Application Note: 10167

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

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Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- Benzoylecgonine
- Cocaine
- 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 Productivity Solution for the confirmation and quantitation of benzoylecgonine (BE) 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 comprehensive Productivity Solution provides high-throughput toxicology laboratories a means of simplifying method development and validation. The Productivity Solution 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

- BE limit of detection and limit of quantitation of 15 ng/mL using a 2 mL sample size (Figure 1)
- BE retention time of 1.13 minutes, and inject-to-inject time of 5.90 minutes (~10 samples per hour)
- Assay linearity from 15 ng/mL to 12,500 ng/mL (Figure 2)
- Intra-day precision of <1% CV (Coefficient of Variation) at 60 ng/mL, and <5% difference from inter-day extractions
- No interference seen from cocaine, ecgonine, ecgonine methyl ester or norcaine, nor from a list of 24 other drugs
- Easy start-up using pre-developed methods

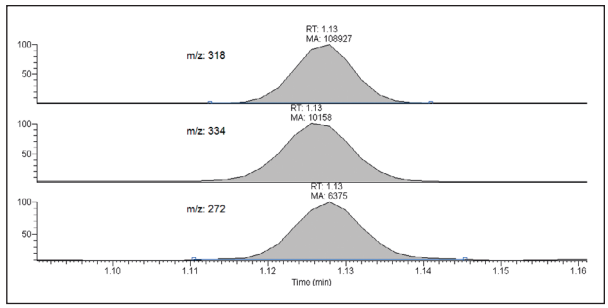

Figure 1: *m/z* 318, 334, and 272 from the 15 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

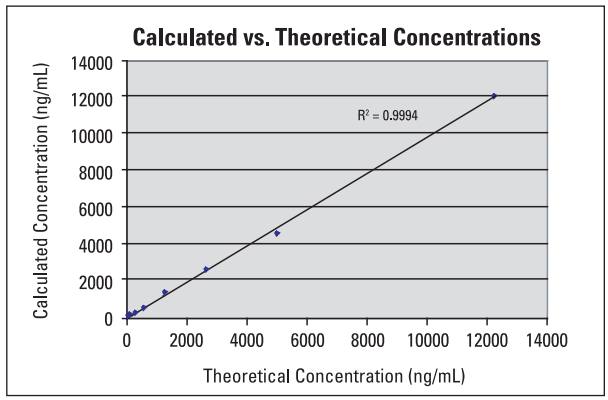

Figure 2: Linearity study results, comparing average concentrations for replicates at 10 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9994 from 15 ng/mL to 12,500 ng/mL.

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 BE were used as controls. BE-D3 was used as the internal standard. Batches included a matrix-matched single point calibrator (at 150 ng/mL), quality control samples set to contain BE at 40% and 125% of the calibrator (60 ng/mL and 187.5 ng/mL respectively), along with an unextracted standard and a negative control, which was blank urine with BE-D3 only. Hypersep™ Verify™-CX solid phase extraction columns from Thermo Fisher Scientific were used for sample extraction, and sample extracts were derivatized with a mixture of HFIP and PFPA. Ethyl acetate was used to bring the final extract volume to 50 µL[1].

The DSQ II was operated in selected ion monitoring mode (SIM), collecting 3 ions for the BE target compound, and 2 ions for BE-D3. 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 TRACE™ TR-DoA5 analytical column was used to enhance separation of BE from matrix components. ToxLab™ 2.0 Intelligent Sequencing Software automated the acquisition and processing of all data, including quantification 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 BE and BE-D3 were established using ± 20% of the ratios calculated for the 150 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 instrumentation into their workflow. The resulting BE assay has 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 15 ng/mL provide sensitive performance for retest and directed assay samples, and ToxLab 2.0 software offers unparalleled intelligent sequencing for optimal productivity and sample throughput.

For detailed information on the instrument and processing parameters, as well as comprehensive coverage of validation results, please visit our Web site at www.thermo.com/gc and request TN10168.

References

1) *High-Throughput GC/MS Confirmation and Quantitation of Benzoylecgonine in Urine Using the DSQ II*. Jason Cole, Matthew Lambing, and Trisa Robarge. Thermo Fisher Scientific Technical Note # TN10168.

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AN10167_E 12/06M