

Multiplexed Online Ion Exchange SPE combined with LC/MS/MS for the Simultaneous Analysis of Common Drugs in Human Plasma

Eishi Imoto¹, Vikki Johnson¹, Logan Miller¹, Daiki Fujimura², Toshiya Matsubara¹

(1) Shimadzu Scientific Instruments, R&D Center, US (2) Shimadzu Corp., LabConsumables Business Unit, Japan

1. Introduction

The multiplexed 4-channel LC/MS/MS system uses 4 multiple streams, which consist of 4 pumps and 4 autosamplers, to keep a single mass spectrometer working continuously. Online solid phase extraction (SPE) reduces matrix effects automatically and enriches the analyte to increase reproducibility and sensitivity without complicated sample pretreatment. However, target compound panel is limited due to the compatibility of the online SPE column. In this study the newly designed columns, weak anion exchange and phenyl type, were implemented to the multiplexed system. Low and high hydrophobic drugs spiked to plasma were used for its quantitative evaluation.

Nexera QX (Multi autosampler configuration)

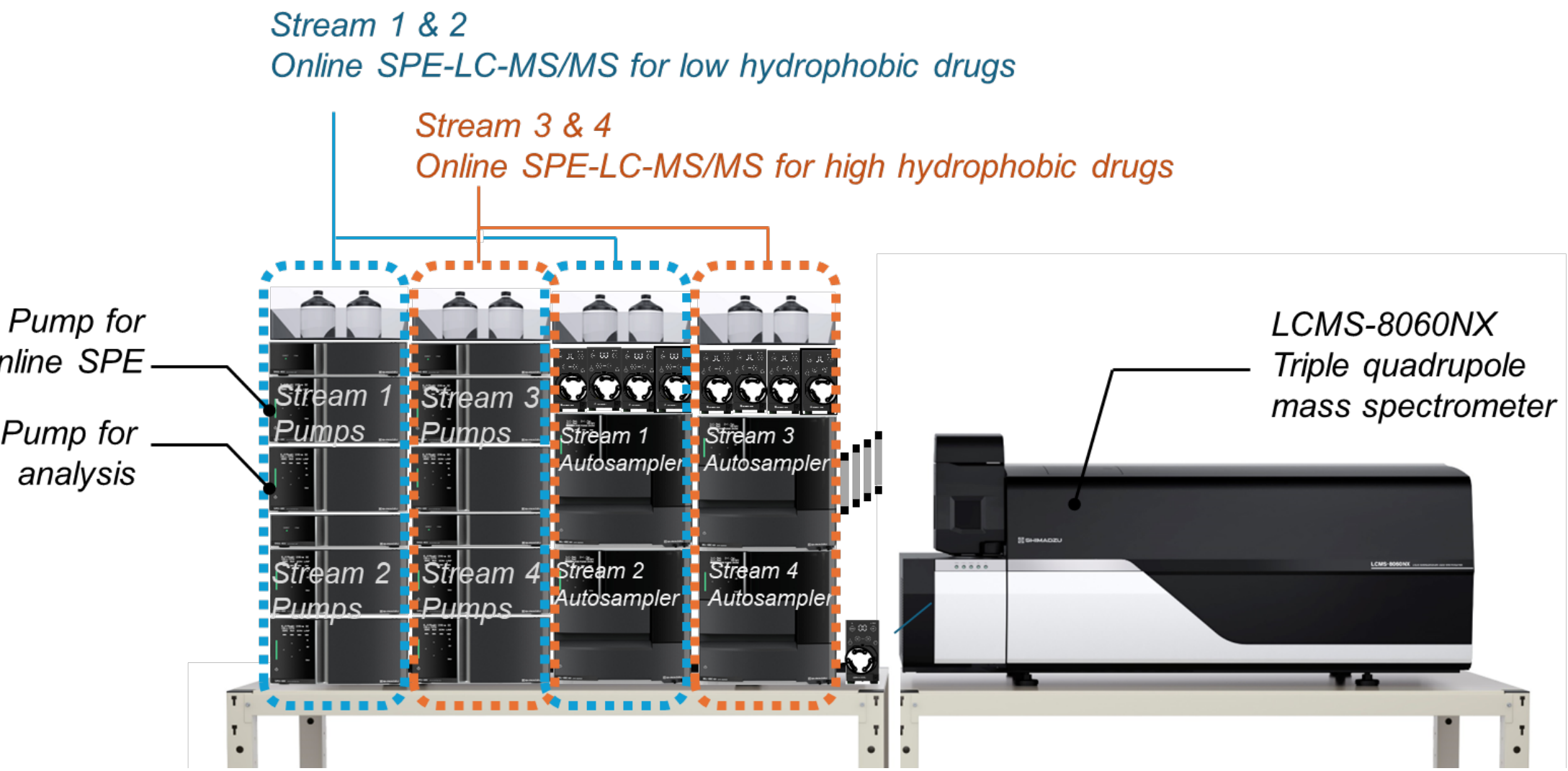


Fig1. Appearance of Nexera QX (left) which has multi autosampler and LCMS-8060NX (right). This system implements 4-channel Online-SPE-LC/MS/MS.

2. Sample Preparation and Analytical conditions

Materials

Standard solutions of amine, opiates and benzodiazepines were purchased from Merck and commercially available human plasma was purchased from Golden West Diagnostics, LLC.

