

A Sensitive and Rapid Approach to the Analysis of Amphetamine and Methamphetamine in Urine on the Finnigan TRACE DSQ

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Overview

Purpose

A method for the rapid analysis of amphetamine (AM) and methamphetamine (MA) in urine on the Finnigan™ TRACE™ DSQ gas chromatograph/mass spectrometer from Thermo Electron Corporation is described, including sample extraction and preparation, as well as analytical parameters. Method linearity, precision, and specificity, along with optimization hints and a discussion of derivative selection, provide a complete picture for the productive analysis of amphetamine and methamphetamine for GC/MS confirmation.

Methods

Known negative urine was spiked with appropriate concentrations of amphetamine and methamphetamine. Commercially prepared controls demonstrated calibration accuracy. A simple liquid-solvent basic extraction, followed by a one-step derivatization process, provided extracts for analysis. Gas chromatography/quadrupole mass spectrometry (GC/MS) operating in selected ion monitoring mode (SIM) provided means for sample acquisition, and a basic forms package offered reporting of results for review and interpretation.

Results

The Finnigan TRACE DSQ provided exceptional precision and linearity for the analysis of AM and MA by GC/MS. Streamlined sample preparation through liquid-solvent extraction and elimination of time-consuming solvent and derivative evaporation steps allowed extracts to be prepared quickly, and fast GC techniques increased sample throughput. GC/MS SIM analysis showed linearity for amphetamine and methamphetamine from 50 ng/mL to 15,000 ng/mL, with precision at a cutoff of 500 ng/mL for both analytes at less than 2%. Quality control results at 40%, -25% and +25% of the cutoff were within +20% of the target value. No carry over was seen following a 12,500 ng/mL sample. Lack of interference indicated method specificity.

Introduction

Amphetamine (AM) and methamphetamine (MA) are stimulants belonging to a class of compounds known as sympathomimetic amines. Subject to abuse, either by prescription or through use of illicit forms, the use of AM and MA is monitored as a component of drug testing programs or through other toxicology testing. In the United States, federal workplace drug testing programs

currently require urine as the matrix of choice. Other biological samples also have utility for testing for AM and MA and are being considered for routine use¹.

Since other members of this drug class can interfere with immunoassay screening methods, a rapid, sensitive, and specific method for confirmation of the screening test is essential to preclude false positive results. The method described here includes a simplified extraction procedure and fast GC/MS analysis, which increases sample throughput and provides sensitivity and dynamic linear range.

Analysis of extracted samples was performed on the Finnigan TRACE DSQ (Figure 1), a quadrupole mass spectrometer that incorporates a curved prefilter, which reduces neutral noise at the detector, thus enhancing sensitivity of the instrument. This increased sensitivity lends itself to low limits of detection and quantitation necessary for confirmation of AM and MA. It also possesses a wide dynamic linear range, which minimizes the need for repeat analysis of concentrated samples that exceed the linearity of the instrument.



Figure 1. The Finnigan TRACE DSQ is shown with the AS 3000 Autosampler.

The Finnigan TRACE DSQ demonstrated exceptional precision and linearity for the analysis of AM and MA by GC/MS. AM and MA were linear from 50 to 15,000 ng/mL, as demonstrated by samples prepared at 50 ng/mL and 15,000 ng/mL and with a three-point calibration range of 250-1000 ng/mL. The method limit of detection was 25 ng/mL for both AM and MA. Precision at the cutoff of 500 ng/mL was 2.5% for AM and 0.9% for MA (coefficient of variation (CV) of concentration, n=7).

Key Words

- Finnigan TRACE DSQ
- Amphetamine
- GC/MS Confirmation
- Methamphetamine
- ToxLab Forms

Precision at 250 ng/mL was 3.2% for AM and 1.8% for MA (CV, n=7). The system was also free from interference due to pseudoephedrine (PSE), as demonstrated by both a spiked and unspiked sample containing 1.0 mg/mL of PSE. Other compounds were evaluated for interference at 3000 ng/mL, and no interference was seen.

