

# High-Throughput GC/MS Confirmation and Quantitation of Amphetamine and Methamphetamine in Urine Using the DSQ II

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## Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- Amphetamines
- Toxicology
- Urine Drug Testing

## Abstract

Amphetamine and methamphetamine are commonly abused synthetic drugs which act as central nervous stimulants and have sympathomimetic properties. Because of their abuse, their use is closely monitored in the United State and elsewhere. Both drugs are also administered legitimately for common ailments. Amphetamine can be taken for the treatment of hypotension, narcolepsy and obesity. The “*d*” isomer of methamphetamine can also be prescribed for obesity, and the “*l*” isomer is commonly taken as a nasal decongestant through the use of Vicks<sup>®</sup> Vapor Inhalers<sup>®</sup> inhalers. When abused, synthesized amphetamine and *d*-methamphetamine are typically ingested or taken intravenously. *l*-methamphetamine has also been abused by its removal from inhalers followed by oral or intravenous administration<sup>1</sup>.

For these compounds, workplace drug testing laboratories analyzing urine will most often analyze for the parent drugs because they are typically found in high concentrations relative to their metabolites. The methodology described below details the confirmation and quantitation of parent amphetamine and methamphetamine (Figure 1) in urine using the DSQ<sup>™</sup> II GC/MS system.

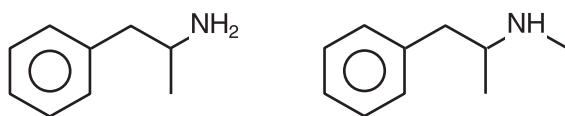


Figure 1: Chemical structures of amphetamine (left) and methamphetamine (right)

For this assay, a 2 mL urine sample size was extracted, with amphetamine-D5 and methamphetamine-D5 used as deuterated internal standards. Samples were extracted using a liquid-liquid extraction technique, followed by derivatization with 4-carbethoxyhexafluorobutyryl chloride (4-CB). The final reaction products were analyzed using a DSQ II single stage quadrupole GC/MS system. For both drugs, a calibrator at 500 ng/mL was used for single point calibration. The resulting method demonstrated excellent precision, no interference for a number of tested compounds and provided linearity from 25 to 25,000 ng/mL, with a limit of detection and limit of quantitation of 25 ng/mL.

## Introduction

The presence of amphetamine or methamphetamine in a urine sample identifies the donor as one who has been exposed to some form of these stimulants. Samples are often derivatized before injection because these drugs are active with commonly used columns and because they produce low-mass ions in electron impact common to many compounds found in a biological matrix, making them prone to interference. The procedure described here includes a derivatization step with 4-CB, which allows for better recovery, better chromatography, less chance of interference and more confidence in identification.

A possible problem when analyzing urine for methamphetamine is the potential for false positives of methamphetamine created from the presence of ephedrine and pseudoephedrine in samples. To protect against this, samples are pretreated with periodate, which oxidizes ephedrine and pseudoephedrine, eliminating them as possible interferents. A further precaution is taken by requiring that amphetamine is present in a positive methamphetamine sample, since amphetamine is a product of methamphetamine metabolism<sup>2</sup>.

A simple liquid-liquid extraction is used for sample preparation. The resulting extracts derivatized with 4-CB proved to be very clean. Furthermore, the ease with which the extraction is performed and the low cost of the materials make this an attractive alternative to other extraction techniques.

The DSQ II, a single stage quadrupole mass spectrometer with a curved prefilter that minimizes background noise derived from excited neutrals, was used for this analysis. Coupled to a TRACE<sup>™</sup> GC Ultra<sup>™</sup> gas chromatograph and an AS3000 autosampler, this GC/MS system represents the industry standard for confirmatory analyses of drug use. ToxLab<sup>™</sup> 2.0 software provided automated sample analysis and quantitation, and the method was fully validated, including assessments of precision, interference, and linearity. This method describes the GC/MS confirmation and quantitation of amphetamine and methamphetamine in urine, and it does not include other matrices or any other drugs or drug classes.

## Methods

To provide a comprehensive view of method development and validation, methods for sample preparation, acquisition, and analysis are described in detail below. Sample preparation plays a critical role in method validation since many certifying bodies recommend or require method validation performed in matrix.

