Quantitative Confirmatory Analysis of the NIDA 5 Panel Using Prelude SPLC System and TSQ Quantum Ultra MS

Haiqiang (Bill) Yu, Kristine Van Natta, Marta Kozak
Thermo Fisher Scientific, San Jose, CA, USA

Thermo SCIENTIFIC

Overview

Purpose: Develop and validate a simple and efficient quantitative LC-MS/MS method for SAMHSA-compliant confirmatory analysis of 5 panel drug using novel HPLC system.

Methods: Human urine containing the drugs were spiked with internal standards, enzymatically hydrolyzed, and diluted.

Results: The LC-MS/MS method was developed and validated to comply with SAMHSA guidelines.

Introduction

Effective on October 2011, the new SAMHSA/NIDA guidelines allow implementation of LC-MS technique to perform NIDA-5 panel, urine quantitative confirmatory analysis. LC-MS/MS methods are often less complicated than the previously implemented GC-MS/MS methods because they do not require derivatization. The NIDA-5 panel requires 6 separate quantitative methods for analysis of THCA, opiates, amphetamines, cocaine, phencyclidine and 6-MAM to confirm immunomethod positive samples. Here we developed 6 methods using a single sample preparation procedure, analytical column, mobile phase and instrument configuration. The methods are implemented on new Thermo Scientific™ dual channel Prelude ™ SPLC online sample preparation-liquid chromatography system, which allows method execution in parallel with a different method on each channel or the same method on both channels multiplexed to a single mass spectrometer.

Serial MS detection of multiplexed methods improves mass spectrometer utilization time, increases laboratory throughput and reduces analysis cost. The syringe pumps and high-pressure, low-volume gradient mixing used in the Prelude SPLC system provide enhanced LC performance including improved peak shape and resolution, stable retention times and reduced solvent consumption.

Methods

Sample Preparation

The sample prep procedure includes glucuronide hydrolysis followed by dilution. For each sample a 200-µL aliquot of urine was spiked with 10 µL of internal standard solution and 100 µL of β-glucuronidase enzyme in ammonium acetate buffer, pH=5.0. The samples were incubated at 60 °C for 2 hours. A 200-µL aliquot of methanol was added to each sample to stop enzymatic reaction. Samples were cooled down, centrifuged and diluted 20-fold with water, except for THCA, which was diluted 2-fold with water. Then 20 µL of sample was injected onto the LC-MS/MS system.

Liquid Chromatography

Chromatographic separations were performed with the Prelude SPLC system by direct injection onto a Thermo Scientific™ Accucore™ PFP 50x2.1mm, 2.6 µm analytical column. The column was maintained at room temperature. Mobile phases A and B consisted of 10 mM ammonium formate with 0.1% formic acid in water and methanol, respectively. Separate methods were set up to analyze 6-MAM, BE, PCP, and THCA. One method was set up for the combination of amphetamine, methamphetamine, MDA, MDEA and MDMA. A final method was used for the opiates morphine and codeine along with hydromorphone, hydrocodone, oxymorphone and oxycodone. Figure 1 shows the LC method for analyzing the opiates.

Mass Spectrometry

MS/MS analysis was carried out on a Thermo Scientific™ Quantum Ultra™ triple quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. MRM transitions for each compound are listed in Table 1.

