

APPLICATIONS

Enantiomeric Analysis of Dextromethorphan & Levomethorphan and Dextrorphan & Levorphanol in Urine and Serum by LC-MS/MS

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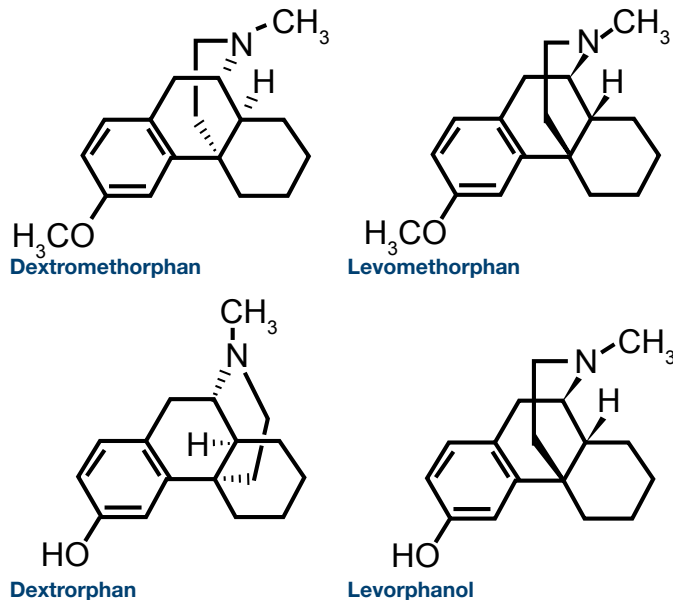


Laura Snow

Outside of the lab, Laura enjoys spoiling her dog Maggie and subjecting her husband to novel methods of torture, such as endless playlists of sad songs and long walks on the beach to catch Pokémon.

Introduction

Dextromethorphan is a drug found in over the counter cough medicine, while its stereoisomer levomethorphan is an opioid and Schedule II Narcotic that has not been marketed. Dextromethorphan is metabolized to dextrorphan, and levomethorphan is metabolized to levorphanol. Levorphanol, stereoisomer of dextrorphan, is also available by prescription for pain management. These sets of enantiomers are indistinguishable by mass spectrometry and not resolved by typical reversed phase chromatography methods. The LC-MS/MS method presented here uses chiral chromatography to identify and quantitate the individual enantiomers present in both urine and serum.



Solid Phase Extraction (SPE)

96-Well Plate: Strata[®]-X-C, 30 mg/well

Part No.: [8E-S029-TGB](#)

Condition: 1 mL methanol

Equilibrate: 1 mL 0.1 % Formic acid in Water

Load: Pretreated sample

Wash 1: 1.0 % Formic acid in water

Wash 2: 1.0 % Formic acid in methanol

Dry: 3-4 minutes at high vacuum

Elute: 2x 0.5 mL 5 % Ammonium hydroxide in 50:50 methanol/ acetonitrile

Dry Down: To dryness at 40 °C under a gentle stream of nitrogen

Reconstitute: 100 µL 40:60 acetonitrile/water

HPLC Conditions

Column: Lux[®] 3 µm AMP

Dimensions: 150 x 4.6 mm

Part No.: [00F-4751-E0](#)

Guard Cartridge: [AJ0-8476](#)

Guard Holder: [KJ0-4282](#)

Mobile Phase: A: 5 mM Ammonium bicarbonate, adjusted to pH 11 with ammonium hydroxide
B: Acetonitrile

Gradient: Time (min) % B

0.0 45

4.5 45

4.6 80

8.0 80

8.1 45

10.0 45

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Injection Volume: 10 µL

System: Agilent[®] 1260

Detection: MS/MS (SCIEX[®] Triple Quad[™] 4500, ESI+)

Backpressure: ~160 bar (250 bar at max)

Note: Column was flushed with pure acetonitrile for 20 minutes following the completion of each set of injections prior to column storage.