### Sample Preparation

Known negative urine was collected and used for sample preparation. A sample size of 2 mL was selected. Calibrators, quality controls, and linearity samples were spiked with appropriate amounts of amphetamine and methamphetamine (Cerilliant, Round Rock, TX). Single point calibration at 500 ng/mL was used for calculation of all quantitative amounts. A commercial control (Medical Analysis Systems, Level G3, Freemont, CA) calibrated to represent 125% of 500 ng/mL (625 ng/mL) was used as the positive control for the batch, and the 40% control (200 ng/mL) was prepared from source material from an alternate source (Alltech Associates, Deerfield, IL). All batches contained an unextracted standard, the calibrator at 500 ng/mL, a negative control, a 40% control and a 125% control. Amphetamine-D5 and methamphetamine-D5 (Cerilliant) were used as the deuterated internal standards, and were added to each sample at a final concentration of 200 ng/mL. An unextracted standard was prepared by adding 100  $\mu$ L of 10,000 ng/mL mixed amphetamine and methamphetamine standard solution and 100  $\mu$ L of 4,000 ng/mL of mixed internal standard solution to a labeled tube, yielding the equivalent of a 500 ng/mL sample, with the internal standards at 200 ng/mL. Also, 10  $\mu$ L of 2% HCl were added to the unextracted standards prior to initial evaporation to increase recovery. The purpose of the unextracted standard is to prep the GC/MS system, and to demonstrate ion ratios and recovery. The unextracted standard is not subjected to the oxidation and extraction steps but instead proceeds directly to evaporation and reconstitution, at which point it rejoins the rest of the samples for derivatization and analysis.

Prior to extraction, samples were brought to a pH of  $9 \pm 0.5$  by adding 2 mL of 2 M sodium phosphate buffer, pH 9. To this, 800  $\mu$ L of 10% (by weight) sodium periodate was added, and the samples were vortexed. After allowing samples to react at room temperature for 15 minutes, 100  $\mu$ L of 10 M potassium hydroxide were added to further alkalinize the samples.

For the extraction, one milliliter of 1-chlorobutane was added to each tube. The contents of the culture tubes were gently rocked for 10 minutes, followed by centrifugation for another 10 minutes. The top organic layer was then transferred to a clean autosampler vial, where 50  $\mu$ L of 2% 4-CB were added. The samples were capped, vortex mixed and incubated at 70  $^{\circ}$ C for 20 minutes. After equilibration to room temperature, the derivatized samples were loaded onto the AS 3000 autosampler for GC/MS analysis. Table 1 summarizes sample prep, extraction, and derivatization steps.

### Instrumental Analysis

The DSQ II mass spectrometer used for this analysis was configured with a 250 L/s turbomolecular pump, and the TRACE GC Ultra was equipped with a standard split/splitless injector. A 5 mm i.d. deactivated glass liner was used in the injector (Thermo Scientific, PN 45350033), and Siltek™ glass wool was used in the liner (Restek, Bellefonte, PA). The split/splitless injector temperature was set to 175  $^{\circ}$ C. A 2  $\mu$ L injection volume was programmed on the AS 3000 autosampler, and a 10:1 split injection was used. The analytical column was a TRACE™ TR-5MS 15 m x 0.25 mm i.d. x 0.25  $\mu$ m film (Thermo Scientific), which was installed 64 mm into the injection port (Figure 2).

The carrier gas flow rate was set to 2.5 mL/min of helium. The initial temperature on the TRACE GC Ultra was set to 140  $^{\circ}$ C. Upon injection, the GC temperature was immediately ramped at 40  $^{\circ}$ C/min to a final temperature of 175  $^{\circ}$ C and held for 3.10 min, for a total run time of 4 minutes, and amphetamine and methamphetamine retention times of 2.45 and 3.29 minutes respectively.