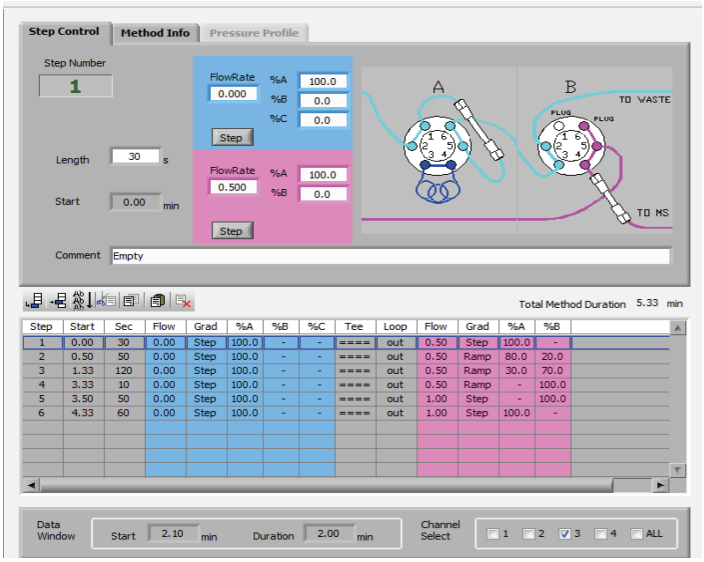
Validation

The calibration standards and quality control (QC) samples were prepared by spiking compounds into blank urine. Samples were processed as described in the Sample Preparation section. Methods were validated in multiplexed mode. Intra- and inter- method precision and accuracy were determined by analyzing a calibration curve along with replicate QCs on three different days. Matrix effects were determined by comparing peak area of samples processed in multiple lots of urine to that of one process in water. Additionally for the opiates, we were able to correlate results obtained with this method to those from a toxicology laboratory validated method.

Data Analysis

Thermo Scientific™ TraceFinder™ software was used for data acquisition and processing. Data were processed with ion ratio confirmation.

FIGURE 1. LC method for separating morphine and codeine.



Results

For each method, performance was within SAMHSA/NIDA guidelines. The quantitation limits (LOQ) for some compounds were lower than required to demonstrate method capability. The linear ranges were 2.5–2000 ng/mL for PCP and THCA; 5–2000 ng/mL for methamphetamine, BE and 6-MAM; 10–2000 ng/mL for morphine, codeine, amphetamine, MDA, and MDMA (Figure 2). The intra-method precision was <13.5%, <3.5%, <14.1%, <6.9%, < 9.6%, <15.9% for PCP, BE, 6-MAM, THCA, opiates and amphetamines respectively. The inter-method precision was <8.9%, <3.6%, <10.9%, <8.8%, <7.0%, <15.3% for PCP, BE, 6-MAM, THCA, opiates and amphetamines respectively. These results are summarized in Table 2. Limited matrix effects were seen and those were largely mediated by deuterated internal standards. The percent recovery for 8 spiked urine donor samples was in range of 80–120% (Table 3). Data collected for opiates with developed methods correlated well with toxicology laboratory data with coefficient of correlation >0.99 (Figure 4). Implementation of the dual channel Prelude SPLC system with syringe pumps improved retention time precision, chromatographic peaks shape and resolution, thus allowing for short, small solvent consumption LC methods while still keeping good data quality.

TABLE 1. List of NIDA 5 compounds MRM transitions, cutoff requirements, LOQ and Linear range

Drug	MRM (Q: Quantifier)	Cutoff (ng/mL)	LOQ (ng/mL)	Linear Range
Amphetamine	136.1–91.3 (Q), 136.2–119.3	250	10	10–5000
Methamphetamine	150.2–91.2 (Q), 150.2–119.2	250	5	5–5000
MDA	180.2–135.2 (Q), 180.2–163.2	250	10	10–5000
MDMA	194.1–163.1 (Q), 194.1–135.1	250	10	10–5000
MDEA	208.1–163.1 (Q), 208.1–135.2	250	10	10–5000
Benzoylecgonine	290.1–168.1 (Q), 290.1–105.1	100	5	5–2000
THCA	354.3–336.3 (Q), 354.3–308.3	15	2.5	2.5–2000
Phencyclidine	244.2 –159.1 (Q), 290.1–105.1	25	2.5	2.5–2000
Morphine	286.11–152.1 (Q), 286.11–165.1	2000	10	10–6000
Codeine	300.2–152.1 (Q), 300.2–165.1	2000	10	10–6000
6-Acetylmorphine	328.1–165.1 (Q), 328.1–211.1	10	5	5–2000

FIGURE 2. Representative calibration curves for BE, THCA, 6-MAM and PCP.

