Application Note: 561

Quantitation of Amphetamines in Urine for SAMHSA Mandated Workplace Drug Testing Using a Triple Stage Quadrupole LC-MS System

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Key Words

- TSQ Quantum Ultra
- Hypersil Gold
- NIDA
- Methamphetamine
- Phentermine

Introduction

Federal employees and public transportation workers are required to pass a pre-employment drug screen known as the NIDA5, which refers to the five drugs of abuse that are required to be tested for by the National Institute of Drug Abuse (NIDA), or the Substance Abuse and Mental Health Services Administration (SAMHSA) panel. The assays are divided into 5 groups: opiates, amphetamines, cocaine (benzoylecgonine), cannabis (THCA) and PCP. In the past, these five groups have been screened by immunoassay and confirmed by gas chromatography-mass spectrometry (GC/MS). In October 2010, SAMHSA approved the use of liquid chromatography-mass spectrometry (LC/MS) for confirmation of workplace drug testing samples. Here we will focus on the amphetamine group which consists of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) and methylenedioxyethylamphetamine (MDEA).

Goal

To develop a specific and robust dilute-and-shoot quantitative method for the confirmation of amphetamine, methamphetamine, MDA, MDMA, MDEA in urine that meets SAMHSA cutoffs. Additionally, the method should be able to discriminate between the structural isomers methamphetamine and phentermine.

Methods

Sample Preparation

Urine was spiked with internal standards and hydrolyzed with β-glucuronidase. While amphetamines do not require hydrolysis, other compounds in the SAMHSA panel such as the opiates and THC do require hydrolysis. Adding this step enables all SAMHSA panel compounds to be processed with one method. Methanol was added to the hydrolysis mixture and the resulting mixture was centrifuged. The supernatant was further diluted and subjected to LC-MS analysis.

HPLC Conditions

Chromatographic analysis was performed using Thermo Scientific Accela 600 HPLC pumps and a Thermo Scientific Hypersil GOLD aQ column (50 x 4.6 mm, 1.9 µm particle size). The mobile phase consisted of 5 mM ammonium formate with 0.1% formic acid in both water and methanol. The flow rate was 1.5 mL/min and the column was maintained at 30 °C. The total run time was 4.5 minutes.

MS Conditions

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. Two selected reaction monitoring (SRM) transitions were monitored for each compound to provide ion ratio confirmations (IRC).

Validation

Standard curves were prepared by fortifying pooled blank human urine with analytes. Quality control (QC) samples were prepared in a similar manner at concentrations corresponding to the low (LQC), middle (MQC) and high (HQC) end of the calibration range. Intra-run variability and robustness were determined by analyzing six replicates of each QC level with a calibration curve on three different days. Matrix effects were investigated by comparing peak area of analytes prepared in multiple lots of urine to those of a sample prepared in water.

Results and Discussion

The limits of quantitation (LOQs) for all compounds meet the SAMHSA confirmation requirements. (Table 1). The method is linear up to 5,000 ng/mL with R2 values > 0.99 for all compounds. Figure 1 shows representative calibration curves for all compounds. Quality control results for the validation are shown in Table 2. Figure 2 shows an SRM chromatogram at LOQ. Peak areas of analytes in samples prepared from seven different lots of blank human urine compared to that of a sample prepared in water were all within 15% for amphetamine, methamphetamine, MDMA and MDEA. The peak areas were within 30% for MDA.

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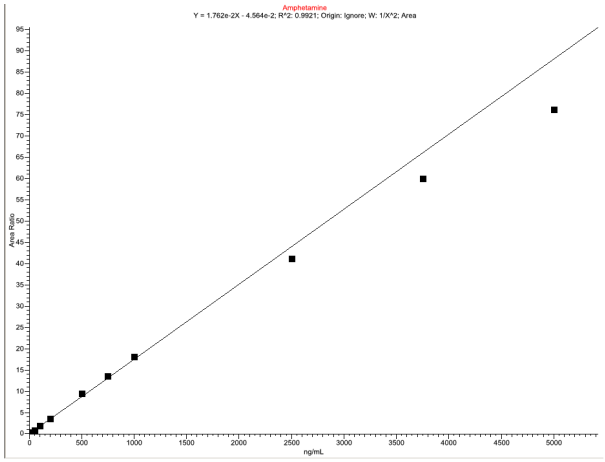
Table 1. Method summary for quantitation of amphetamines in urine

