

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, TX, USA

Abstract

Codeine and morphine are drugs derived from opium which are listed in the United States as Schedule II narcotic substances because they are considered highly addictive and have a high potential for abuse; however, both also have legitimate uses by prescription for physical ailments. Codeine is often prescribed for cough, diarrhea or mild pain relief, while morphine's main uses include relief of more severe pain and as an anesthetic before and after surgery. Because of the high potential for abuse, the illegitimate use of codeine and morphine is closely monitored in the United State and elsewhere.

For these compounds, workplace drug testing laboratories analyzing urine will most often quantitate parent drugs after the hydrolysis of the glucuronide conjugated metabolites. The methodology described here details the confirmation and quantitation of parent codeine and morphine (Figure 1) in urine using the DSQ™ II GC/MS system.

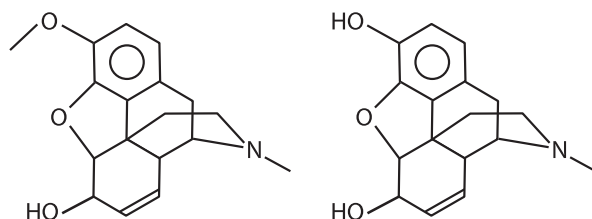


Figure 1: Chemical structures of codeine (left) and morphine (right)

For this assay, a 2 mL urine sample size was used, with codeine-D6 and morphine-D6 used as deuterated internal standards. Samples were extracted using solid phase extraction, followed by derivatization with acetic anhydride in pyridine. The final reaction products were analyzed using a DSQ II single stage quadrupole GC/MS system. For both drugs, a calibrator at 2000 ng/mL was used for single point calibration. The resulting method demonstrated excellent precision, no interference for a number of tested compounds, and provided linearity from 60 to 50,000 ng/mL for codeine and 100 to 50,000 ng/mL for morphine, with limits of detection and quantitation of 60 ng/mL for codeine and 100 ng/mL for morphine.

Introduction

The presence of codeine and/or morphine in a urine sample identifies the donor as one who has been exposed to some form of these drugs. To improve the chromatographic performance of the analysis, these compounds are typically derivatized. A derivative can also be chosen to eliminate interference from other compounds which share common ions and coelute with codeine or morphine. The procedure described here includes a derivatization step with acetic anhydride in pyridine, which gives good chromatographic peak shapes and no coelution with the potential interferents tested. Combined with solid phase extraction, this procedure gave a clean background free from interference.

Another important step in preparing these analytes for confirmation is the hydrolysis of glucuronide conjugates to free drugs. This step is critical because approximately 90% of morphine is excreted in urine as its conjugated metabolites, and codeine can be present in urine as its conjugated form at concentrations over five times that of unconjugated codeine.¹ The two most common approaches to this hydrolysis are high temperature acid hydrolysis and enzyme hydrolysis. Acid hydrolysis was chosen for this method for a number of reasons. First, the acid hydrolysis step was performed successfully in one hour, whereas enzyme hydrolysis with β -glucuronidase typically takes hours or overnight, making enzymatic sample hydrolysis a time-consuming process. In addition, using a commonly-produced acid like hydrogen chloride rather than an enzyme reduces the possibility of significant variation in performance across manufacturers and lot numbers. Finally, a recent study raised questions about the efficiency of β -glucuronidase in the conversion of the glucuronide conjugates, particularly morphine-6-glucuronide and codeine-6-glucuronide.² In this study, recoveries from patient samples hydrolyzed with the enzyme from *Patella vulgata* gave an average response relative to acid hydrolysis of 21% for codeine and 64% for morphine. It should be noted that acid hydrolysis converts heroin and 6-acetylmorphine to morphine, so an additional, separate test for 6-acetylmorphine, a metabolite of heroin, must be conducted to determine whether the morphine present was from the use of heroin.³ A reflex test for 6-acetylmorphine is typically performed in cases of positive morphine results, since morphine is the major urinary metabolite of heroin.¹

Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- Codeine
- Morphine
- Opiates
- Urine Drug Testing

Another important decision in developing this method is the choice of internal standards. Both codeine and morphine produce many minor ions that can interfere at high relative concentrations of internal standard to analyte or vice versa. This can significantly limit the effective dynamic range of the assay. During method development, both BSTFA and acetic anhydride were tried in conjunction with the D-3 and D-6 deuterated analogs. The D-6 analogs were chosen in combination with the acetic anhydride derivative because these ions resulted in minimal interference between analyte and internal standard.

