Identification and quantification of 12 drugs in sewage using on-line solid phase extraction on the SCIEX Triple Quad 4500 system

The Power of Precision

Zhang Chong, Sun Xiaojie, Liu Bingjie, and Guo Lihai
SCIEX Asia Pacific Application Support Center, Beijing, China

ABSTRACT

The concentration determination of toxic substances and their metabolites in sewage is the key to evaluate drug use by sewage analysis. The basic idea of the method is to detect drugs and metabolites in sewage, but drug metabolites are mixed with domestic sewage after entering the sewage system. As a result, the analyte concentration can be diluted thousands of times, resulting in final concentrations down to the ng/L level. At the same time, the complex matrix in sewage also puts increased pressure on the uptime and robustness of the instrument. Compared with the traditional off-line solid phase extraction, on-line SPE has the advantages of high sample utilization and lower sample requirements, full volume automatic on-line extraction, automatic desorption to sample injection, and high throughput, which can greatly reduce manpower, analysis time and cost. In addition to these benefits, the use of on-line SPE greatly reduce cross contamination of samples during pretreatment. Therefore, this approach is very popular with front-line testers in the sewage drug testing industry. Here, an on-line SPE method coupled directly to MS analysis was developed for qualitative and quantitative analysis of 12 drugs and metabolites in sewage.

INTRODUCTION

Determining the concentration of toxic substances and their metabolites in sewage is key to monitoring drug use by domestic sewage analysis. This is challenging because compounds are diluted thousands of times, resulting in concentration levels down to the ng/L levels, and the complex sewage matrix stresses instrument uptime. Compared with traditional off-line solid phase extraction (SPE), on-line SPE achieves high sample utilization with less sample volume, automation of extraction, desorption and injection with good throughput. The use of on-line SPE can therefore reduce manpower, improve uptime and shorten time to results. On-line SPE is often implemented by frontline labs that perform drug testing in sewage. To meet this need, we developed an on-line SPE-MS/MS system for qualitative and quantitative analysis of 12 drugs and metabolites in sewage.

MATERIALS AND METHODS

Sample Preparation:
Isotope-labeled internal standards were added to 10 mL of sewage (25 ng/L solution), then centrifuged at 10,000 rpm for 10 min. The supernatant was injected for HPLC-MS/MS analysis.

HPLC Conditions:
An ExionLC system coupled with CTC PAL3 autosampler was used for separation. A 15-minute gradient on a Kinetex Biphenyl column (2.1 x 100 mm, 2.6 µm, 100 Å, Phenomenex) was used to achieve good separation and a HLB column (2.1 x 30 mm, 20 µm, 100 Å, Waters) was used for the SPE process. The mobile phases for elution were 0.02% formic acid and 2mM ammonium formate in water and 0.02% formic acid and 2mM ammonium formate in acetonitrile, respectively. The mobile phase for SPE was water/methanol.

MS/MS Conditions:
The supernatant was injected into the SCIEX Triple Quad 4500 system equipped with a Turbo V ion source and electrospray ionization (ESI) was used in positive mode. Source conditions were optimized as follows: curtain gas 30 psi, collision gas medium, nebulizing gas 55 psi, heater gas 60 psi, ion spray voltage 3000 V and source temperature 600ºC. 12 drugs were monitored using 2 MRM transitions per compound to allow quantification and identification based on the ratio of quantifier and qualifier transitions as defined by regulation 2002/657/EC.

RESULTS

In this study, a sensitive and reliable HPLC-MS/MS approach for the simultaneous, rapid, qualitative and quantitative analyses of drugs and metabolites in sewage was developed, optimized and validated. A method for quantification and identification of 12 drugs: amphetamine (AM), benzoylecgonine (BZE), ketamine (K), methamphetamine (MA), methylenedioxymethamphetamine (MDMA), norketamine (NK), morphine 6-acetate hydrochloride (O6), morphine (Mor), cocaine (Coc), codeine (Cod), and cotinine (Cot) was developed as shown in Figure 1.

