Application Note: 52098

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

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Key Words

- ISQ Single Quadrupole GC-MS
- ToxLab Forms
- Forensic Toxicology
- Opiate
- Selected Ion Monitoring

Overview

Opioids compose a chemical class of both naturally occurring compounds produced from the opium poppy plant (*Papaver somniferum*) and similar synthetic analogues. Substances within this class are commonly recognized as potential drugs of abuse.[1] Biological matrices containing heroin (*diacetyl morphine*), codeine, morphine, hydrocodone, hydromorphone, oxycodone and oxymorphone and thebaine are regularly monitored by drugs of abuse analysis laboratories. Structural similarities of opiate metabolites introduce challenges to the accurate separation, confirmation and quantitation of these substances.[2]

A forensic toxicology method for the confirmation and quantitation of codeine, morphine, hydrocodone, hydromorphone, oxycodone and oxymorphone in human urine was developed using the Thermo Scientific ISQ single quadrupole GC-MS system. Multiple analytical procedures can be replaced by this method, which combines a dual derivatization technique with the selection of the correct analytical column.

Methods

All samples were prepared as batches using a 2 mL sample size. Standard materials were obtained for calibration, and separate sources of the opiates were used as controls. Deuterated internal standards were employed. Batches included a matrix-matched single point calibrator (300 ng/mL), quality control samples set to contain each target compound at 40% and 125% of the calibrator (120 ng/mL and 375 ng/mL respectively), and a negative control, which was blank urine with internal standard only.

A hydrolysis step was performed to release the bound drug from the glucuronide. Samples underwent a first derivitization with hydroxylamine to convert the keto moieties to their oxime derivatives. Thermo Scientific HyperSep Verify-CX solid-phase extraction columns (200 mg, 10 mL) were used for sample extraction. Sample extracts were derivatized a second time with Thermo Scientific BSTFA and 1% TMCS.

A Thermo Scientific AS 3000 II autosampler and a Thermo Scientific TRACE GC Ultra gas chromatograph, equipped with a split/splitless injection port, provided sample introduction into the ISQ mass spectrometer. A 15 m × 0.25 mm I.D. × 0.25 µm Thermo Scientific TraceGOLD TG-1MS analytical column was used to enhance separation of the target opioid class compounds from each other and from matrix components (Figure 1). The ISQ™ mass spectrometer system was operated in selected ion monitoring mode (SIM), collecting three ions for each target compound, and two ions for each deuterated internal standard (Table 1 and Figure 2). Thermo Scientific ToxLab Forms software provided automated acquisition and processing of all data, including quantitation and ion ratio confirmation calculations. The method was assessed for specificity, linearity, precision, recovery and interference.

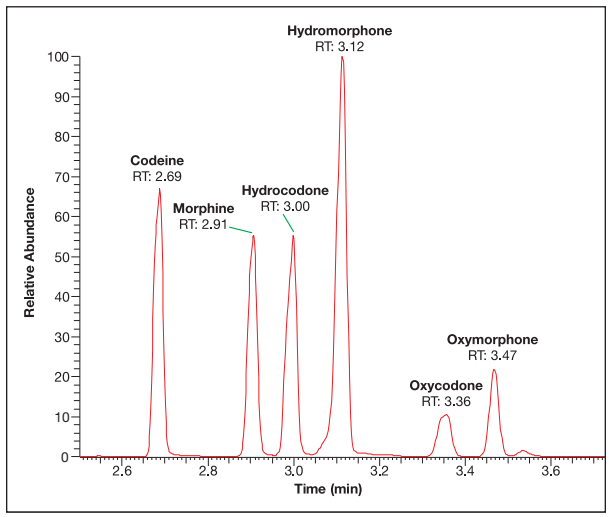

Figure 1: Total ion chromatogram of the six opiate compounds from an extracted urine sample at the cutoff (300 ng/mL)

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Analyte	Retention Time (min)	Quan Ion *(m/z)*	Qual Ion(s) *(m/z)*
Codeine-D6	2.67	377	349
Codeine	2.69	371	372, 313
Morphine-D6	2.90	435	435
Morphine	2.91	429	414, 401
Hydrocodone-D6	2.98	392	377
Hydrocodone	3.00	386	371, 297
Hydromorphone-D6	3.10	450	435
Hydromorphone	3.12	444	429, 355
Oxycodone-D6	3.34	480	465
Oxycodone	3.36	474	459, 475
Oxymorphone-D3	3.46	535	520
Oxymorphone	3.47	532	533, 517

Table 1: Retention times and ions monitored for the opioid analytes and their deuterated internal standards

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 300 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 were required for each analyte.

