

Trace analysis of abused drugs in waste water sample by AOE system coupled with LCMS-8060

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1. Introduction

Pesticides, drugs, pharmaceuticals are widely used in daily life and cause great pollution to water. The development of analytical methods was mainly dictated by their low concentrations found in water. In order to meet the requirement of high sensitivity, sample preparation plays a crucial role in the analytical method. Most of the reported methods used solid-phase extraction when pretreated water samples. However, the offline SPE procedures are time-consuming and error-prone, meaning they require a large amount of organic solvents and have the potential to lose a certain number of analytes

The online SPE-LCMS systems, which allowed the entire analytical procedure to be performed in a closed system, reduced sample loss while obtaining high accuracy and repeatability. In this study, an online cross used SPE-UHPLC-MS/MS system named AOE systems (Automatic Online Extraction/Enrichment system), which allowed the automatic cross-utilization of two SPE columns, was developed, validated and applied to analyze 12 kinds of abused drugs in waste water samples. All the methods were fully validated and proved to be accurate, sensitive, and showed good linearity and recovery. The developed method was successfully applied to the real waste water samples analysis.

2. Methods

The AOE system coupled with tandem mass spectrometer (LC-8060) was consisted of a CBM-20A system controller, two LC-30AD pumps, a LC-20AD pump with LPGE unit, SIL-30AC autosampler for UHPLC system sample injection, a SIL-16P for SPE system sample injection, a CTO-20AC column oven equipped with a ten-pot switching valve, and two DGU-20A5 online degassers. Data acquisition and processing were performed with Labsolutions Version 5.91 and above.



Figure 1 AOE system coupled with LCMS-8060 triple quadrupole mass spectrometer

AOE Analytical Conditions	
SPE Column	: Oasis HLB direct connect HP(20 μm, 2.1 mm × 30 mm) × 2
SPE loading solvent	: Milli-Q water
SPE cleaning solvent	: 0.1%(v/v) formic acid in methanol/water (1/1, v/v) and methanol/acetonitrile/2-propanol (1/1/1, v/v/v)
Separation column	: Inert Sustain AQ-C18 (1.9 μm, 2.1 mm × 100 mm)
Mobile phase	: A: 0.1%(v/v) formic acid in water : B: Acetonitrile
Injection volume	: 3 mL

MS Analytical Conditions

Ionization: ESI, Positive MRM mode	Interface Temperature: 300 °C
Heating Gas Flow: 10.0 L/min	Heat block Temperature: 400 °C
DL Temperature: 200 °C	Nebulizer Flow: 3.0 L/min
Dry Gas: 10.0 L/min	

3. Results

3-1. System working process

The AOE system consisted of two statuses during the analysis, which were shown in Figure 2. The first status (Figure 2-A) was "extraction of the SPE column 1 and equilibration of the chromatographic column". During this status the sample solution, which was injected into the sample loop before the end of the previous analysis, was driven into the SPE column1 by the loading solvent from pump C. Then the target analytes were adsorbed while the impurities were removed by the washing solvent transfused by pump C. At the same time, the chromatographic column was equilibrated to the initial conditions. As the ten-port valve switched, SPE column 1 was connected to the UHPLC system and the second status "desorption of SPE column 1, chromatographic separation and preparation of SPE column 2 started (shown in Figure 2-B). Pump A and B transfused the gradient mobile phase to desorb the analytes from SPE column 1, meanwhile the analytes were transported to the UHPLC column for chromatographic separation. During the chromatographic separation, SPE column 2 was cleaned up and equilibrated by the solvents transfused by pump C. This online system cross used two SPE columns and allowed the SPE desorption to work with the chromatographic separation simultaneously, which reduced sample loss and speeded up the analysis.