Post spiked calibrators and QC samples

100 μ L of plasma was mixed with 300 μ L of acetonitrile, then vortexed 1min. After centrifugation for 10 minutes at 10,000 rpm, 270 μ L of supernatant was transferred to a new plastic tube. 30 μ L of standard was added to the tube and transferred to an LC vial.

Multiplexed online SPE-LC-MS/MS system

The 4-channel online SPE-LC-MS/MS system was composed of Nexera QX and LCMS-8060NX. MAYI 2 WCX was used for basic drugs, such as amine and opiates, which have low hydrophobicity and high pKa, and MAYI 2 Phenyl was used for benzodiazepines which have high hydrophobicity. The flow diagram in each steps and analytical conditions are shown on Table1, 2 and Figure2.

Table1. LC conditions

System:	Nexera QX	
Stream	Stream 1 & 2	Stream 3 & 4
Online SPE Column	MAYI 2 WCX (prototype) (1.0 x 50 mm)	MAYI 2 Phenyl (prototype) (1.0 x 50 mm)
Mobile Phase A for online SPE	Water	0.1% formic acid in Water
Mobile Phase B for online SPE	0.1% formic acid in 20% methanol	Methanol
Analytical column	Shim-pack Scepter C18-120 (2.1 x 50 mm, 3 μ m)	
Mobile Phase A for analysis	0.1% formic acid in Water	
Mobile Phase B for analysis	Methanol	
Injection volume:	10 μ L	

Table2. MS conditions

System:	LCMS-8060NX
Ionization:	Heated ESI
Interface Temp	400°C
DLTemp:	250°C
Heat Block Temp	500°C
Nebulizing Gas	3.0 L/min
Heating Gas	10 L/min
Drying Gas	10 L/min