The DSQ II, a single stage quadrupole mass spectrometer with a curved prefilter that minimizes background noise derived from excited neutrals, was used for this analysis. Coupled to a TRACE GC Ultra™ gas chromatograph and an AS3000 autosampler, this GC/MS system represents the industry standard for confirmatory analyses of drug use. ToxLab™ 2.0 software provided automated sample analysis and quantitation, and the method was fully validated, including assessments of precision, interference, and linearity. This method describes the GC/MS confirmation and quantitation of codeine and morphine in urine, and it does not include other matrices or any drugs other than codeine and morphine.

Methods

To provide a comprehensive view of method development and validation, methods for sample preparation, acquisition, and analysis are described in detail below. Sample preparation plays a critical role in method validation since many certifying bodies recommend or require method validation performed in matrix.

Sample Preparation

Known negative urine was collected and used for sample preparation. A sample size of 2 mL was selected. Calibrators, quality controls, and linearity samples were spiked with appropriate amounts of codeine and morphine (Cerilliant, Round Rock, TX). Single point calibration at 2000 ng/mL was used for calculation of all quantitative amounts. A commercial control (Medical Analysis Systems, Level G3, Fremont, CA) containing 375 ng/mL of morphine-6-glucuronide was used as the hydrolysis control for the

batch. Also, a 40% (800 ng/mL) and a 125% (2500 ng/mL) control were prepared from stock material from an alternate source (Alltech Associates, Deerfield, IL). All batches contained an unextracted standard, the calibrator at 2000 ng/mL, a negative control, a 40% control, a 125% control and a hydrolysis control. Codeine-D6 and morphine-D6 (Cerilliant) were used as deuterated internal standards, and were added to each sample at a final concentration of 2000 ng/mL. An unextracted standard was prepared by adding 100 µL of 40 µg/mL mixed codeine and morphine standard solution and 100 µL of 40 µg/mL of mixed internal standard solution to a labeled tube, yielding the equivalent of a 2000 ng/mL sample, with the internal standards at 2000 ng/mL. The unextracted standard is used to prep the GC/MS system, and to demonstrate ion ratios and extraction recovery. The unextracted standard is not subjected to the extraction steps but instead proceeds directly to the dry-down step, at which point it rejoins the rest of the samples for derivatization and analysis.

For hydrolysis, 400 µL of hydrochloric acid (HCl) were added to each sample. The samples were then transferred to a heating block set at 120 °C for one hour to accelerate hydrolysis. The vials were then removed from the heating block and allowed to cool to room temperature. 3 mL of 0.5 M, pH 6 phosphate buffer were added, followed by the addition of 400 µL of ammonium hydroxide (NH₄OH) to neutralize the acid. The samples were then adjusted to pH 6.5 ± 0.5 by drop-wise addition of NH₄OH or HCl.

Labeled solid phase extraction columns were loaded onto an extraction manifold. Columns were conditioned with 3 mL of methanol, followed by 3 mL of deionized (DI) water, and finally 3 mL of 0.1 M, pH 6 phosphate buffer. Next, the hydrolyzed, pH-adjusted samples were applied to the columns under low vacuum. The columns were then rinsed sequentially with 3 mL of DI water, 3 mL of 0.1 M, pH 4.5 acetate buffer, and 3 mL of methanol. The columns were dried under high vacuum (> 10 in. Hg) for ≥ 5 minutes. Culture tubes labeled with the appropriate sample ID were then placed under the columns, and the analytes were eluted with 3 mL of a methylene chloride: isopropanol: ammonium hydroxide (78:20:2 by volume) solution.