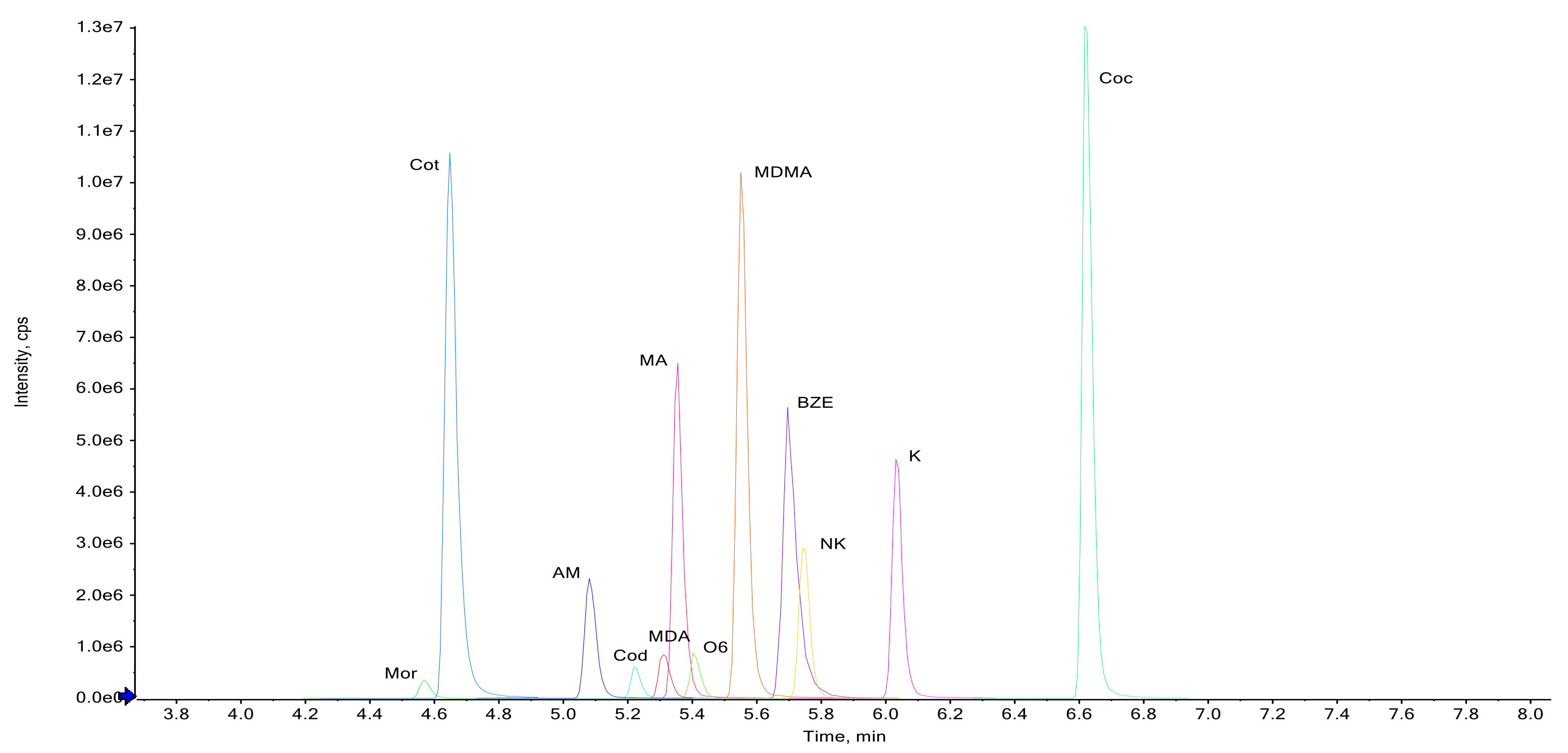


Figure 1. Detection of 12 drugs by LC-MS/MS analysis.

Table 1. LOQ of detected drugs.

Mycotoxin	LOQ (ng/L)
AM	0.1
BZE	0.05
K	0.1
MA	0.05
MDA	0.2
MDMA	0.02
NK	0.2
O6	0.2
Mor	0.2
Coc	0.05
Cod	0.5
Cot	0.2

