

A Single Platform LC-MS/MS System for the Simultaneous Analysis of Opiates and Benzodiazepines in Urine

Christopher L. Esposito, Matthew Berube, Francois Espourteille

Thermo Fisher Scientific, Franklin, MA, USA



Overview

The intent of this study was to develop a two channel on-line extraction and liquid chromatography (LC) separation system for simultaneous analysis of opiates and benzodiazepines in forensic toxicology testing. A Thermo Scientific Aria TLX-2 LC system based on Thermo Scientific TurboFlow technology was used to run two LC-MS/MS methods, one for each class of compound. While different TurboFlow™ and analytical columns were used for each method, the system utilized one set of solvents. The multiplexing technology and data windowing allows for these methods to be run simultaneously and robustly, leading to increased throughput with minimal operator intervention.

Introduction

LC-MS/MS is rapidly getting adopted in the analysis of drugs in urine and plasma. The relative complexity of biological extraction procedures and the inherent sample variability is a major stumbling block for a field that is early in the adoption of this technology. Developing assays that incorporate on-line biological extraction with rugged and high resolution chromatography is essential for a successful transition. The day-to-day variability in number of samples for any one bioassay and differences in total samples that a site must process also create the need for a highly flexible LC-MS/MS platform.

Methods

Urine samples are spiked with a deuterated internal standard mix. Opiate samples are acidified to hydrolyzed metabolites. Then all samples are centrifuged. A 10-20 µL injection is made onto a two dimensional multiplexing LC system and analyzed by a Thermo Scientific TSQ Quantum Access triple stage quadrupole mass spectrometer. TurboFlow method was followed by analysis with a PFP analytical column for opiates and a C18 column for benzodiazepines. Ionization was accomplished with heated electrospray ionization (H-ESI) and detection in positive mode. Chromatography was optimized for both panels and their metabolites, with run times from 5-7 minutes and data collection durations around 3 minutes.

FIGURE 1. Aria™ TLX-2 TurboFlow Method Parameters (benzodiazepines/opiates)

TurboFlow Columns:	TurboFlow Cyclone-P; TurboFlow Cyclone+ pre-mixing column
Analytical Column:	Thermo Scientific Hypersil GOLD aQ 3x50 mm 5µm Hypersil GOLD™ PFP 3x100 mm 3µm
Aria TLX-2 System Plumbing:	Focus Mode/Focus Mode
Transfer Loop Volume:	0.200 mL/0.200 mL
Column and Sample Temperatures:	Ambient/Ambient
Aria Operating System Software Version:	1.6.2

Loading Pump Mobile Phases

Mobile Phase A:	50 mM Ammonium Acetate +1.0% HCOOH (aq)
Mobile Phase B:	6.25 mM Ammonium Acetate pH to 9.5 w/Ammonium Hydroxide
Mobile Phase C:	60 mM Ammonium Acetate in Methanol
Mobile Phase D:	40/40/20 Acetonitrile/Isopropyl Alcohol/Acetone

Eluting Pump Mobile Phase

Mobile Phase A:	6.25 mM Ammonium Acetate pH 7.5 (aq)
Mobile Phase B:	10 mM Ammonium Acetate in Methanol

FIGURE 2. TSQ Quantum Access™ Mass Spectrometer (MS) Parameters (benzodiazepines and opiates)

Ion Polarity:	Positive
Ionization Source:	H-ESI
Spray Voltage:	4200 Volts
Vaporizer temperature:	375 °C
Capillary temperature:	270 °C
Sheath Gas pressure (N₂):	50 arb units
Auxiliary Gas pressure (N₂):	40 arb units
Ion Sweep Gas Pressure (N₂):	3.0 arb units
Skimmer Offset:	N/A
Collision Pressure:	1.2 mTorr
Chrom Filter Peak Width:	10.0 s
Scan type:	SRM
Scan time:	0.5 s
Scan width:	0.01 m/z
Peak Width Q1 Da. (FWHM):	0.700
Peak Width Q3 Da. (FWHM):	0.700

Opiate LC Method Details

Step 1	Load analytes onto TurboFlow column, dispose of matrix -30 seconds, in aqueous environment in turbulent flow regime (2 ml/minute).
Step 2	Elutes the analytes from the TurboFlow column to the analytical column
Steps 3 to 5	Wash the TurboFlow column with an organic cocktail to minimize carry-over and remove matrix biofilm. Simultaneously, analytes are eluted by gradient from the analytical column to the detector.
Steps 6 and 7	Loop filling with the appropriate elution mobile phase for the next sample. End of gradient and wash for the analytical column.
Step 8	Re-equilibration of both columns to initial conditions.

Table 1. Analytes and their positive SRM transition ions.