Sample Preparation	Extraction	Derivatization
1. Label 13 x 125 mm screw top culture tubes	1. Condition SPE columns sequentially with	1. Blow down samples under N ₂ stream
2. Add 100 µL of working internal standard to each tube	a. 3 mL methanol	2. Add 100 µL of acetic anhydride:pyridine (50:50)
3. Add 2 mL of blank urine, QC or donor specimen	b. 3 mL DI water	3. Cap vials and vortex
4. Spike calibrator and alternate source QC's	c. 1 mL 0.1 M, pH 6 phosphate buffer	4. incubate at 60 °C for 20 minutes
5. Add 400 µL of HCL	2. Apply samples at low vacuum	5. Remove from heat and let cool
6. Cap and mix gently	3. Rinse SPE columns sequentially with	6. Blow down access reagent under N ₂
7. Incubate at 120 °C for one hour	a. 3 mL DI water	7. Reconstitute with 150 µL ethyl acetate
8. Allow to cool to room temperature	b. 3 mL 0.1 M acetate buffer, pH 4.5	8. Transfer samples to labeled autosampler vials
a. Add 3mL 0.5 M, pH 6 phosphate buffer	c. 3 mL methanol	9. Move vials to autosampler tray for GC/MS analysis
b. Add 400 µL of NH ₄ OH	4. Dry columns under high vacuum for 5 minutes	
9. Adjust sample pH to 6.5 ± 0.5 by drop-wise addition of NH ₄ OH or HCl	5. Elute with 3 mL methylene chloride: isopropanol:ammonium hydroxide (78:20:2)	

Table 1: Sample Prep, Extraction and Derivatization Summary

The extracts were evaporated under nitrogen at 40 °C until dry, taking care not to overdry the samples. Next, 100 µL of a solution of acetic anhydride in pyridine (50:50 by volume) were added to each sample vial, which were vortexed and placed into a heating block at 60 °C for 20 minutes for derivatization. Following derivatization, the samples were brought to room temperature and then evaporated under nitrogen to remove excess derivatization reagent. The resulting residues were reconstituted in 150 µL of ethyl acetate, vortexed and transferred to labeled autosampler vials, which were then capped and loaded onto the AS 3000 autosampler for GC/MS analysis. Table 1 summarizes these sample prep, extraction, and derivatization steps.

Instrumental Analysis

The DSQ II mass spectrometer used for this analysis was configured with a 250 L/s turbomolecular pump, and the TRACE GC Ultra was equipped with a standard split/splitless injector. A 5 mm i.d. deactivated glass liner was used in the injector (Thermo Scientific, P/N 45350033), and a plug of Siltek™ glass wool (Restek, Bellefonte, PA) was placed approximately 40 mm from the top of the liner. The split/splitless injector temperature was set to 250 °C. A 1 µL injection volume was programmed on the AS 3000 autosampler, and a 15:1 split injection was used. The analytical column was a

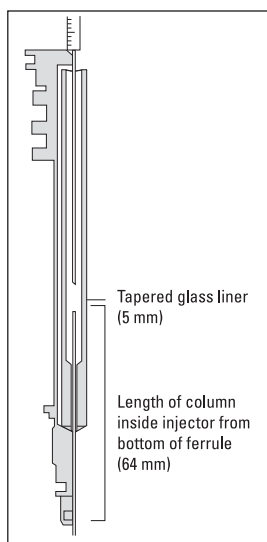


Figure 2: Column installation in GC split/splitless injection port (not to scale)

TRACE™ TR-5MS 15 m x 0.25 mm i.d. x 0.25 µm thickness film (Thermo Scientific, PN 260F130P), which was installed 64 mm into the injection port (Figure 2).

The carrier gas flow rate was set to 2.5 mL/min of helium. The initial temperature on the TRACE GC Ultra was set to 190 °C. The high temperature at the beginning of the analytical run allows the analytes to elute from the column as quickly as possible. Due to the high boiling point of derivatized codeine and morphine, it is not necessary to use the solvent to recondense the sample at the head of the column. Upon injection, the GC temperature was immediately ramped at 40 °C/min to a temperature of 240 °C, with a 2.56 minute hold time. The temperature was then ramped at 40 °C/min to a final temperature of 285 °C and held for 0.3 min, to allow heavy matrix compounds time to elute. The total run time is 5.18 minutes, with codeine and morphine retention times of 2.76 and 3.75 minutes respectively.

The DSQ II source temperature was set to 300 °C, and the mass spectrometer was tuned using default *AutoTune* parameters. These tune settings were used for acquisition, with a detector gain of 3×10^5 and an emission current of 120 µA set in the method. For initial mass spectrometer method development, high concentrations of derivatized analytes and their internal standards were injected and analyzed in electron ionization (EI) full scan to determine masses for EI selected ion monitoring (SIM). The set of SIM masses and dwell times used are shown in Table 2. m/z 341 was used as the quantitation mass for codeine, and m/z 347 was the quantitation mass for its internal standard. For morphine, m/z 327 was used as the quantitation ion and m/z 333 for the internal standard quantitation ion. The narrow SIM width used enhances sensitivity and builds on the mass stability and resolution of the DSQ II, while a short dwell time provides quantitative precision across the GC peaks. Table 2 summarizes instrument parameters for the validated method.