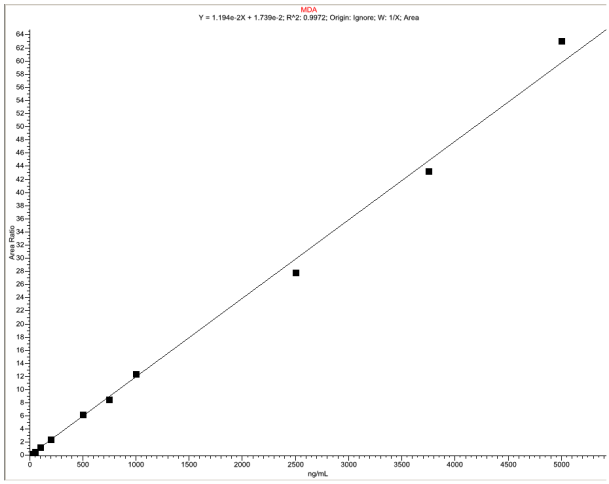
Compound	LOQ	ULOQ	SAMHSA Cutoff
Amphetamine	10 ng/mL	5000 ng/mL	250 ng/mL
Methamphetamine	5 ng/mL	5000 ng/mL	250 ng/mL
MDA	20 ng/mL	5000 ng/mL	250 ng/mL
MDMA	5 ng/mL	5000 ng/mL	250 ng/mL
MDEA	5 ng/mL	5000 ng/mL	250 ng/mL
Phentermine	Not quantitated, but chromatographically well-separated from isomeric methamphetamine.		
Total run time: 4.5 minutes			

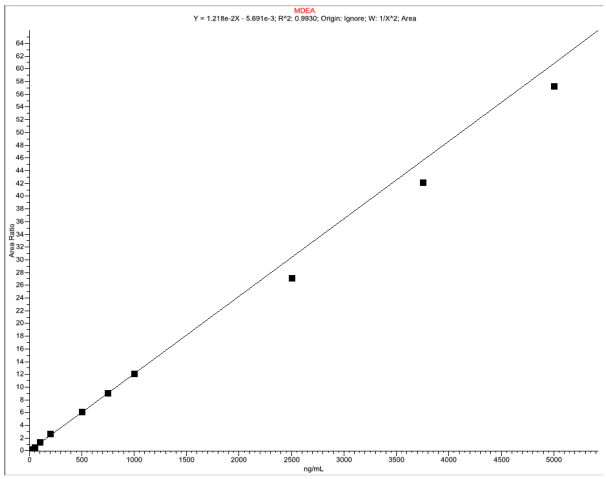
Table 2. %CV/%Bias for QCs analyzed during validation of amphetamines in urine

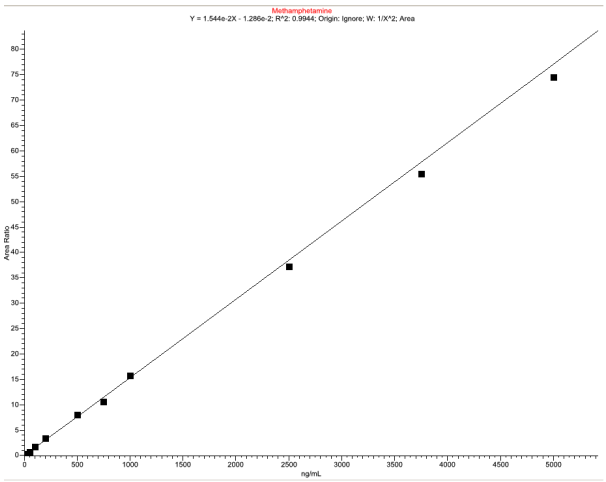
Compound	LQC (10 ng/mL)	MQC (100 ng/mL)	HQC (500 ng/mL)
Amphetamine	10.9/-2.24	4.45/6.39	2.56/0.431
Methamphetamine	7.03/0.420	3.02/7.78	4.26/1.67
MDA	NA	5.97/3.46	4.17/-0.196
MDMA	5.88/0.737	3.31/7.88	4.95/3.45
MDEA	4.51/3.35	2.96/8.20	4.34/2.54

NA: LQC concentration is below LOQ for MDA; data not reported.









