Application Note: 10160

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

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Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- THC
- 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 Productivity Solution for the confirmation and quantitation of drugs of abuse 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 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

- Assay linearity from 1.5 ng/mL to 1000 ng/mL (Figure 1)
- THCA limit of detection and limit of quantitation of 1.5 ng/mL (Figure 2)
- THCA retention time of 1.73 minutes, and inject-to-inject time of 5.65 minutes (~11 samples per hour)
- Intra-day precision of <2% CV (Coefficient of Variation) at 6 ng/mL, and <2% difference from inter-day extractions
- No interference seen from Δ-9-THC, 11-hydroxy-Δ-9-THC, cannabidiol, Δ-8-THC, ibuprofen, or cannabinol, nor from a list of 24 other drugs
- Easy start-up using pre-developed methods

Methods

All validation studies were prepared in urine using a 3 mL sample size. Standard materials were obtained for calibration and separate sources of THCA were used as controls. THCA-D9 was used as the internal standard. All batches included a matrix-matched single point calibrator (at 15 ng/mL), quality control samples set to contain THCA at 40% and 125% of the calibrator (6 ng/mL and 18.75 ng/mL respectively), along with an unextracted standard and a negative control, which was blank urine with THCA-D9 only. Thermo Scientific HyperSep™ Verify™ CX solid phase extraction columns were used for sample extraction, and sample extracts were derivatized with BSTFA with 1% TMCS. Ethyl acetate brought the final extract volume to 125 µL, and extracts were transferred to autosampler vials for GC/MS analysis.[1]

The DSQ II mass spectrometer was operated in selected ion monitoring mode (SIM), collecting three ions for the THCA target compound, and two ions for THCA-D9. 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

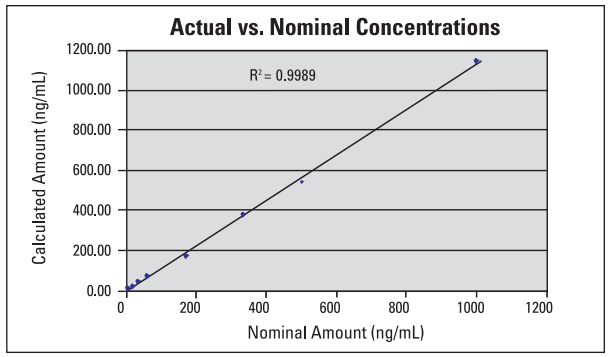

Figure 1: Linearity study results, comparing average concentrations for replicates at 11 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9989 across all 11 levels.

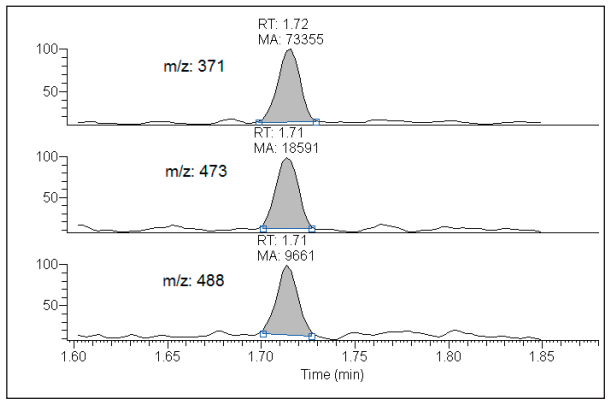

Figure 2: m/z 371, 473, and 488 from the 1.5 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

methodology. A 15 m x 0.2 mm i.d. x 0.33 µm TRACE™ TR-35MS analytical column was used to enhance separation of THCA from matrix components. ToxLab™ 2.0 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 THCA and THCA-D9 were established using ±20% of the ratios calculated for the 15 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% was required for the calculated amounts, 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.[2] The resulting THCA 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 1.5 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 the complete Technical Note for this application, please visit our web site at www.thermo.com/gc and request TN10161.

References

1. *High-Throughput GC/MS Confirmation and Quantitation of a THC Metabolite in Urine Using the DSQ II*. Jason Cole, Matthew Lambing, and Trisa Robarge. Thermo Fisher Scientific Technical Note 10161.
2. *Toxicology Productivity Kits Product Specification Sheet*. Thermo Fisher Scientific.

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