

# Simultaneous Analysis of Opiates and Benzodiazepines in Urine in Under 3 Minutes per Sample Using LC-MS/MS

*Forensic Toxicology Use Only*

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## Introduction

A two-channel liquid chromatography separation method has been developed for the simultaneous analysis of opiates and benzodiazepines in urine for forensic use. A Thermo Scientific Transcend TLX-2 system powered by multiplexing and automated online sample preparation technology was used to run two LC-MS/MS methods, one for each class of compounds. The multiplexing technology and data windowing of the system increase throughput with minimal operator intervention.

## Experimental Conditions

### Sample Preparation

Urine samples were spiked with a deuterated internal standard mix. Opiate samples were acidified to hydrolyze the metabolites, and then all samples were centrifuged.

### HPLC

HPLC analysis was performed using the Transcend™ TLX-2 system. Samples were separated from the matrix using Thermo Scientific TurboFlow Cyclone-P polymer columns. Chromatographic separation was performed using a Thermo Scientific Hypersil GOLD C18 column (50 x 3 mm; 5 μm) for benzodiazepines and a Hypersil GOLD™ PFP column (100 x 3 mm; 3 μm) for opiates.

### Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization source (H-ESI). The selective reaction monitoring (SRM) mode was used for mass spectrometry detection.

## Results and Discussion

The analysis of directly-injected urine is accomplished for both drug classes. Seven benzodiazepines and internal standards and seven opiates and internal standards were analyzed. Figures 1 and 2 display data-windowed runs for selected benzodiazepines and opiates, respectively. Table 1 provides calibration curve statistics for several benzodiazepines and opiates.

## Conclusion

The Transcend TLX-2 system with its unique multiplexing technology successfully runs two totally independent channels for forensic use. Limits of detection were 1 ng/mL (25 ng/mL for morphine). Quantitative analysis ranges were 5-5000 ng/mL for benzodiazepines and 50-25,000 ng/mL for opiates. Multiplexing both channels for analysis of benzodiazepines and opiates produces very significant time savings. The total MS data collection run times are efficiently reduced to less than 3 minutes per sample, inclusive of online sample preparation, thus

resulting in more than 50% time savings versus running the analyses separately.

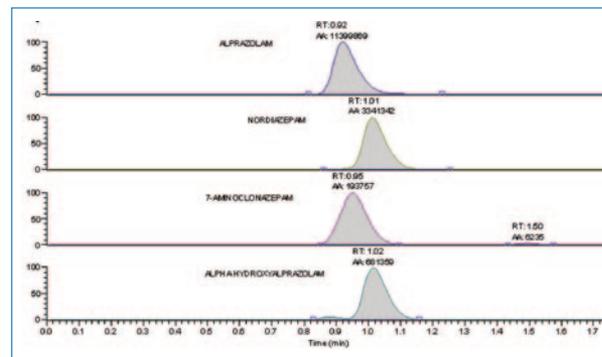


Figure 1: Data-windowed run for selected benzodiazepines

## Assay performance summary

Target Analytes	Benzodiazepines	Opiates
Matrix	Urine	Urine
LOD	1 ng/mL	1 ng/mL (25 ng/mL morphine)
LOQ	5 ng/mL	50 ng/mL
Assay Linearity	1 ng/mL – 5 μg/mL	1 ng/mL – 25 μg/mL
Precision (%CV)	±15% (20% at LLOQ)	±15% (20% at LLOQ)
Sample Volume	10 μL	20 μL
Analysis Time	5.5 minutes, with a 2.5 minute data collection window	7 minutes, with a 3 minute data collection window

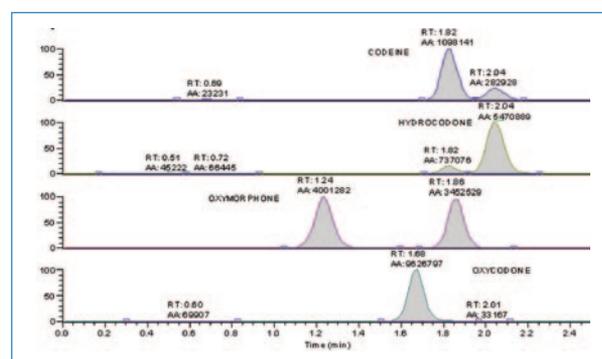


Figure 2: Data-windowed run for selected opiates

Table 1: Calibration curve statistics of 4 analytes

Analyte	R <sup>2</sup> (1/x weighing)	Range (ng/mL)	LOD (ng/mL)
Nordiazepam	0.9900	5-5000	1
Clonazepam	0.9960	5-5000	1
Oxycodone	0.9903	50-25000	1
Hydromorphone	0.9950	50-25000	1

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