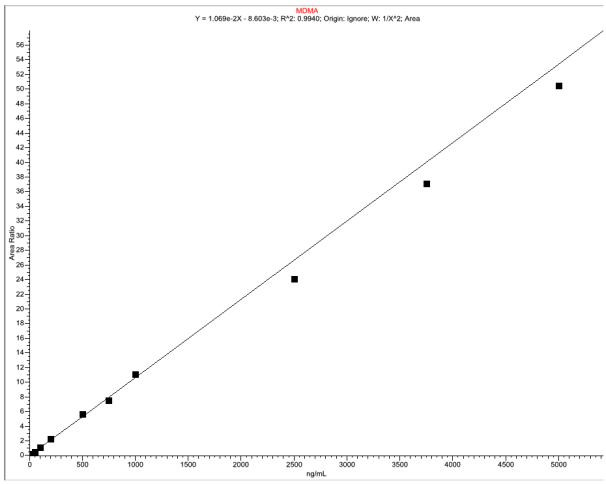


Figure 1. Representative calibration curves for amphetamine, methamphetamine, MDA, MDMA, MDEA in urine

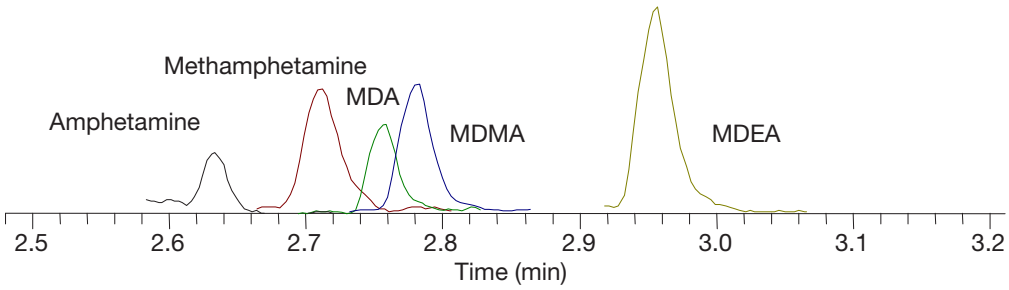


Figure 2. SRM chromatogram of amphetamine, methamphetamine, MDA, MDMA and MDEA in urine at their respective LOQs

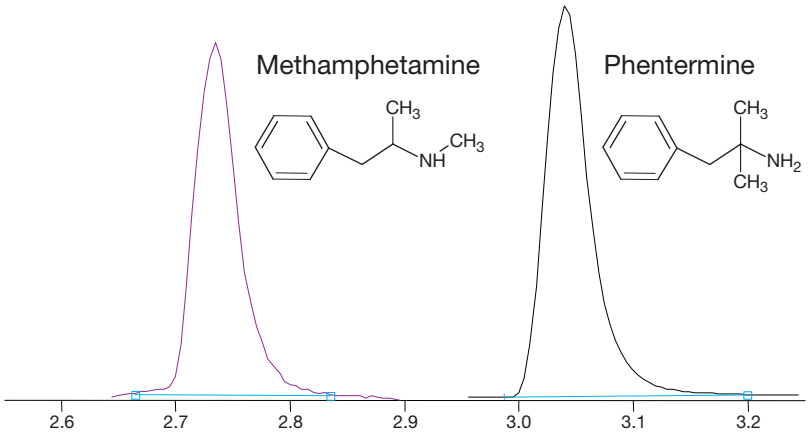


Figure 3. SRM chromatogram showing excellent resolution between structural isomers methamphetamine and phentermine

Methamphetamine and phentermine (an anti-obesity drug) are structural isomers with identical molecular masses and similar fragments. To avoid false positives, they must be separated chromatographically. As seen in Figure 3, these two compounds are well-resolved and will not interfere with each other.

Conclusion

A method with simple dilute-and-shoot sample preparation for the confirmation of amphetamines in urine was developed. This method is suitable for SAMHSA-mandated workplace drug testing, meeting cutoff and specificity requirements within a 4.5-minute run. The sample processing method also enables all SAMHSA panels to be processed at once.

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