Application Note: 10162

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

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Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- PCP
- 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 for confirmation of phencyclidine (PCP) in urine that encompassed linearity, carryover, inter- and intra-day precision, and specificity, using extracted urine samples.

Results

- PCP limit of detection and limit of quantitation of 5 ng/mL (2 mL sample size)
- PCP retention time of 1.77 minutes, and inject-to-inject time of 6.9 minutes (~9 samples per hour)
- Assay linearity from 5 ng/mL to 5,000 ng/mL (Figure 1)
- Intra-day precision of <3% CV (Coefficient of Variation) at 10 ng/mL, and <4% difference from inter-day extractions
- No interference seen from venlafaxine, dextromethorphan or diphenhydramine, nor from a list of 24 other drugs
- Easy start-up using pre-developed methods

Methods

All validation samples were prepared as batches using a 2 mL sample size. Standard materials were obtained for calibration, and separate sources of PCP were used as controls. PCP-D5 was used as the internal standard. Batches included a matrix-matched single point calibrator (at 25 ng/mL), quality control samples set to contain PCP at 40% and 125% of the calibrator (10 ng/mL and 31.25 ng/mL respectively), along with an unextracted standard and a negative control, which was blank urine

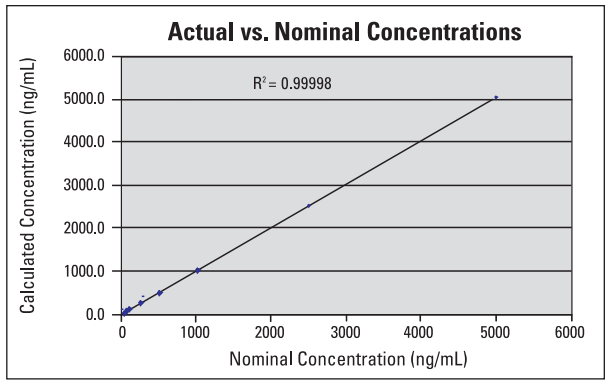

Figure 1: Linearity study results, comparing average concentrations for replicates at 9 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.99998 across all 9 levels.

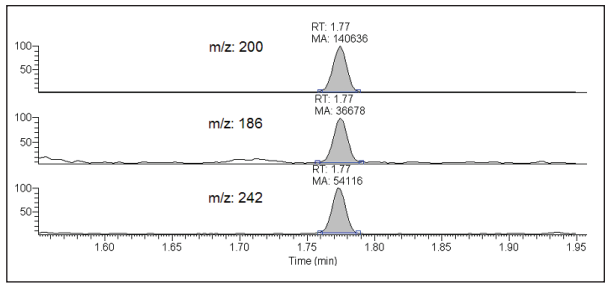

Figure 2: m/z 200, 186, and 242 from the 5 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

with PCP-D5 only. Thermo Scientific HyperSep™ Verify™ CX solid phase extraction columns were used for sample extraction, and sample extracts were analyzed without derivatization. Ethyl acetate was used to bring the final extract volume to 100 µL.[1]

The DSQ II system was operated in selected ion monitoring mode (SIM), collecting 3 ions for the PCP target compound, and 2 ions for PCP-D5. 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-5MS analytical column was used to enhance separation of PCP from matrix components. ToxLab™ 2.0 software automated the acquisition and processing of all data, including quantification and ion ratio confirmation calculations.

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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 PCP and PCP-D5 were established using ±20% of the ratios calculated for the 25 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.[2] The resulting PCP assay has broad linearity to cover a wide range of analyte concentrations, with excellent specificity and precision throughout the concentration range (Figure 2). Limits of detection and quantitation at 5.0 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 TN10163.

References

1. *High-Throughput GC/MS Confirmation and Quantitation of Phencyclidine (PCP) in Urine Using the DSQ II*. Jason Cole, Matthew Lambing, and Trisa Robarge. Thermo Fisher Scientific Technical Note # TN10163.
2. *Toxicology Productivity Kits Product Specification Sheet*. Thermo Fisher Scientific.

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