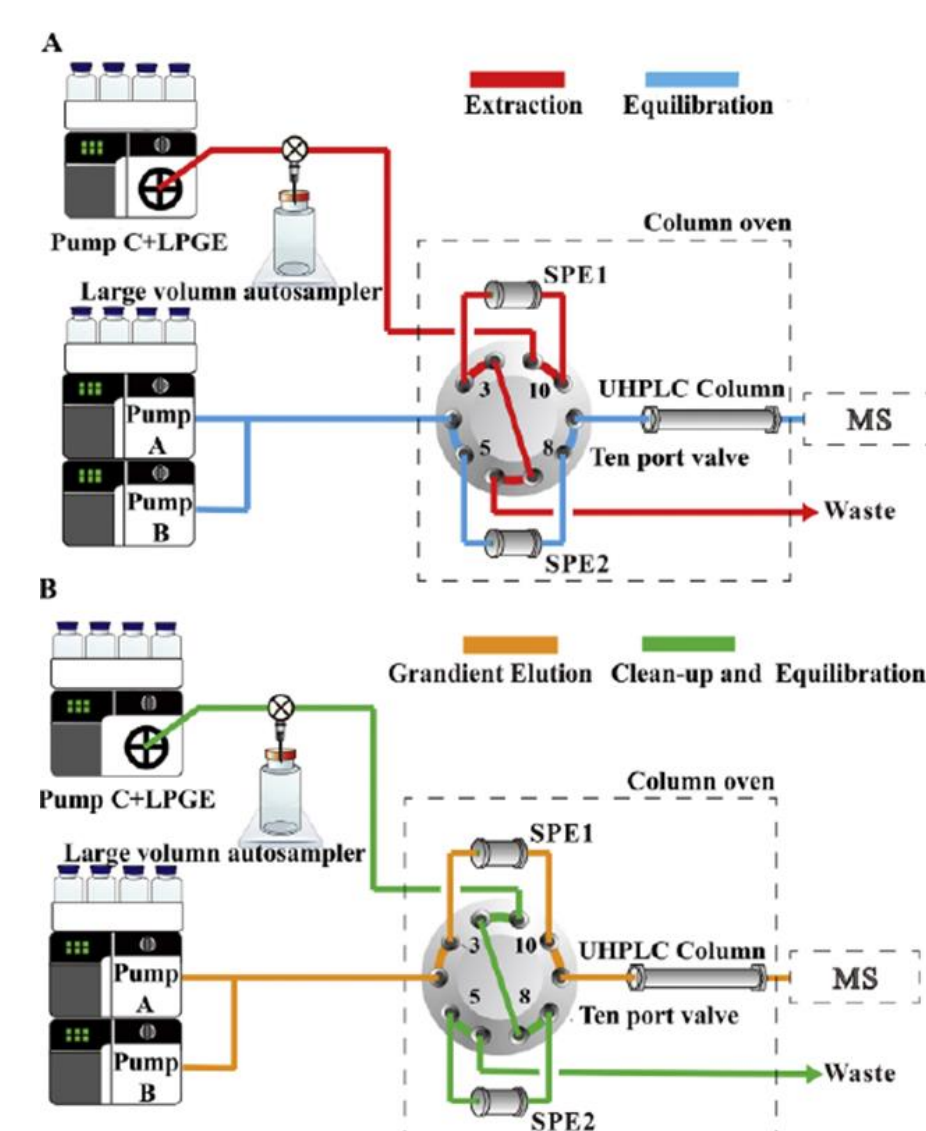


Figure 2 Schematic diagrams of the online cross used SPE-UHPLC-MS/MS system, including two statuses: extraction of the SPE column 1 and equilibration of the chromatographic column (A) and desorption of the SPE column 1, chromatographic separation and preparation of the SPE column 2 (B).

3-2. Method validation

13 kinds of abused drugs and its metabolites standards solutions were prepared by dilution in buffer solution of pH 7. Typical chromatograms obtained under optimized conditions were shown in Figure 3.