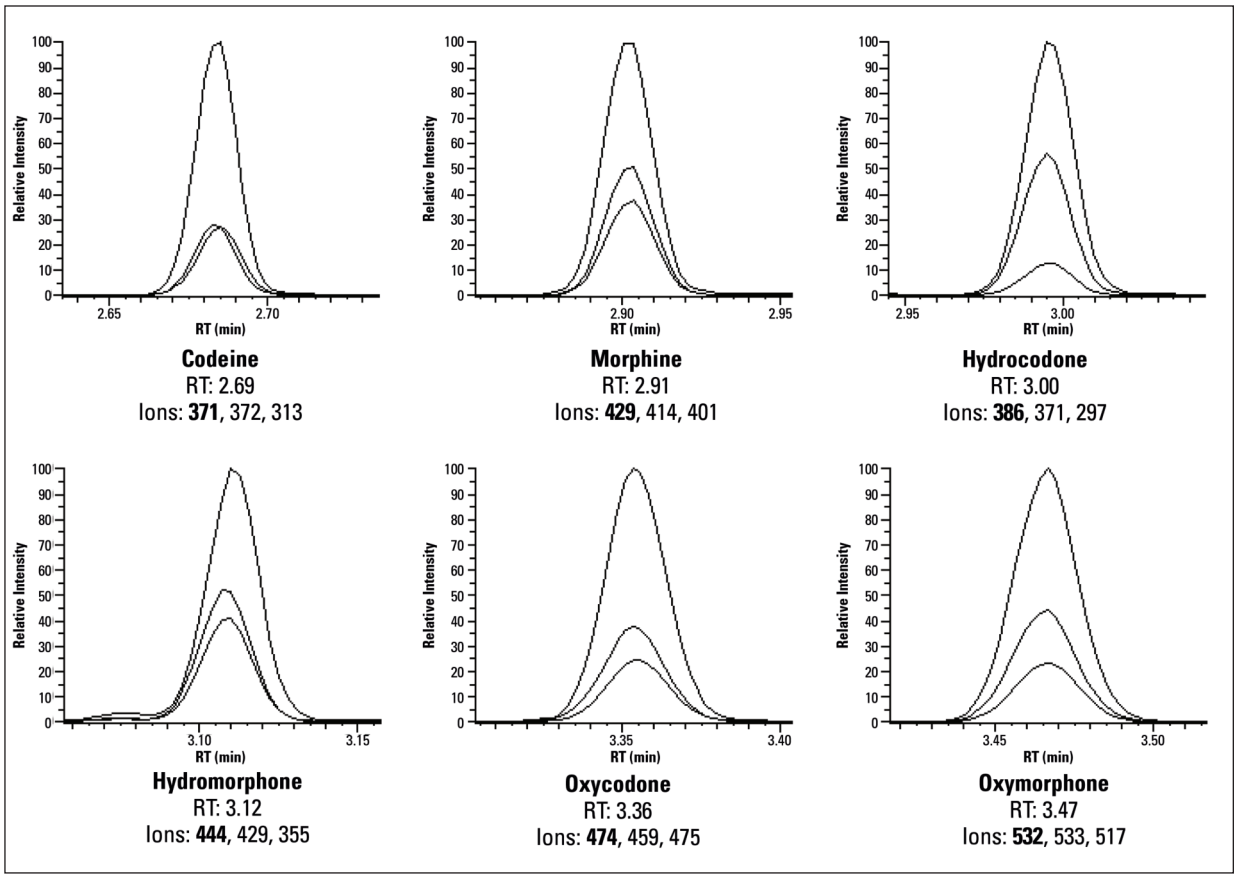

Figure 2: Extracted ion overlays of the six opiate compounds at the cutoff (300 ng/mL). Note that no interference is seen from coeluting matrix ions.

Results

- The dual derivatization technique led to the successful separation of all six opiate compounds (Figure 1).
- Optimized instrument parameters and the selection of the 1 phase column versus a 5 phase analytical column eliminates compound coelution and interference (Figure 3).
- Linearity ranged between 30–5000 ng/mL for codeine, morphine, hydrocodone, hydromorphone, oxycodone and 30–1200 ng/mL for oxymorphone (Figure 4). This supports both directed assay and retest samples while also reducing the need to re-extract concentrated samples.
- Correlation coefficients (R^2) better than 0.9960 for all analytes based on a one point calibration further demonstrates sample concentration range capability.
- Intra and inter-batch precision of <5% CV (Coefficients of Variation) at 120 ng/mL and 375 ng/mL ensure confidence in the measured result and achieve reliable results day in and day out.
- Limit of quantitation at 30 ng/mL using a 2 mL sample size allows the analyst to achieve required detection limits with limited sample volume.

