Screening and Quantitation of Targeted and Non-targeted Environmental Pollutants in Water Samples

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OVERVIEW

A wide range of Pharmaceuticals and Personal Care Products (PPCP) were determined in river water samples using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Different water samples were injected directly into the LC-MS/MS to quantify PPCP at low parts-per-trillion levels (ng/L). Multiple Reaction Monitoring was used for detection on an AB SCIEX QTRAP® 5500 system for highest sensitivity and selectivity with the *Scheduled* MRM™ algorithm activated for best accuracy and reproducibility. In addition high resolution and accurate mass MS and MS/MS data acquired on an AB SCIEX TripleTOF® 5600 system was screened for non-target and unexpected pollutants.

INTRODUCTION

PPCP encompass a wide range of pollutants, including Endocrine Disrupting Compounds (EDC), pesticides, hormones, antibiotics, drugs of abuse, x-ray contrast agents, drinking water disinfection by-products to name a few. In order to properly assess the effects of these compounds on our environment, it is necessary to accurately monitor their presence. The diversity of chemical properties of these compounds makes method development challenging. LC-MS/MS is able to analyze polar, semi-volatile, and thermally labile compounds covering a wide molecular weight range. In addition, state-of the-art LC-MS/MS instruments operated in selective Multiple Reaction Monitoring (MRM) mode, offer unmatched selectivity and sensitivity to quantify PPCP reproducibly at trace levels without time consuming and extensive sample preparation.[1-4]

A method is presented for analyzing 80 EDC and PPCP compounds using LC-MS/MS. This method is a straight forward approach for the analysis and identification of these compounds with excellent sensitivity and ruggedness.[5]

More recently there is a growing interest from environmental researchers to also screen for and identify non-targeted compounds in environmental samples, including metabolites and degradates, but also completely unexpected pollutants. The AB SCIEX TripleTOF® 5600 ystem is capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also performing selective and characteristic MS/MS scanning for further compound identification and, therefore, is the instrument of choice for this challenging task. General unknown screening workflows do not use a target analyte list and compound detection is not based on any a priori knowledge, including retention times and information on possible molecular and fragment ions. Therefore, acquired chromatograms are very rich in information and can easily contain thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore such data to identify the unexpected compound.

Data processing workflows are presented to successfully identify unexpected environmental pollutants using non-target peak finding, statistical data analysis, empirical formula finding, and MS/MS fragment interpretation.[6]

EXPERIMENTAL

Sampling and Sample Preparation
More than 70 water samples in different cities and countries from different type of waters, including drinking water, creeks, rivers, lakes, sea etc were collected by different scientist. Samples were kept frozen until analysis.

LC Separation
A Shimadzu UFLC$_{XR}$ system was used with a Phenomenex LUNA 2.5u C18(2)-HST 100 x 3 mm column and fast gradients of water and acetonitrile with 0.1% formic acid at a flow rate of 0.8 mL/min. An sample volume of 100 µL was injected directly.

MS/MS Detection
The AB SCIEX QTRAP® 5500 LC/MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe was used. The mass spectrometer was operated in MRM mode using the *Scheduled* MRM™ algorithm. The AB SCIEX TripleTOF® 5600 LC/MS/MS system with DuoSpray™ source operated in ESI mode. An IDA experiment was used with a TOF-MS survey (100 ms) and up to 10 dependent MS/MS scans (accumulation time of 50 ms) using Collision Energy Spread (35 V ± 15 V).

RESULTS AND DISCUSSION

Targeted Quantitation using the QTRAP® 5500 system
The combination of a small particle size column (2.5 µm), high flow rate (0.8 mL/min), and large injection volume (100 µL), with high sensitivity MS/MS detection allowed the direct injection of water samples and detection of PPCP with Limits of Detection (LOD) in the low parts per trillion (ppt) range. Two MRM transitions were monitored for each of the 80 analytes to quantify and identify using MRM ratio calculation. The *Scheduled* MRM™ algorithm automatically adjusts dwell times for best Signal-to-Noise (S/N) based on retention time and targeted cycle time input. Two MRM transitions were monitored for each analyte, the most sensitive, first MRM transition was used for quantitation while the second MRM transition was used for qualitative identification based on ion ratio calculation.