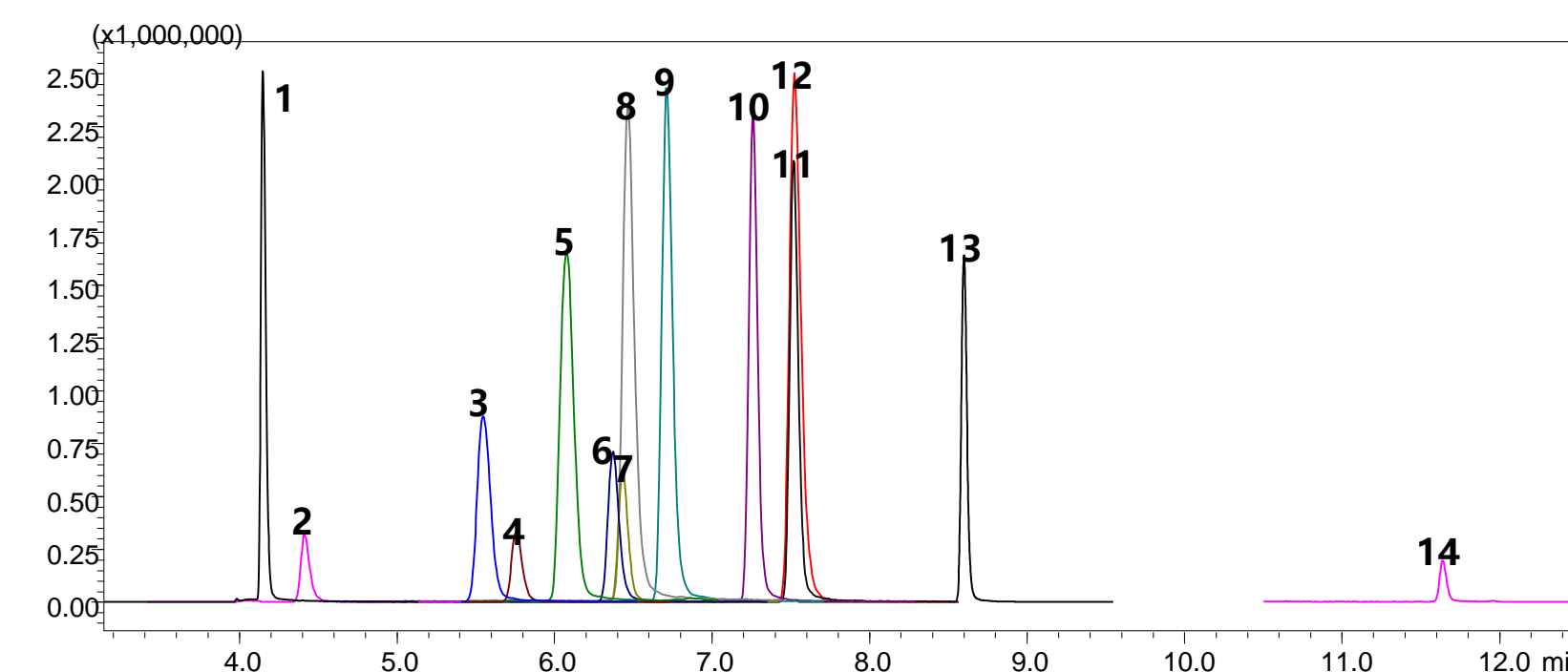


Figure 3 Typical chromatograms of abused drugs and Cotinine (1- Cotinine, 2- Morphine, 3- MC, 4- Codeine, 5- Amphetamine, 6- MDA, 7- O⁶-Monoacetylmorphine, 8- Methamphetamine, 9- MDMA, 10- Norketamine, 11- Benzoylcegonine, 12- Ketamine, 13- Cocaine, 14- THC-COOH)

Linear calibration curves were generated using standard concentrations ranged from 0.5 ng/l to 250 ng/L considering of method detection limit. All the target compounds showed good linearities with the linear correlation coefficients (r) higher than 0.995. The RSDs% of intra-day precision were within 6.90%.

