Non-target screening for the comprehensive monitoring of organic contaminants in large rivers and small streams impacted by agricultural activities in Jaén

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INTRODUCTION

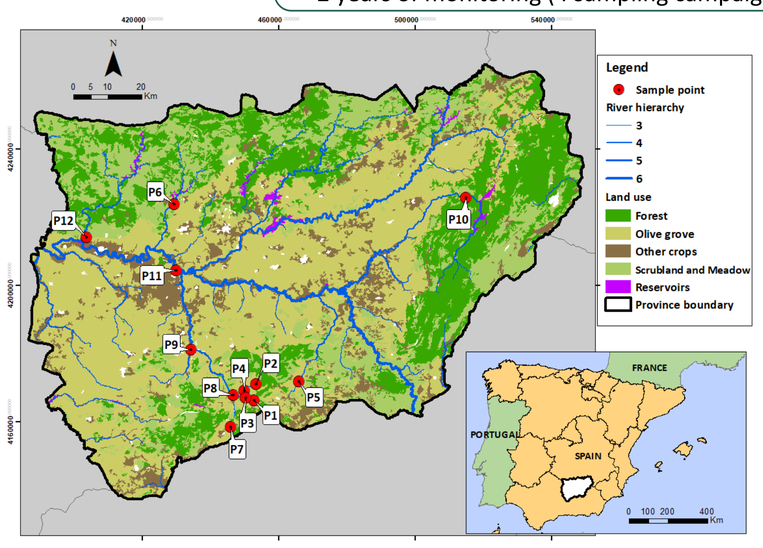
Surface waters are continuously exposed to organic contaminants (OCs) and their transformation products (TPs), which may be more mobile, toxic, and persistent than parent compounds. In rural areas, where agriculture is the main economic activity, pesticides are one of the main sources of contamination. Likewise, wastewater treatment plants of small populations have poorly upgraded secondary or tertiary wastewater treatments to completely remove OCs at trace levels, so pharmaceuticals or personal care products may be dumped into surface waters. In recent years, the scientific community has mainly addressed the monitoring of environmental contamination by applying analytical techniques based on liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-HRMS/MS). Moreover, the development of novel non-target screening (NTS) approaches based on LC-HRMS has expanded the scope of analysis beyond target compounds, allowing the discovery and monitoring of unknown contaminants and their qualitative and semi-quantitative details.

In this work, an NTS strategy using LC-HRMS and MS-DIAL as open-source software was applied to investigate the occurrence of OCs and their TPs in large rivers and small streams of the province of Jaén, located in the southeast of Spain, where olive oil production is the main economic activity. Software pre-processing settings were carefully optimized using 20 target compounds. MassBank of North America (MoNA) was used as a spectral library database for compound annotation. This strategy was successfully applied to retrospectively analyze 37 water samples collected from 12 sampling points over 2 years of monitoring. A total of 37 OCs, comprising pesticides, pharmaceuticals, and TPs were tentatively identified with high confidence, proving the applicability of the developed NTS strategy for monitoring contaminants out of the scope of analysis.

MATERIALS AND METHODS

Area of study

- 12 sampling points (rivers and streams)
- 37 water samples
- 2 years of monitoring (4 sampling campaigns)

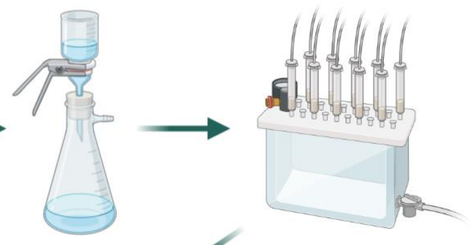


Sample preparation

Sample collection (river water)

Filtration 0.45 µm (cellulose)

SPE procedure Oasis HLB (200 mg)



Evaporation
N_2 stream (15 psi)
Water bath (37 °C)

Final preconcentration factor x 20

UHPLC-Orbitrap-MS

UHPLC parameters:

- Column: ZORBAX Eclipse Plus C18 2.1 mm x 50 mm, 1.8 µm (Agilent)
- Mobile phase: A: H_2O (0.1% FA), B: MeOH (0.1% FA)

MS analysis:

- Ion source: HESI
- Polarity: +/- (individual analysis)
- Full MS + DDA, NCE

MS-DIAL optimization: 20 target compounds

Steps	Optimized parameters				
Data collection	MS1 tolerance	MS2 tolerance	Max charged number		
Peak detection	Min peak height	Mass slice width	Smoothing method	Smoothing level	Min peak width
Spectrum deconvolution	Sigma window value	MS2 abundance cut off			
Identification	MS1 tolerance	MS2 tolerance	% Match spectrum	Min # of matched spectra	
Adduct selection	Positive ions	Negative ions			
Alignment	RT tolerance	MS1 tolerance			

NTS workflow

Data conversion
Raw → Abf (Reifycs Abf Converter)

Data pre-processing
- MS1 tolerance: 0.002 Da
- MS2 tolerance: 0.05 Da
- Blank subtraction

Prioritization of peaks
- Min height > 50000
- RT Alignment (0.2 min)

Library match
- MS1 tolerance: 0.002 Da
- MS2 tolerance: 0.05 Da
- ≥ 4 ion products (>80% score)

Tentative identification
- MS1, MS2
- Structure
- Literature

Confirmation with analytical standard
- RT (± 0.2 min)
- MS2 spectra

Automatic processing

MS-DIAL (v 5.1)

MoNA - MassBank of North America

Manual inspection

Xcalibur (v 3.0)

RESULTS AND DISCUSSION

Non-target screening results

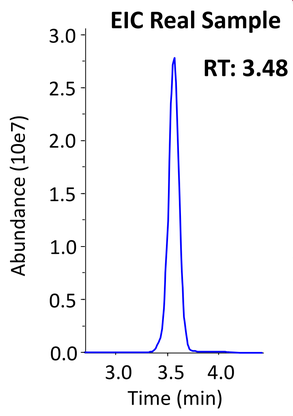
Pre-processing 27215 features → **Prioritization of peaks** 5338 features → **Library match** 296 features → **Tentative identification** 37 candidates

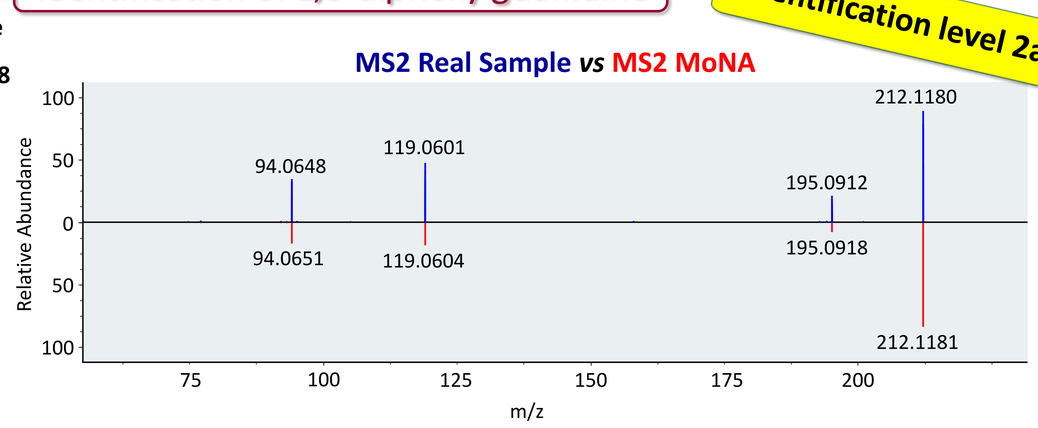
11 analytical standards acquired → **9 True positives** ✓ / **2 False positives** ✗