Analyte	Precursor Ion (Q1)	Product Ions (Q3)
Morphine	288.14	165.09, 201.103, 181.09
Oxycodone	302.08	283.97, 226.92, 160.90
Hydromorphone	286.13	185.08, 157.101
Codeine	300.14	165.10, 215.12
Oxycodone	316.13	298.145, 241.11, 256.14
Hydrocodone	300.13	199.10, 171.12, 128.12
Dihydrocodeine	302.10	284.13, 227.10

Analyte	Precursor Ion (Q1)	Product Ions (Q3)
7- Aminoclonazepam	286.06	222.10, 250.06
Alpha-Hydroxyalprazolam	324.99	215.13, 296.20
Lorazepam	320.99	274.90
Oxazepam	287.04	240.95
Alprazolam	309.04	281.01
Nordiazepam	271.05	165.21
Clonazepam	316.05	214.05, 270.06

FIGURE 3. Full LC run vs. data windowed run for benzodiazepines

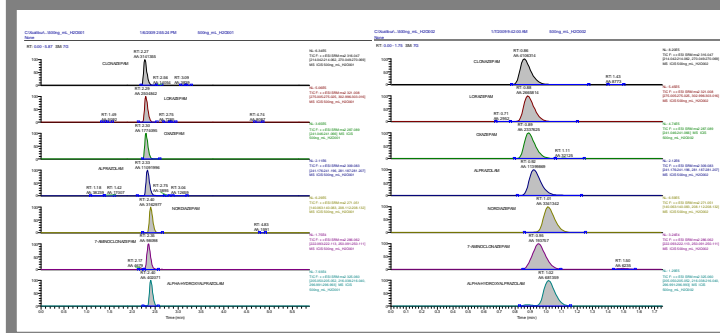
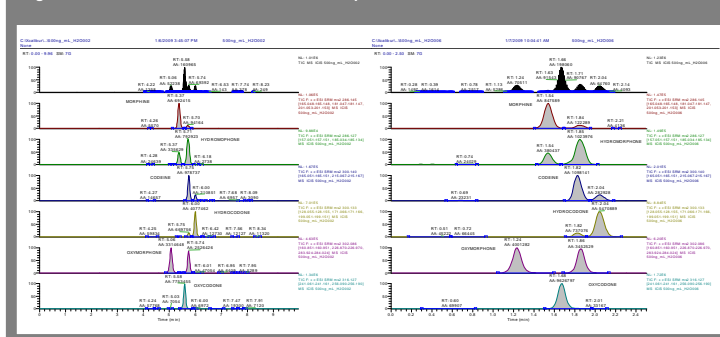


Figure 4. Full LC run vs. data windowed run for opiates



Results

The analysis of opiates and benzodiazepine (two very common drug classes in forensic toxicology testing) with a multiplexed LC-MS system is a good example of a rugged and reliable methodology and a system with the flexibility to work in most environments. Utilizing a triple quadrupole mass spectrometer and a 2-channel TurboFlow method with TurboFlow Cyclone-P extraction columns, analysis of direct injected urine is accomplished for both drug classes. Seven benzodiazepines plus IS's are analyzed using a 10µL injection volume. Linearity ranging from 0.990-0.999 is achieved within a range of 1ng/mL-5µg/mL. Total run time is 5.5-minutes with a 2.5-minute data collection window. For the opiates, a 20µL injection volume is used in this 7-minute method with a 3-minute data collection window. Seven opiates plus IS's are analyzed from 1ng/mL-20µg/mL. Again, linearity from 0.99-0.999 is achieved. CV's for both drug classes all fall within +/- 15% for standards and OQ's with +/-20% for LLOQ's.

Table 2. Calibration Curve Statistics of the 14 Analytes and representative curves

Analyte	R ² (1/x weighing)	Range (ng/mL)	LOD (ng/mL)
7 Aminoclonazepam	0.99	5-5000	1
Alpha-Hydroxyalprazolam	0.997	5-5000	1
Lorazepam	0.999	5-5000	1
Oxazepam	0.998	5-5000	1
Alprazolam	0.993	5-5000	1
Nordiazepam	0.99	5-5000	1
Clonazepam	0.996	5-5000	1
Morphine	0.9951	50-25000	25
Oxycodone	0.9903	50-25000	1
Hydromorphone	0.995	50-25000	1
Codeine	0.9981	50-25000	1
Oxycodone	0.99	50-25000	1
Hydrocodone	0.888	50-25000	1
Dihydrocodeine	0.9993	50-25000	1

Conclusions

- A robust multiplex method for simultaneous analysis for opiates and benzodiazepines based on TurboFlow extraction technology.
- LOD of 1ng/mL for all compounds (25ng/mL for morphine).
- Quantitative analysis range of 5-5000ng/mL and 50-25,000ng/mL for benzodiazepines and opiates, respectively.
- Run time of <3 minutes per sample including on-line extraction.
- The Aria TLX-2 system with TurboFlow technology and multiplexing enables complete automation for simultaneous analysis of benzodiazepines and opiates in urine.

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