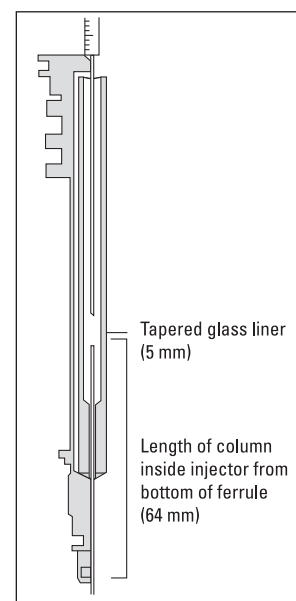


Figure 2: Column installation in GC split/splitless injection port (not to scale)

#### Sample Preparation

1. Label 13 x 125 mm screw top culture tubes
2. Add 100  $\mu$ L of working AM/MA internal standard to each tube
3. Add 2 mL of blank urine, QC or donor specimen
4. Spike calibrator and low QC with AM/MA
5. Add 2 mL of 2 M pH 9 phosphate buffer
6. Add 800  $\mu$ L of 10% sodium periodate
7. React for 15 minutes

#### Extraction

1. Add 100  $\mu$ L of 10M potassium hydroxide
2. Add 1 mL of 1-chlorobutane
3. Rock sample gently for 10 minutes
4. Centrifuge for 10 minutes
5. Transfer top layer into autosampler vials

#### Concentration and Derivatization

1. Add 50  $\mu$ L of 2% 4-CB in 1-chlorobutane
2. Cap vials and vortex
3. incubate at 70  $^{\circ}$ C for 20 minutes
4. Remove from heat and let cool
5. Transfer vials to autosampler tray for GC/MS analysis

Table 1: Sample Prep, Oxidation, Extraction and Derivatization Summary

The DSQ II source temperature was set to 300 °C, and the mass spectrometer was tuned using default *AutoTune* parameters. These tune settings were used for acquisition, with a detector gain of  $3 \times 10^5$ . For initial mass spectrometer method development, high concentrations of derivatized analytes and their internal standards were injected and analyzed in electron impact (EI) full scan to determine masses for EI selected ion monitoring (SIM). The set of SIM masses and dwell times used are shown in Table 2. Mass 294 was used as the quantitation mass for amphetamine, and mass 298 was the quantitation mass for its internal standard. For methamphetamine, mass 308 is used as the quantitation ion and mass 312 for the internal standard quantitation ion. The narrow SIM width enhances sensitivity and builds on the mass stability and resolution of the DSQ II, while a short dwell time provides quantitative precision across the narrow GC peaks. Table 2 summarizes instrument parameters for the validated method.

### Sample Processing and Result Derivation

For sample acquisition, peak detection and quantitation, ToxLab 2.0 software was utilized. By incorporating all of the vital components of analyses into a unified workflow-oriented application, ToxLab 2.0 provides an integrated solution to amphetamine and methamphetamine GC/MS confirmation. To make use of ToxLab 2.0 for method validation, an instrument method was created for the mass spectrometer, autosampler, and GC. A processing method for component identification and quantitation was also developed. In ToxLab 2.0, these methods were integrated into a single master method, which also allows the user to establish criteria specific to the method. Batch creation was performed through the *Batch Wizard* function of ToxLab 2.0, which greatly simplified and streamlined sample entry, particularly for the longer validation batches (Figure 3). This highlights the applicability of this software to routine analysis of toxicological samples<sup>3</sup>.

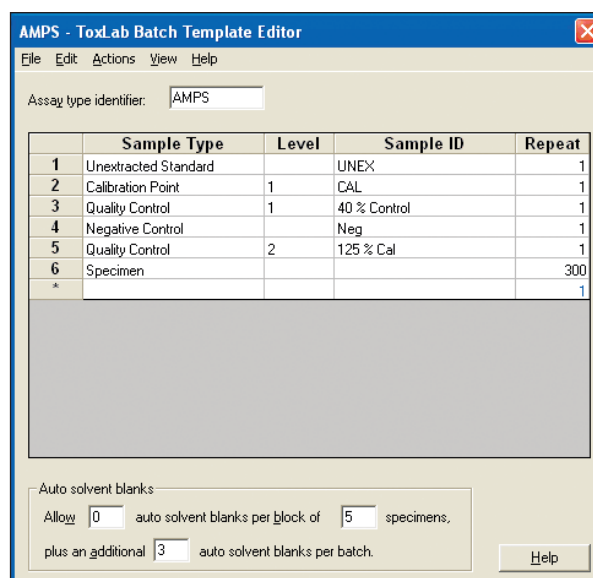


Figure 3: ToxLab 2.0 Batch Template Editor, showing framework for amphetamine and methamphetamine batches.