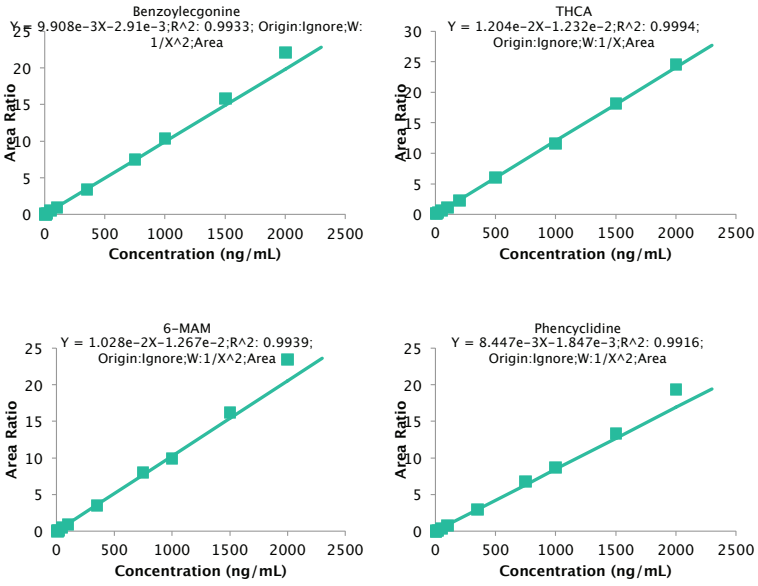


FIGURE 3. Example chromatograms for each method at respective LOQs.

