Application Note: 10177

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

Jason Cole, Matthew Lambing, Trisa Robarge, Thermo Fisher Scientific, Austin, Texas, USA

Key Words

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

Overview

Optimizing the gas chromatographic/mass spectrometric (GC/MS) confirmation and quantitation of drugs of abuse in urine often requires balancing sample throughput with assay performance – including linearity, sensitivity, and instrument longevity. By taking advantage of a complete package that covers hardware, software, and sample preparation, a method for the confirmation and quantitation of amphetamine and methamphetamine in human urine was developed using the DSQ™ II GC/MS system. Based upon guidelines published by the United States Substance Abuse and Mental Health Services Administration (SAMHSA), the College of American Pathologists (CAP), the Society of Forensic Toxicologists (SOFT) and the European Workplace Drug Testing Society (EWDTS), this method provides high-throughput toxicology laboratories a means of simplifying method development and validation. The final method was incorporated into a Productivity Solution designed specifically for the GC/MS analysis of drugs of abuse in urine. The Productivity Solution approach was used to perform a complete method validation that encompassed linearity, carryover, inter- and intra-day precision, and specificity, using extracted, derivatized urine samples.

Results

- Assay linearity from 25 ng/mL to 50,000 ng/mL for amphetamine and 25 ng/mL to 25,000 ng/mL for methamphetamine (Figure 1)
- Amphetamine and methamphetamine limits of detection and quantitation of 25 ng/mL using a 2 mL sample size (Figure 2)
- Inject-to-inject time of 6.12 minutes (~10 samples per hour)
- Intra-day precision of < 2% CV (Coefficient of Variation) at 200, 500 and 625 ng/mL for both drugs
- Interference study shows that high levels of pseudoephedrine, ephedrine, dextromethorphan, phenylpropanolamine, phentermine, MDA, MDMA, MDEA, phenthylamine, phensuximide, caffeine, and a list of 31 other drugs do not effect quantitation near the 500 ng/mL cutoff
- Easy start-up using pre-developed methods

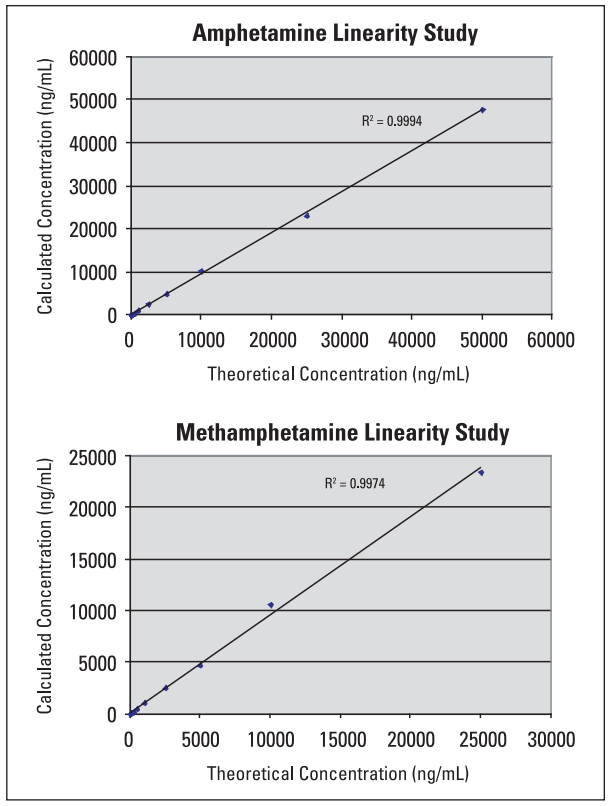

Figure 1: Linearity study results for amphetamine (top) and methamphetamine (bottom), comparing average concentrations for replicates at 10 different levels to the expected amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9994 across 11 levels for amphetamine and 0.9974 across 10 levels for methamphetamine.

