Application Note 601

Quantitative Forensic Analysis of Opiates, Opioids, and Their Metabolites in Human Urine Without Hydrolysis

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Key Words

Opiates, opioids, metabolites, sample preparation liquid chromatography (SPLC), mass spectrometry (MS), forensic toxicology

Goal

To develop a quantitative forensic method for analysis of opiates, opioids, and their metabolites in human urine without the time-consuming step of hydrolysis.

Introduction

Analysis of opiate and opioid metabolites in urine is most often done with a hydrolysis step that make total sample preparation time up to 24 hours. The method described here eliminates the hydrolysis step by analyzing the conjugated metabolites intact using a Thermo Scientific™ Prelude SPLC™ system for sample preparation and a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer for analysis.

Experimental

Sample Preparation

Urine samples, which were free of opiates, were diluted two-fold with water and methanol (95:5) containing internal standards. There were a total of 10 deuterated internal standards in solution at a concentration of 50 ng/mL. After the addition of the internal standards, 50 µL of each sample were injected onto the analytical column at a temperature of 27 °C.

Calibration standards containing all 19 compounds at concentrations ranging from 5 to 500 ng/mL were prepared in urine. Quality control (QC) samples were also prepared in urine at three levels: 12, 225, and 400 ng/mL.

SPLC Method Parameters

Instrumentation	Prelude SPLC system (Figure 1)
Analytical column	Thermo Scientific™ Accucore™ aQ column (100 x 2.1 mm, 2.6 µm particle size)
Mobile phase A	0.1% formic acid in water (Fisher Chemical brand)
Mobile phase B	0.1% formic acid in methanol (Fisher Chemical brand)
Gradient	Refer to Table 1

Figure 1. Prelude SPLC system with TSQ Endura triple quadrupole mass spectrometer

Table 1. Gradient details

Step	Start (min)	Time (s)	Flow (mL/min)	Grad.	%A	%B
1	0.00	20	0.40	Step	100.0	0.0
2	0.33	5	0.40	Step	92.0	8.0
3	0.42	50	0.40	Step	92.0	8.0
4	1.25	5	0.40	Step	75.0	25.0
5	1.33	130	0.40	Ramp	65.0	35.0
6	3.50	45	0.40	Step	0.0	100.0
7	4.25	100	0.40	Step	100.0	0.0

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MS Method Parameters

Instrumentation	TSQ Endura triple quadrupole MS
Ion source	Heated electrospray (HESI II)
Ionization polarity	Positive
Cycle time	0.200 s
Peak width (Q1)	0.7 Da
Peak width (Q3)	0.7 Da
Chrom peak filter width	3.0
Spray voltage	4500 V
Vaporizer temperature	400 °C
Sheath gas pressure	30 (arbitrary units)
Ion sweep gas pressure	1.0 (arbitrary units)
Aux gas pressure	15 (arbitrary units)
Capillary temperature	325 °C
Collision gas pressure	1.5 mTorr
SRM parameters	Refer to Table 2

