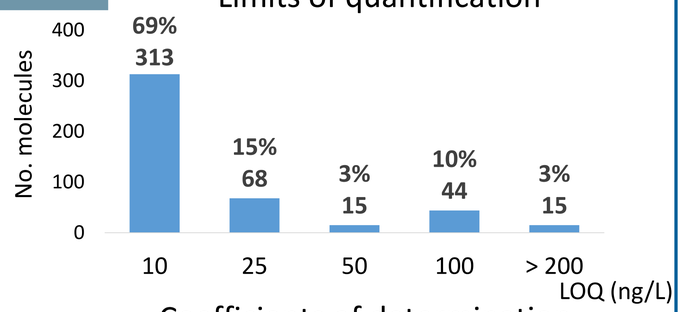
Evaluation protocole:

- Matrix: **drinking water** (Evian)
- Range:
  - 1 - 1000 or 5 - 5000 ng/L
- 6 replicates per level

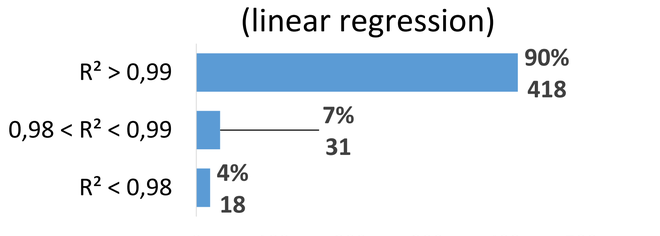
LOQ estimation requirements:

- **S:N ratio** ≥ 10
- **Δm/z** ≤ 5 ppm or 1 mDa
- **RSD (%)** ≤ 20%

Limits of quantification



Coefficients of determination (linear regression)



→ **440 molecules with LOQ ≤ 100 ng/L**

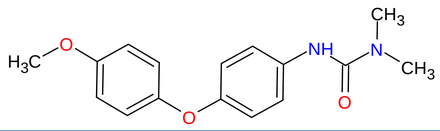
Response factors (RF)

356 molecules of interest:

- **$[M+H]^+$**
- Pesticides, metabolites and drugs
- Matrix: **drinking water** (Evian)
- **8 series** over a **year**

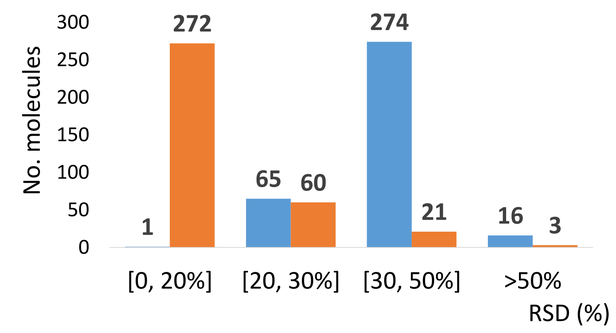
Reference compound:

- **Difenoxuron**
- Mid LC gradient (Rt = 7.8 min)
- Highly sensitive
- Allows **R**elative **I**onization **E**fficiency (**RIE**) calculation



$$RF = Slope_{compound} \qquad RIE = \frac{RF}{RF_{ref}}$$

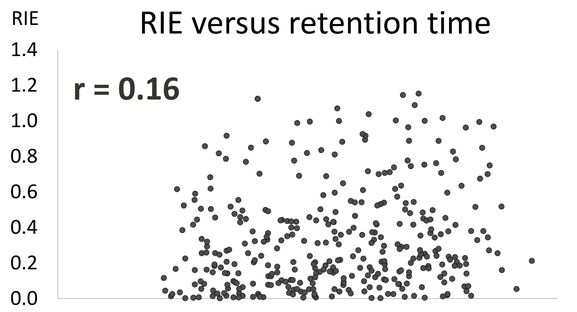
RSD (%) distribution over a year for the RF and RIE



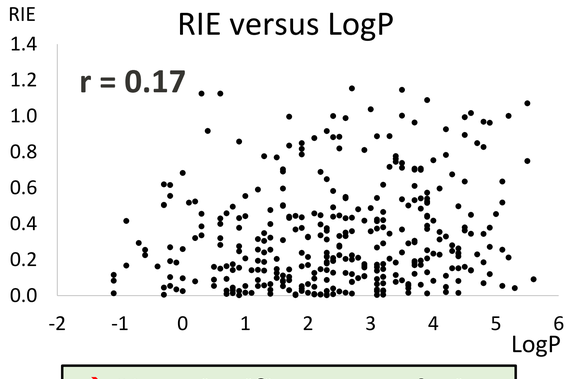
→ **Good RIE stability over a year**

RIE vs Rt and LogP

RIE versus retention time



RIE versus LogP



→ **Non-significant correlation**

Semi-quantitative approach on surface water proficiency test sample

ESI+ : 48 molecules (pesticides and metabolites) in concentration range 12 – 768 ng/L

2D structure similarity

Based on chemical fingerprint:

- Comparison of different **fingerprint types**
- R script using **C**hemistry **D**evelopment **K**it library (**RCDK**)
- Input data : compounds SMILES
- Similarity classification determined by the **Tanimoto coefficient**
- Semi-quantified with **top 1** similar molecule from in-house database (**341 molecules**)

Prediction of ionization efficiency

Based on online machine learning tools:

- Identifying the **chemical descriptors** of interest to predict **Ionization Efficiency** (**IE**)
- Using **machine learning** tools to train the models to predict IE

Semi-Quantification of Emerging Pollutants(v3.0)[1]:

- **7** significant descriptors (ESI+)
- Ant Colony Optimization-MLR (ACO-MLR)

Quantem[2]:

- **450** significant descriptors (ESI+)
- Eluent composition and LC gradient
- Random forest regression

	2D structure similarity					Prediction of ionization efficiency	
Fingerprint type	Standard	MACCS	Pubchem	ECFP2	**FCFP2**	Semi-quant EP[1]	**Quantem[2]**
Mean **E**rror **F**actor (**MEF**)	4.8	6.1	5.9	3.6	**3.6**	5	**2.6**
No. molecules with MEF > 10	4	6	4	5	**3**	6	**1**

MACCS: **M**olecular **ACC**ess **S**ystem – **ECFP**: **E**xtended-**C**onnectivity **F**inger**P**rints – **FCFP**: **F**unctional-**C**lass **F**inger**P**rints

MEF = MAX($C_{predicted}/C_{PT\ assigned\ value}$; $C_{PT\ assigned\ value}/C_{predicted}$) PT: Proficiency Test

Conclusions and perspectives

This work led to the development of a sensitive LC-Q-TOF quantitative screening method for pesticides, drugs, and their metabolites. Indeed, the reference concentration level of 0.1 µg/L was reached for more than 400 compounds in drinking water. The method enables suspect screening using in-house databases. Due to the lack of analytical standards, several semi-quantitative approaches were evaluated in a proficiency test sample. The best results were obtained with Quantem with a mean error of a factor 2.6, which uses machine learning to predict ionization efficiency. Also, 2D structure similarity FCFP fingerprint approach showed good performance, whereas approaches based on retention time or LogP did not correlate well with RIE. Further semi-quantification tests are being carried out on other samples.

References:

[1] R. Aalizadeh, V. Nikolopoulou, N. Alygizakis, J. Slobodnik and N. S. Thomaidis. Semi-Quantification of Emerging Pollutants (v.3.0). App developped by R. Aalizadeh at University of Athens (2021-2022).

[2] Quantem. Available at https://quantem.co/

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