High-Throughput GC/MS Confirmation and Quantitation of Codeine and Morphine 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**
- **Codeine**
- **Morphine**
- **Opiates**
- **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), the Productivity Solution approach provides high-throughput toxicology laboratories a means of simplifying method development and validation. The Productivity Solution was used to perform a complete method validation for the analysis of codeine and morphine in urine that encompassed linearity, carryover, inter- and intra-day precision, and specificity, using extracted, derivatized urine samples.

Results

- Codeine and morphine limits of detection quantitation of 60 ng/mL and 100 ng/mL respectively using a 2 mL sample (Figure 1)
- Inject-to-inject time of 7.73 minutes (~8 samples per hour)
- Assay linearity from 60 ng/mL to 50,000 ng/mL for codeine and 100 ng/mL to 50,000 ng/mL for morphine (Figure 2)
- Intra-day precision of < 1% CV (Coefficient of Variation) at 800, 2000 and 2500 ng/mL for both drugs
- No effect on quantitation shown in interference study for high levels of hydromorphone, hydrocodone, oxycodone, norcodeine, normorphine, thebaine, oxymorphone, methadone and a list of 24 other drugs
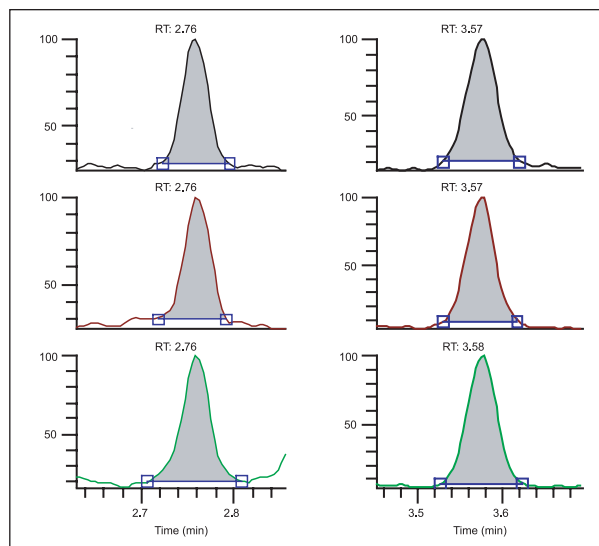- Easy start-up using pre-developed methods

Figure 1: Quant and qual ions for codeine (left) at 60 ng/mL and morphine (right) at 100 ng/mL, showing good chromatography and signal intensity at the limits of detection for this method.
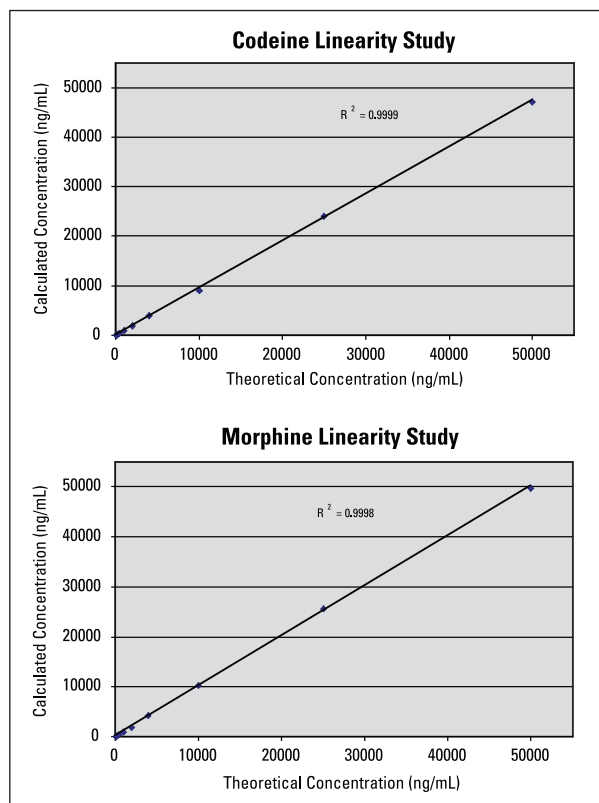

Figure 2: Linearity study results for codeine (top) and morphine (bottom), comparing average concentrations for replicates at each concentration level. A regression analysis gave a correlation coefficient of 0.9999 across 10 levels for codeine (60 to 50,000 ng/mL) and 0.9998 across 9 levels for morphine (100 to 50,000 ng/mL).

Thermo
SCIENTIFIC

Methods

All validation samples were prepared as batches using a 2 mL sample size. Standard materials were obtained for calibration and separate sources of codeine and morphine were used as controls. Codeine-D6 and morphine-D6 were used as the internal standards. Batches included a matrix-matched single point calibrator (at 2000 ng/mL), quality control samples set at 40% and 125% of the calibrator (800 ng/mL and 2500 ng/mL respectively), along with an unextracted standard, a hydrolysis control of morphine-3-glucuronide at 375 ng/mL, and a negative control, which was blank urine with internal standard only. The samples were first hydrolyzed with hydrochloric acid under high heat, before being extracted using solid phase extraction. Samples were then derivatized with acetic anhydride before injection onto the DSQ II.

The DSQ II was operated in selected ion monitoring mode (SIM), collecting 3 ions for 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 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 codeine and morphine were established using ± 20% of the ratios calculated for the 2000 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.[1] The incorporated codeine/morphine 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 60 ng/mL for codeine and 100 ng/mL for morphine provides 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 methods and results, please visit *www.thermo.com* and request TN10178.

Reference

1. *High-Throughput GC/MS Confirmation and Quantitation of Codeine and Morphine in Urine Using the DSQ II.* Jason Cole, Matthew Lambing, and Trisa Robarge. Thermo Fisher Scientific Technical Note # TN10178

Thermo SCIENTIFIC