Concentration calculations were based on a single point calibrator at 500 ng/mL. Calibration curves consisted of a line including the origin and the calibrator, and calculated amounts were based on this curve. All validation batches had to conform to quality control (QC) criteria, including quantitative and qualitative bounds checking.

Quantitative criteria for the batch included acceptable quantitation ranges for all samples in each batch. All calculated amounts for QC samples and study samples had to fall within  $\pm 20\%$  of the expected concentration in order to accept the sample. Failure of a QC sample within a batch would mean the entire batch would need to be repeated. In addition to this quantitative window, negative controls were evaluated based on two additional criteria. One means of assessing a negative control is a quantitative value of less than the method limit of detection (LOD), which in this case is 25 ng/mL for both drugs. An alternate

DSQ II	TRACE GC Ultra	AS3000	
Source Temp (°C):	300	Oven Method	
Detector Gain:	$3 \times 10^6$	Initial Temp (°C):	140
Amphetamine Start Time (min)	2.2	Initial Time (min):	0.0
Amphetamine Masses (m/z):	294.0 266.0 248.0	Rate (°C /min):	40
Amphetamine-D5 Masses (m/z):	298.0 270.0	Final Temperature (°C):	175
Methamphetamine Start Time (min):	2.75	Final Hold Time (min):	3.10
Methamphetamine Masses (m/z):	308.0 262.0 280.0	SSL Method	
Methamphetamine-D5 Masses (m/z):	312.0 266.0	Temperature (°C):	175
Acquisition End Time (min):	3.6	Mode:	Split
Width (amu):	0.5	Split Ratio:	10:1
Dwell Time (ms):	20	Split Flow:	25
		Constant Septum Purge:	on
		Carrier Flow (mL/min):	2.5
		Gas Saver:	off
		Vacuum Compensation	on
		Transfer Line Temp (°C):	200
		Sample Volume (µL):	2
		Viscous Sample:	Yes
		Sampling Depth in Vial:	Bottom
		Injection Depth:	Standard
		Pre-Inj Dwell Time (sec):	0
		Post-Inj Dwell Time (sec):	0
		Sample Rinses:	0
		Plunger Strokes:	5
		Pre-Injection Solvent Rinses:	0
		Pre-Inj Solvent Rinses	
		Solvent A (50:50 EtOAc:MeCl <sub>2</sub> ):	1
		Solvent B (50:50 EtOAc:MeCl <sub>2</sub> ):	1
		Post-Inj Solvent Rinses	
		Solvent C (98:2 1-chlorobutane:4-CB):	5
		Solvent D (50:50 EtOAc:Me:Cl <sub>2</sub> ):	5

Table 2: Instrument method summary for the SIM analysis of amphetamine and methamphetamine on the DSQ II

criterion for negative controls is that the calculated amount must be less than a pre-determined percentage of the method cutoff. For this method, a level of 5% of the cutoff (also 25 ng/mL) was used as a second criterion, and all negative controls were evaluated for compliance to both criteria.

Qualitative criteria included ion ratio and retention time target ranges based on the calibrator, along with peak shape considerations. These criteria were applied to all sample types. Ion ratio ranges for the batch were developed based on the appropriate ratios from the 500 ng/mL calibrator. Ratios were defined as follows:

$$\text{ion ratio} = \frac{\text{area of qual ion}}{\text{area of quant ion}} \times 100\%$$

Ratios were calculated for amphetamine (266:294 and 248:294) and amphetamine-D5 (270:298), as well as methamphetamine (262:308 and 280:308) and methamphetamine-D5 (266:312), and for each ratio, an acceptable range of  $\pm 20\%$  was established. Similarly, the target retention time for amphetamine, methamphetamine and their internal standards was set using a  $\pm 2\%$  retention time window based on the calibrator retention time. Peak symmetry requirements required the peaks to be  $>90\%$  symmetrical at 50% peak height.

Each validation batch was reviewed for compliance with these criteria, and for a study batch to be accepted, it had to comply with all of these QC criteria. For the purposes of this study, the limit of detection is defined as that concentration at which the compound could be reliably identified but not necessarily at which it could be accurately quantified. The method LOD is the concentration at which either AM or MA met the ion ratio criteria but for which the calculated amount may or may not have fallen within  $\pm 20\%$  of the expected concentration. The limit of quantitation is defined as that concentration at which the compound could be reliably identified and quantified.