Table1. Linearity parameters of compounds

NO.	Analytes	RT min	Linear Range ng/L	Correlation Coefficient
1	MDA	6.368	0.5-250	0.9996
2	Norketamine	7.267	0.5-250	0.9996
3	MC	5.544	0.5-250	0.9990
4	Morphine	4.414	0.5-250	0.9993
5	O ⁶ -Monoacetylmorphine	6.436	0.5-250	0.9989
6	Codeine	5.759	0.5-250	0.9980
7	Methamphetamine	6.466	0.5-250	0.9996
8	MDMA	6.711	0.5-250	0.9994
9	Amphetamine	6.073	0.5-250	0.9999
10	Benzoylcegonine	7.524	0.5-250	0.9996
11	Ketamine	7.529	0.5-250	0.9998
12	Cocaine	8.61	0.5-250	0.9994
13	THC-COOH	11.649	0.5-250	0.9983
14	Cotinine	4.147	5-2000	0.9998

The AOE system was applied to the analyses of real waste water. The waste water should be kept in low pH by adding HCl. After adding inter-standard, adjusting pH to about 7 and filtrating by 0.22 μm membrane, the waste water could be analyzed by the online SPE system. All kinds of the samples were analyzed in 3 replicates. The recoveries of all the target analytes obtained from the spiked waste water samples at three concentration levels were in the range of 88.75% ~ 120.05% with RSDs% less than 3.74%..

