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Use of Non-Targeted Workflows for the Analysis of Pharmaceutical and Illicit Drug Occurrence in Environmental Samples

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Overview

- High resolution LC-MS/MS methods for targeted and non-targeted workflows were applied to the analysis of wastewater and river water samples taken from a heavily urbanized tidal river catchment area (London, UK).
- Targeted and non-targeted workflows used a standardized LC-MS/MS method to increase reporting confidence in compound identification (reported analytes agree with a precursor mass accuracy error, isotopic pattern, retention time and library verification with product ion spectra).

1. Introduction

Quantitative monitoring of large panels of contaminants of emerging concern (CECs) in environmental samples is a key enabling tool to assess the impact of human exposure from prescription pharmaceuticals, lifestyle chemicals and illicit drugs. Although targeted mass spectrometry workflows have been successfully used in wastewater-based epidemiology (WBE) the challenge is to work with inherently complex samples and changing CEC usage.

In this work, both targeted and non-targeted workflows were used to identify a number of illicit drug and pharmaceutical compounds in river water and wastewater samples. Each target was confirmed using accurate mass, isotopic distribution, retention time and accurate mass fragment spectrum data. For non-targeted or suspect screening analysis, a series of tools were used including component detection, suspect screening search lists (to match molecular ion features) and to provide evidence for identification (fragment ion matching with external data bases using a fragment structure assignment application).

2. Materials and Methods

Samples of river water and waste were prepared by filtering using a PTFE 0.2um filter (Millex-FG hydrophobic PTFE membrane, SLFGR04NL) and injected directly into a HRMS LC-MS/MS (LCMS-9030, Shimadzu Corporation, Japan). The same samples were also quantified using a validated triple quadrupole LC-MS/MS method (LCMS-8060, Shimadzu Corporation).

Table 1. HRMS LC-MS/MS parameters.

HRMS LC-MS/MS method | LC parameters	
Sample injection	Direct injection; 40 uL
Column	Shim-pack Velox Biphenyl (2.1 mm x 100 mm, 2.7um).
Mobile phase A	2 mM ammonium formate +0.002% formic acid
Mobile phase B	Methanol + 2 mM ammonium formate +0.002% formic acid
Flow rate	0.3 mL/min
HRMS LC-MS/MS method | MS parameters	
Cycle time	0.9 seconds for all mass scans
TOF survey	100-920 Da; 100 msecs; positive ion
DIA-MS/MS	40-920 Da; 25 msecs for each mass scan; 32 DIA-MS/MS mass scans; variable isolation width; CE 5-55V
Mass calibration	External mass calibration
Data processing	LabSolutions 5.99 and Insight 3.8 research application

3. Results

3.1 Non-Targeted Workflows

The workflow involves the following steps:

1. **Detecting Components,** with Insight Analyze chromatographic deconvolution algorithm. This step generates a list of components as m/z, RT and ion abundance.
2. **Matching detected components with a search list** based on expected m/z, isotopic distribution (and within an expected RT window) within the search list.
3. **Verifying identified targets:** cross-referencing results to a highly curated high-resolution mass spectrometry library (table 2) generating a DotProd score.
4. **Reporting criteria:**
   - **Precursor ion:**
     - **Quantitation mass accuracy** < 5 ppm
     - **Isotope distribution score** > 30
     - **RT** < 0.5 min
   - **Product ion spectra (DIA-MS/MS mass scans):**
     - **Library similarity score (Similarity Index; SI)** > 40 (default settings applied to DotProd weightings)

Table 2. Summary of Library Screening.

Toxicology and Pesticide libraries:	
Spectra in libraries	>1300 combined chromatographically separated authentic standards
CE spread	5-55 V
Precursor isolation	1 Da width (targeted MS/MS)
RT	Standardized LC with a Shim-pack Velox Biphenyl column
Product ion spectra	MS/MS verified with Assign fragment annotation tool and curated for spectrum noise
Freely editable	Scalable to build crowd sourced libraries

3.2 Targeted workflows

Compounds identified in the non-targeted workflow were validated and quantified using authentic standards confirming identification/FPR/FNR:

1. Using a targeted QTOF method, previously identified components were used as a search list and quantified using authentic standards (Table 3). An example of the workflow and identification of cocaine in wastewater is shown in Figure 1.
2. Quantitative results from the Q-TOF were cross compared to results from an established validated triple quadrupole LC-MS/MS MRM method(1).

Comparison of quantitative results showed close agreement between both QTOF and LC-MS/MS measurements; plotting the analyte concentrations determined by the QTOF v TQ resulted in a linear regression analysis with a slope close to unity (Figure 2).

Figure 1. Screenshot of LabSolutions Insight software highlighting cocaine detected in the wastewater sample which met the reporting criteria.

4. Conclusions

- Non-target workflows using a standardized LC-MS/MS method with DIA-MS/MS mass scans can be highly effective in screening environmental samples. In this study, metformin, cocaine and its primary metabolite benzoylecgonine were detected in both waste and river water samples at high concentrations. Interestingly levamisole, a known cutting agent was also detected. CECs from the suspect screening experiment included clozapine, citalopram, fluoxetine and sertraline.
- As the data acquired are data independent, retrospective analysis for new or emerging analytes is possible for research purposes. A new or emerging analyte can be added to the search list or compound list and the mass accuracy, isotopic pattern, RT and product ion fragments are used to find suspect identifications.

Table 3. Comparison of component concentration in wastewater and river water quantitated with QTOF method.

Compound	Concentration (ng/L) Wastewater	River water
Amitriptyline	77	
Benzatropine	134	96
Benzoylecgonine	1079	11
Carbamazepine	222	64
Citalopram	308	
Clothianidin		11
Clozapine	75	
Cocaine	484	
Diclofenac	86	78
Fluoxetine	25	
Imidacloprid	27	16
Ketamine	54	
Ketoconazole	101	
Levamisole	33	33
Lidocaine	67	15
MDMA	102	
Metformin	>ULOQ	526
Miconazole	37	
Morphine	350	
Nicotine	2236	
Oxamyl		63
Oxycarboxin	21	9
Propranolol	50	20
Sertraline	187	
Temazepam	17	
Terbutryn	24	
Tramadol	214	78
Trimethoprim	176	21
Venlafaxine	194	57
Verapamil	6	

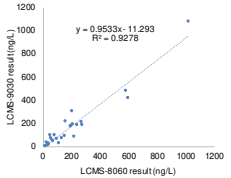

Figure 2. Comparison of quantitative results across two LC-MS/MS platforms: triple quadrupole (LCMS-8060) and Q-TOF (LCMS-9030) showed good correspondence.

5. References

(1) KT Ng et al., *J Hazardous Materials, 398, 2020, 122933*