DSQ II			TRACE GC Ultra		AS3000	
Source Temp (°C):	300		Oven Method		Sample Volume (µL):	1
Emission Current:	120 µA		Initial Temp (°C):	190	Viscous Sample:	Yes
Detector Gain:	3×10^5		Initial Time (min):	0.0	Sampling Depth in Vial:	Bottom
Codeine Start Time (min):	2.5		Rate (°C/min):	40	Injection Depth:	Standard
	<i>m/z</i>	<i>Dwell (ms)</i>	Hold Temp (°C):	240	Pre-Inj Dwell Time (sec):	0
Codeine SIM Masses:	341.0	15	Hold Time (min):	2.5	Post-Inj Dwell Time (sec):	0
	282.0	15	Final Temp (°C):	285	Sample Rinses:	0
	298.0	100	Final Hold Time (min):	0.3	Plunger Strokes:	5
Codeine-D6 SIM Mass:	347.0	15	SSL Method		Pre-Injection Solvent Rinses:	0
	288.0	15	Temp (°C):	250	Pre-Inj Solvent Rinses:	
Morphine Start Time (min):	3.3		Mode:	Split	Solvent A (50:50 EtOAc:MeCl ₂):	1
	<i>m/z</i>	<i>Dwell (ms)</i>	Split Ratio:	15:1	Solvent B (50:50 EtOAc:MeCl ₂):	1
Morphine SIM Masses:	327.0	20	Split Flow:	38	Post-Inj Solvent Rinses:	
	369.0	20	Constant Septum Purge:	on	Solvent A (50:50 EtOAc:MeCl ₂):	4
	310.0	20	Carrier Flow (mL/min):	2.5	Solvent B (50:50 EtOAc:MeCl ₂):	4
Morphine-D6 SIM Masses:	333.0	20	Gas Saver:	off		
	375.0	20	Vacuum Compensation:	on		
Acquisition End Time (min):	3.7		Transferline Temp (°C):	280		
Width (amu):	0.5					

Table 2: Instrument method summary for the SIM analysis of codeine and morphine on the DSQ II

Sample Processing and Result Derivation

For sample acquisition, peak detection and quantitation, ToxLab 2.0 software was utilized. By incorporating all of the vital components of analyses into a unified workflow-oriented application, ToxLab 2.0 provides an integrated solution to codeine and morphine GC/MS confirmation. To make use of ToxLab 2.0 for method validation, an instrument method was created for the mass spectrometer, autosampler, and GC. A processing method for component identification and quantitation was developed. In ToxLab 2.0, these methods were integrated into a single master method, which also allows the user to establish criteria specific to the method. Batch creation was performed through the *Batch Wizard* function of ToxLab 2.0, which greatly simplified and streamlined sample entry, particularly for the longer validation batches (Figure 3). This highlights the applicability of this software to routine analysis of toxicological samples.⁴

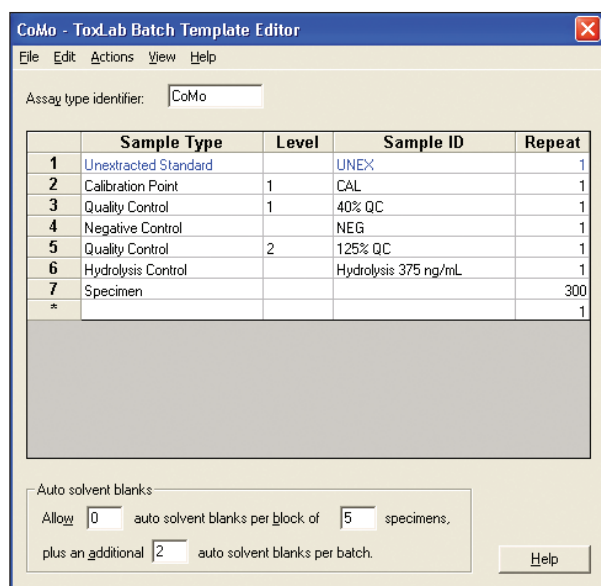


Figure 3: ToxLab 2.0 Batch Template Editor, showing framework for codeine and morphine batches

Concentration calculations were based on a single point calibrator at 2000 ng/mL. Calibration curves consisted of a line including the origin and the calibrator, and calculated amounts were based on this curve. All validation batches had to conform to quality control (QC) criteria, including quantitative and qualitative bounds checking.

