High-Throughput Identification of Buprenorphine, Norbuprenorphine, Ethyl-Glucuronide, and Ethyl Sulfate in Urine on a Solvent-Conservative Multichannel HPLC

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Overview

Purpose: Evaluate the performance of running LC-MS/MS forensic methods used to detect buprenorphine and ethanol use on a four-channel UHPLC system that utilizes positive-displacement pumps.

Methods: Reversed-phase liquid chromatography was used to separate analytes with corresponding stable-isotope internal standards eluting from up to four UHPLC channels. A triple quadrupole mass spectrometer with a heated electrospray ionization (HESI) source was used to measure urine levels of the forensic compounds buprenorphine (Bup) and norbuprenorphine (Norbup) after hydrolysis and ethyl-glucuronide (EtG) and ethyl sulfate (EtS) after dilution.

Results: Running Bup/Norbup batches on two channels while EtG/EtS batches ran on the remaining two channels yielded a throughput of 17 injections per hour for each batch, which totaled 34 injections per hour. The four-channel system with positive-displacement pumps reduced solvent consumption by at least 60%. Results from this system were within ±15% of those previously determined on a conventional multichannel system using reciprocating pumps.

Introduction

Many forensic laboratories run several different LC-MS methods in series on a single-channel LC-MS system. If the methods involve different ion sources, columns, and mobile phases, the changeover is time consuming, labor intensive, and increases the risk of mistakes and contamination. A four-channel UHPLC system multiplexed into one mass spectrometer permits parallel batches of up to four different methods—each utilizing a common ion source and unique column and mobile phases—to be completed in a fraction of the time and effort. However, conventional systems utilizing reciprocating pumps needlessly consume mobile-phase solvents between injections. The Thermo Scientific™ Prelude™ SPLC system, which utilizes positive-displacement pumps, does not waste solvents between injections. A modified version of this multichannel UHPLC system (an LX-4 configuration) was tested by running batches for two forensic methods: Bup/Norbup and EtG/EtS.

Methods

Consumables

Fisher Scientific™ HPLC-grade solvents, reagents, and other consumables were used. Buprenorphine and norbuprenorphine and their corresponding deuterated internal standards, as well as ethyl sulfate and ethyl glucuronide and their corresponding deuterated internal standards, were from Cerilliant (Round Rock, TX). Calibrators were made by mixing these standards with synthetic urine. Liquid urine controls were from Biochemical Diagnostics (Edgewood, NY). β-glucuronidase powder was purchased from Sigma-Aldrich (St. Louis, MO).

Sample Preparation

Urine specimens and corresponding calibrators and QCs to be analyzed for Bup/Norbup were hydrolyzed by incubating a mixture of 150 µL of β-glucuronidase solution (10,000 U/mL, pH 5) with 200 µL of specimen, and 50 µL of IS solution containing buprenorphine-D3 & norbuprenorphine-D4 for 1.5 hours at 60 °C. Each preparation was then mixed with 200 µL of cold methanol and refrigerated for 10 minutes before centrifugation. Then, 20 µL injections of supernatants from each preparation were made into the UHPLC systems.

Urine specimens and corresponding calibrators and QCs to be analyzed for EtG/EtS were diluted 1:10 with water and then spiked with 50 µL of IS solution containing EtG-D5 & EtS-D5. Then, 20 µL was injected into the UHPLC system.

Liquid Chromatography

A Prelude SPLC system was modified to permit injections across four channels, similar to a Thermo Scientific™ Transcend™ LX-4 multichannel system. Thermo Scientific™ Accucore™ RP-MS, 2.6 µm, 50 x 2.1 mm HPLC columns were used for the Bup/Norbup method. Mobile phase conditions for this method are described in Figure 1. Thermo Scientific™ Synchronis™ aQ, 3 µm, 100 x 3.0 mm HPLC columns were used for the EtG/EtS method. Mobile phase conditions for this method are described in Figure 2.

