Highly sensitive quantification of 12 drugs of abuse in wastewater using a simple dilution approach

Using the SCIEX Triple Quad 7500 system, powered by SCIEX OS software

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Wastewater analysis is an alternative method of monitoring population drug consumption by measuring excreted drug residues in raw wastewater. Unlike toxicology analysis, the study of drugs of abuse and their metabolites in wastewater provides a non-invasive measure of geographical drug consumption while protecting individual's anonymity. In addition, wastewater analysis provides a robust method of monitoring drug consumption trends across a specific community or location which could be subsequently relevant to identify new substance use and emerging hot spots of drug abuse. For these reasons, analysis of wastewater provides a robust drug market surveillance system comparable to traditional epidemiological approaches.

Generally, the residual concentration of drugs and their metabolites is relatively high and easily detectable in human excrement. However, following ingestion, the concentration of drugs and their metabolites in human excrement is diluted significantly in wastewater. As a result, the analytical sensitivity needed for the analysis of drugs and their metabolites in sewage water is much higher than the requirements for general biological materials commonly screened by conventional toxicology approaches. By the time water sampling occurs at the wastewater treatment plan, drugs and their residuals are found in extremely low (sub ppm to ppt) levels. As a result, there is a need for rapid and robust drug screening methods capable of accurately quantifying drugs of abuse and their metabolites with a high level of selectivity and sensitivity.

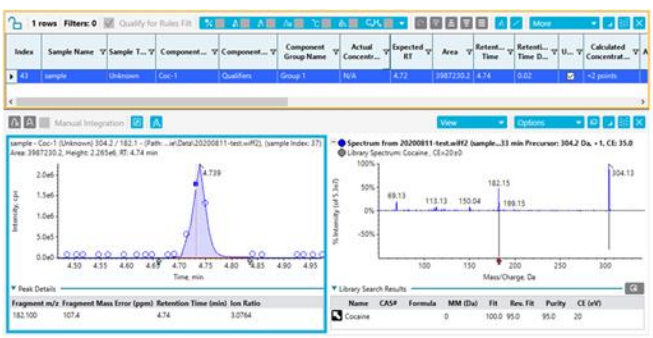

Figure 1. Accurate identification of cocaine using the QTRAP system functionality of the SCIEX 7500 system. Extracted ion chromatogram (XIC) and MS/MS spectrum showing confident identification of cocaine in one of the wastewater samples. MRM XIC provides quantitative data and the full scan MS/MS is matched to the library spectrum for confident confirmation.

In this technical note, the sensitivity of the SCIEX 7500 system[1] was investigated for the detection of 12 drugs of abuse in wastewater. To this end, sub-pg/mL levels of drugs and metabolites were accurately detected with high quantitative metrics (accuracy, precision, linear response) even with a simple dilution approach.

Key advantages of the SCIEX 7500 system for wastewater drug monitoring

- Hardware improvements on the SCIEX 7500 system provide significant gains in sensitivity and quantitative performance[1]
- Instrument sensitivity is leveraged to streamline and simplify sample preparation, negating the need for laborious, costly and time-consuming sample cleanup techniques such as online or offline solid-phase extraction (SPE)
- Improved ion generation and sampling on the SCIEX 7500 system enables accurate and sensitive quantification of all 12 drugs of abuse down to sub-pg/mL levels
- The QTRAP system functionality of the SCIEX 7500 system was leveraged to perform simultaneous identification and confirmation of drugs using the full scan MS/MS data and automated MS/MS library searching
- The results highlight the impressive levels of sensitivity, robustness and accuracy provided by the SCIEX 7500 system for wastewater drug monitoring

Experimental details

Target analytes and solutions: A total of 12 drugs of abuse were targeted for this workflow: amphetamine, benzoylecgonine, ketamine, methamphetamine, MDA, MDMA, norketamine, morphine, methadone, codeine, morphine and cocaine. Two solutions were prepared in water: a 10 ng/mL standard mixture containing the 12 target analytes and a 1 ng/mL internal standard mixture containing the 12 deuterated internal standards.

Calibrator preparation: The 10 ng/mL standard mixture containing the 12 target analytes was used to fortify 10 mL of blank wastewater. This freshly spiked mixture was used to prepare a series of 8 calibrator solutions covering concentrations ranging from 1 to 500 pg/mL. The final IS mixture concentration in the wastewater samples was 25 pg/mL.