In their native states, AM and MA generate EI mass spectra that lack high mass ions and exhibit indistinct spectral patterns (Figure 2). Therefore, most GC/MS confirmation methods involve derivatization of the sample extracts. Derivatization serves to increase the volatility of the compounds and generates higher mass ions for monitoring. Several derivatives have been described for analysis of AM and MA, including BSTFA, TFAA, and 4-CB. Each of these derivatives offers benefits to the user, but both BSTFA and TFAA require the sample extract to be dried down prior to derivatization. BSTFA also yields low-mass ions that are less suitable for confirmation analyses. TFAA also requires that the derivative be evaporated again before analysis to prevent system damage. The procedure described here utilizes 4-carbomethoxyhexafluorobutylchloride (4-CB) to form stable high mass derivatives of AM and MA.

The 4-CB derivative of MA is shown in Figure 2 with the SIM masses circled. This figure shows that derivatizing the components creates distinctive high mass ions that can be monitored using the Finnigan TRACE DSQ in SIM mode. Derivatization using 4-CB was described in 1989 as a means of derivatizing AM and MA². A modification of that method simplifies the extraction and derivatization procedure, and this streamlined procedure is described here. By ensuring that the derivative is present at extremely dilute concentrations, column damage typically associated with acid chloride derivatives is minimized.

Methods

Instrumentation:

A Finnigan TRACE DSQ with a 70 L/sec turbomolecular pump was used for analysis. The Finnigan TRACE GC Ultra was equipped with a split/splitless injection port and an AS 3000 autosampler. The Finnigan TRACE DSQ was equipped with an optional vacuum probe interlock, which allows users to perform basic source maintenance without venting the analyzer. A 15 m x 0.25 mm i.d. x 0.25 μ m TR-5MS column (Thermo Electron Corporation, Waltham, MA) was used as the analytical column.