That is, the method LOQ is the concentration at which either AM or MA met the ion ratio criteria and at which the calculated amount falls within  $\pm 20\%$  of the expected concentration.

## Results

The analysis of amphetamine and methamphetamine in urine using the DSQ II GC/MS system was thoroughly validated through determination of linear range, carryover, precision, and specificity. Six separate batches were prepared and analyzed: one for linearity/carryover, one for specificity, and four for precision. Each batch included the appropriate quality controls and calibration standards, along with validation samples prepared according to Table 3. Batch acceptability was determined by applying the QC standards described above. Carryover was assessed during the course of the linearity study. Precision analyses were performed on four separate batches analyzed on two separate days, while specificity assessed potential interference from a number of compounds. The DSQ II demonstrated excellent intra- and inter-day precision, linearity from 25 to 25,000 ng/mL, with carryover below the QC limits following 25,000 ng/mL, and the sample ran during the interference study passes all QC criteria. With 6.12 minute inject-to-inject times, the method also provides a productive means of performing this confirmation.

### Linear Range Determination

The determination of assay linearity was performed at concentrations across a broad dynamic range. The linearity batch, as with every validation batch, included an unextracted standard, a negative control (blank urine and internal standard), the 500 ng/mL calibrator, a 40% control sample (200 ng/mL) and a 125% commercial control sample (625 ng/mL). To evaluate method linearity, samples at 25, 50, 100, 250, 500, 1000, 2500, 5000, 10000, 25000 and

Linearity	Precision		Interference
1. Unextracted (500 ng/mL)	<b>Batch 1</b>	<b>Batch 2</b>	1. Unextracted (500 ng/mL)
2. Calibrator (500 ng/mL)	1. Unextracted 500 ng/mL)	1. Unextracted (500 ng/mL)	2. 40% Control (Alltech)
3. 40% Control (Alltech)	2. Calibrator (500 ng/mL)	2. 40% Control (Alltech)	3. Calibrator (500 ng/mL)
4. Negative	3. 40% Control (Alltech)	3. Calibrator (500 ng/mL)	4. Negative
5. 125% Control (MAS)	4. Negative	4. Negative	5. 125% Control (MAS)
6. 25 ng/mL x 7	5. 125% Control (MAS)	5. 125% Control (MAS)	6. Negative w/ Interference #1
7. 50 ng/mL x 7	6. 80 ng/mL x 7	6. 80 ng/mL x 7	7. 200 ng/mL w/ Interference #1
8. 100 ng/mL x 7	7. 200 ng/mL x 7	7. 200 ng/mL x 7	8. 625 ng/mL w/ Interference #1
9. 250 ng/mL x 7	8. 250 ng/mL x 7	8. 250 ng/mL x 7	9. Negative w/ Interference #2
10. 500 ng/mL x 7	9. Unextracted 500 ng/mL)	9. Unextracted (500 ng/mL)	10. 200 ng/mL w/ Interference #2
11. 1,000 ng/mL x 7	10. Calibrator (500 ng/mL)	10. 40% Control (Alltech)	11. 625 ng/mL w/ Interference #2
12. 2,500 ng/mL x 7	11. 40% Control (Alltech)	11. Calibrator (500 ng/mL)	12. Repeat for remaining interferents
13. 5,000 ng/mL x 7	12. Negative	12. Negative	
14. 10,000 ng/mL x 7	13. 125% Control (MAS)	13. 125% Control (MAS)	
15. 25,000 ng/mL x 7	14. 200 ng/mL x 7	14. 200 ng/mL x 7	
	15. 500 ng/mL x 7	15. 500 ng/mL x 7	
	16. 625 ng/mL x 7	16. 625 ng/mL x 7	

Table 3: Validation study sample preparation guide for BE confirmation in urine.

