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Determination Of Pharmaceutical Compounds from Drinking and Surface Water at Low ng/L Levels Using Direct Aqueous Injection Triggered MRM Triple Quad Mass Spectrometry

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Introduction

Pharmaceuticals are "emerging contaminants" and characteristically they do not need to persist in the environment to cause adverse effects, because of the continuously-increasing consumption of pharmaceuticals worldwide due to improving health care and longer life expectancies. In addition we have to consider components that are being released into the environment mainly from disposal of unused products, manufacturing processes and excreta. The European Union Water Framework Directive (2000/60/EC) promotes sustainable water use, including the long term-reduction of contaminant discharges to the aquatic environment. Implementation of the Directive requires the development of sensitive, accurate, robust and reliable analytical methods.

Two primary ion transitions (a "target" and "qualifier") are often used in multiple reaction monitoring (MRM) to confirm the presence of target analytes in samples based upon their retention time and qualifier/quantifier ratio. In triggered MRM analysis, up to 10 MRM transitions (primaries and secondaries) may be defined for each target analyte in the method. The primary transitions are acquired for all analytes, but when one of the primary transitions exceeds a user-defined threshold, the secondary transitions are "triggered" and acquired for a specified number of scans. The key advantage of tMRM acquisition is that the combined MRM MS data result in a pseudo MS product ion spectrum, which can be used to create (and subsequently search) an MS reference library of target compounds.

The aim of the study was to determine the sensitivity, linearity and reproducibility of the LC/MS/MS method, and to determine compound concentrations in a real water sample. Furthermore, the reliable confirmation of the target compounds in a real water sample using triggered MRM acquisition mode was evaluated.

Experimental

Sample preparation

The analyzed water sample was filtered before injection (0.2 µm). A 10 ng/mL water-based standard mix containing 20 environmentally relevant compounds (pharmaceuticals, an X-ray contrast agent, drugs of abuse, and drug metabolites) was used to prepare a calibration series covering the concentration range 0.25 ng/L – 1000 ng/L at 12 different levels.

Experimental

Sample solvent was tap water (Waldbronn, Germany), which was tested prior to usage. Confirmation of target compounds was done by comparing the qualifier/quantifier ratios and by using a library match score from a tMRM library containing spectra for the pharmaceutical compounds.

Agilent 1290 Infinity UHPLC

Column: Waters HSS T3 2.1x150 mm, 1.8 µm

Flow rate: 0.4 mL/min

Solvents: A = 0.5% formic acid with 5 mM ammonium formate; B = neat methanol

Injection volume: 20 µL (draw speed: 100 µL/min; equilibration time: 2 s; needle wash in flush port: methanol-acetonitrile 1:1, 8 s)

Gradient: 0 min – 0% B; 0.3 min – 0 % B, 1.2 min – 35 % B, 9.0 min 95 % B. Stop time: 11.0 min; Post time: 2 min

Injection volume: 20 µL (needle wash in flush port: methanol-acetonitrile 1:1, 8 seconds)

Agilent 6490 Triple Quadrupole MS

Scan type: Triggered and Dynamic MRM (MassHunter Optimizer software was used to define MRM parameters).

tMRM parameters: Two primary transitions for the 20 target compounds were included, while additional compounds were added from the Forensics tMRM DB. The confirmatory ions were measured over 3 acquisition cycles, while the cycle time was set to 500 ms. The tMRM thresholds were compound-specific and were set to 50% of the ion count for the lowest calibration standard; Resolution: MS1/MS2: Unit/Wide (Q1:0.7 *m/z* /Q2:1.2 *m/z*).

Software: Agilent MassHunter Data Acquisition QQQ B.06.00., Qualitative Analysis B.06.00. and Quantitative Analysis B.06.00.

Ion source: AJS, positive polarity.

Parameters: Drying gas temperature: 150°C, Drying gas flow: 15 L/min, Nebulizer pressure: 35 psig, Capillary voltage: 3000 V, Nozzle voltage: 400 V, Sheath gas temperature: 375°C, Sheath gas flow: 12 L/min, High Pressure Funnel RF: 120 V, Low Pressure Funnel RF: 80 V, ΔEMV: 300 V.

Results and Discussion

Method performance and characterization

A method for the analysis of environmentally relevant compounds (pharmaceuticals, an X-ray contrast agent, drugs of abuse, metabolites) using the new tMRM acquisition mode has been developed for drinking and surface water. Linearity was evaluated for each target compound (0.25 ng/L – 1000 ng/L) using external calibration approach.

Calibration curves were visualized using normal and log-log and linearity was demonstrated (R^2>0.99) with low qualifier ratio variance for the target compounds over the evaluated analytical range. Water analysis using direct injection of the water sample seems to be a very trivial approach, but as shown in Figure 1, the chemical properties of the target compounds may significantly affect the precision and the accuracy of the measurement results.