Codeine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch CV
120 ng/mL	1.5%	1.8%	1.7%
375 ng/mL	2.7%	3.1%	2.9%
Morphine Concentration	**CV for Batch 1**	**CV for Batch 2**	**Inter-batch CV**
120 ng/mL	4.3%	2.4%	3.9%
375 ng/mL	2.5%	2.5%	3.9%
Hydrocodone Concentration	**CV for Batch 1**	**CV for Batch 2**	**Inter-batch CV**
120 ng/mL	1.5%	2.4%	2.2%
375 ng/mL	2.1%	3.3%	3.0%
Hydromorphone Concentration	**CV for Batch 1**	**CV for Batch 2**	**Inter-batch CV**
120 ng/mL	1.4%	3.1%	3.5%
375 ng/mL	2.5%	2.8%	3.8%
Oxycodone Concentration	**CV for Batch 1**	**CV for Batch 2**	**Inter-batch CV**
120 ng/mL	1.7%	2.8%	3.4%
375 ng/mL	2.6%	3.3%	4.3%
Oxymorphone Concentration	**CV for Batch 1**	**CV for Batch 2**	**Inter-batch CV**
120 ng/mL	1.5%	2.1%	2.4%
375 ng/mL	1.8%	2.2%	2.5%

Table 2: Results of the precision study for 120 ng/mL and 375 ng/mL quality control samples.
Note: Individual quantitative values from each batch combined for calculation of inter-batch CV.

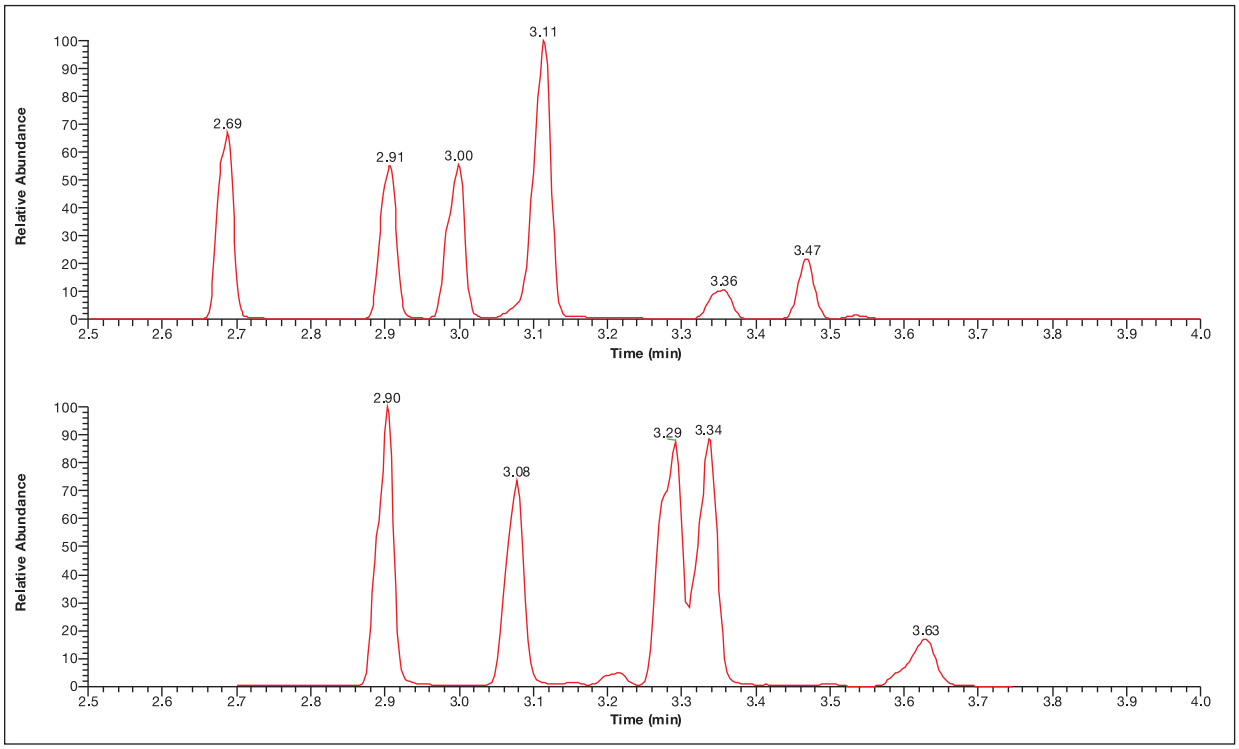

Figure 3: Comparison of chromatography using a TG-1MS (top) to a TG-5MS (bottom) analytical column