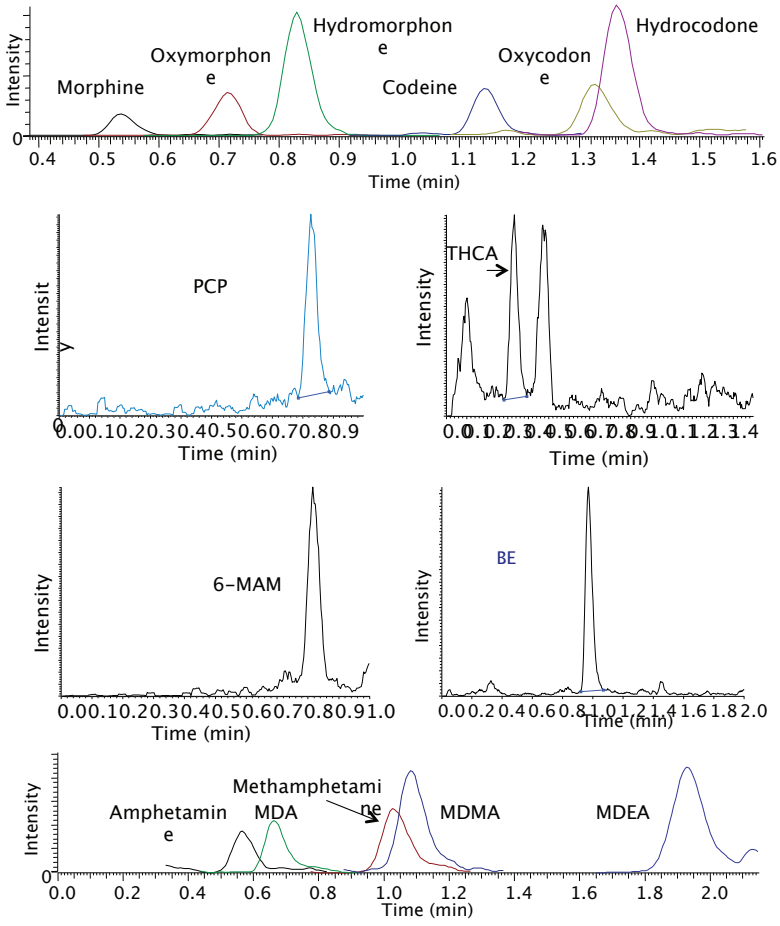


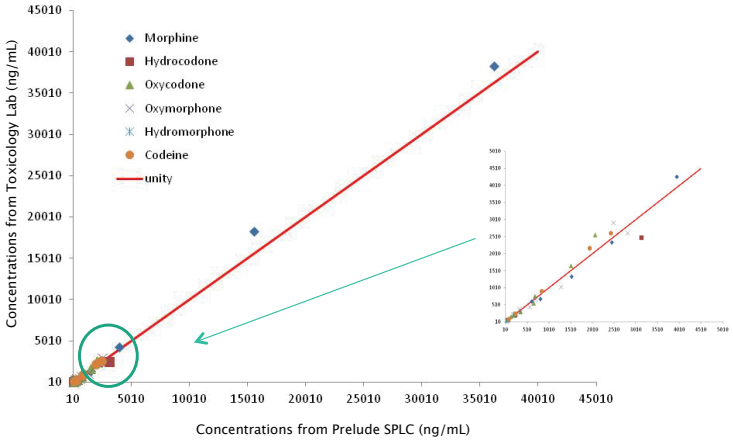
TABLE 2. Intra-method and Inter-method Precision.

Compound	Precision (RSD%)					
	Intra-method			Inter-method		
	LQC	MQC	HQC	LQC	MQC	HQC
Amphetamine	<15.9	<3.68	<2.86	15.33	3.23	2.32
MDEA	<5.33	<3.46	<5.24	3.65	2.88	3.62
MDA	<6.66	<4.15	<11.89	5.84	2.83	2.52
MDMA	<5.52	<4.34	<3.26	4.68	3.31	3.46
Methamphetamine	<5.47	<4.52	<16.63	6.2	4.33	3.79
Benzoylecgonine	<2.21	<2.35	<2.53	1.84	1.8	2.2
Phencyclidine	<6.88	<3.56	<4.33	8.8	3.57	3.63
6-Acetylmorphine	<5.87	<3.39	<4.11	4.69	3.51	3.67
THCA	<7	<2.8	<2.3	8.3	2.5	3.3
Morphine	<8.2	<10.8	<2.2	8.2	4.8	3
Codeine	<7.35	<5.20	<3.68	5.8	3.99	3.77

TABLE 3. Recovery of 11 drugs in 6 different urine lots.

Urine Lot	1	2	3	4	5	6
Amphetamine	100	103	98.3	95.8	101	103
MDEA	94.8	99.9	101	98.3	98.5	94.9
MDA	99.6	107	101	100	102	98.7
MDMA	101	100	97.9	99.3	103	102
Methamphetamine	99.8	101	102	96.1	105	98.5
Benzoylecgonine	106	111	97.6	107	109	106
Phencyclidine	88	84.2	81	83.5	85.6	85.9
6-Acetylmorphine	117	109	104	108	104	105
THCA	95.8	90.2	91.2	93.7	97.8	106
Morphine	96.1	99.8	91	90.7	93.9	92.3
Codeine	102	100	102	103	99.7	104

FIGURE 4. Correlation of data acquired with Prelude-Ultra method compared with data from a toxicology research laboratory validated method.



Conclusion

- An LC-MS/MS method for confirmatory analysis of the 11 drugs in the NIDA 5 panel using the Prelude SPLC and TSQ Quantum Ultra MSwas developed and validated.
- The method has LOQs that satisfy the SAMSHA cutoff requirements for these 11 drugs.
- No matrix interference were observed.
- The method is simple and fast.
- Two-channel multiplexing on Prelude SPLC would allow two different methods multiplexing in two channels and 3 minutes for a sample.

Acknowledgements

We would like to thank Kent Johnson from Pacific Hospital of Long Beach for supplying the comparison samples.

www.thermoscientific.com

Thermo Fisher Scientific, San Jose, CA USA is ISO 9001:2008 Certified.

Africa-Other +27 11 570 1840
Australia +61 3 9757 4300
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 10 8419 3588
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland/Norway/Sweden +46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9434
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Russia/CIS +43 1 333 50 34 0
South Africa +27 11 570 1840
Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific

ASMS13_SAMSHA_Prelude_E 06/13S