Quantitative criteria for the batch included acceptable quantitation ranges for all samples in each batch. All calculated amounts for QC samples and study samples had to fall within $\pm 20\%$ of the expected concentration in order to accept the sample. Failure of a QC sample within a batch would mean the entire batch would need to be repeated. In addition to this quantitative window, negative controls were evaluated based on two additional criteria. One means of assessing a negative control is a quantitative value for less than the method limit of detection (LOD), which in this case is 60 ng/mL for codeine and 100 ng/mL for morphine. An alternate criterion for negative controls

is that the calculated amount must be less than a pre-determined percentage of the method cutoff. For this method, a level of 5% of the cutoff (100 ng/mL) was used as a second criterion, and all negative controls were evaluated for compliance to both criteria.

Qualitative criteria included ion ratio and retention time target ranges based on the calibrator, along with peak shape considerations. These criteria were applied to all sample types. Ion ratio ranges for the batch were developed based on the appropriate ratios from the 2000 ng/mL calibrator. Ratios were defined as follows:

$$\text{ion ratio} = \frac{\text{area of qual ion}}{\text{area of quant ion}} \times 100\%$$

Ratios were calculated for codeine (282:341 and 298:341) and codeine-D6 (288:347), as well as morphine (369:327 and 310:327) and morphine-D6 (375:333), and for each ratio, an acceptable range of $\pm 20\%$ was established. Similarly, the target retention time for codeine, morphine and their internal standards was set using a $\pm 2\%$ retention time window based on the calibrator retention time. Peak symmetry requirements required the peaks to be $> 50\%$ symmetrical at 50% peak height.

Each validation batch was reviewed for compliance with these criteria, and for a study batch to be accepted, it had to comply with all of these QC criteria.

Results

The analysis of codeine and morphine in urine using the DSQ II GC/MS system was thoroughly validated through determination of linear range, carryover, precision, and specificity. Four separate batches were prepared and analyzed: one for linearity/carryover, one for specificity, and two for precision. Each batch included the appropriate quality controls and calibration standards, along with validation samples prepared according to Table 3. Batch acceptability was determined by applying the QC standards described above. Carryover was assessed during the course of the linearity study. Precision analyses were performed on two separate batches analyzed on two separate days, while specificity assessed potential interference from a number of compounds. The DSQ II demonstrated excellent intra- and inter-day precision, and linearity from 60 to 50,000 ng/mL for codeine, and 100 to 50,000 ng/mL for morphine with no significant carryover even following the 50,000 ng/mL sample. Also, all samples passed the relevant QC criteria. With 7.73 minute inject-to-inject times, the method also provides a productive means of performing this confirmation.

Linearity

1. Unextracted (2000 ng/mL)
2. Calibrator (2000 ng/mL)
3. 40% Control (Alltech)
4. Negative
5. 125% Control (Alltech)
6. 375 ng/mL Hydrolysis (MAS)
7. 60 ng/mL x 7
8. 100 ng/mL x 7
9. 200 ng/mL x 7
10. 400 ng/mL x 7
11. 1000 ng/mL x 7
12. 2000 ng/mL x 7
13. 4000 ng/mL x 7
14. 10,000 ng/mL x 7
15. 25,000 ng/mL x 7
16. 50,000 ng/mL x 7

Precision

Batch 1

1. Unextracted (2000 ng/mL)
2. Calibrator (2000 ng/mL)
3. 40% Control (Alltech)
4. Negative
5. 125% Control (Alltech)
6. 375 ng/mL Hydrolysis (MAS)
7. 800 ng/mL x 7
8. 2000 ng/mL x 7
9. 2500 ng/mL x 7

Batch 2

1. Unextracted (2000 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (2000 ng/mL)
4. Negative
5. 125% Control (Alltech)
6. 375 ng/mL Hydrolysis (MAS)
7. 800 ng/mL x 7
8. 2000 ng/mL x 7
9. 2500 ng/mL x 7

Interference

1. Unextracted (2000 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (2000 ng/mL)
4. Negative
5. 125% Control (Alltech)
6. 375 ng/mL Hydrolysis (MAS)
7. Negative w/ Interference #1
8. 800 ng/mL w/ Interference #1
9. 2500 ng/mL w/ Interference #1
10. Negative w/ Interference #2
11. 800 ng/mL w/ Interference #2
12. 2500 ng/mL w/ Interference #2
13. Repeat for remaining interferents