FIGURE 1. Bup/norbup LC conditions.

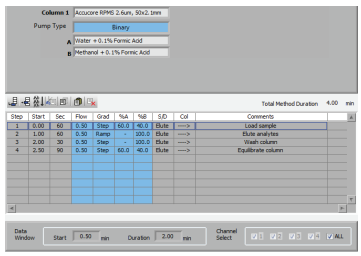
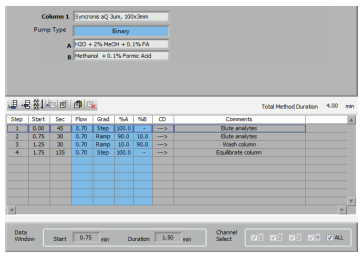


FIGURE 2. EtG/EtS LC conditions.



Mass Spectrometry

The Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer was used with HESI. Ion source conditions common to both methods are described in Figure 3 and the MS/MS method transitions in Figure 4.

System Control and Data Analysis

Thermo Scientific™ TraceFinder™ software was used with Thermo Scientific™ Aria™ MX software to control the modified Prelude system (LX-4 configuration) and TSQ Endura system, submit batches to desired channels, analyze data, and report results.

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FIGURE 3. Common HESI source conditions.

Parameter	Setting	Parameter	Setting
Ion Source Type:	HESI	Cycle Time (secs):	0.5
Spray Voltage:		Use Calibrated RF Lens:	False
Positive Ion (V):	3500	Q1 Resolution (FWHM):	0.7
Negative Ion (V):	1000	Q3 Resolution (FWHM):	0.7
Sheath Gas (Arb):	50	CID Gas (mTorr):	2
Aux Gas (Arb):	12	Source Fragmentation (V):	10
Sweep Gas (Arb):	2	Chrom Filter (secs):	3
Ion Transfer Tube Temp (°C)	350		
Vaporizer Temp (°C)	400		

FIGURE 4. Mass spectrometry acquisition methods.

Bup/Norbup Acquisition Method:

SRM Table							
Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
NorBup	0	1.5	Positive	414.3	243.1	30	150
NorBup	0	1.5	Positive	414.3	340.2	30	150
NorBup-d3	0	1.5	Positive	417.3	246.1	30	150
NorBup-d3	0	1.5	Positive	417.3	343.2	30	150
Bup	0	1.5	Positive	468.35	396.3	40	170
Bup	0	1.5	Positive	468.35	414.3	35	170
Bup-d4	0	1.5	Positive	472.35	243.05	40	170
Bup-d4	0	1.5	Positive	472.35	400.2	40	170

EtG/EtS Acquisition Method:

SRM Table							
Compound	Start Time (min)	End Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
EtS	0	1.5	Negative	125.1	80.1	35	60
EtS	0	1.5	Negative	125.1	97.05	15	60
EtS-d5	0	1.5	Negative	130.1	80.1	35	65
EtS-d5	0	1.5	Negative	130.1	98.05	15	65
EtG	0	1.5	Negative	221.1	75.2	15	75
EtG	0	1.5	Negative	221.1	85.1	15	75
EtG-d5	0	1.5	Negative	226.1	75.2	15	75
EtG-d5	0	1.5	Negative	226.1	85.1	15	75

Results

Batches of calibrators, QCs, and at least 20 specimens were prepared and run by multichanneling Bup/Norbup across two channels. EtG/EtS was run on the other two channels, and allowed 23 Bup/Norbup and 12 EtG/EtS injections/hour. Using two channels for each will not always increase throughput but can ensure completion of all batches, even if one channel stops because of leakage or over-pressurization. Multichanneling batches across four channels using these two different forensic methods is illustrated in Figure 5.