50000 ng/mL were prepared and extracted, along with the calibrator and controls. These samples were then injected 7 times each, and the resulting 77 data points were quantified based on the 500 ng/mL calibrator. All 77 quantitative values were within  $\pm 20\%$  of their target concentrations. However, contribution from methamphetamine to its internal standard caused its internal standard ion ratio to fail at 50,000 ng/mL. As such, amphetamine linearity was accepted up to 50,000 ng/mL and methamphetamine to 25,000 ng/mL. At the lowest level for both drugs, 25 ng/mL, the calculated amount of amphetamine was 26.8 ng/mL with a coefficient of variation (CV) of 2.3%, For methamphetamine, the average calculated amount was 25.1 ng/mL, with a CV of 1.7%. Chromatography for the quantitation ion and all qualifiers was good at the LOD, as shown in Figure 5.

An additional component of the linearity study included a determination of the carryover limit for the method. To do so, a negative control was injected following each set of linearity samples starting with the 500 ng/mL cutoff. These negatives were evaluated for acceptability according to the batch criteria described above. Under these constraints, there was no carryover failing the QC criteria even following the 7 injections of the 50,000 ng/mL level. The use of a gas-tight syringe coupled with syringe rinse steps ensures minimal carryover.

Finally, for the batch to be considered acceptable, the quality control for the batch had to meet QC standards described above. For the 40% amphetamine control, the calculated value was 221 ng/mL, a 10% deviation from the target and within the  $\pm 20\%$  quantitation range, and the ion ratios were also within the  $\pm 20\%$  target range. The 125% control was calculated to be 635 ng/mL, a 2% deviation from expected. For the 40% methamphetamine control, the calculated value was 206 ng/mL, a 3% deviation from the expected concentration. The 125% control was calculated to be 651 ng/mL, a 4% deviation from the theoretical. For this method, the LOD is 25 ng/mL for

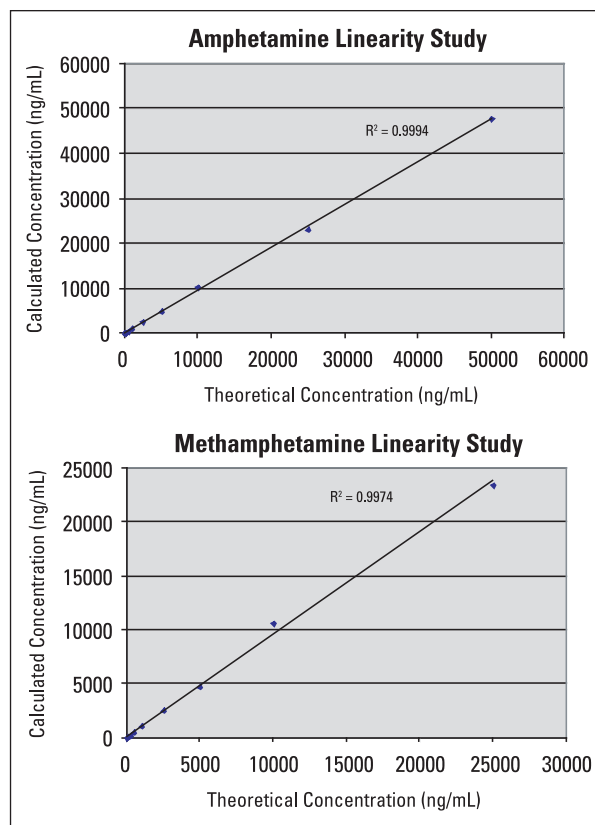


Figure 4: Linearity study results for amphetamine (top) and methamphetamine (bottom), comparing average concentrations for replicates at 10 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9994 across 11 levels for amphetamine and 0.9974 across 10 levels for methamphetamine.

both drugs, making the negative threshold 25 ng/mL. The negative control passed based on this QC criteria. As such linearity batch was accepted. Table 4 includes a summary of the linearity/carryover study for amphetamine and methamphetamine on the DSQ II.