Table 3: Validation study sample preparation guide for codeine and morphine confirmation in urine

Linear Range Determination

The determination of assay linearity was performed at concentrations across a broad dynamic range. The linearity batch, as with every validation batch, included an unextracted standard, a negative control (blank urine and internal standard), the 500 ng/mL calibrator, a 40% control sample (800 ng/mL) and a 125% commercial control sample (2500 ng/mL). To evaluate method linearity, samples at 60, 100, 200, 400, 1000, 2000, 4000, 10000, 25000 and 50,000 ng/mL were prepared and extracted, along with the calibrator and controls. These samples were then injected 7 times each, and the resulting 70 data points were quantified based on the 2000 ng/mL calibrator. All 70 quantitative values were within $\pm 20\%$ of their target concentrations for both drugs. At 60 ng/mL, morphine was not accepted due to peak shape criteria, although it passed QC criteria for quantitation accuracy and ion ratios. The calculated amount of codeine at its 60 ng/mL LOQ/LOD was 62.2 ng/mL with a coefficient of variation (CV) of 3.8%. For morphine at its 100 ng/mL LOQ/LOD, the average calculated amount was 88.8 ng/mL, with a CV of 1.8%. Chromatography for the quantitation ion and all qualifiers was good at the LOD, as shown in Figure 5.

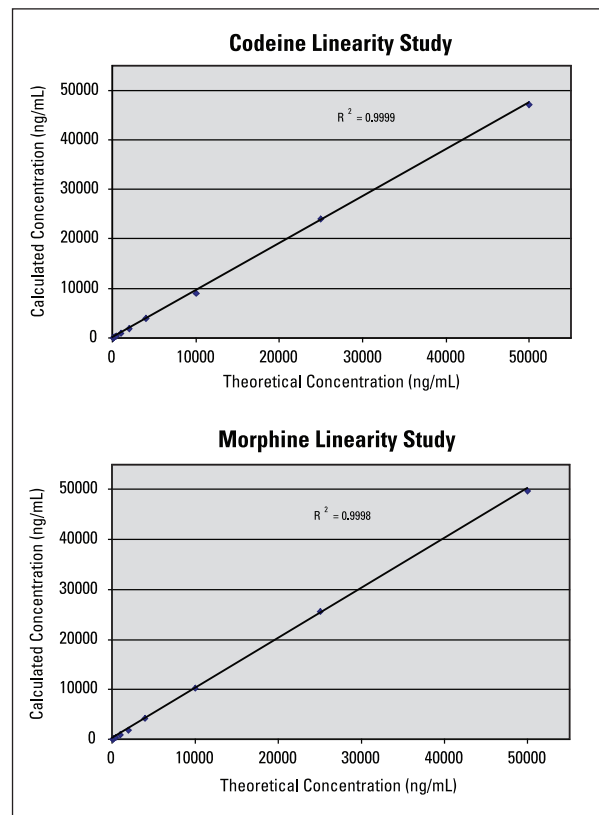


Figure 4: Linearity study results for codeine (top) and morphine (bottom), comparing average concentrations for replicates at each concentration level. The regression analysis for this study gave a correlation coefficient of 0.9999 across 10 levels for codeine (60 to 50,000 ng/mL) and 0.9998 across 9 levels for morphine (100 to 50,000 ng/mL).

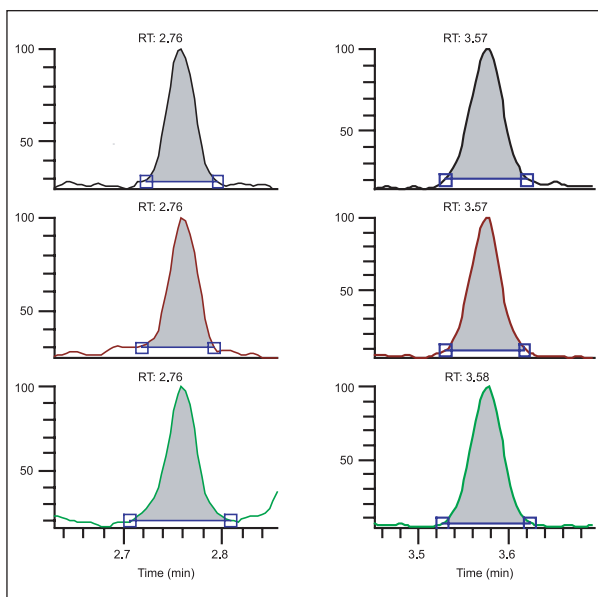


Figure 5: Quant and qual ions for codeine (left) at 60 ng/mL and morphine (right) at 100 ng/mL, showing good chromatography and signal intensity at the limits of detection for this method