Sample preparation: A simplified and fast dilution approach was used for this analysis. Wastewater samples spiked with various concentrations of the drugs of abuse were prepared using the preparation procedure summarized in Figure 2.

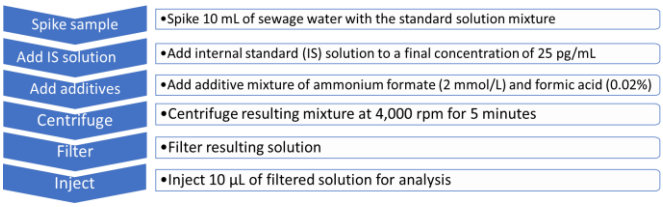

Figure 2. Simplified wastewater sample preparation procedure. A 6-step direct injection method consisting of a dilution, centrifugation and filtration steps was used to prepare the wastewater samples for analysis on the SCIEX 7500 System.

Liquid chromatography: HPLC separation was performed on an ExionLC system using a Phenomenex Kinetex Biphenyl column (100 × 3.0 mm, 2.6 µm, 00D-4622-Y0). The flow rate was 0.45 mL/min. The injection volume was 10 µL and the total LC runtime was 12 minutes. Table 1 summarizes the LC gradient used for the separation.

Table 1. LC gradient.

Time (min)	B (%)
0	*5*
0.5	*5*
7.5	*90*
9.0	*90*
9.1	*5*
12	*End*

Table 2. OptiFlow Pro Ion Source parameters.

Parameter	Value
Collision Gas (CAD)	*9 psi*
Curtain Gas (CUR)	*32 psi*
Ion Source Gas (GS1)	*40 psi*
Ion Source Gas (GS2)	*70 psi*
IonSpray Voltage (IS)	*1500 v*
(Temperature) TEM	*500°C*

Mass spectrometry: A SCIEX 7500 system equipped with an OptiFlow Pro ion source with an analytical probe and E Lens probe was used. The ionization source was operated with electrospray ionization (ESI) in positive mode using generic settings (Table 2). For each transition, voltages were optimized per analyte for compound-specific parameters such as collision energy (CE). A single acquisition method consisting of 48 MRM transitions (24 for the drugs of abuse and 24 for the internal standards) was created using the Scheduled MRM algorithm in SCIEX OS software 2.0. Two MRM transitions were monitored for each of the targeted analytes.

Data analysis: Data processing was performed using SCIEX OS software. Detection and integration of the peaks from the background was accomplished within the viewing window using the MQ4 algorithm. Quantitative analysis was performed in the Analytics module of the software. Here calibration curves, concentration calculations, assay precision and accuracy statistics were automatically generated.

Method development and optimization

A diluted, 500 ng/mL neat standard mixture containing the 12 drugs of abuse and internal standards was used for initial method development. Retention times for each of the 12 analytes were determined, then a final optimization method was built using the Scheduled MRM algorithm in SCIEX OS software to optimize data sampling across each peak while maintaining optimal dwell times for each MRM transition. Figure 3 shows the elution profile for the 12 targeted drugs resulting from the optimized data acquisition method. The optimized LC conditions and MS acquisition method ensured reliable integration, quantification and confirmation of the peak for each target analyte.