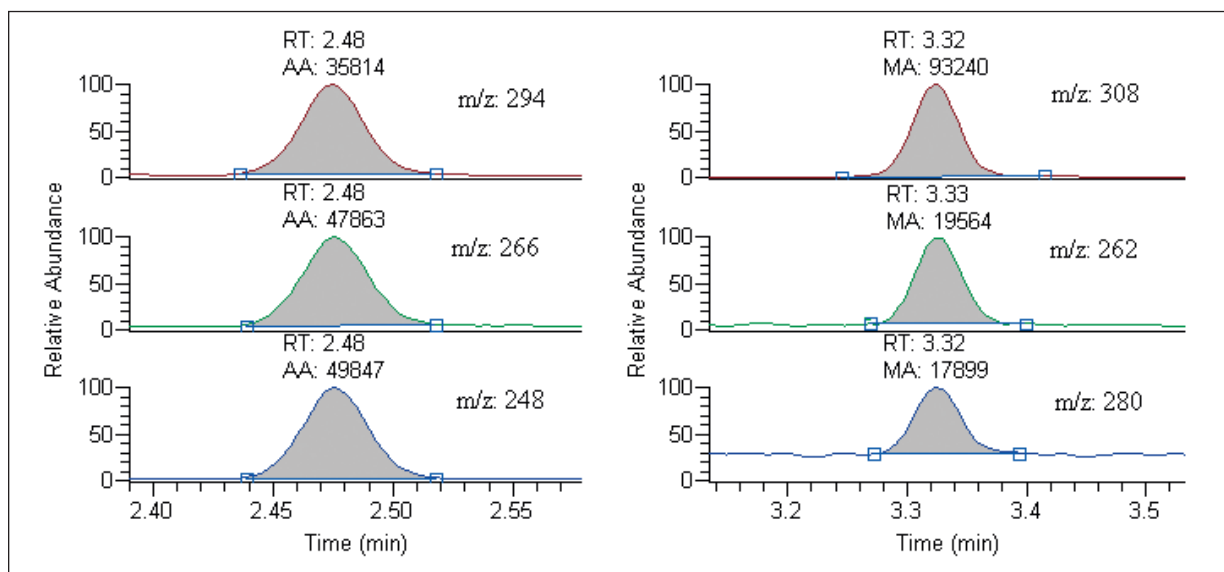


Figure 5: Quant and Qual ions for amphetamine (left) and methamphetamine (right) at 25 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

AMPHETAMINE		METHAMPHETAMINE	
Expected AM Concentration (ng/mL)	Average Calculated AM Concentration (ng/mL)	Expected AM Concentration (ng/mL)	Average Calculated AM Concentration (ng/mL)
25	26.8	25	25.1
50	49.2	50	46.3
100	106	100	101
250	254	250	248
500	509	500	499
Negative	0	Negative	0
1000	1100	1000	1070
Negative	0	Negative	0
2500	2630	2500	2550
Negative	0.6	Negative	1.6
5000	4930	5000	4710
Negative	1.2	Negative	2.6
10,000	10,300	10,000	10,600
Negative	1.8	Negative	2.2
25,000	23,100	25,000	23,400
Negative	8.2	Negative	5.3
50,000	47,700		
Negative	4.6		

Table 4: Results of linearity/carryover study for amphetamine (left) and methamphetamine (right). Calculated concentrations representing points on the linearity curve were obtained by averaging seven injections made at that concentration.

### Intra- and Inter-day Precision

Instrument precision and method precision for both drugs were measured by extracting two separate precision batches at the 500 ng/mL cutoff and running these batches on two different days. Under SAMHSA guidelines, the presence of amphetamine is required at 200 ng/mL in order to report a positive methamphetamine result. Therefore, an additional precision study was performed on two batches at 200 ng/mL for amphetamine. The precision study was designed to indicate precision at the 40% level, at the cutoff of 500 ng/mL (200 ng/mL for the second amphetamine batch set) and at the 125% level. Coefficients of variation (CV) were

calculated for the average concentrations at each level, and these CVs had to be less than 10% for each concentration. As with the linearity batch, the precision batches had to comply with the QC criteria, and all controls were acceptable. To gauge inter-day precision, the CV for the combined data of the two batches was required to be less than 10%.

The method described above provides excellent quantitative precision, with intra-batch CVs all less than 2% and inter-batch CVs all less than 5%. Table 5 includes a summary of the precision results for amphetamine and methamphetamine on the DSQ II.