List of findings:
Level 1: 9
Level 2a: 26
Level 3: 2

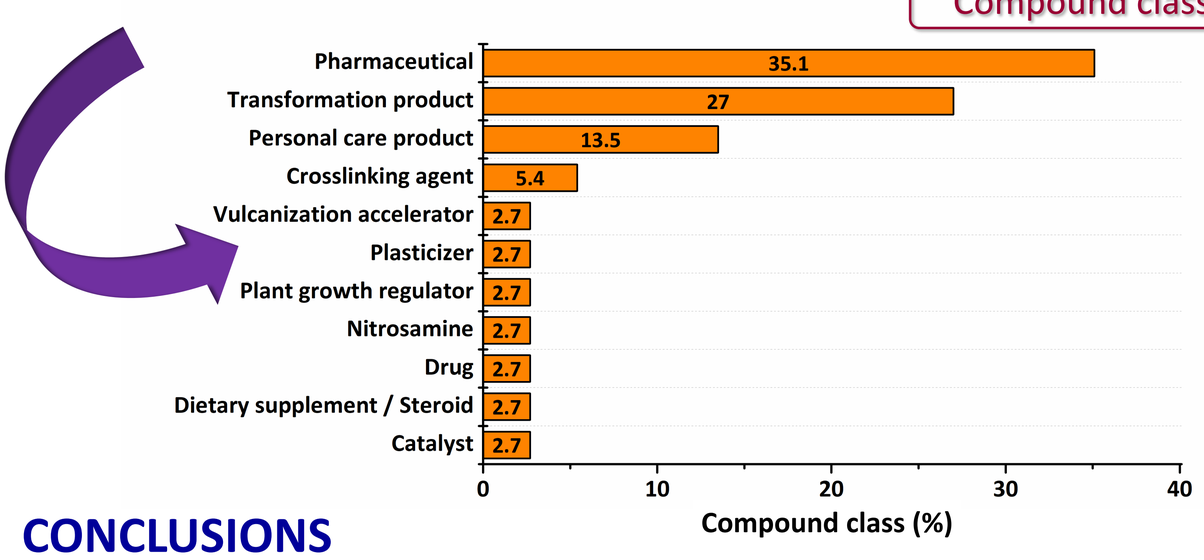
Identification of 1,3-diphenylguanidine

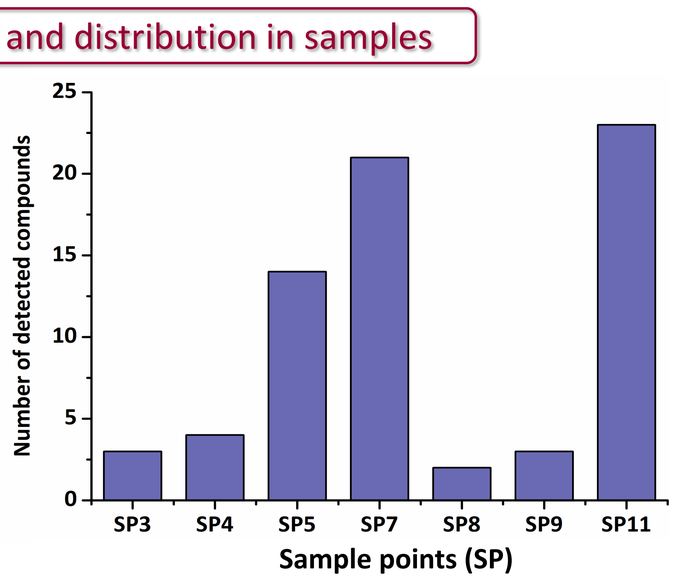
Identification level 2a





Compound class and distribution in samples





- Any compounds were detected in **headwater** samples (SP1, SP2, SP6, SP10).
- SP11 (Guadalquivir River) exhibited the highest number of OCs. It is the **largest river** within the study, receiving the inflow of numerous tributaries, which contribute to the **accumulation effect**.
- Although SP7 is classified as a lower-order stream, the **absence of WWTPs** in the villages upstream may account for a higher number of detected compounds.

CONCLUSIONS

- An open-science NTS strategy has been applied for the (tentative) identification of OCs in 37 surface water samples from the Guadalquivir river basin.
- A total of 37 OCs were tentatively identified; 9 of them were confirmed with analytical standards, 26 were identified at high confidence level (2a), and 2 compounds at level 3. Two false positives were found after purchasing the analytical standard.
- More than 60% of the tentatively identified analytes were pharmaceuticals of different therapeutic classes and their TPs.
- Headwaters (SP1, SP2, SP6, SP10) presented a lower number of detected compounds than samples taken downstream. The Guadalquivir river (SP11) showed the highest number of detected compounds presumably due to accumulation effects.
- This study emphasizes the wide variety of OCs that can be present in surface waters, and evidences the contamination variation between small streams and large rivers of the Guadalquivir basin.

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