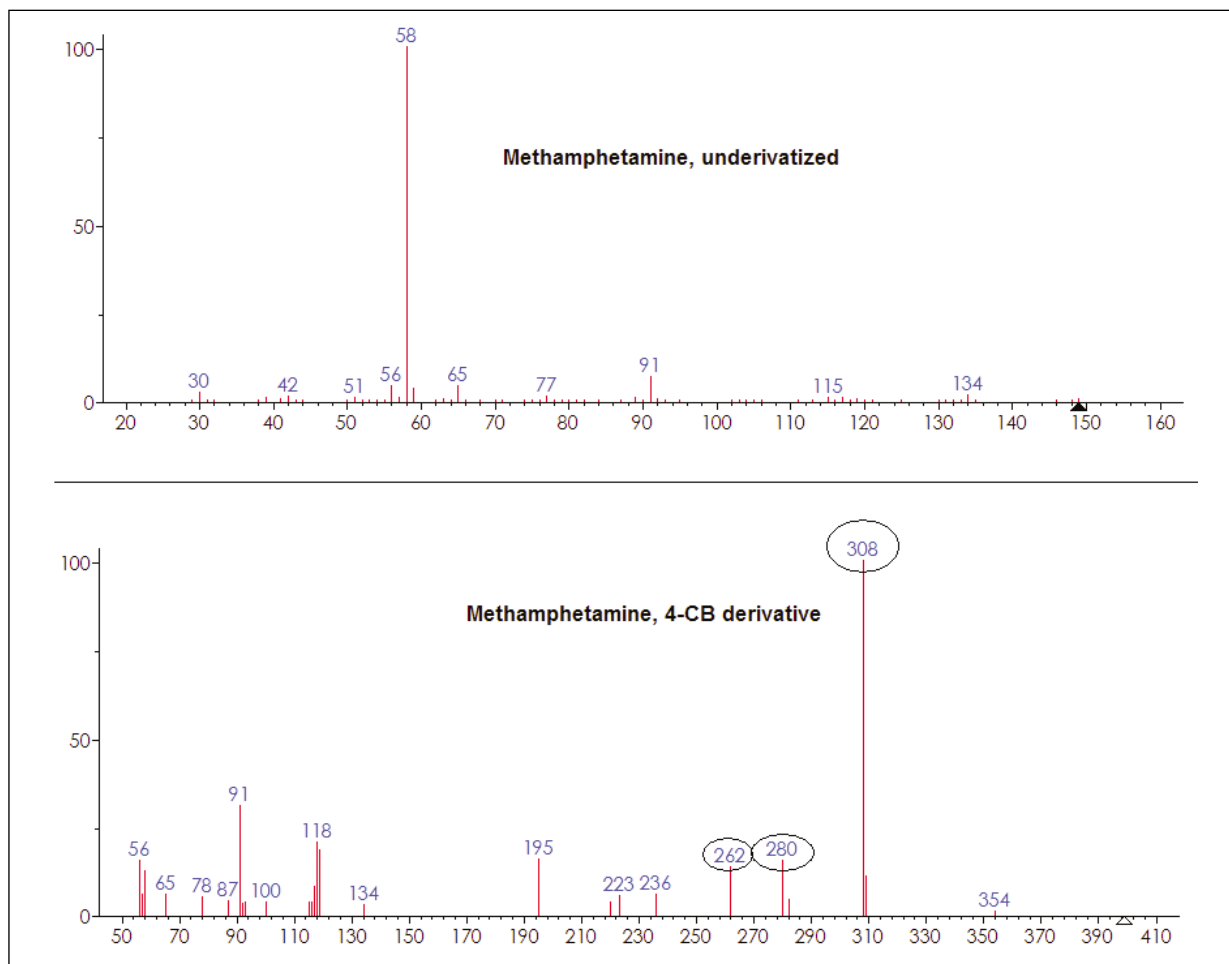


Figure 2. NIST Library mass spectral data for methamphetamine. The top spectrum reflects the underivatized, native state of the drug, while the bottom spectrum depicts the 4-CB derivative. The circled quantities denote SIM masses monitored for MA. Corresponding masses for the deuterated analogs were also collected for internal standardization.

The Finnigan TRACE DSQ was operated in electron impact mode with two segments containing five SIM masses each. The timing of the groups corresponded to the retention times of AM and MA, respectively. The SIM masses and settings for SIM width and dwell times are listed in Table 1. Increased sensitivity can be gained by increasing the dwell times and by decreasing the SIM width. However, the increased dwell time needs to be balanced by the GC peak width to ensure a minimum number of points across the peak. At a dwell time of 80 ms, m/z 308, the quantitation mass for MA, had approximately six points across the peak, which provides sufficient precision for quantitation balanced by sensitivity needs.

The method for the Finnigan TRACE GC Ultra is also described in Table 1. The oven temperature was held at 190°C isothermal through the elution of MA and then ramped at 40°C/min to 270°C to clear the column of any non-volatile components. The total GC run time was 4.0 minutes, and cycle time (sample to sample) was approximately 7.0 minutes. A 2.0 mL/min flow rate for carrier gas (He) along with a 30 mL/min split flow yielded a 15:1 split injection, with a 1.0 µL injection volume. The AS 3000 was operated in standard mode, utilizing a hot needle injection technique that included 3.0 second pre- and post-injection delays. Other AS 3000 settings are summarized in Table 1.

Samples, QCs, and Calibrators:

Drug standards for AM, MA and their D5 analogs (label on side chains) were obtained from Cerilliant Corporation (Round Rock, TX). MAS® DOA GC/MS liquid assayed controls at levels below (Level G2) and above (Level G3) the 500 ng/mL cutoff were received from Medical Analysis Systems, Inc. (Camarillo, CA). d-PSE was obtained from Sigma Aldrich (St. Louis, MO), as were sodium carbonate and 1-chlorobutane. 4-CB was received from United Chemical Technologies, Inc. (Bristol, PA). Standards containing a variety of drugs for interference testing

were obtained from Alltech Associates, Inc. (Deerfield, IL). Drug-negative urine was collected and used to prepare samples and calibrators. The sample size was 2 mL, which represents conservative usage of collected specimens.

