Application Note: 51996

Confirmation and Quantitation of Benzoylecgonine in Urine Using the ISQ Single Quadrupole GC-MS

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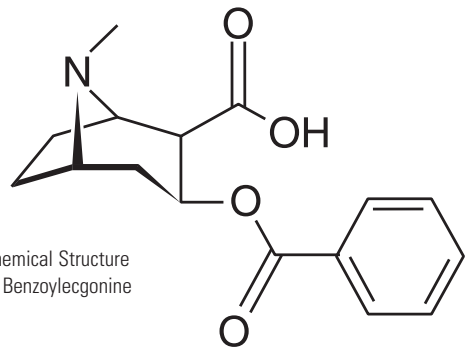
Key Words

- ISQ Single Quadrupole GC-MS
- Benzoylecgonine
- SAMHSA Workplace Drug Testing
- Selected Ion Monitoring
- ToxLab Forms

Overview

The United States Substance Abuse and Mental Health Service Administration (SAMHSA) has released a revision to the Mandatory Guidelines for Federal Workplace Drug Testing Programs.[1] As part of the new guidelines, changes have been made for the confirmation of the cocaine metabolite, benzoylecgonine (BE) in urine. Scheduled to become effective in 2010, SAMHSA has lowered the confirmatory cutoff concentration level for the regulated benzoylecgonine to 100 ng/mL from the previous level of 150 ng/mL.



Chemical Structure of Benzoylecgonine

A forensic toxicology method for the confirmation and quantitation of benzoylecgonine in human urine was developed using the Thermo Scientific ISQ Single Quadrupole GC-MS system. This method adheres to guidelines published by SAMHSA, the College of American Pathologists (CAP), the Society of Forensic Toxicologists (SOFT) and the European Workplace Drug Testing Society (EWDTS).

Methods

All validation samples were prepared as batches using a 2 mL sample size. Standard materials were obtained for calibration, and separate sources of parent benzoylecgonine were used as controls. Deuterated BE-D3 was employed as the internal standard. Batches included a matrix-matched single point calibrator (at 100 ng/mL), quality control samples set to contain BE at 40% and 125% of the calibrator (40 ng/mL and 125 ng/mL respectively), and a negative control, which was blank urine with internal standard only. Thermo Scientific HyperSep Verify-CX solid phase extraction columns (200 mg, 10 mL, P/N: 60108-742) were used for sample extraction. Samples were derivatized with hexafluoroisopropanol (HFIP) and pentafluoropropionic acid (PFPA or PFAA).

The ISQ™ mass spectrometer system was operated in selected ion monitoring mode (SIM), collecting 3 ions for the target compound, and 2 ions for the deuterated internal standard. A Thermo Scientific AS3000 II autosampler and a Thermo Scientific TRACE GC Ultra gas chromatograph, equipped with a split/splitless injection port, provided sample introduction and separation. A 15 m × 0.25 mm ID × 0.25 µm film thickness Thermo Scientific TraceGOLD TG-5MS (P/N: 26098-1300) analytical column was used to enhance separation of the target compound from matrix components (Figures 1 and 2). Thermo Scientific ToxLab Forms 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 demonstrated quantitative results within ± 20% of their expected (theoretical) concentration. Additionally, ion ratio ranges for qualifier ions for target compounds were established using ± 20% of the ratios calculated for the 100 ng/mL calibration standard. These ranges were used to assess ion ratio performance. ToxLab™ Forms 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 quality control amounts was required, and inter-day percent differences of calculated amounts also had to be less than 10%.

Results

- Assay linearity ranged from 10 ng/mL to 12,500 ng/mL for benzoylecgonine (Figure 3)
- Limits of detection and quantitation of 10 ng/mL using a 2 mL sample size
- Intra- and inter-day precision of <10% CV at the quality control levels of 40 ng/mL and 125 ng/mL
- Correlation coefficient (R^2) better than 0.9990 for benzoylecgonine based on a one point calibration