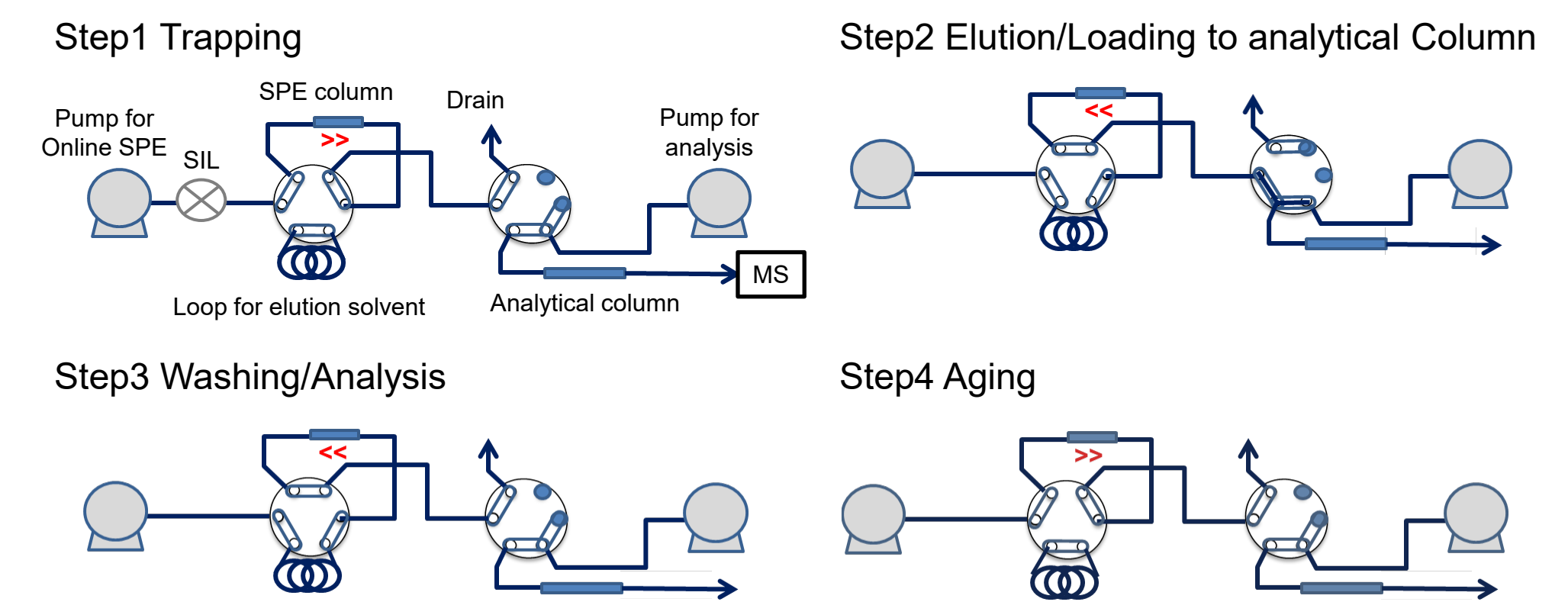


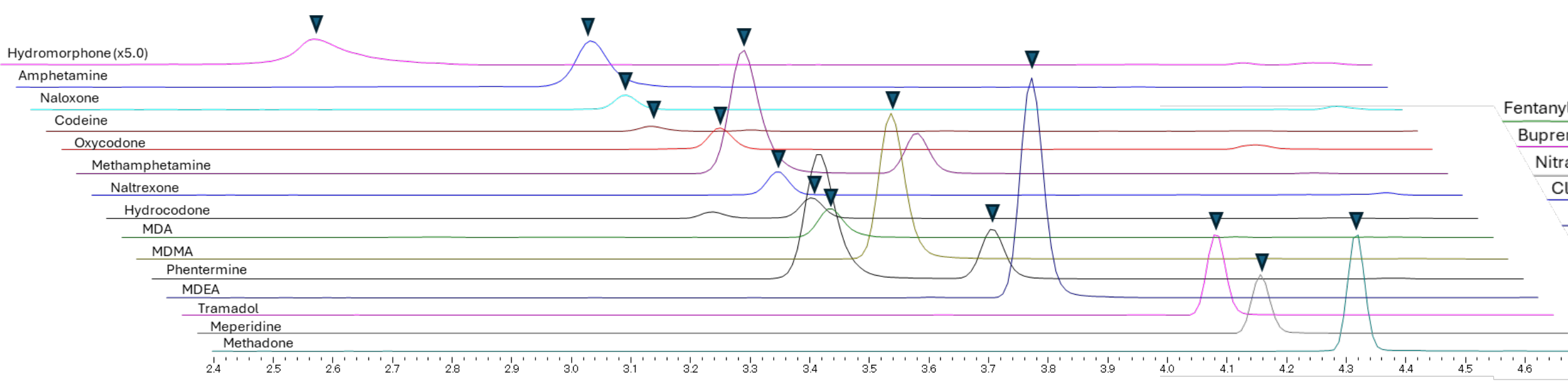
Fig2. The schematic diagram of online SPE-LC/MS/MS in each step

3. Results and Discussion

Online SPE-LC-MS/MS for drugs

Amine and opiates were selected to evaluate the efficiency of trap and elute with MAYI 2 WCX. Benzodiazepines were selected to evaluate MAYI 2 Phenyl. The composition of elution solvent which filled in loop was optimized and Fig3 indicates that those solvent worked in both assay.