A working standard solution of AM and MA was prepared at a concentration of 10,000 ng/mL of each component. 100 µL of this solution, spiked into 2 mL of blank urine, yielded 500 ng/mL of each compound. Subsequent spike amounts were appropriate for the concentration desired, and the same standard was used to prepare all samples, except for the 10,000 and 12,500 ng/mL linearity standards. For these, stock standards at 1.0 mg/mL were used to spike the blank urine to the appropriate concentrations. To prepare the PSE interference challenge samples, 25.0 mg of d-PSE were dissolved into 25 mL of urine to yield a final concentration of 1.0 mg/mL of PSE. Other interference compounds were tested by spiking the appropriate amounts of stock solution to achieve 3,000 ng/mL final concentration of each component. This interference sample also contained 3,000 ng/mL each of AM and MA.

After all samples were prepared appropriately, internal standard was added at a final concentration of 250 ng/mL each of amphetamine-D5 (AM-D5) and methamphetamine-D5 (MA-D5). 1.0 mL of saturated sodium carbonate and approximately 50 mg of sodium carbonate powder were added to each sample. This made the samples basic, with a pH >12. Then, 1.0 mL of 1-chlorobutane was added to each sample. The tubes were capped and mixed gently by rocking for 10 minutes. The mixed samples were centrifuged for 5 minutes, and the upper organic layers were transferred to appropriately labeled glass screw-capped tubes. 50 µL of a dilute solution of 4-CB in 1-chlorobutane (20 µL of 4-CB solution to 2.0 mL 1-chlorobutane, mixed and made fresh before use) were dispensed into each tube, which were then capped, vortexed and derivatized at 50°C for 25 minutes. After derivatization, the samples were removed from the heat and allowed to return to room temperature.

FINNIGAN TRACE DSQ		FINNIGAN TRACE GC ULTRA		AS 3000 AUTOSAMPLER	
Source Temp:	250°C	Oven Method		Sample Volume (µL):	1.00
Solvent Delay:	1.50 min	Initial Temperature (°C):	190	Plunger Strokes:	5
Segment 1		Initial Time (min):	2.00	Viscous Sample:	Yes
Detector Gain:	1 x 10 ⁵	Ramp Rate (deg/min):	40.0	Sampling Depth in Vial:	Bottom
Width (amu):	0.4	Final Temperature (°C):	270	Injection Depth:	Standard
Dwell (ms):	80	Final Hold Time (min):	0.00	Pre-Inj Dwell Time (s):	3
Mass:	248.00	Left SSL Method		Post-Inj Dwell Time (s):	3
Mass:	266.00	Base Temperature (°C):	175	Sample Rinses:	1
Mass:	270.00	Mode:	Split	Post-Inj Solvent:	A+B
Mass:	294.00	Split Flow (ml/min):	30	Post-Inj Solvent Cycles:	10
Mass:	298.00	Constant Purge:	On	Syringe Type:	10 µL cone-tip syringe
Segment 2		Left Carrier Method			
Detector Gain:	1 x 10 ⁵	Constant Flow (ml/min):	2.00		
Width (amu):	0.4	Vacuum Compensation:	On		
Dwell (ms):	80	Auxiliary Zones			
Mass:	262.00	MS Transfer Line (°C):	280		
Mass:	266.00				
Mass:	280.00				
Mass:	308.00				
Mass:	312.00				
Emission Current:	100 µA				

Table 1: Instrument method settings summary for the Finnigan TRACE DSQ, Finnigan TRACE GC Ultra, and AS 3000

When samples reached room temperature, the resulting extracts were transferred to appropriately labeled autosampler vials, capped and transferred to the Finnigan TRACE DSQ for analysis.

Results

The Finnigan TRACE DSQ allowed the rapid confirmation and quantitation of AM and MA, with detection limits considerably lower than the maximum assay limits required by the NLCP program, even at the proposed lower cutoff levels.¹ Figure 3 shows a total ion chromatogram, demonstrating a run time of 4.0 minutes, with AM and MA eluting at 2.00 and 2.48 minutes respectively. A three-point calibration curve demonstrated linearity, with r^2 values of 0.9975 for AM and 0.9983 for MA.

