

Quantitation and Identification of Pharmaceuticals and Personal Care Products (PPCP) 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.

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

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.



Water samples were injected directly after filtration without additional cleanup.

LC Separation

A Dionex Ultimate 3000 Rapid Separation LC system was used with a Phenomenex LUNA 2.5u C18(2)-HST 100 x3 mm column and fast gradients of water and acetonitrile with 0.1% formic acid at a flow rate of 0.8 mL/min. An injection volume of 100 μ L was used.

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. MRM mode allowed screening and quantifying targeted compounds with highest selectivity and sensitivity by monitoring the transition from the precursor ion (filtered in Q1) to a product ion (generated in a collision cell Q2 and filtered in Q3). The *Scheduled* MRM[™] algorithm monitors transitions automatically during a short retention time window only. This allows many more MRM transitions to be monitored in a single LC run, while still maintaining maximized dwell time and optimized cycle time.

Results and Discussion

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 on a QTRAP[®] 5500 equipped with Turbo V[™] source allowed the direct injection of water samples and detection of PPCP with Limits of Detection (LOD) in the low parts per trillion 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.

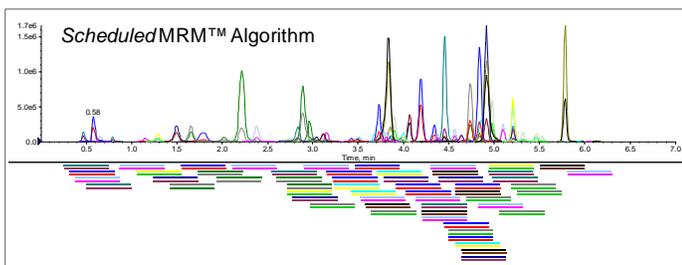


Figure 1 The *Scheduled MRM*[™] Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

An LC-MS/MS example chromatogram of 80 PPCP at a concentration of 1 $\mu\text{g/L}$ is shown in Figure 2.

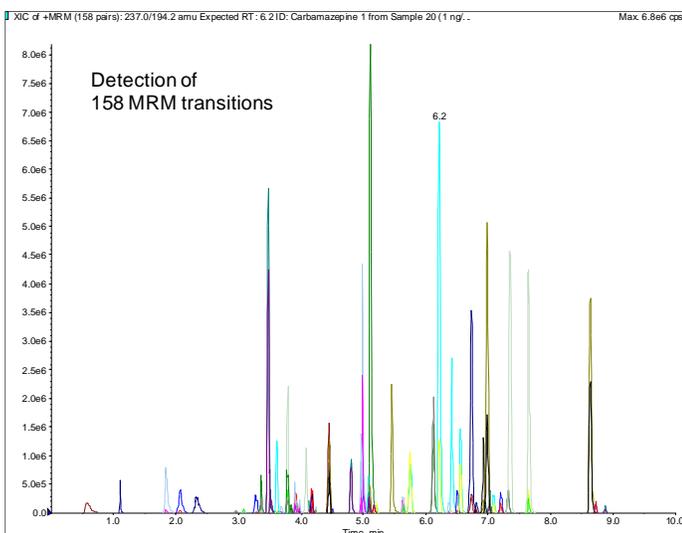


Figure 2. LC-MS/MS Detection of 80 PPCP at 1 $\mu\text{g/L}$

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.

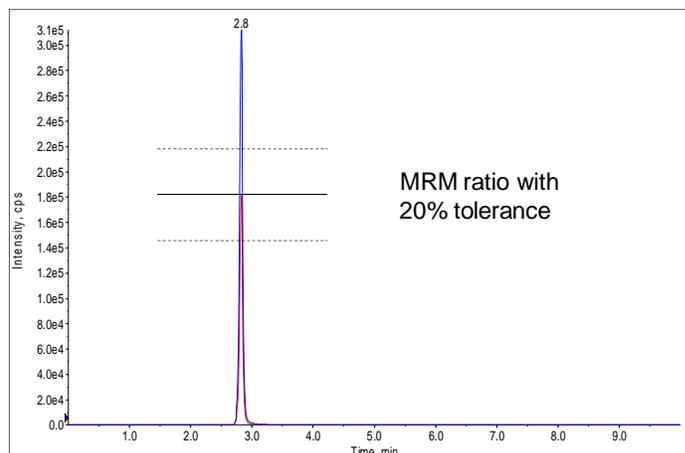


Figure 3. Identification based on MRM ratio calculation with tolerance levels of 20%

Example chromatograms of 15 selected analytes at a concentration of 10 ng/L (10 ppt) are presented in Figure 4. The superior sensitivity of the new QTRAP[®] 5500 system is highlighted by S/N values between 10 and 1500 (calculated with an algorithm using 3 times the standard deviation of the noise). Such low LOD allow the detection of PPCP in water samples by direct injection without additional cleanup or time consuming and extensive concentration.