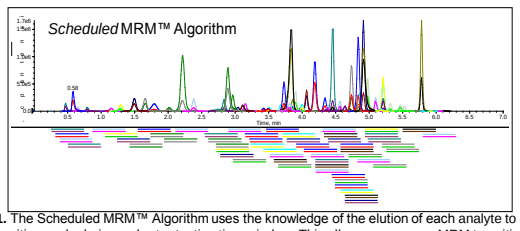


Figure 1. The Scheduled MRM™ Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

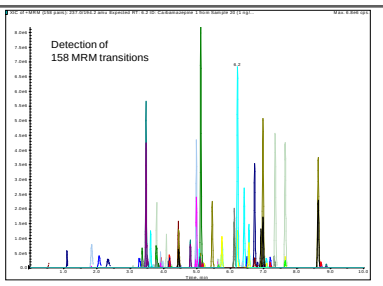


Figure 2. LC-MS/MS Detection of 80 PPCP at 1 µg/L

The developed LC-MS/MS method was used to screen collected water samples. Results of quantified PPCP are shown in Figures 3 and 4. All findings were identified by comparing the MRM ratio of the unknown sample with the average MRM ratio of standard injections.

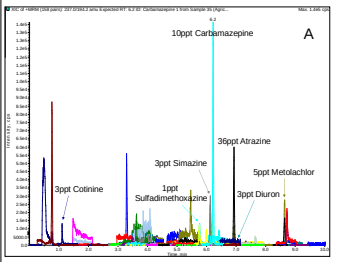


Figure 3. Quantitation of PPCP in a water samples from an agricultural area

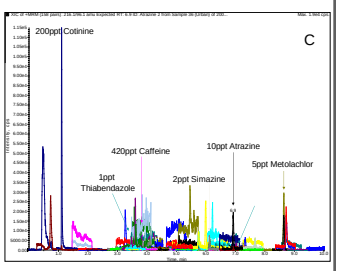


Figure 4. Quantitation of PPCP in a water samples from an urban area

Figure 6 shows the quantitative results of Benzoylecgonine, a metabolite of Cocaine, in the studied water samples. All drinking water samples had a concentration of benzoylecgonine below 5 ng/L. As expected, Benzoylecgonine was found in rivers running through major cities at concentrations of up to 200 ng/L indicating the abuse of cocaine. The concentration of Benzoylecgonine in water samples collected in less urban and wilderness areas was much lower with the exception of one creek and one lake in popular vacation destinations. The concentration of benzoylecgonine reflects the amount collectively excreted in urine and can be used the estimate drug consumption. Also when analyzed over time drug consumption habits can be investigated.[7]

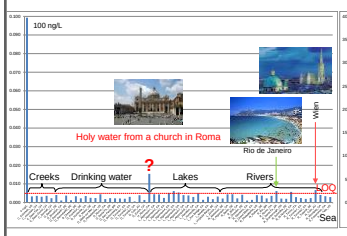


Figure 6. Findings of Benzoylecgonine, a metabolite of cocaine and indicative for cocaine abuse, in various water samples

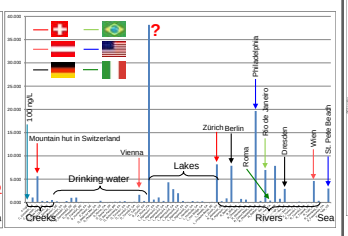


Figure 7. Findings of caffeine in various water samples

Figures 7 and 8 show the findings of caffeine and Sildenafil in studied waters. The water sample with a question mark was collected from a holy water basin in a church in Roma, Italy. The summary of detected compounds in the holy water sample is presented in Table 1.