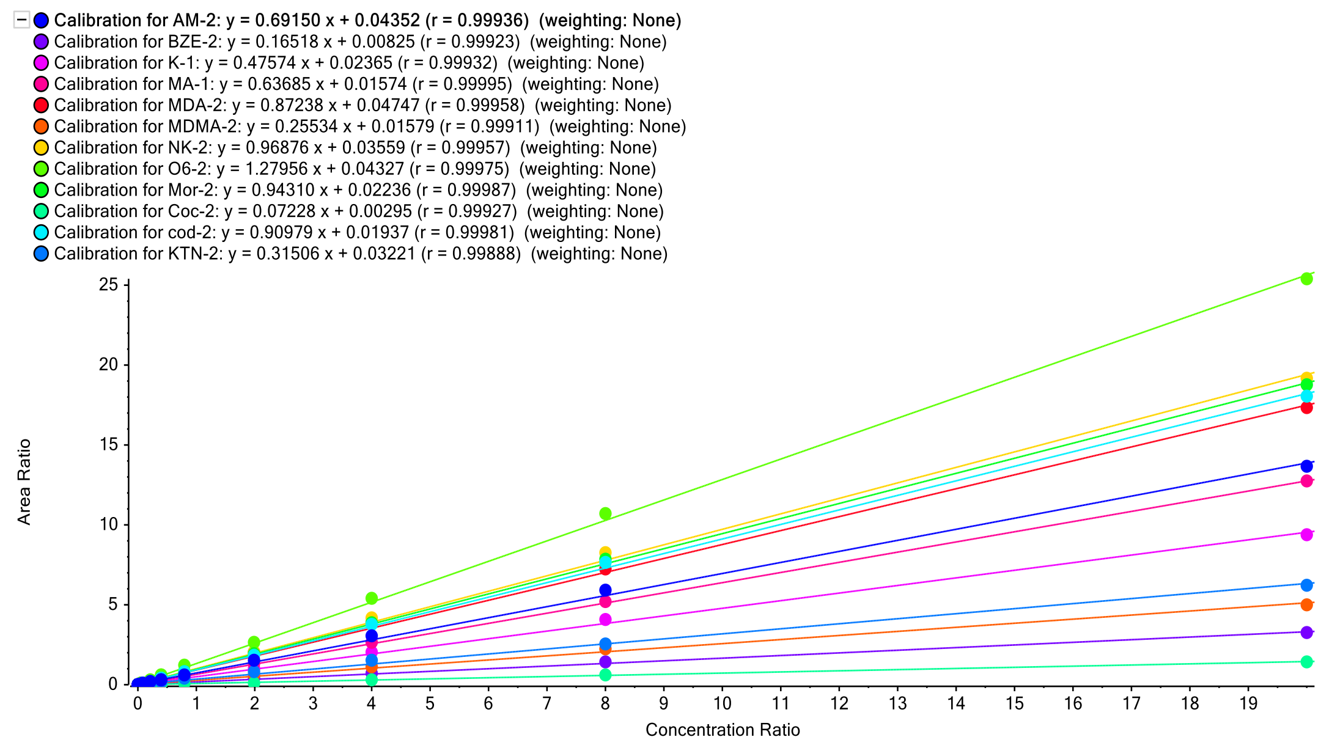
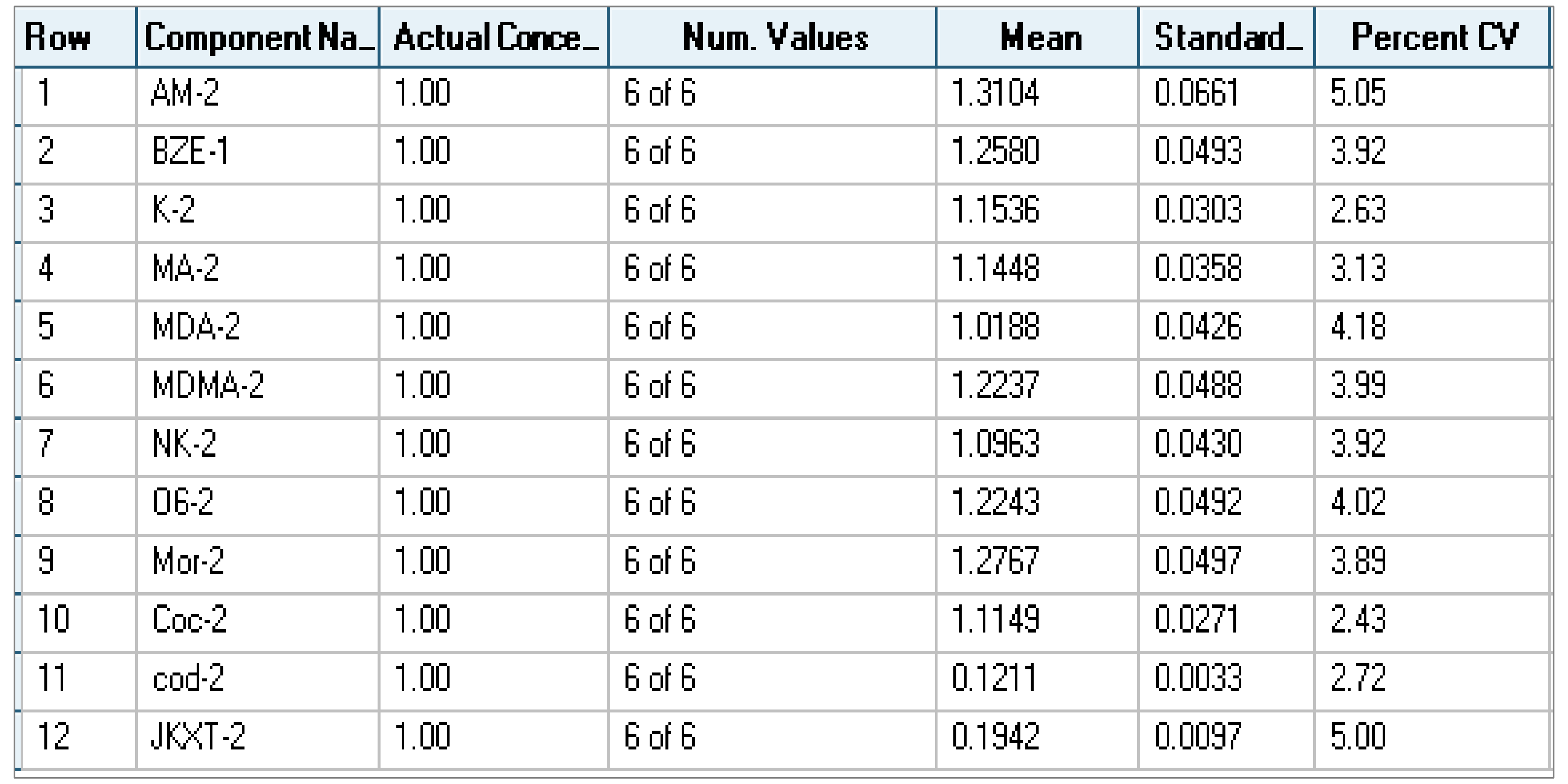