Stream 1 and 2 MAYI 2 WCX



Stream 3 and 4 MAYI 2 Phenyl

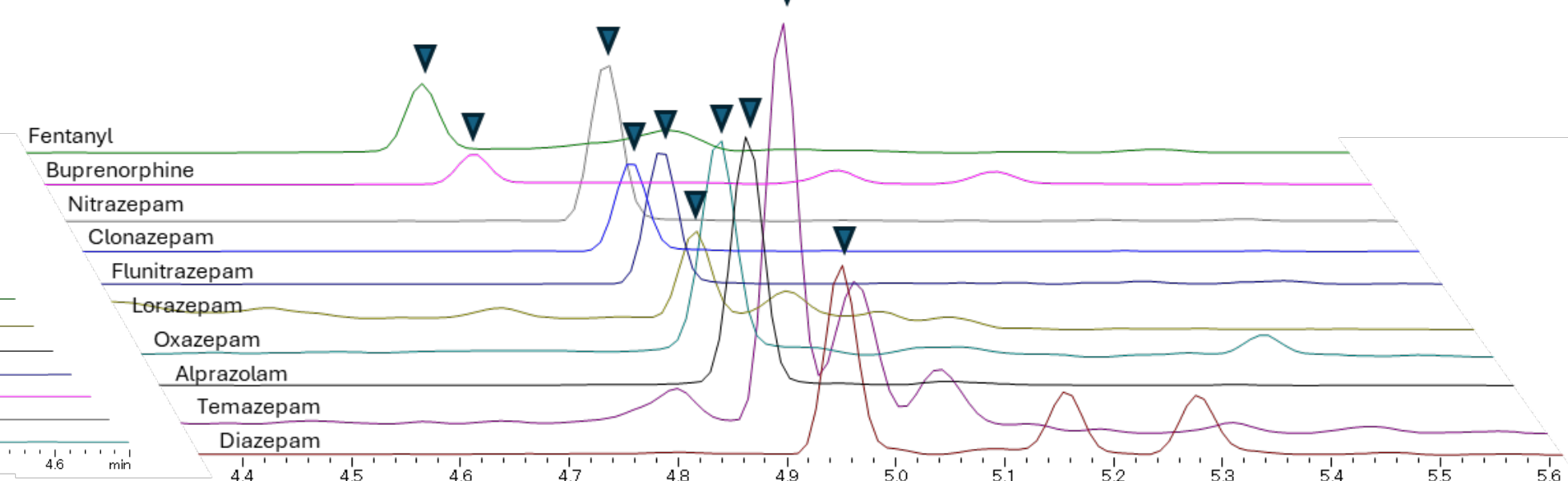


Fig3. MS chromatogram (left) of 15 drugs, such as amine and opiates. Stream1 and 2 use MAYI 2 WCX for online SPE. MS chromatogram (right) of 10 drugs, benzodiazepines. Stream3 and 4 use MAYI 2 Phenyl for online SPE.

Quantification results of drugs spiked to human plasma

The linear range of the drugs in post spiked sample was evaluated from 0.05 to 200 pg/ μ L using calibrators. Quantitative accuracies were confirmed by measuring controls. The linear regression values of R² were grater than 0.998 in both assays and all accuracies were within 100 \pm 15%. (See Table3 and 4).

Table3. Quantitative results of 15 drugs using MAYI 2 WCX

Compounds	Linear Range (pg/ μ L)	R2	LQC			MQC			HQC		
			Spiked Conc (pg/ μ L)	Measured Conc (pg/ μ L)	Accuracy (%)	Spiked Conc (pg/ μ L)	Measured Conc (pg/ μ L)	Accuracy (%)	Spiked Conc (pg/ μ L)	Measured Conc (pg/ μ L)	Accuracy (%)
Amphetamine	0.2-200	0.999	1.5	1.40	93	15	15.7	105	150	145	97
Codeine	0.2-200	0.999	1.5	1.63	109	15	16.4	109	150	144	96
Hydrocodone	0.2-200	0.998	1.5	1.40	94	15	14.1	94	150	143	96
Hydromorphone	0.5-200	0.999	1.5	1.40	94	15	14.8	98	150	144	96
MDA	0.5-200	0.998	1.5	1.62	108	15	16.3	109	150	144	96
MDEA	0.05-50	0.999	0.15	0.14	93	1.5	1.38	92	15	14.6	98
MDMA	0.05-100	0.999	0.15	0.13	88	1.5	1.37	91	15	14.6	97
Meperidine	0.1-200	0.999	0.15	0.16	108	1.5	1.36	91	15	14.2	95
Methadone	0.05-50	0.998	0.15	0.15	102	1.5	1.31	87	15	14.9	100
Methamphetamine	0.05-100	0.999	0.15	0.15	101	1.5	1.40	93	15	15.2	101
Naloxone	0.5-200	0.999	1.5	1.30	87	15	15.0	100	150	142	95
Naltrexone	0.5-200	0.998	1.5	1.34	89	15	16.5	110	150	146	97
Oxycodone	0.5-200	0.999	1.5	1.42	95	15	14.6	98	150	139	93
Phentermine	0.05-200	0.999	0.15	0.16	103	1.5	1.45	97	15	15.7	105
Tramadol	0.05-100	0.999	0.15	0.15	101	1.5	1.36	91	15	14.9	100

Throughput improvement

The measurement time in single stream with MAYI 2 WCX was 8.0 min. 2 streams measurement took 4.6 min/sample. The measurement time using MAYI 2 Phenyl was also improved from 8.2 min to 4.8 min.