An additional component of the linearity study included a determination of the carryover limit for the method. To do so, a negative control was injected following each set of linearity samples starting with the 2000 ng/mL cutoff. These negatives were evaluated for acceptability according to the batch criteria described above. Under these constraints, there was no carryover failing the QC criteria for either drug even following the 7 injections of the 50,000 ng/mL level. The use of a gas-tight syringe coupled with syringe rinse steps ensures minimal carryover.

Finally, for the linearity study to be accepted, the quality controls for the batch had to meet QC standards described above. For the 40% codeine control, the calculated value was 797 ng/mL, a 0.3% deviation from the target and within the $\pm 20\%$ quantitation range, and the ion ratios were also within the $\pm 20\%$ target range. The 125% control was calculated to be 2605 ng/mL, a 4.2% deviation from expected. For the 40% morphine control, the calculated value was 806 ng/mL, a 0.8% deviation from expected. The 125% control was calculated to be 2730 ng/mL, a 9.2% deviation from target. Also, the 375 ng/mL hydrolysis control of morphine-3-glucuronide quantitated at 343 ng/mL, an 8.4% deviation, demonstrating good recovery of the conjugated species. For this method, the LOD is 60 ng/mL for codeine and 100 ng/mL for morphine, making the negative threshold 60 ng/mL and 100 ng/mL respectively. The negative control passed based on this QC criteria. As such linearity batch was accepted. Table 4 includes a summary of the linearity/carryover study for codeine and morphine on the DSQ II.

Expected Concentration (ng/mL)	Average Calculated Concentration (ng/mL)
60	62
100	90
200	180
400	370
1000	890
2000	1900
Negative	0
4000	3900
Negative	0
10,000	9100
Negative	13
20,000	24,000
Negative	32
50,000	47,000
Negative	37

Expected Concentration (ng/mL)	Average Calculated Concentration (ng/mL)
100	89
200	180
400	370
1000	890
2000	1900
Negative	0
4000	3900
Negative	0
10,000	9100
Negative	8
25,000	24,000
Negative	48
50,000	47,000
Negative	56

Table 4: Results of linearity/carryover study for codeine (top) and morphine (bottom). Calculated concentrations representing points on the linearity curve were obtained by averaging seven injections made at that concentration.

Intra- and Inter-day Precision

Instrument precision and method precision for both drugs were measured by extracting two separate precision batches and running these batches on two different days. The precision study was designed to indicate precision at the 40% level, at the cutoff of 2000 ng/mL, and at the 125% level. Coefficients of variation (CV) were calculated for the average concentrations at each level, and these CVs had to be less than 10% for each concentration. As with the linearity batch, the precision batches had to comply with the QC criteria, and all controls were acceptable. To gauge inter-day precision, the percent difference in the average quantitation amounts at each level had to be less than 10%.

The method described above provides excellent quantitative precision, with CVs all less than 2%, and percent differences all less than 9%. Table 5 includes a summary of the precision results for codeine and morphine on the DSQ II.

Codeine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch Percent Difference
800 ng/mL	0.2%	0.9%	1.4%
2000 ng/mL	0.8%	0.3%	1.4%
2500 ng/mL	0.8%	0.9%	3.3%

Morphine Concentration	CV for Batch 1	CV for Batch 2	Inter-batch Percent Difference
800 ng/mL	0.9%	0.7%	1.0%
2000 ng/mL	0.5%	0.8%	1.8%
2500 ng/mL	0.9%	0.8%	5.5%

Table 5: Results of the cutoff precision study for codeine (top) and morphine (bottom)

Specificity

To determine assay specificity, an interference study was performed. A number of compounds with potential to interfere with the immunoassay screening test for codeine and morphine were included in this test, as were a range of other compounds. Drugs that were tested individually were hydromorphone, hydrocodone, oxycodone, norcodeine, normorphine, thebaine, and oxymorphone. The remaining drugs were tested together as a mix. Table 6 describes the drugs and their respective concentrations. For each interference test, the potential interferent was spiked into a blank urine sample, an 800 ng/mL sample and a 2500 ng/mL sample at the concentration specified. All negatives met the negative control criteria for both drugs, and each 40% and 125% control quantified within 20% of the target concentration, showing that none of the potential interferents tested affected quantitation. Also, all ion ratios were checked against the ion ratios of the calibrator and each were within 20% of the calibrator ion ratios, showing no interference with the confirming ions. Retention times also fell within the specified window of $\pm 2\%$ of the calibrator retention time. The interference batch also complied with all applicable QC criteria, and the results of the specificity batch were accepted as demonstrating the assay to be free of interference from the tested compounds.