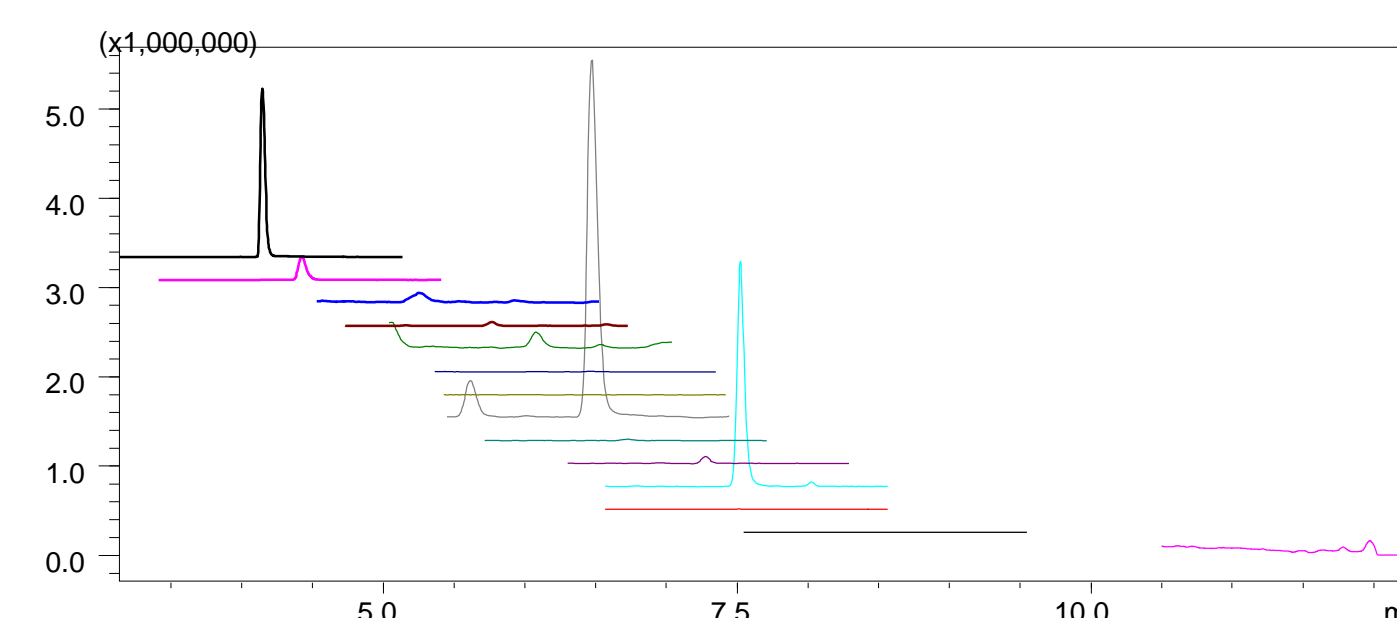


Figure 4 Typical chromatograms of waste water samples

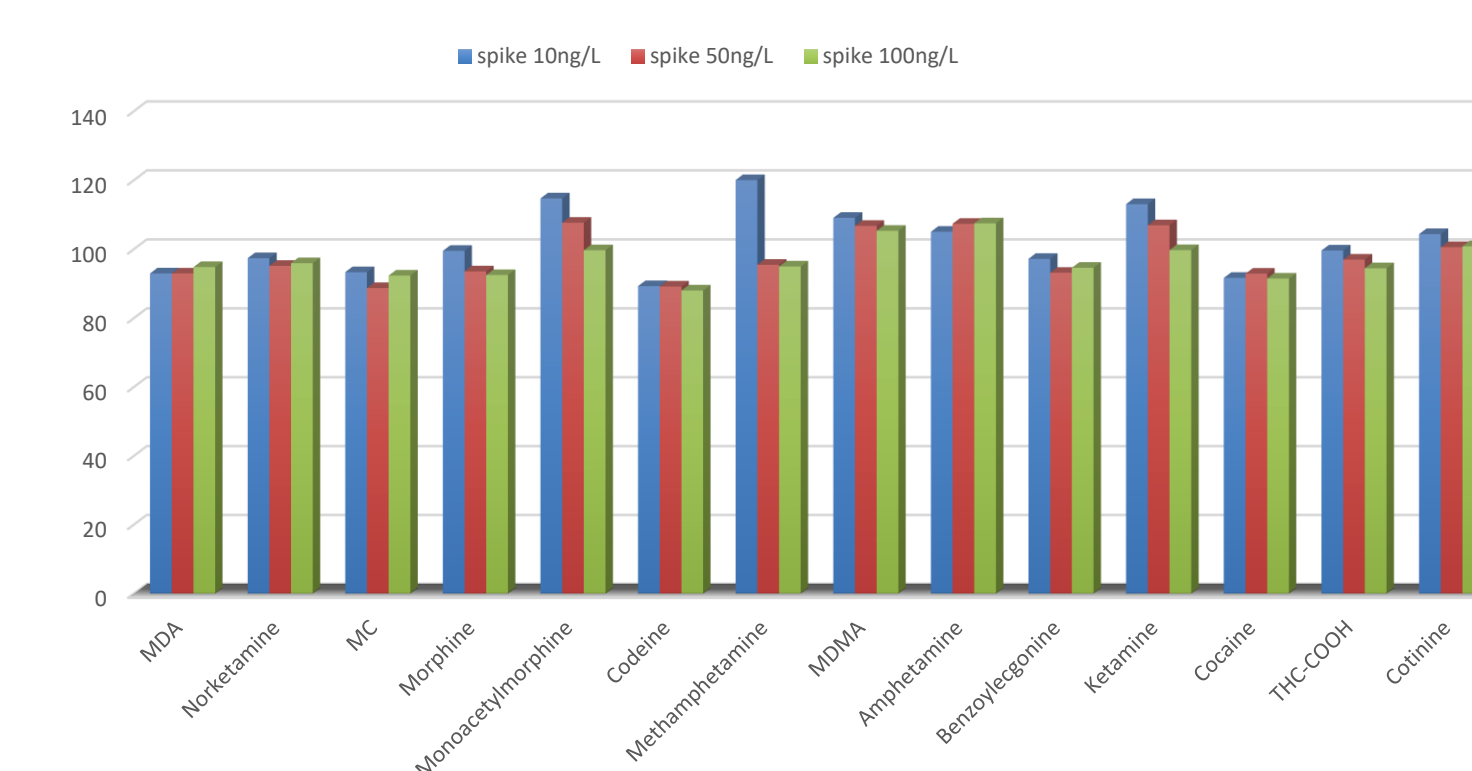


Figure 5 Recoveries of waste water samples

3-3. Comparison

Compared with the offline SPE-UHPLC-MS/MS method, the method using AOE system showed significant advantages in analytical speed, and the analysis efficiency was improved about 80%. The same sample was analyzed offline and online solid phase extraction, and by comparing the test results, it was found that the two methods showed good consistency in the detection items and detection concentrations. So the online solid phase extraction technology can replace the traditional solid phase extraction analysis method in waste water sample analysis.