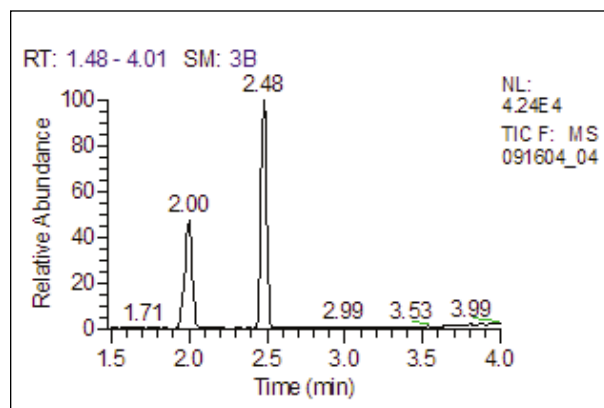


Figure 3. Total ion chromatogram of 1000 ng/mL standard, showing peaks for AM and MA, along with AM-D5 and MA-D5. The total GC run time was 4.0 minutes, and sample-to-sample time was approximately seven minutes.

The limit of detection, determined experimentally as the lowest amount that could be detected that also met the ion ratio criteria of the method, was 25 ng/mL for both compounds. The limit of quantitation, determined as the amount of each that could both be detected with acceptable ion ratios and quantitated within $\pm 20\%$ of the nominal value, was 50 ng/mL. Figure 4 shows the quantitation ions for AM and MA at 50 ng/mL, along with the corresponding calibration curves for each compound.

Along with providing excellent sensitivity, the Finnigan TRACE DSQ demonstrated considerable dynamic linear range. From a LOD of 25 ng/mL, the upper limit of linearity for both AM and MA extended to 15,000 ng/mL, and the carry over limit of the method was 12,500 ng/mL.

Instrument and method precision were determined by evaluating the retention times of the internal standards over the course of the run ($n=51$). For AM-D5, the retention time variation was 0.17% RSD, and for MA-D5, the variation was 0.07%. Precision at the current cutoff of 500 ng/mL was determined through six replicate injections of a 500 ng/mL sample. Additional precision analyses at the proposed new cutoff of 250 ng/mL reinforce the suitability of this method for confirmation of AM and MA by GC/MS. Accuracy of the calibrator preparation was determined through use of assayed commercial controls. These controls come from the manufacturer with a certificate of analysis, along with gravimetric reference concentrations. Table 2 summarizes the quantitative results for AM and MA.