Figure 2. Linear calibration curve of 12 drugs and metabolites.

Recoveries, linearity, precision and sensitivity were assessed to validate the method. The application of on-line SPE yielded good method validation results. The recoveries in 4 sewage samples ranged from 81.3% to 110.7%. Lower limits of quantification (LLOQs) were determined and ranged from 0.05 to 500 µg/L (Table 1). Different ranges of linear calibration curves were established for each of the different drugs, each with $R^2 > 0.997$. The relative standard deviations (RSDs) of intra-day precisions (n=6 replicates) for the analytes ranged from 2.43% to 5.05% (Table 2).

Table 2. Reproducibility index of 6 consecutive injections.



Row	Component Na_	Actual Conce_	Num. Values	Mean	Standard_	Percent CV
1	AM-2	1.00	6 of 6	1.3104	0.0661	5.05
2	BZE-1	1.00	6 of 6	1.2580	0.0493	3.92
3	K-2	1.00	6 of 6	1.1536	0.0303	2.63
4	MA-2	1.00	6 of 6	1.1448	0.0358	3.13
5	MDA-2	1.00	6 of 6	1.0188	0.0426	4.18
6	MDMA-2	1.00	6 of 6	1.2237	0.0488	3.99
7	NK-2	1.00	6 of 6	1.0963	0.0430	3.92
8	O6-2	1.00	6 of 6	1.2243	0.0492	4.02
9	Mor-2	1.00	6 of 6	1.2767	0.0497	3.89
10	Coc-2	1.00	6 of 6	1.1149	0.0271	2.43
11	cod-2	1.00	6 of 6	0.1211	0.0033	2.72
12	JKXT-2	1.00	6 of 6	0.1942	0.0097	5.00

The proposed method was applied for the accurate quantification of 12 drugs and metabolites in sewage from different sewage treatment plants. The concentrations of these compounds were highly variable. Some drug types were detected in samples at concentrations lower than 1 ng/L. In some samples, however, morphine was detected at concentrations as high as 5 ng/L, suggesting that additional monitoring of morphine administration, for example, might be needed.

Sample number	Mor (ng/L)	MA (ng/L)	MDA (ng/L)	K (ng/L)
589	2.13	NA	0.51	0.21
602	7.82	0.16	1.7	0.23
617	5.50	NA	0.49	0.34
572	4.38	NA	0.27	0.44

CONCLUSIONS

A sensitive and reliable HPLC-MS/MS approach for the simultaneous, rapid, qualitative and quantitative analyses of 12 drugs and metabolites in sewage was developed, optimized and validated. The chromatographic conditions, mass spectrometric conditions and pretreatment method were systematically optimized.

The application of on-line SPE yielded good method validation results. Lower limits of quantification (LLOQs) were obtained, ranging from 0.05 to 500 µg/L. Most of the recoveries in 4 sewage samples ranged from 81.3% to 110.7%.

Overall, this methodology proved to be simple, reliable and highly efficient for the detection of the 12 drugs and metabolites in sewage, indicating that this methodology might be implemented to detect drugs in sewage.

REFERENCES

1. 2002/657/EC: Commission Decision (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32002D0657).

TRADEMARKS/LICENSING

To receive a copy of this poster:

- Scan the code with your phone camera
- Complete the form