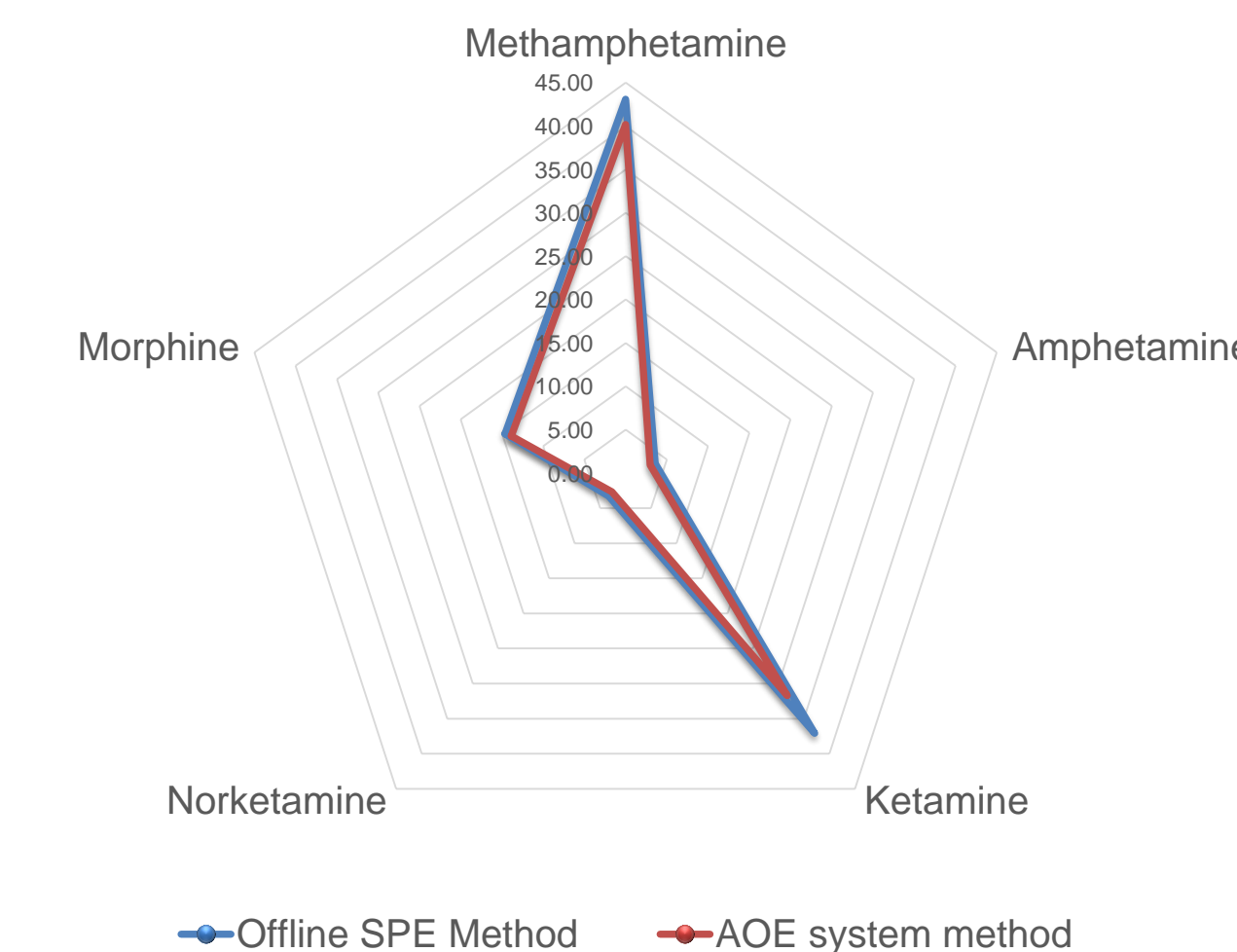


Figure 6 Detection result comparison of two method

4. Conclusions

- In this study, an automated online cross used SPE-UHPLC-MS/MS system was developed for the simultaneous determination of abused drugs in waste water at trace levels.
- Under the optimal conditions, this online system offered low LODs and satisfactory repeatability. The validated method was successfully applied to analyze the real water samples.
- In conclusion, this AOE system, which was an automatic, sensitive and efficient technique, showed its possibility for the trace analysis of organic compounds in aqueous samples.