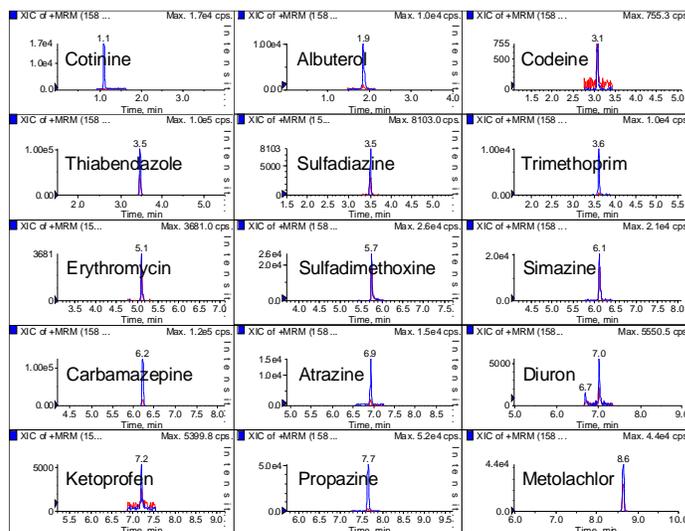
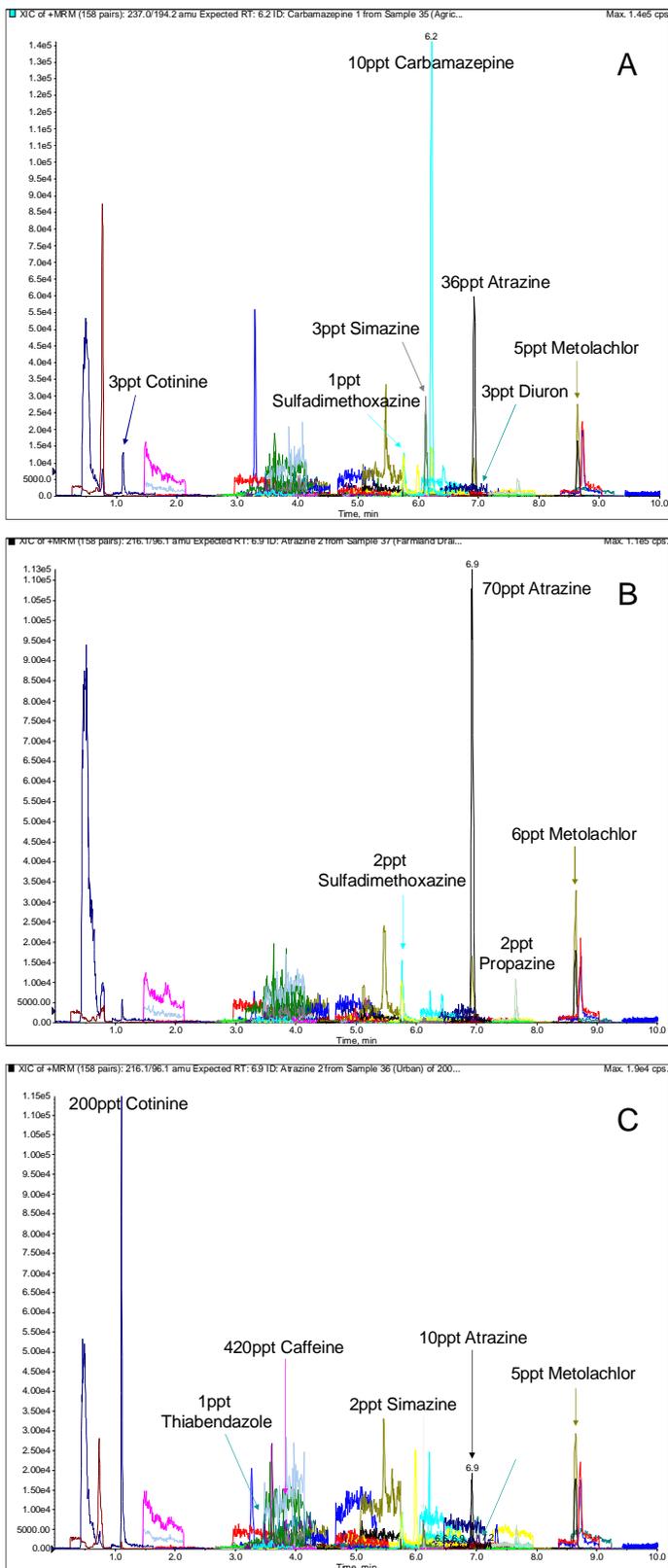


Figure 4. Example chromatograms of 15 selected PPCP at a concentration of 10 ng/L