### Specificity

To determine assay specificity, an interference study was also performed. A number of compounds with potential to interfere with the immunoassay screening test for amphetamine and methamphetamine were included in this test, as were a range of other compounds. Drugs that were tested individually were dextromethorphan, phenylpropanolamine, phentermine, MDA, MDMA, MDEA, phenethylamine, pseudoephedrine and ephedrine. The other drugs were tested together as a mix. Table 6 describes the drugs and their respective concentrations. For each interference test, the potential interferent was spiked into a blank urine sample, a 200 ng/mL sample and a 625 ng/mL sample at the concentration specified. All negatives met the negative control criteria for both drugs, and each 40% and 125% control quantified within 20% of the target concentration, showing that none of the potential interferents tested affected quantitation. Also, all ion ratios were checked against the ion ratios of the calibrator and each were within 20% of the calibrator ion ratios, showing no interference with the confirming ions. Retention times also fell within the specified window of  $\pm 2\%$  of the calibrator retention time. The interference batch also complied with all applicable QC criteria, and the results of the specificity batch were accepted as demonstrating the assay to be free of interference from the tested compounds.

Amphetamine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch CV
200 ng/mL	1.2%	0.5%	1.6%
500 ng/mL	1.3%	1.3%	1.9%
625 ng/mL	0.3%	0.6%	1.3%

Methamphetamine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch CV
200 ng/mL	1.0%	0.5%	4.3%
500 ng/mL	0.4%	1.0%	4.6%
625 ng/mL	0.6%	0.6%	4.4%

Amphetamine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch CV
80 ng/mL	1.3%	1.4%	2.2%
200 ng/mL	0.5%	1.7%	1.2%
250 ng/mL	0.7%	1.6%	2.1%

Table 5. Results of the cutoff precision study for amphetamine (top) and methamphetamine (middle), and the 200 ng/mL precision study for amphetamine (bottom).

Drug	Concentration (ng/mL)
Dextromethorphan	10,000
Phenylpropanolamine	10,000
Phentermine	10,000
MDA	10,000
MDMA	10,000
MDEA	10,000
Phenethylamine	10,000
Pseudoephedrine	1,000,000
Ephedrine	1,000,000
Phensuximide	10,000
Caffeine	2,000
Methadone	1,500
Cocaine	1,500
Codeine	2,500
6-Monoacetylmorphine	3,750
Diacetylmorphine	3,750
Caffeine	5,000
Phenobarbital	5,000
Methadone	5,000
Carbamazepine	5,000
Glutethimide	5,000
EDDP	5,000
Cocaine	5,000
Lidocaine	5,000
Methaqualone	5,000
Desipramine	5,000
Barbital	50,000
10,11-Dihydrocarbamazepine	50,000
Ethosuximide	50,000
Mephentoin	50,000
Metharbital	50,000
4-Methylprimidone	50,000
Methsuximide	50,000
PEMA	50,000
Phensuximide	50,000
Carbamazepine	50,000
Phenytoin	50,000
Ethotoin	50,000
Mephobarbital	50,000
Methyl PEMA	50,000
$\alpha$ -Methyl- $\alpha$ -propylsuccinimide	50,000
N-Normethsuximide	50,000
Phenobarbital	50,000
Primidone	50,000

Table 6: List of compounds tested for potential interference, along with concentrations tested.

## Conclusion

The analysis of amphetamine and methamphetamine on the DSQ II was completed with a total run time of four minutes. The validated method described above is one that is sensitive and has a wide dynamic range, ranging from 25 to 50,000 ng/mL for amphetamine and 25 to 25,000 ng/mL for methamphetamine. All samples tested in this range gave calculated amounts that were within 20% of the nominal values, based on a one-point calibration curve at 500 ng/mL. Across these ranges, all samples also gave ion ratios which were within 20% of the ion ratios of the calibrator. A series of replicate injections at the reported LOD of 25 ng/mL gave a coefficient of variation of 2.3% and an average calculated value of 26.8 ng/mL for amphetamine, and an average value of 25.1 ng/mL and a CV of 1.7% for methamphetamine, demonstrating remarkable sensitivity even when using a split injection technique without an evaporation/concentration step.

Method precision and specificity were also excellent, with intra-day coefficients of variation all less than 2% at three different concentrations. Because all method development and validation were performed in extracted urine matrix, the results demonstrate that the DSQ II is able to handle matrix when a sufficient amount of sample preparation is done. These results also accurately reflect method development and validation as they would be performed within a working laboratory.

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