Non-Targeted Screening using the TripleTOF® 5600 system
Full scan MS and MS/MS allow retrospective screening and identification of non-target and unexpected compounds. Acquired chromatograms are very rich in information and can easily contain thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore such data to identify the unexpected compound. Two data processing workflows are possible. **1)** Comparison of two similar samples (sample and control) and **2)** Statistical comparison of a huge set of samples (metabolomic profiling)

Compound	µg/L	LOQ (µg/L)
Acetaminophen	9.1	0.010
Benzoylecgonine	0.47	0.001
Caffeine	38	0.010
Carbamazepine	0.21	0.001
Codeine	0.050	0.001
Dextromethorphan	0.021	0.001
Diazepam	0.003	0.001
EDDP	0.001	0.001
Erythromycin	1.7	0.050
Morphine	0.15	0.005
Sildenafil	0.015	0.005
Thiabendazole	0.016	0.001

Table 1. Compounds detected in a holy water sample

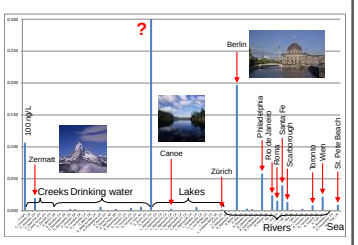


Figure 8. Findings of the virility regulator Sildenafil in various water samples

1) The MasterView™ software was used to compare two different water samples. A non-target peak finding algorithm is used to automatically generate extracted ion chromatograms (XIC). The user can then define an intensity threshold to compare both samples. Figure 10 shows an example of comparing two lake water samples. A total of 36 signals were found in the urban lake water which were 10x higher than in the control water. DEET was automatically identified using MS/MS library searching (Figure 9),

empirical formula finding, and ChemSpider searching, the structure found in ChemSpider was automatically fragmented and compared against the MS/MS spectrum (Figure 10).
2) In the case that a proper control sample is not available or that contaminations have to be identified at lower concentrations a statistical approach is needed. A large set of water samples was compared using principal components analysis (PCA) in MarkerView™ software and DEET was identified using empirical formula calculation follow by an online database search. (Figure 11)[6]

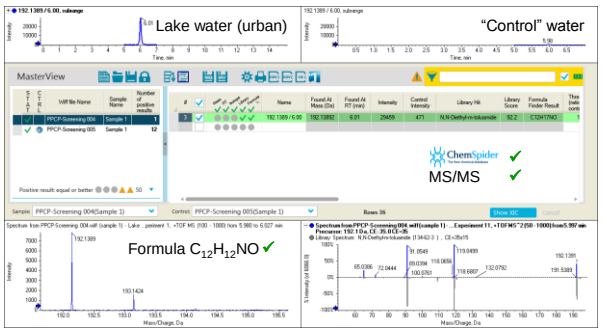


Figure 9. Comparison of two water sample using the MasterView™: DEET was identified using automatic MS/MS library searching (FIT = 92.2)

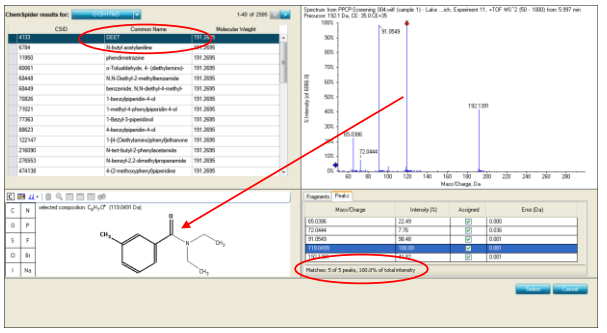
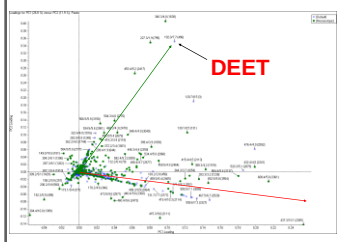
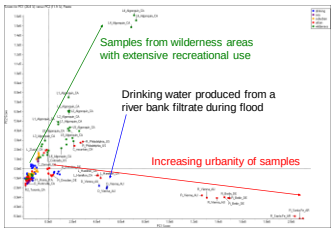


Figure 10. Further identification of DEET based on empirical formula calculation followed by ChemSpider searching





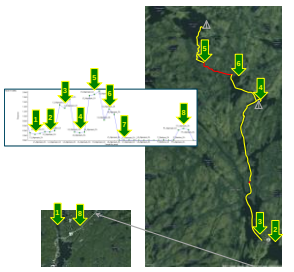


Figure 11. Principal Components Analysis to identify unexpected water contaminants: the insect repellent DEET was identified in samples from wilderness areas and DEET profile measured in Algonquin Provincial Park (ON)

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TRADEMARKS/LICENSING

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