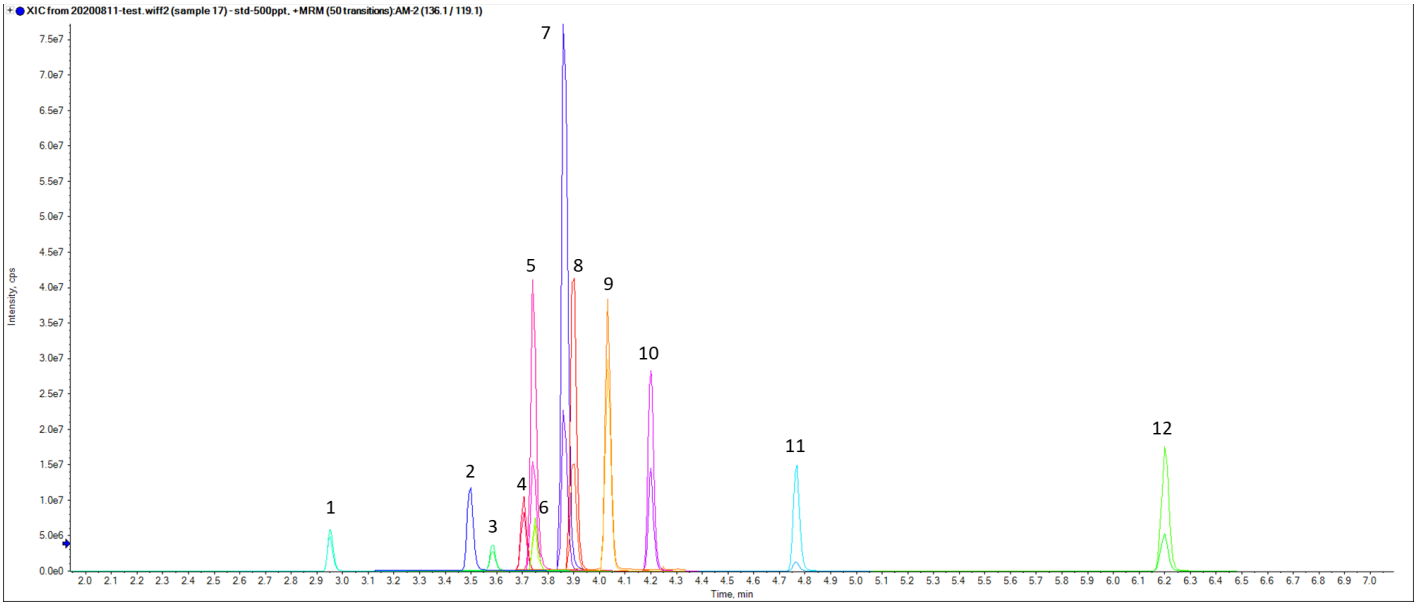

Figure 3. Chromatographic profile of the 12 drugs of abuse targeted in this study. Shown is an extracted ion chromatogram (XIC) resulting from the optimized LC conditions and data acquisition method using a 500 ng/mL neat standard mixture containing the 12 drugs of abuse included in the panel. The numbered peaks are assigned as follows: 1. morphine, 2. amphetamine, 3. codeine, 4. MDA, 5. methamphetamine, 6. oxycodone, 7. benzoylecgonine, 8. MDMA, 9. norketamine, 10. ketamine, 11. cocaine, 12. methadone.

Optimized data collection using the Scheduled MRM algorithm enables robust drug quantification

The ability to accurately detect low levels of drugs of abuse from wastewater is critical to inform the extent of drug consumption in the geographic location in which the sample was collected. The series of calibrator solutions ranging from 1 to 500 pg/mL was injected to evaluate the quantitative performance of the system and its ability to accurately measure low levels of drugs of abuse from wastewater with a high level of precision and accuracy.

Figure 4 shows representative extracted ion chromatograms (XIC) for the two MRM transitions monitored for ketamine and MDA. The series of XIC overlays display both the quantifier and qualifier ions for a blank injection (left) and for concentrations ranging from 1 pg/mL (LLOQ) to 500 pg/mL. The majority of the targeted drugs showed detectable signal below the LLOQ and down to sub pg/mL levels. In addition, the robustness and quantification performance of the SCIEX 7500 System were demonstrated with calculated concentration accuracy within 20% of 100%, precision (%bias) below 20% across the calibration range for all 12 analytes targeted in this panel.

Calibration curves were generated for each of the analytes. Figure 5 shows the resulting calibration curves plotted across the eight calibrator levels. The calibration curves demonstrated excellent linearity with R^2 greater than 0.99 for all the drugs of abuse targeted in the panel. Overall, the assay showed excellent reproducibility, precision, accuracy and linearity, proving the robustness of the method and the quantitative performance of the SCIEX 7500 system.

Fast and robust sample preparation procedure leads to high drug recovery

One of the challenges associated with wastewater analysis is the presence of many matrix components that can potentially affect the detection performance of the assay. As a result, a reliable and efficient sample preparation procedure is key to extract the analytes of interest from the matrix interferences and achieve the desired reproducibility of the assay. Here, the sensitivity of the SCIEX 7500 system allows use of a large dilution of sample, reducing the matrix components while achieving robust detection and quantification of low levels of drugs of abuse.

