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Multi-Method Drug Analysis in Human Plasma Using Fault-Tolerant High-Throughput LC-MS/MS System

Yuki SUZUKI[1], Eishi IMOTO[2], Logan MILLER[1], Shohei SATO[1], Vikki JOHNSON[1]
(1) Shimadzu Scientific Instruments, R&D Center, MD, US; (2) Shimadzu Corporation, MS Business Unit, Kyoto, Japan

1. Introduction

Forensic and clinical research laboratories require high-throughput workflows, yet carryover and instrument downtime remain major obstacles. We have developed a high-throughput LC-MS/MS system featuring multiple independent LC flow paths.
By incorporating four autosamplers for a single mass spectrometer, each flow path (stream) is completely isolated, thereby reducing cross-contamination. This reduces carryover and minimizes downtime associated with cleaning, enabling increased throughput while maintaining analytical stability and quantitative precision. Additionally, integrated online SPE automates sample preparation, mitigating matrix effects and enhancing sensitivity without complex manual steps.
In this study, we performed simultaneous analysis of drugs spiked into plasma samples to evaluate the system's sample throughput and quantitative performance.

2. Sample and Analysis Conditions

Samples

Standard samples for 26 target compounds (Merck) were spiked into pooled human plasma (Golden West Diagnostics). Protein precipitation was performed by adding 300 µL of acetonitrile to 100 µL of plasma. After mixing and centrifugation (10,000 rpm, 10 min), 90 µL of the supernatant was mixed with 10 µL of the standard to prepare QC samples.

Online SPE and LC-MS/MS analysis

Analyses were performed using a multiplex 4-channel LC–MS/MS system, consisting of a Nexera QX (four sets of binary pumps and autosamplers) coupled with an LCMS-8060RX mass spectrometer.(Figure 1 left). The system was collectively managed via QX Solution software, which simplifies operation of the multiplexed LC-MS/MS system (Figure 1 right).
For online SPE, MAYI 2 C18-60 and MAYI 2 WCX columns were selected based on compound characteristics.
Detailed analytical parameters and valve configurations are provided in Table 1 and Figure 2.

Fig. 1 Layout of the multiplexed 4-channel LC-MS/MS system (left) and control software “QX Solution” (right).

Table 1 Analytical Conditions

HPLC (Nexera QX system)	ODS Condition	WCX Condition
Trap column	MAYI 2 C18-60 (50 × 1.0 mm I.D.)	MAYI 2 WCX (50 × 1.0 mm I.D.)
Mobile phase A	0.1% formic acid in water	Water
Mobile phase B	Methanol	0.2% formic acid in 20% acetonitrile
Analytical column	Shim-pack Scepter C18-120 (50 × 2.1 mm I.D., 3.0 µm)	
Mobile phase A	0.1% formic acid in water	
Mobile phase B	Methanol	
Column temp.	40 ℃	

MS (LCMS-8060RX)	
Ionization	ESI
Nebulizing gas flow	3.0 L/min
Drying gas flow	10.0 L/min
Heating gas flow	10.0 L/min
DL temp.	250 ℃
Heat block temp.	500 ℃
Interface temp.	400 ℃

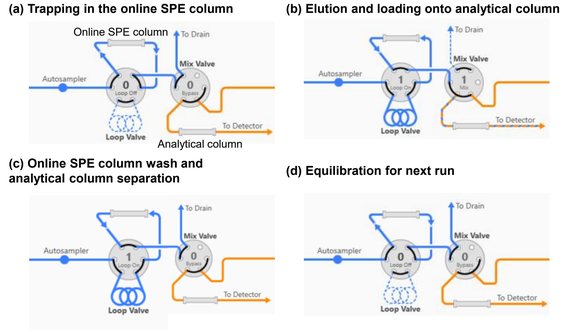
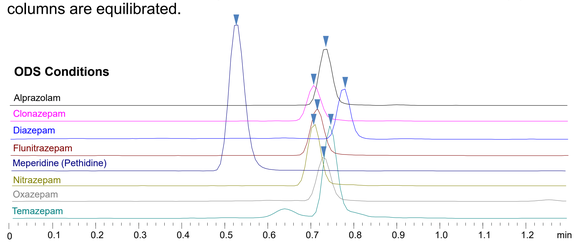


Fig. 2 Schematic of online SPE-LC-MS/MS analysis.
(a) Analytes are trapped in the ion exchange online SPE column. (b) Analytes are eluted with acidic solvent in the loop and transferred to the analytical column. (c) Gradient separation is performed with the analytical column. The online SPE column is washed, and the loop refilled with the solvent for the next run. (d) Both columns are equilibrated.



3. Results

Calibration curves and QC checks

MS chromatograms under ODS conditions for standard samples at 1000 ng/L are shown in Figure 3 left, and those under WCX conditions are shown in Figure 3 right.
Calibration curves were generated for the 26 target drugs by diluting standard samples with methanol across the concentration ranges shown in Table 2. The coefficients of determination (r^2) were greater than 0.99 for all compounds, demonstrating excellent linearity for each calibration curve. Quantitative precision was evaluated using QC samples at two concentration levels. The results for each QC sample are summarized in Table 2. All compounds met the criteria with accuracies ranging from 80 - 115%, indicating robust and reliable performance.

