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

Sarah Fair Wandland, Kerry Hassell, Joseph Herman, Thermo Fisher Scientific, Franklin, MA

#### **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<sup>™</sup> Prelude SPLC<sup>™</sup> system for sample preparation and a Thermo Scientific<sup>™</sup> TSQ Endura<sup>™</sup> 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  $\mu$ L 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 <sup>™</sup> Accucore <sup>™</sup> aQ column (100 x 2.1 mm, 2.6 µm particle size), catalog # 17326-102130
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.	% <b>A</b>	%В
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



## **MS Method Parameters**

Instrumentation	TSQ Endura triple quadrupole MS
lon 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)
lon 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)
Nevereventine	272.0	165.0	59	95
Normorphine		209.0	40	95
Marphina 2h aluguranida	462.1	286.1	52	148
Morphille 3b glucurorlide		185.2	58	139
Ovumarahana 2h gluauranida	478.1	284.1	47	147
Oxymorphone 3b gluculonide		302.1	42	147
Hydromorphone 3b alucuronide	462.1	185.2	58	139
		286.1	52	148
Morphine 6b alucuronide	462.1	286.1	52	148
		185.2	58	139
Codeine 6h alucuronide	476.2	300.2	31	114
		215.2	39	114
6-Acetylmorphine	328.1	165.0	58	112
0-Acetyinioi pinine		211.0	39	112
6 Acotylcodoino	342.1	225.1	27	109
0-Acelyicoueine		165.1	47	109
Dibydromorphipo	288.1	185.1	48	95
Diriyuromorphine		165.0	59	95
Morphipo	286.1	165.1	64	90
NOLDUNE		185.0	44	119
Outmorphono	302.0	227.0	40	116
Oxymorphone		199.1	55	116
ludromorphono	286.1	185.0	44	119
nyuronnorphone		165.1	64	90
Codoino	300.0	171.0	40	119
Codellie		199.1	43	119
Dibudracadaina	302.0	201.1	42	93
Dinyarocodenie		199.0	52	93
Managada ta a	286.1	165.1	64	90
Norcodeine		181.6	49	90
Owned	316.0	241.1	41	119
Uxycodone		256.0	40	119
Manageralana	302.1	227.0	41	116
Noroxycodone		187.0	40	116
Neveudropodeno	286.1	199.0	39	119
Nornydrocodone		241.1	35	119
lludraaadana	300.0	171.1	40	119
пушосоцоне		181.1	51	94
Noroxycodone-D <sub>3</sub>	305.1	190.1	25	116
Norhydrocodone-D <sub>3</sub>	298.1	152.1	62	116
6acetylmorphine-D <sub>6</sub>	334.1	165.1	38	116
Morphine 6b glucuronide-D <sub>3</sub>	465.1	298.1	32	140
Morphine-D <sub>3</sub>	289.1	152.1	61	116
Dihydrocodeine-D <sub>6</sub>	308.1	202.1	34	116
Codeine-D <sub>6</sub>	306.1	165.1	43	116
Hydromorphone-D <sub>6</sub>	292.1	185.1	32	116
Morphine-3b-glucuronide-D <sub>3</sub>	465.1	289.1	31	140
Oxycodone-D <sub>6</sub>	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



## 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.

For Forensic Use Only

#### www.thermoscientific.com

©2014 Thermo Fisher Scientific Inc. All rights reserved. ISO is a registered trademark of the International Organization for Standardization (Organisation Internationale De Normalization). All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change Not all products are available in all countries. Please consult your local sales representative for details.

Japan +81 45 453 9100

Latin America +1 561 688 8700

Middle East +43 1 333 50 34 0

Netherlands +31 76 579 55 55

New Zealand +64 9 980 6700

Russia/CIS +43 1 333 50 34 0

Norway +46 8 556 468 00



Singapore +65 6289 1190 Spain +34 914 845 965 Sweden +46 8 556 468 00 Switzerland +41 61 716 77 00 UK +44 1442 233555 USA +1 800 532 4752



400 650 5118 AN64030\_E 06/14S BN0911146

China 800 810 5118 (free call domestic)

Africa +43 1 333 50 34 0

Austria +43 810 282 206

Belgium +32 53 73 42 41

Canada +1 800 530 8447

Australia +61 3 9757 4300

Denmark +45 70 23 62 60 Europe-Other +43 1 333 50 34 0 Finland +358 9 3291 0200 France +33 1 60 92 48 00 Germany +49 6103 408 1014 India +91 22 6742 9494 Italy +39 02 950 591