To assess the efficiency of the direct injection method used in this experiment, the recovery was calculated by spiking a blank wastewater sample with the standard mixture at three concentration levels (1, 10 and 100 pg/mL) before and after the sample preparation procedure. Three replicate injections were performed for each of the three concentrations. The direct injection method used in this experiment demonstrated averaged recovery values ranging between 80.1 and 110.7% for the 12 targeted drugs of abuse (data not shown).

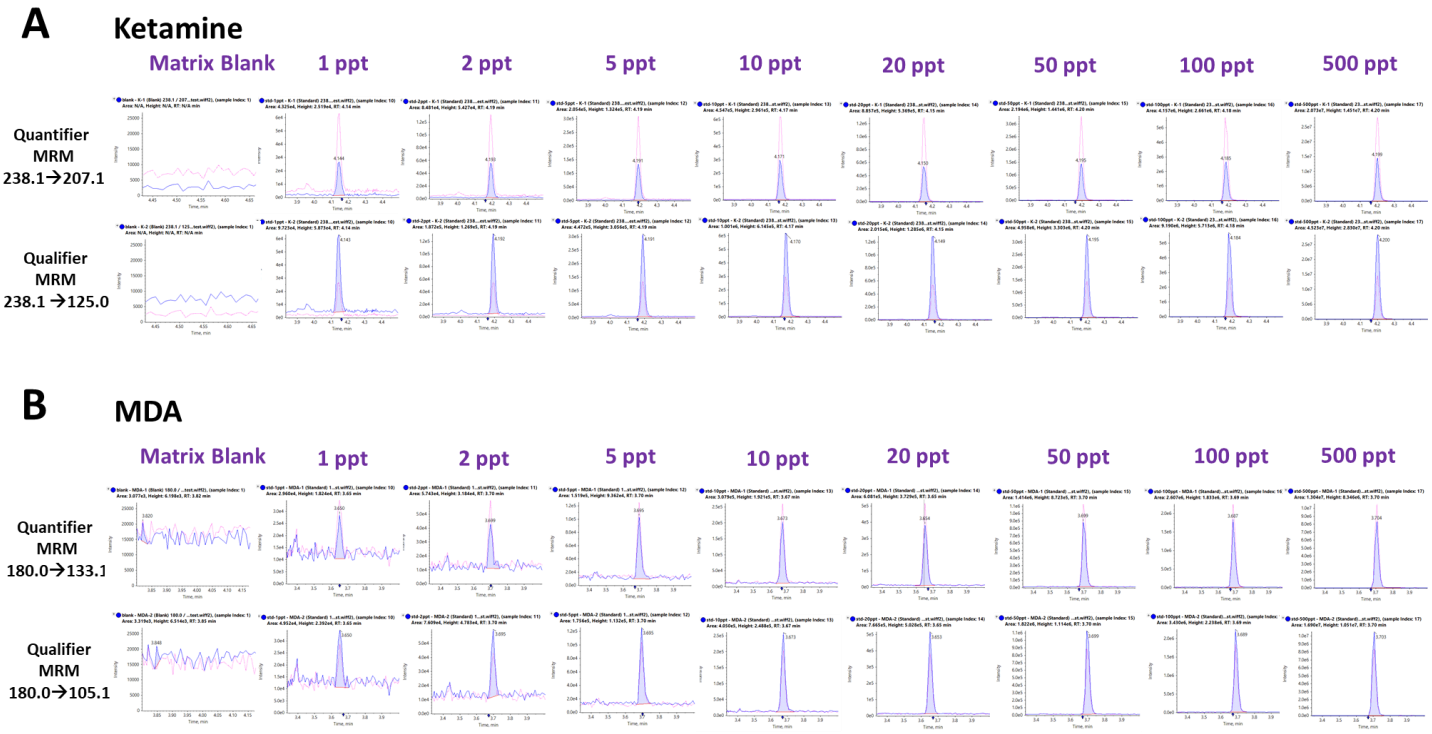

Figure 4. Accurate quantification of two drugs of abuse from wastewater samples using the SCIEX 7500 system. Extracted ion chromatogram (XIC) overlays of the quantifier and qualifier ions for a blank injection (left) and for concentrations ranging from 1 (LLOQ) to 500 pg/mL for A) ketamine and B) MDA. The use of the Scheduled MRM algorithm in SCIEX OS software enabled collection of optimal data quality to ensure accurate quantification of the 12 drugs.