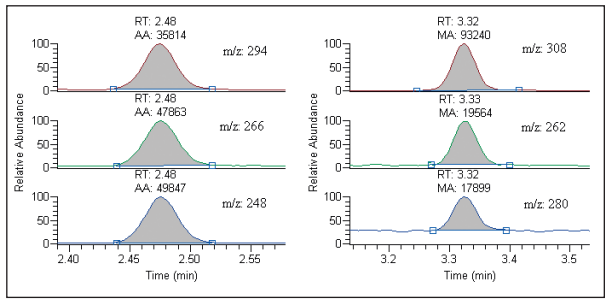

Figure 2: Quant and Qual ions for amphetamine (left) and methamphetamine (right) at 25 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

Thermo SCIENTIFIC

Methods

All validation samples were prepared as batches using a 2 mL urine sample size. Standard materials were obtained for calibration and separate sources of amphetamine and methamphetamine were used as controls. Amphetamine-D5 and methamphetamine-D5 were used as the internal standards. Batches included a matrix-matched single point calibrator (at 500 ng/mL), quality control samples set at 40% and 125% of the calibrator (200 ng/mL and 625 ng/mL respectively), along with an unextracted standard and a negative control, which was blank urine with internal standard only. The samples were first treated with periodate for oxidation before isolating the target analytes with a liquid-liquid extraction with 4-chlorobutane. Samples were then derivatized with 4-carbethoxyhexafluorobutyryl chloride (4-CB) before injecting into the DSQ II. No evaporation step is required when using this derivative.

The DSQ II was operated in selected ion monitoring mode (SIM), collecting 3 ions for the both target compounds, and 2 ions for the deuterated internal standards. A TRACE GC Ultra™ equipped with a split/splitless injection port and an AS3000 autosampler provided sample introduction and separation, along with the requisite fast chromatography required for the high-throughput methodology. A 15 m x 0.25 mm i.d. x 0.25 µm film thickness TRACE™ TR-5MS analytical column was used to enhance separation of the analytes from matrix components. ToxLab™ 2.0 software automated the acquisition and processing of all data, including quantitation and ion ratio confirmation calculations.

Batches were reviewed for conformance to quality control criteria regarding both quantitative and qualitative performance, based on accrediting agency guidelines. All quality controls within a batch had to have quantitative results within ± 20% of their expected (theoretical) concentration. Additionally, ion ratio ranges for qualifier ions for amphetamine and methamphetamine were established using ± 20% of the ratios calculated for the 500 ng/mL calibrator sample. These ranges were used to assess ion ratio performance. Retention time criteria were also implemented, using ± 2% of the calibrator's retention time. ToxLab™ 2.0 performed ion ratio confirmations, retention time checking, and quality control conformance automatically as a part of batch acquisition and processing. For precision analyses, a coefficient of variation (CV) of < 10% of the average calculated amount was required, and inter-day percent differences of calculated amounts had to be less than 10%.

Conclusion

By using a Productivity Solution that encompasses the hardware, software, and methodologies developed specifically for GC/MS confirmation and quantitation of drugs of abuse in urine, high-throughput toxicology laboratories can move easily into implementation of the DSQ II GC/MS system into their workflow. The amphetamine/methamphetamine assay included in the Productivity Solution offers broad linearity to cover a wide range of analyte concentrations, with excellent specificity and precision throughout the concentration range. Limits of detection and quantitation at 25 ng/mL ensure sensitive performance for retest and directed assay samples, and ToxLab 2.0 software offers unparalleled intelligent sequencing for optimal productivity and sample throughput.

For more detailed method information and a comprehensive description of the validation results, please visit *www.thermo.com/gc* and request TN10176.[1]

References

1. *High-Throughput GC/MS Confirmation and Quantitation of Amphetamine and Methamphetamine in Urine Using the DSQ II.* Jason Cole, Matthew Lambing, and Trisa Robarge. Thermo Fisher Scientific Technical Note # TN10176

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 532 4752
China
+86 10 5850 3588
Denmark
+45 70 23 62 60
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Netherlands
+31 76 587 98 88
South Africa
+27 11 570 1840
Spain
+34 91 657 4930
Sweden/Norway/ Finland
+46 8 556 468 00
Switzerland
+41 61 48784 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Thermo Electron Corporation, Austin, TX USA is ISO Certified.

AN10177_E 12/06M