Drug	Concentration (ng/mL)
Hydromorphone	5000
Hydrocodone	5000
Oxycodone	5000
Norcodeine	5000
Normorphine	5000
Thebaine	5000
Oxymorphone	5000
Methadone	5000
Caffeine	5000
Cocaine	5000
Lidocaine	5000
Barbital	5000
Phenobarbital	5000
Methbarbital	5000
Mephobarbital	5000
Ethosuximide	5000
Methsuximide	5000
Phensuximide	5000
N-Normethsuximide	5000
α -Methyl- α -propylsuccinimide	5000
Carbamazepine	5000
10,11-Dihydrocarbamazepine	5000
Mephenytoin	5000
Phenytoin	5000
Ethotoin	5000
Primidone	5000
4-Methylprimidone	5000
Methyl PEMA	5000
PEMA	5000
Methaqualone	5000
EDDP	5000
Glutethimide	5000

Table 6: List of compounds tested for potential interference, along with concentrations tested

Conclusion

The analysis of codeine and morphine on the DSQ II was completed with a total run time of 5.2 minutes. The validated method is sensitive and has a wide dynamic range, ranging from 60 to 50,000 ng/mL for codeine and 100 to 50,000 ng/mL for morphine. All samples tested in this range gave calculated amounts that were within 20% of the nominal values, based on a one-point calibration curve at 2000 ng/mL. Across these ranges, all samples also gave ion ratios which were within 20% of the ion ratios of the calibrator. A series of seven replicate injections at the reported LOD of codeine at 60 ng/mL gave a coefficient of variation of 3.8% and an average calculated value of 62 ng/mL. Seven injections of morphine at 100 ng/mL gave an average value of 89 ng/mL and a CV of 1.8%, demonstrating remarkable sensitivity even when using a split injection technique. Method precision and specificity were also excellent, with intra-day coefficients of variation all less than 1% at three different concentrations. Because all method development and validation were performed in extracted hydrolyzed urine matrix, the results reflect vigorous method validation and demonstrate that the DSQ II GC/MS system provides a robust and reliable means of performing this confirmation. These results also accurately reflect method development and validation as they would be performed within a working laboratory.

References

1. *Disposition of Toxic Drugs and Chemicals in Man. Sixth Edition.* Randall C. Baselt. Biomedical Publications. Foster City, California. 2002. pp 253–257, 720–724.
2. Incomplete Recovery of Prescription Opioids in Urine using Enzymatic Hydrolysis of Glucuronide Metabolites. P. Wang et al. *Journal of Analytical Toxicology*, 30, pp 570–575. October, 2006.
3. *Forensic Application of Mass Spectrometry.* Jehuda Yinon. CRC Press. Boca Raton, Florida. 1995. pp 21–22.
4. *AN10108: ToxLab 2.0: Evaluation of Intelligent Sequencing Software for Use in High-Throughput Laboratory Settings.* Trisa Robarge, Jim Edwards and Meredith Conoley, Thermo Fisher Scientific, Austin, TX.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Australia

+61 2 8844 9500

Austria

+43 1 333 50340

Belgium

+32 2 482 30 30

Canada

+1 800 532 4752

China

+86 10 5850 3588

Denmark

+45 70 23 62 60

France

+33 1 60 92 48 00

Germany

+49 6103 408 1014

India

+91 22 6742 9434

Italy

+39 02 950 591

Japan

+81 45 453 9100

Latin America

+1 608 276 5659

Netherlands

+31 76 587 98 88

South Africa

+27 11 570 1840

Spain

+34 91 657 4930

Sweden/Norway/ Finland

+46 8 556 468 00

Switzerland

+41 61 48784 00

UK

+44 1442 233555

USA

+1 800 532 4752

www.thermo.com



Thermo Fisher Scientific, Austin, TX USA is ISO Certified.

©2007 Thermo Fisher Scientific Inc. All rights reserved. Siltek is a trademark of the Restek Corporation. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

TN10178_E 04/07M