Table 2. SRM parameters

Analyte	Precursor Ion (Q1)	Product Ions (Q3)	CE (V)	S-lens (V)
Normorphine	272.0	165.0	59	95
		209.0	40	95
Morphine 3b glucuronide	462.1	286.1	52	148
		185.2	58	139
Oxymorphone 3b glucuronide	478.1	284.1	47	147
		302.1	42	147
Hydromorphone 3b glucuronide	462.1	185.2	58	139
		286.1	52	148
Morphine 6b glucuronide	462.1	286.1	52	148
		185.2	58	139
Codeine 6b glucuronide	476.2	300.2	31	114
		215.2	39	114
6-Acetylmorphine	328.1	165.0	58	112
		211.0	39	112
6-Acetylcodeine	342.1	225.1	27	109
		165.1	47	109
Dihydromorphine	288.1	185.1	48	95
		165.0	59	95
Morphine	286.1	165.1	64	90
		185.0	44	119
Oxymorphone	302.0	227.0	40	116
		199.1	55	116
Hydromorphone	286.1	185.0	44	119
		165.1	64	90
Codeine	300.0	171.0	40	119
		199.1	43	119
Dihydrocodeine	302.0	201.1	42	93
		199.0	52	93
Norcodeine	286.1	165.1	64	90
		181.6	49	90
Oxycodone	316.0	241.1	41	119
		256.0	40	119
Noroxycodone	302.1	227.0	41	116
		187.0	40	116
Norhydrocodone	286.1	199.0	39	119
		241.1	35	119
Hydrocodone	300.0	171.1	40	119
		181.1	51	94
Noroxycodone-D_3	305.1	190.1	25	116
Norhydrocodone-D_3	298.1	152.1	62	116
6acetylmorphine-D_6	334.1	165.1	38	116
Morphine 6b glucuronide-D_3	465.1	298.1	32	140
Morphine-D_3	289.1	152.1	61	116
Dihydrocodeine-D_6	308.1	202.1	34	116
Codeine-D_6	306.1	165.1	43	116
Hydromorphone-D_6	292.1	185.1	32	116
Morphine-3b-glucuronide-D_3	465.1	289.1	31	140
Oxycodone-D_6	322.1	218.1	43	116

Method Validation

Accuracy and precision were tested by using five replicates of three levels of quality controls over four days and quantitating them using calibration curves at the beginning and end of the batch run. The fourth day of accuracy and precision was performed in real urine to cross-verify the use of real matrix. Carryover was calculated by dividing the total analyte signal of the lower limit of quantitation (LLOQ) by the total analyte signal found in the matrix blank after the upper limit of quantitation (ULOQ). This number could not exceed 20% of the total LLOQ signal. Additionally, autosampler stability (24 hours at 4 °C) was determined by running QC samples that were refrigerated overnight in the autosampler to a new calibration curve the following day.

Results and Discussion

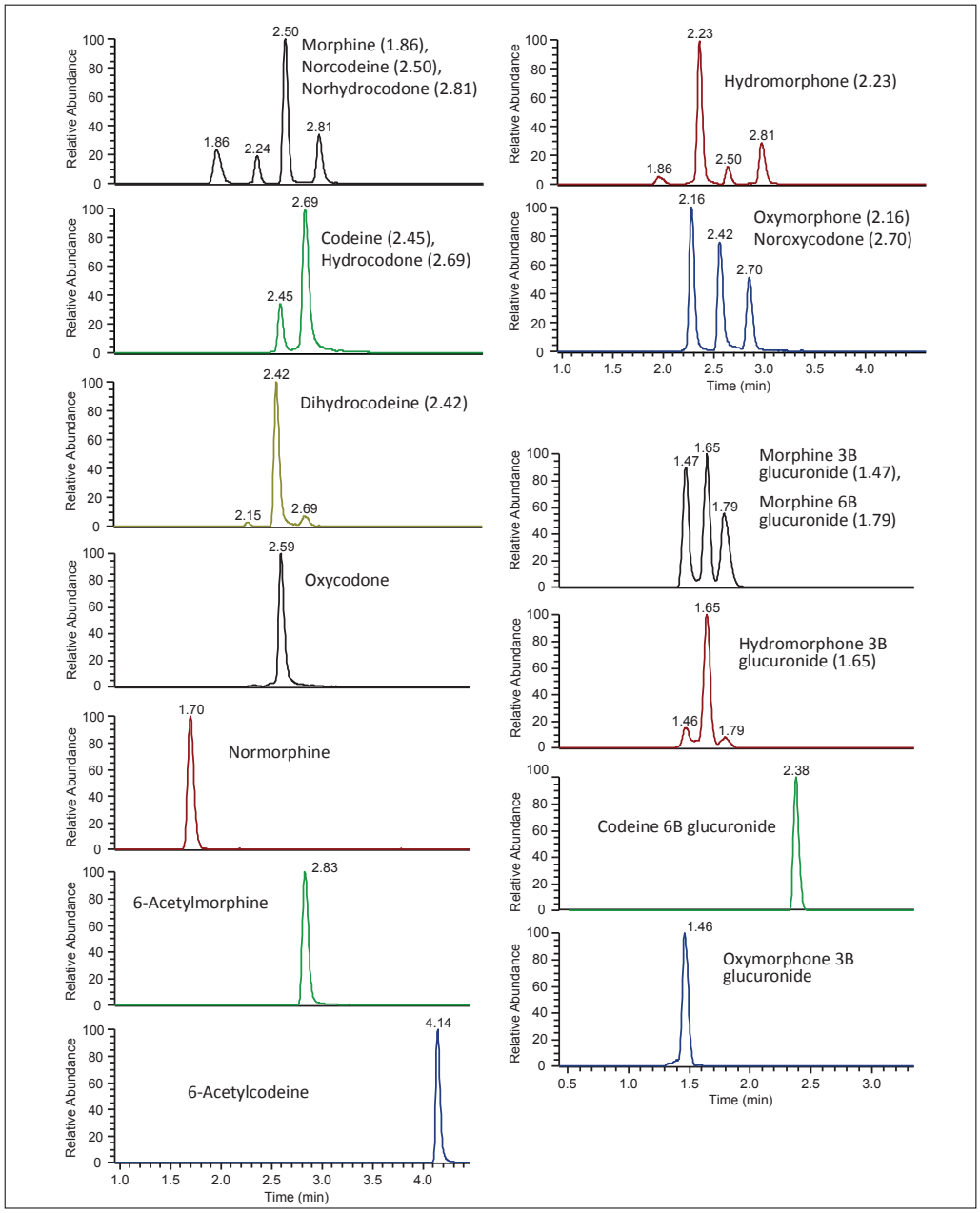
The assay precision had %RSD values that were within 20.0% at the LLOQ and low QC, and within 15.0% for all other QC and calibration standard levels. Additionally, accuracy was within 20.0% at the LLOQ and low QC, and within 15% for all other QC and calibration standard levels. All of these results are shown in Table 3.