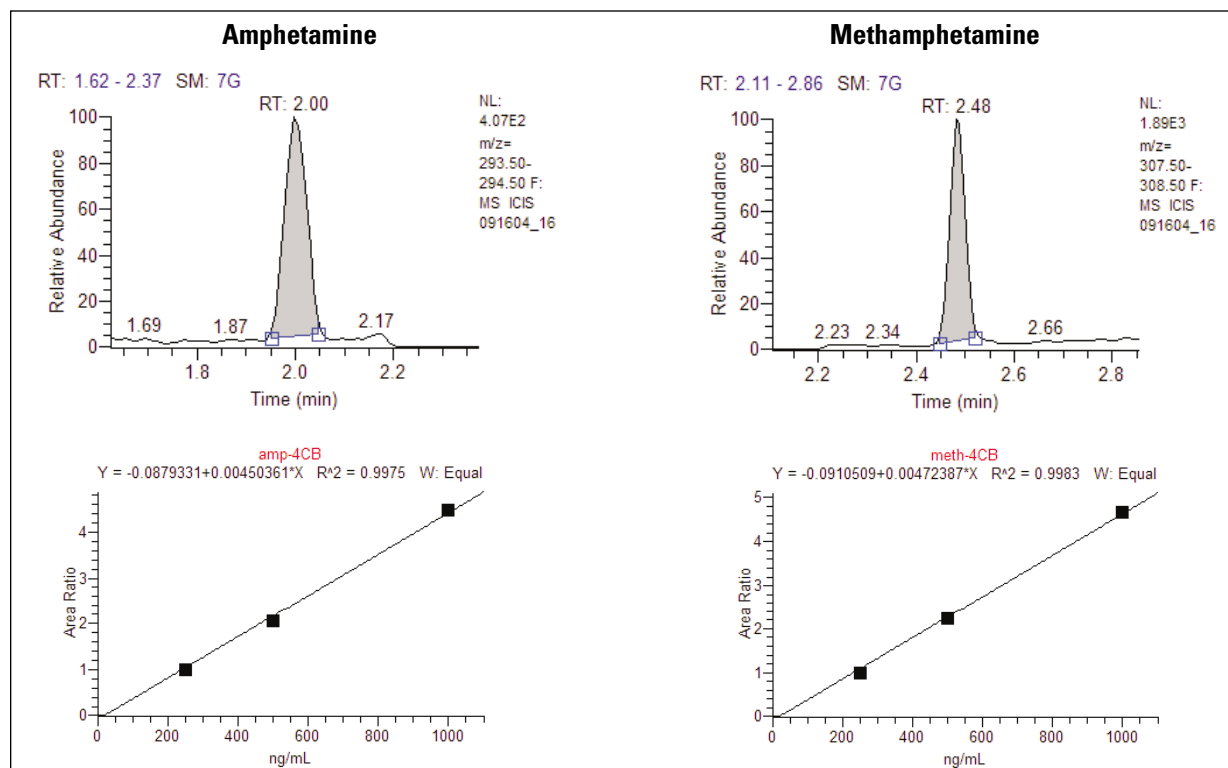


Figure 4. Extracted ion chromatograms for the quantitation ions for AM and MA (m/z 294 and 308, respectively) at the 50 ng/mL concentration level. Also shown are the corresponding calibration curves. A linear least squares fit model that included the origin was used for both compounds and provided linearity from 50 to 15,000 ng/mL over a calibration range of 250-1000 ng/mL.

SAMPLE DESCRIPTION	AM		MA	
	ACTUAL (NG/ML)	% DIFFERENCE	ACTUAL (NG/ML)	% DIFFERENCE
50 ng/mL LOQ sample (average concentration, n=7)	57.7	+15%	54.9	+9.8%
MAS DOA Level G2 (375 ng/mL)	375.5	+0.1%	370.5	-1.2%
MAS DOA Level G3 (625 ng/mL)	614.9	-1.6%	608.9	-2.5%
100 ng/mL (proposed 40% control) (average concentration, n=6)	101.6 (CV=1.6%)	+1.6%	99.4 (CV=2.9%)	-0.5%
200 ng/mL (current 40% control)	186.8 (CV=2.6%)	-6.5%	188.3 (CV=2.4%)	-5.8%
250 ng/mL precision (proposed cutoff) (average concentration, n=6)	227.5 (CV=3.2%)	-9.0%	235.1 (CV=1.8%)	-5.9%
500 ng/mL precision (current cutoff) (average concentration, n=6)	456.0 (CV=2.4%)	-8.7%	467.8 (CV=0.9%)	-6.4%
500 ng/mL + PSE control	475.9	-4.8%	475.2	-4.9%
3,000 ng/mL Interference	2,963	-1.2%	3,003	+0.1%
5,000 ng/mL Linearity Sample	5,214	+4.2%	5,332	+6.6%
10,000 ng/mL Linearity sample	11,780	+17%	10,672	+6.7%
12,500 ng/mL Linearity Sample	12,797	+2.3%	13,129	+5.0%
15,000 ng/mL Linearity sample	17,597	+17%	16,784	+11%

Table 2: Quantitative Results Summary: The quantitative and precision results are summarized in the above table. All calculated amounts were within $\pm 20\%$ of the nominal concentration. (CV = Coefficient of variation = standard deviation/average amount * 100)

Assay specificity was evaluated for a number of compounds. Table 3 lists the drugs included in interference testing. None of the compounds were noted to interfere with this confirmatory method, as determined by the quantitative accuracy of the result (Table 2). Lack of interference by PSE, as well as absence of conversion of PSE to MA, was also demonstrated by including both spiked (500 ng/mL of AM and MA) and unspiked samples with 1,000,000 ng/mL of PSE in each sample. Figure 5 shows the trace for the quant ion for MA (m/z 308) for the unspiked sample.