Improved throughput

A simulation of throughput when analyzing the 2 conditions using 2 streams each (4 streams total) is presented in Figure 4. While a conventional LC–MS method requires 9.3 minutes per sample, use of this system reduces the time needed for system washing and equilibration, enabling approximately a 60% improvement in throughput.

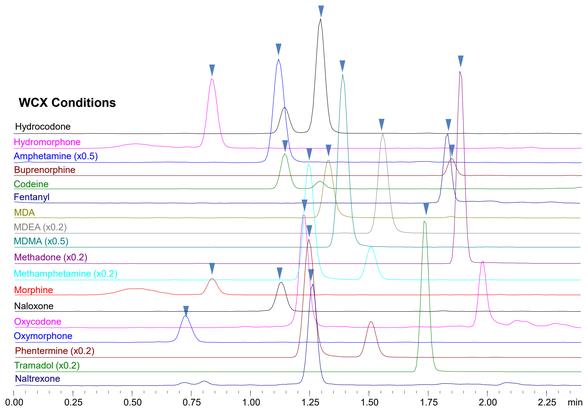


Fig. 3 MS Chromatograms under ODS Conditions (left) and WCX Conditions (right).
Streams 1 and 2 utilize MAYI 2 ODS for online SPE, while Streams 3 and 4 use MAYI 2 WCX.
The numbers in parentheses ( ) indicate the magnification factor for each zoomed chromatogram to optimize the graphic scaling.

Table 2 Quantitative results under ODS Conditions (top) and WCX Conditions (bottom)

			LQC			HQC		
ODS Conditions	**Linear Range (ppt)**	**r2**	**Spiked Conc. (ppt)**	**Measured Conc. (ppt)**	**Accuracy (%)**	**Spiked Conc. (ppt)**	**Measured Conc. (ppt)**	**Accuracy (%)**
Alprazolam	50-10000	0.999	150	128.98	**86.0**	1500	1379.43	**92.0**
Clonazepam	100-10000	0.998	150	124.21	**82.8**	1500	1497.21	**99.8**
Diazepam	100-10000	0.992	150	135.51	**90.3**	1500	1463.67	**97.6**
Flunitrazepam	50-10000	0.999	150	120.01	**80.0**	1500	1330.67	**88.7**
Meperidine (Pethidine)	50-10000	0.999	150	146.18	**97.5**	1500	1457.23	**97.1**
Nitrazepam	50-10000	0.999	150	137.49	**91.7**	1500	1325.35	**88.4**
Oxazepam	100-5000	0.997	150	145.47	**97.0**	1500	1461.06	**97.4**
Temazepam	100-10000	0.993	150	144.63	**96.4**	1500	1283.61	**85.6**
WCX Conditions								
Hydrocodone	20-10000	0.998	150	144.78	**96.5**	1500	1513.85	**100.9**
Hydromorphone	10-10000	0.998	150	136.60	**91.1**	1500	1500.61	**100.0**
Amphetamine	20-10000	0.998	150	140.75	**93.8**	1500	1538.29	**102.6**
Buprenorphine	50-10000	0.995	150	130.06	**86.7**	1500	1654.30	**110.3**
Codeine	50-10000	0.997	150	136.25	**90.8**	1500	1550.49	**103.4**
Fentanyl	5-1000	0.996	150	140.00	**93.3**	1500	1571.56	**104.8**
MDA	50-10000	0.998	150	139.86	**93.2**	1500	1579.46	**105.3**
MDEA	20-10000	0.998	150	134.02	**89.3**	1500	1481.09	**98.7**
MDMA	5-10000	0.998	150	137.21	**91.5**	1500	1540.32	**102.7**
Methadone	10-2000	0.998	150	131.60	**87.7**	1500	1496.50	**99.8**
Methamphetamine	100-5000	0.997	150	149.88	**99.9**	1500	1500.44	**100.0**
Morphine	100-10000	0.998	150	124.44	**83.0**	1500	1507.95	**100.5**
Naloxone	50-10000	0.996	150	146.62	**97.7**	1500	1581.06	**105.4**
Oxycodone	20-10000	0.998	150	139.32	**92.9**	1500	1547.49	**103.2**
Oxymorphone	20-10000	0.997	150	146.46	**97.6**	1500	1595.93	**106.4**
Phentermine	10-10000	0.998	150	142.89	**95.3**	1500	1587.20	**105.8**
Tramadol	5-2000	0.998	150	143.75	**95.8**	1500	1589.72	**106.0**
Naltrexone	50-10000	0.996	150	144.11	**96.1**	1500	1704.22	**113.6**

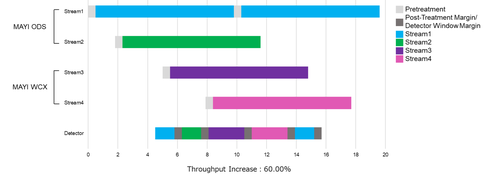


Fig. 4 Analytical Simulation in Multi-Method.

4. Conclusion

This study established a high-throughput simultaneous analysis system using a 4-channel multiplex LC–MS/MS system to selectively retain 26 compounds on two distinct online SPE columns. By executing two different methods in parallel, we eliminated the need for column or mobile phase exchange, significantly increasing throughput over conventional methods. The integrated online SPE further enhanced quantitative precision and robustness, making this system highly effective for high-volume sample processing in forensic and clinical research laboratories.