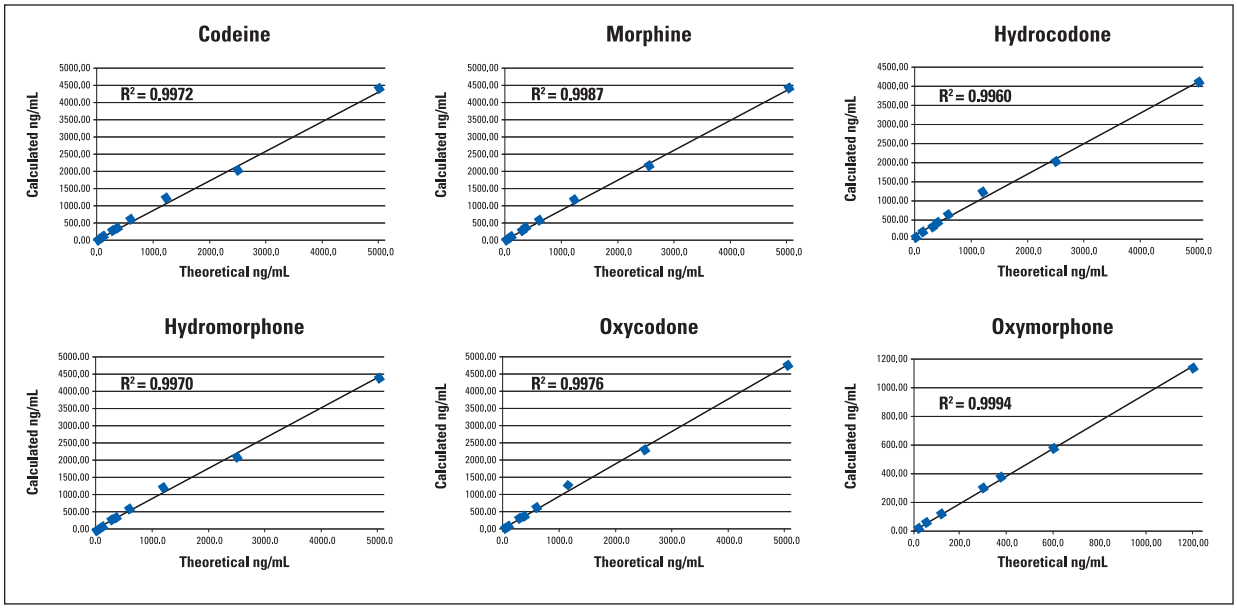

Figure 4: Linearity study results for six opiates comparing calculated concentrations to the expected amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9960 or higher for each analyte.

Conclusion

A method was developed to demonstrate the performance of the ISQ GC-MS system for the confirmation and quantitation of six opiate compounds in a urine matrix. The chromatographic performance of the TraceGOLD™ TG-1MS analytical column with the optimized parameters of the ISQ GC/MS provided fast run times without coelution interference, ensuring accurate confirmation and quantitation. The assay described offers broad linearity to cover a wide range of analyte concentrations, with R^2 values of 0.9960 or higher for all analytes. The coefficient of variation for intra- and inter-batch precision was shown to be less than 5% for 120 ng/mL and 375 ng/mL concentrations, demonstrating excellent precision. Limits of detection and quantitation at 30 ng/mL ensure sensitive performance for retest and directed assay samples. With the final analyte, oxymorphone, eluting at a retention time of less than five minutes, the methodology offers a productive means for confirming the six-opiate panel. Laboratories save time and money by using a single extraction, single injection and a fast analysis time, while achieving results that are quantitatively accurate and precise across a broad concentration range.

References

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2. Ropero-Miller, J.D.; Lambing, M.K.; Winecker, R.E. Simultaneous Quantification of Opiates in Blood by GC-EI-MS Analysis Following Deproteination, Detautomerization of Keto Analytes, Solid Phase Extraction and Trimethylsilyl Derivatization. *J. Anal. Toxicol.* 2002, 26, 524–528.

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