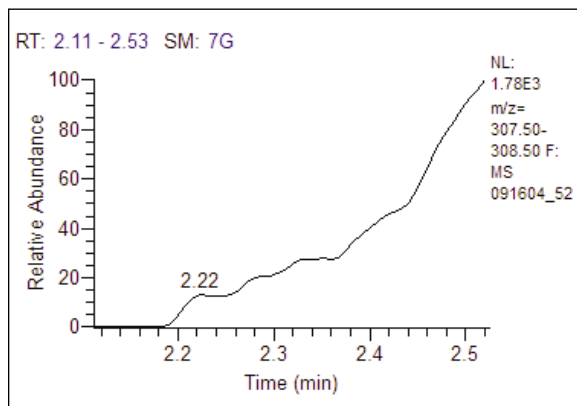


Figure 5: Interference Sample Results: m/z 308 extracted ion chromatogram for 1.0 mg/mL PSE sample. Expected retention time of MA is 2.48 minutes

DRUG	CONCENTRATION (NG/ML)
10,11-dihydrocarbamazepine	3,000
4-methylprimidone	3,000
a-methyl-a-propylsuccinimide	3,000
Amitriptyline	3,000
Amobarbital	3,000
Barbital	3,000
Butobarbital	3,000
Carbamazepine	3,000
Cocaine	3,000
Codeine	3,000
Desipramine	3,000
Doxepin	3,000
Ethosuximide	3,000
Ethotoin	3,000
Glutethimide	3,000
Imipramine	3,000
Meperidine	3,000
Mephentoin	3,000
Mephobarbital	3,000
Methadone	3,000
Methaqualone	3,000
Metharbital	3,000
Methsuximide	3,000
Methyl PEMA	3,000
N-normethsuximide	3,000
Oxycodone	3,000
PEMA	3,000
Pentazocine	3,000
Pentobarbital	3,000
Phencyclidine	3,000
Phenobarbital	6,000
Phensuximide	3,000
Phenytoin	3,000
Primidone	3,000
Pseudoephedrine	1,000,000
Secobarbital	3,000

Table 3: List of drugs tested for interference, including concentration in ng/mL. None of the drugs tested interfered with the confirmation of AM and MA by GC/MS using this methodology.

Ion ratio confirmation enhances confirmatory results and increases confidence in identifications. The use of ToxLab™ Forms software for evaluating the data from the method validation allowed an evaluation of compliance with ion ratio criteria. For this method, a relative window of $\pm 20\%$ provided the range, and ion ratio ranges were calculated based on a weighted average of the ratios for the three calibrators. This weighted average allowed the ion ratios for the cutoff calibrator (500 ng/mL) to carry the greatest weight in establishing the ranges. For all samples, the ion ratios fell within the established ranges.

Conclusion

The method described offers a rapid, sensitive and robust method for GC/MS confirmation of AM and MA in urine on the Finnigan TRACE DSQ. Linearity from 50 to 15,000 ng/mL for both AM and MA provides wide dynamic range to cover a large distribution of sample concentrations, and method specificity was demonstrated using a wide variety of drugs. Excellent reproducibility at both the current and proposed cutoff values show that the Finnigan TRACE DSQ is a valuable tool for this analysis, both now and in the event of future regulatory changes. High throughput is achieved by minimizing sample preparation techniques, using an extraction method that does not involve lengthy evaporation steps. Combined with rapid GC run times and optimized syringe rinse steps, this method allowed for the analysis of 69 samples in seven hours.

The use of ToxLab Forms provided reports for determining sample results and compliance with ion ratio acceptance criteria. These forms can be generated both in real-time and through reprocessing and allow the user to save reports in several report formats. To enhance sample throughput and streamline data review, ToxLab 2.0 software can be incorporated into this analysis. ToxLab 2.0 offers intelligent sequencing, enhanced data review, and automated sample suitability checking that offers true “walk-away” operation of the Finnigan TRACE DSQ.

Overall, the Finnigan TRACE DSQ provides a sensitive, robust and reliable means of confirming AM and MA in urine. It satisfies the demanding requirements for the “gold standard” of drug screen confirmations by offering precision, ease of use, and retention time reproducibility that enhance confidence in the analytical results.

References

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