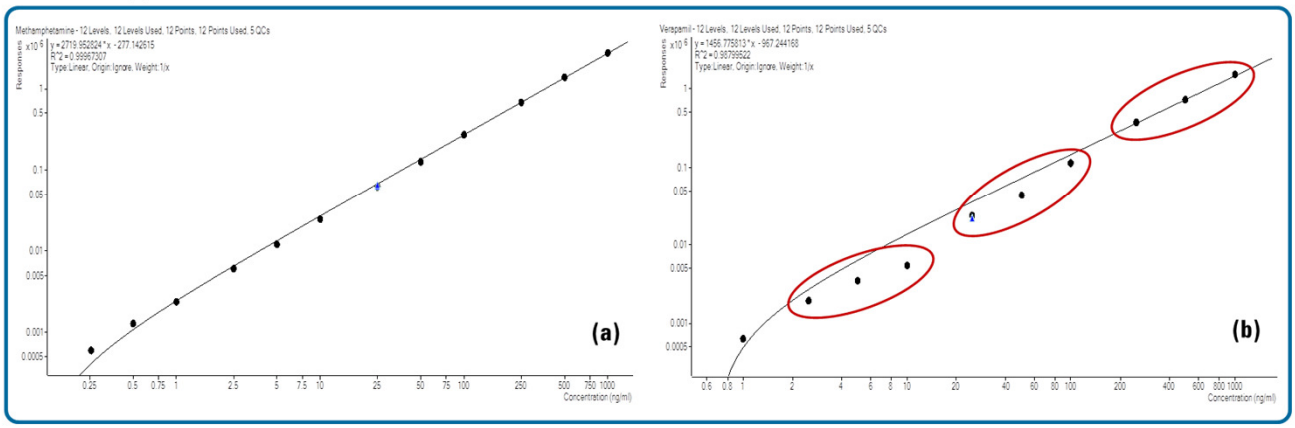

Figure 1: Calibration log-log plots for methamphetamine (a) and verapamil (b). The dilution groups are highlighted with red circles in the case of the verapamil calibration plot.

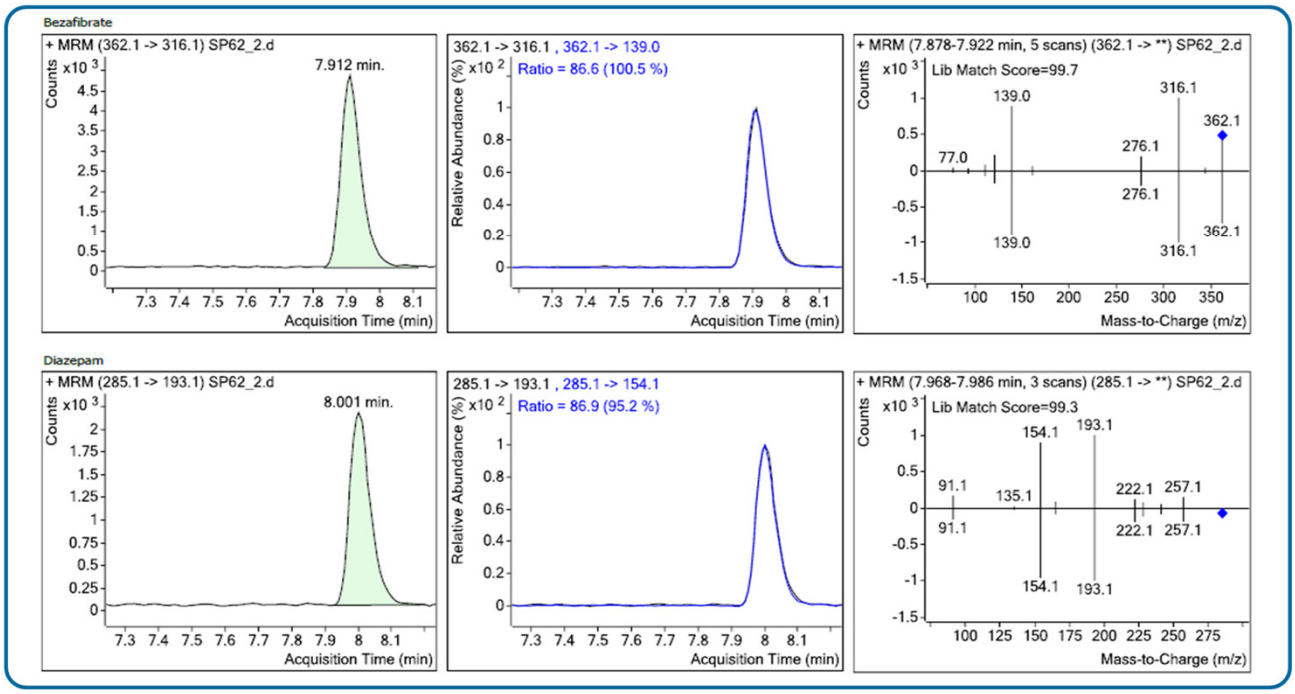

Figure 2: Example primary MRM chromatograms with tMRM spectra of bezafibrate (tMRM confirmation only) and diazepam (18.5 ng/mL) detected in a real water sample.