4. Conclusion

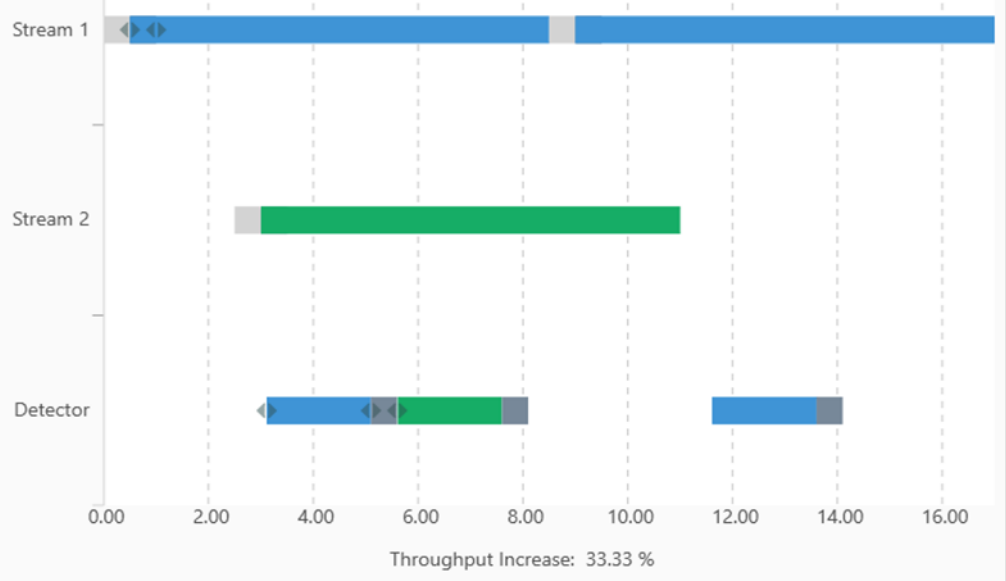
Multiplexed system which has multi autosampler is capable of measuring both low and high hydrophobic drugs without exchanging columns and mobile phases. This system increase the throughput and keep achieving extraordinary quantitative results.

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Stream 1 and 2 MAYI 2 WCX



Stream 3 and 4 MAYI 2 Phenyl

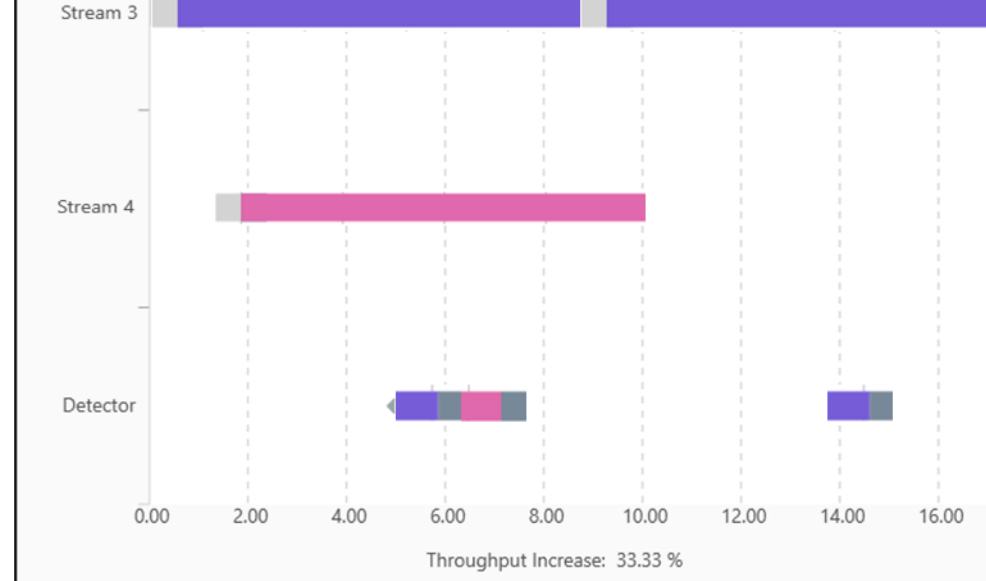


Fig4. QX Solution multiplexing software timing calculator