Typical calibration plots from the modified Prelude system (LX-4 configuration) used with the TSQ Endura system are shown in Figure 6. Quantitation ranges were consistently linear ($r^2 > 0.99$ with 1/X weighting), whether calibrators were injected into one channel or across all channels. Differences in calculated amounts averaged less than ±5% within a maximum of ±15%.

FIGURE 5. Multichanneling Bup/Norbup and EtG/EtS batches.

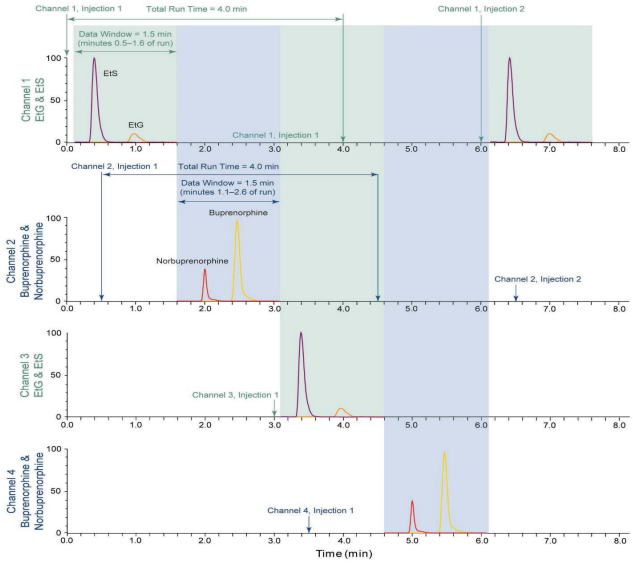
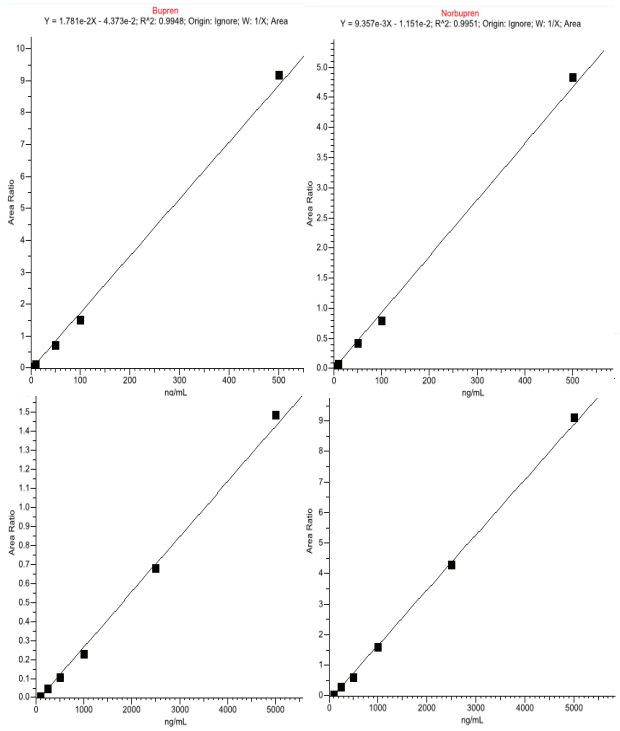


FIGURE 6. Calibration plots from modified Prelude system (LX-4 configuration) used with the TSQ Endura system.



Conclusion

- The modified Prelude system (LX-4 configuration) used with the TSQ Endura system, which utilized unique positive-displacement LC pumps, produced results for Bup/Norbup and EtG/EtS methods that were consistent with those produced by conventional LC-MS.
- The maximum throughput of the four-channel systems was 34 injections per hour when multiplexing the two forensic methods across all four channels.
- The modified Prelude system (LX-4 configuration) reduced solvent consumption by at least 60% when compared to conventional multichannel systems. Other benefits of using this system were the following:
  - Avoided pulsations of reciprocating pumps
  - Easier to use, purge, prepare, and maintain
  - Smaller footprint compared to Transcend LX4 system

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