Results and Discussion

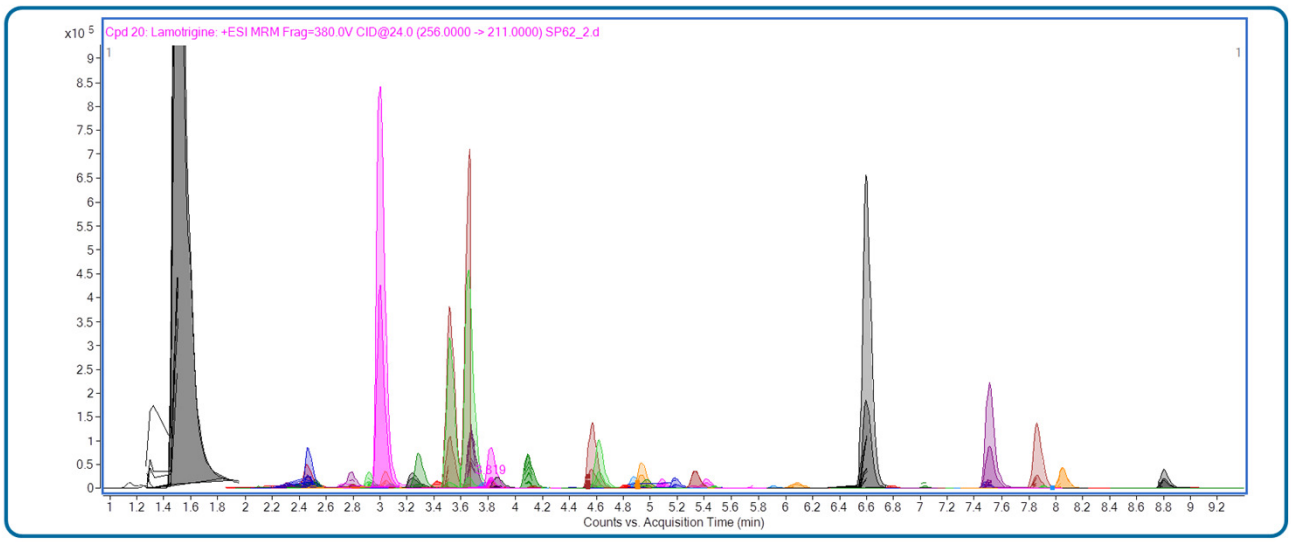

Figure 3: UHPLC/MS/MS chromatogram of a real water sample containing the detected environmentally relevant compounds acquired using tMRM with primary and secondary transitions.

The log-log plot revealed that for verapamil the distribution of the calibration data points resembled a clustered profile, which corresponds to the used dilution groups during the external calibration process (10x, 20x and 40x). Although further research is needed to improve the scientific understanding of this phenomenon, we believe that this behavior is related to surface effects or solubility factors. The same clustered behavior of the data points was also observed for clarithromycin, erythromycin, fluoxetine and diazepam, but the biasing effects were less significant.

Due to the high sensitivity of the QQQ MS system it is very important to have a closer look at carry-over (concentration range < 1 ng/L) after the injection of a high concentration standard. We observed slight "memory-effects" for lidocaine and ciprofloxacin after the injection of the top standard, but with 2 blank injections the contamination was flushed out or the response was less then the 20% of the lowest calibration standard.

Reproducibility was also studied by performing repeatability studies (n=5 @ 25 ng/L), expressed as %RSD and satisfactory precision was obtained for all target compounds. The calculated lowest value was observed for methamphetamine (0.8%), while the highest for amidotrizoic acid (6.0%).

We could reach a lower limit of quantitation of 0.25 ng/L for certain compounds (e.g.: metformin, sotalol, sulpride, methamphetamine, lidocaine...) by using only 20 µL direct aqueous injection. For compounds with higher lower limit of quantitation the injection volume can be increased up to 40 µL by loop exchange, or to 120 µL by installing the multiple range provided by the UHPLC system.

Pre-screening of the real water sample showed that for certain compounds the measured response is greater than the response observed for the top standard (1000 ng/L), thus 10x and 5x sample dilutions were prepared using tap water (Waldbronn, Germany). In the case of sotalol, ciprofloxacin fluoxetine, erythromycin and clarithromycin the calculated final concentration for the diluted samples was either negatively or positively biased (slightly) compared to the undiluted results, which is probably due to the tap water analyzed. The matrix background profile of the tap water was altered, thus the separation and ionization of the target compounds might have been affected.

Conclusions

tMRM is a data dependent acquisition mode which increases throughput and provides quantitative and qualitative information in one run. The tMRM product ion spectrum for the environmentally relevant compounds (pharmaceuticals, an X-ray contrast agent, drugs of abuse, and drug metabolites) was generated while quantitation was performed. Precision data for replicate injections show that quantitation has not been compromised and no compounds were missed when triggering additional transitions. Since tMRM spectra are acquired with optimal collision energies for each individual fragment ion, sensitivity was significantly better than for a data dependent product ion scan approach.