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

Figure 6 shows the quantitative results of Benzoylcegonine, a metabolite of Cocaine, in the studied water samples. All drinking water samples had a concentration of benzoylcegonine below 5 ng/L. As expected, Benzoylcegonine was found in rivers running through major cities at concentrations of up to 200 ng/L indicating the abuse of cocaine. The concentration of Benzoylcegonine 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 benzoylcegonine reflects the amount collectively excreted in urine and can be used to estimate drug consumption. Also when analyzed over time drug consumption habits can be investigated.⁶

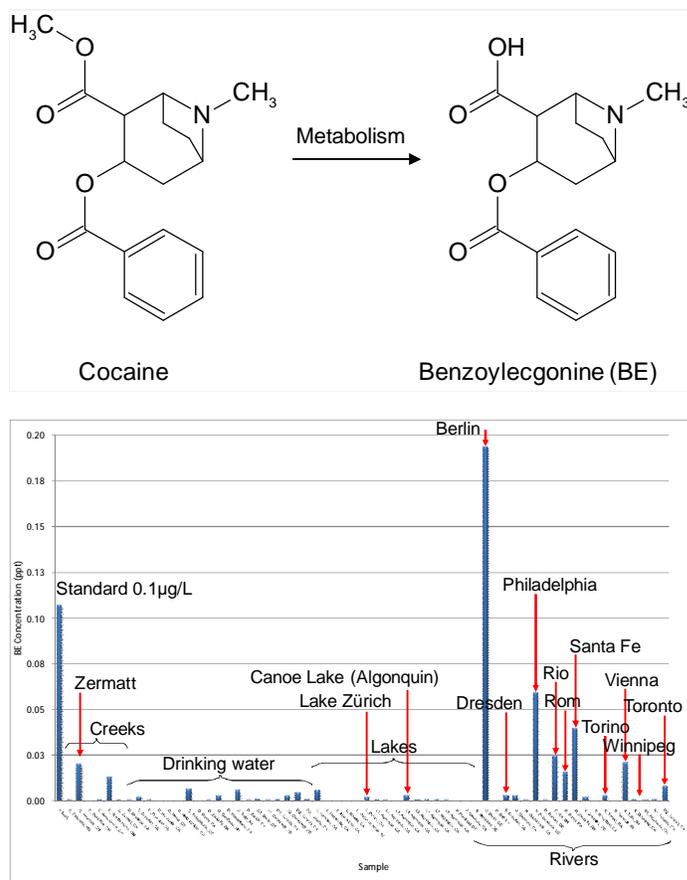


Figure 6. Findings of Benzoylcegonine, a metabolite of cocaine and indicative for cocaine abuse, in various water samples

Figure 5. Quantitation of PPCP in different water samples, A: sample from an agricultural area, B: sample from a farmland drain, C: sample from an urban area

Figure 7 shows the quantitative results of Atrazine in the studied water samples. The herbicide was found in two river samples collected in Canada at a concentration above 100 ng/L. Atrazine was detected in several water samples, including drinking water, in samples, collected throughout the world at concentrations above 10 ng/L. Atrazine was also found in a river water sample from Italy at a concentration of 12 ng/L although Atrazine is banned in the European Union.

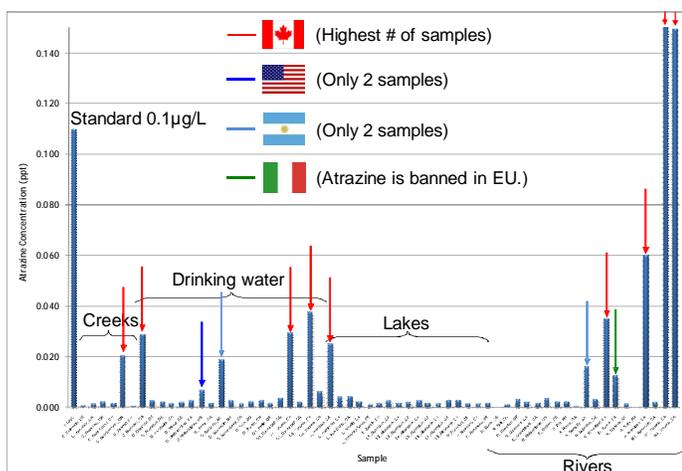


Figure 7. Findings of the herbicide Atrazine in various water samples

Summary

A method using fast LC coupled MS/MS using the *Scheduled* MRMT™ algorithm for the quantitation of 80 PPCP in environmental water samples was developed and successfully applied to real samples.

The AB SCIEX QTRAP® 5500 system operated in MRM mode offers superior selectivity and sensitivity allowing the direct injection of water to quantify PPCP with Limits of Detection in the low ppt range. Quantified compounds were further identified using a quantifier and qualifier ratio.

As an example, results of findings of benzoylecgonine, a cocaine metabolite, and Atrazine were discussed.

Acknowledgement

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