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Chromatography

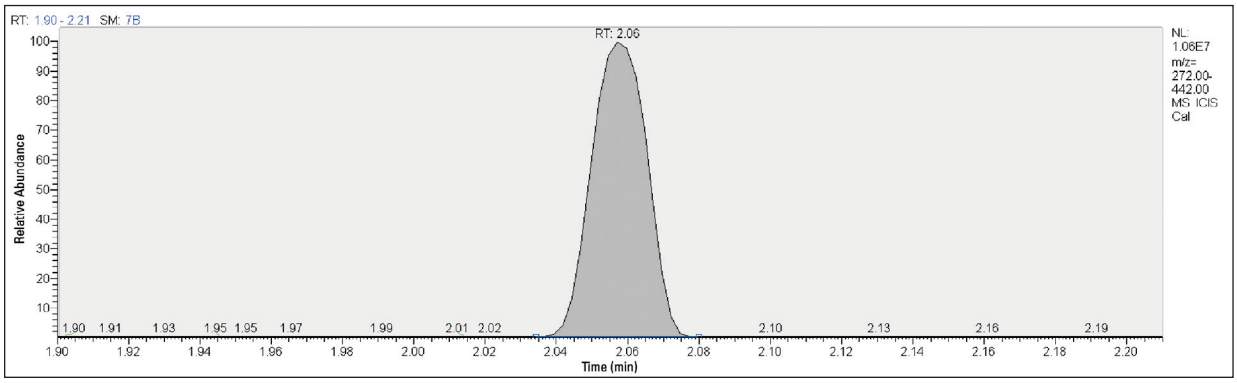

Figure 1: Total ion chromatogram of BE from an extracted urine sample at the cutoff (100 ng/mL)

Extracted Ions

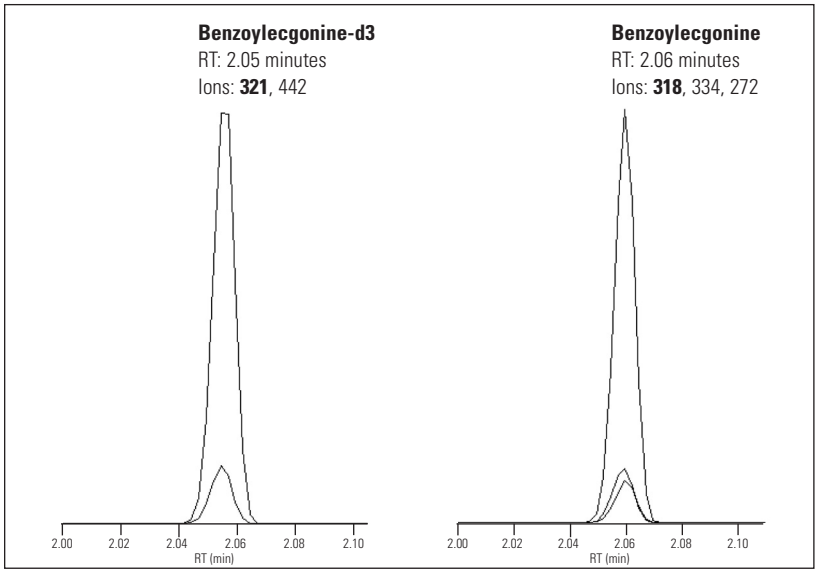

Figure 2: Extracted ion overlays of the BE-d3 and BE at the cutoff (100 ng/mL). Note that no interference is seen from coeluting matrix ions

Linearity Study

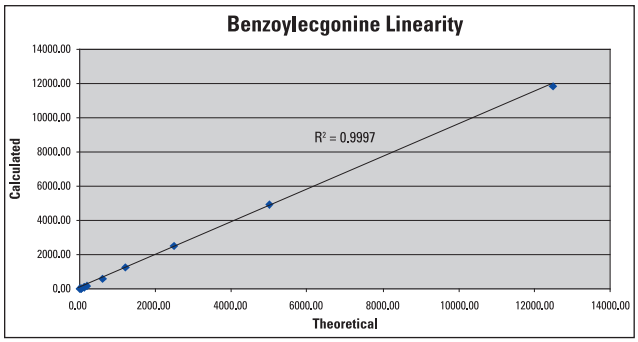

Figure 3: Linearity study results for Benzoylecgonine comparing calculated concentrations to the expected amounts at each level

Conclusions

A method was developed to demonstrate the performance of the ISQ GC-MS system for the confirmation and quantitation of benzoylecgonine in a urine matrix. The assay described offers a broad linearity (10 - 12,500 ng/mL) to cover a wide range of analyte concentrations, thus, reducing the need for dilutions. Excellent precision was also demonstrated around the 100 ng/mL cutoff, with CV measurements of 10% or less over the study. Limits of detection and quantitation at 10 ng/mL ensure sensitive performance for retest and directed assay samples. The methodology described offers a means for a forensic toxicology laboratory to confirm and quantify benzoylecgonine in human urine.

References

1. Mandatory Guidelines for Federal Workplace Drug Testing Programs, Revised Mandatory Guidelines, Fed. Reg. 71857-71907 (Nov. 25, 2008)

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