The short 4.25 minute analytical method provided ample resolution for all isobaric compounds. All the analytes passed acceptance criteria for carryover, matrix effects, and autosampler stability. Example chromatograms for each of the compounds are shown in Figure 2.

Table 3. Accuracy and precision results

Analyte	Accuracy	Precision (%RSD)	
		Intra-Assay	Inter-Assay
Normorphine	94.6	<14.3	<5.7
Dihydromorphine	102	<14.1	<8.2
Morphine	99.2	<8.8	<4.8
Oxymorphone	103	<10.3	<3.5
Hydromorphone	102	<14.1	<5.8
Norcodeine	98.6	<9.6	<4.1
Dihydrocodeine	99.5	<11.1	<5.3
Codeine	99.2	<13.6	<5.7
Norhydrocodone	98.2	<13.5	<9.2
Oxycodone	99.4	<14.1	<5.8
Noroxycodone	100	<11.6	<10.4
Hydrocodone	95.2	<7.4	<5.0
6-Acetylmorphine	103	<9.7	<4.4
Codeine 6B glucuronide	102	<8.5	<4.1
Oxymorphone 3B glucuronide	100	<14.4	<4.4
Hydromorphone 3B glucuronide	108	<7.9	<5.7
Morphine 3B glucuronide	98.5	<14.9	<4.1
Morphine 6B glucuronide	99.0	<10.8	<3.7
6-Acetylcodeine	102	<6.1	<6.9

Figure 2. Representative chromatograms for all 19 compounds



Conclusion

A forensic method for analysis of opiates, opioids, and their metabolites without hydrolysis has been developed using the Prelude SPLC system and TSQ Endura MS. By eliminating the hydrolysis step, the sample preparation time and analysis cost was drastically reduced. The LC method on the Prelude SPLC system/TSQ Endura MS provided ample resolution for all isobaric compounds and an outstanding increase in overall speed of analysis. The high sensitivity that the TSQ Endura MS provided allowed for low limits of quantitation of even the least responsive analytes, like the gluronidated metabolites. The fast SRM acquisition rate yielded a successful, simultaneous analysis of 19 compounds with 10 internal standards.

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