In addition, the assay showed excellent reproducibility across the three injections for the three concentration levels with RSD values between 2.2% and 5.78% for all the drugs targeted used in this study (data not shown). The excellent recovery and RSD values demonstrate both the overall robustness and reproducibility of the direct injection method as well as the quantitative performance of the SCIEX 7500 system.

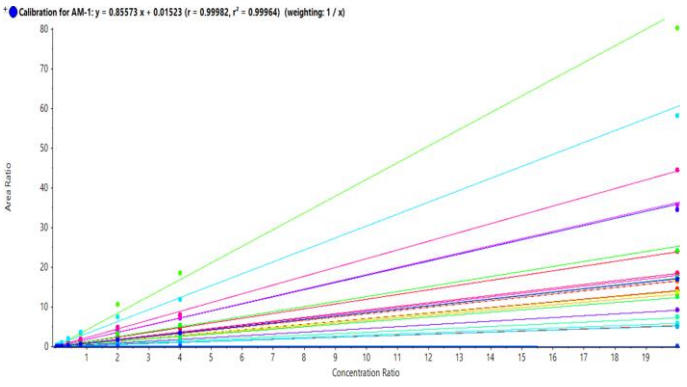

Figure 5. Excellent linearity for the 12 drugs of abuse in wastewater samples. Linear regression curves resulting from the calibration series from 1 to 500 pg/mL for the 12 drugs targeted in this study. R^2 values greater than 0.99 were observed for all the drugs of abuse in the panel.

Highly sensitive detection of drugs of abuse from wastewater

The robustness of the method was further investigated by analyzing two wastewater samples: a domestic sewage and a mixed sewage. The two samples were subjected to the direct injection method and screening using the developed method. Morphine, methamphetamine and cocaine were detected in the two wastewater samples analyzed. The concentration of each of the three drugs detected in the two wastewater samples are summarized in Table 3. The concentration of morphine in these two wastewater samples is higher than the other drugs detected, suggesting that morphine is the most prevalent drug in the two areas where the wastewater was sampled.

Table 3. Results table from the analysis of two sewage samples.

Wastewater Source	Morphine (pg/mL)	Methamphetamine (pg/mL)	Cocaine (pg/mL)
Domestic sewage	*4.39*	*0.48*	*0.84*
Mixed sewage	*8.97*	*0.11*	*0.77*

Compound identification using full scan MS/MS and spectral library searching

The QTRAP system functionality of the SCIEX 7500 system was used for the analysis of the sewage water samples to increase analyte identification confidence. Figure 1 shows the extracted ion chromatogram (XIC) and the MS/MS spectrum with MS/MS matched library spectrum for cocaine, one of the drugs detected in the two wastewater samples analyzed. The acquisition of the MS/MS spectrum enabled positive identification of cocaine through spectral library matching. A fit score of 100% and a purity score of 95% were calculated in SCIEX OS software which provided excellent measures of the confident identification of cocaine in the sewage water samples.

Conclusions

The combination of a simplified sample preparation with a direct injection method on the SCIEX 7500 system enabled accurate identification and sensitive quantification of drugs of abuse in wastewater. The developed workflow enabled confident identification and accurate quantification of drugs in real wastewater samples, demonstrating the applicability of the method for drug market surveillance comparable to traditional epidemiological approaches.

- A direct injection method consisting of a dilution, centrifugation and filtration steps was used to prepare the wastewater samples, reducing sample preparation time, cost and labor while greatly improving laboratory efficiency and throughput
- The high sensitivity of the SCIEX 7500 system enabled accurate and sensitive quantification of 12 common drugs of abuse from wastewater, with an LLOQ of 1 pg/mL with sub-pg/mL detection limits for the drugs in the panel
- Drug recoveries were found to be between 80.1% and 110.7%
- Excellent reproducibility was observed across the three concentration levels with RSD values between 2.2% and 5.78% (data not shown)
- The MRM-triggered MS/MS capability of the QTRAP system enabled confident accurate identification of the analytes using spectral library searching.

References

1. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A.

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