MS/MS Conditions

Q1 Mass (Da)	Q3 Mass (Da)	ID	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
272	215	Dextromethorphan/ Levomethorphan 1	90	10	33	13
272	147	Dextromethorphan/ Levomethorphan 2	90	10	40	13
258	157	Dextrorphan/ Levorphanol 1	90	10	48	13
258	133.2	Dextrorphan/ Levorphanol 2	90	10	38	13
275	213	Dextromethorphan-D3	100	10	36	13
261	157	Dextrorphan-D3	100	10	47	13

Note: The declustering potential (DP) was detuned to 170 volts for urine samples to accommodate the analytical measuring range (AMR).

Parameter	Setting
Curtain Gas (CUR)	25 psi
Collision Gas (CAD)	6 psi
IonSpray Voltage (IS)	5500 V
Temperature (TEM)	600 psi
Ion Source Gas 1 (GS1)	50 psi
Ion Source Gas 2 (GS2)	50 psi

Sample Preparation Pretreatment

Urine: Combine 200 µL urine, 200 µL 100 mM ammonium acetate buffer (pH 4.0), 20 µL internal standard solution (250 ng/mL), and 20 µL of beta-glucuronidase (>100,000 units/mL) and incubate for 1 hour at 55 °C. Centrifuge for 10 minutes at 13,000 rcf. Dilute 400 µL supernatant 1:1 with 0.1 % aqueous formic acid.

Serum: Combine 250 µL serum, 250 µL 0.1 % Formic acid, and 20 µL internal standard solution (250 ng/mL).

Results and Discussion

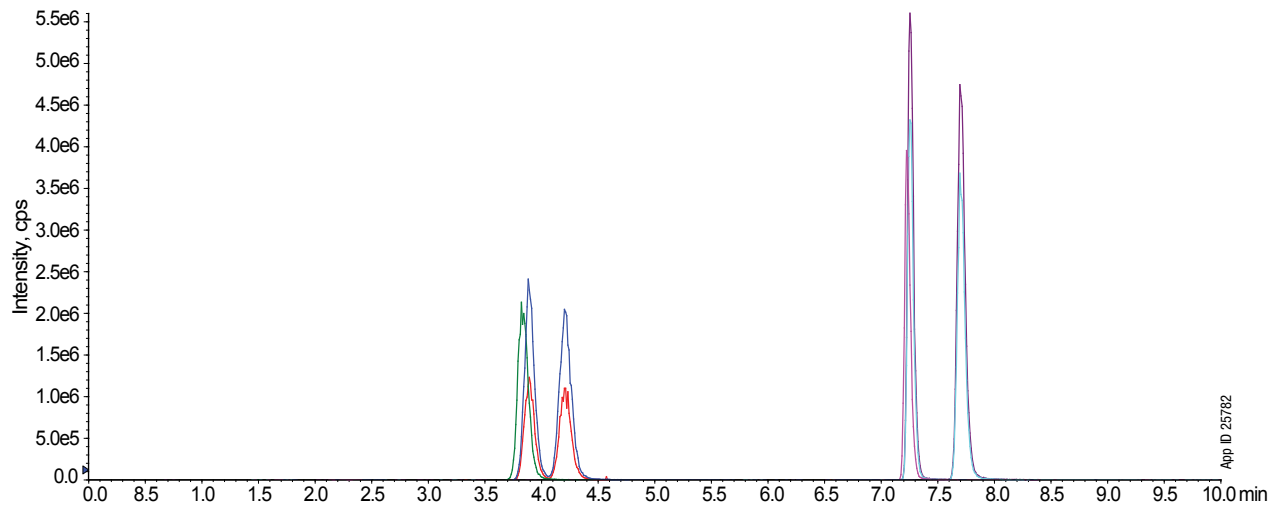
All enantiomers were baseline resolved or better and chromatographically separated in 8 minutes (**Figure 1**). Use of an acetonitrile mobile phase produced much better peak shape compared to methanol. Although the column chemistry is stable from pH 1 to 11.5, following the completion of each set of injections, the column was flushed with pure acetonitrile for 20 minutes prior to column storage.

Signal-to-noise was greater than 10 for the lowest calibrator at 0.1 ng/mL (**Figure 2**). A linear regression with 1/x weighting was used for all calibration curves. The linear range for serum was 0.1 ng/mL to 50 ng/mL (**Table 1**). The declustering potential was detuned to

attain a linear range of 5 ng/mL to 500 ng/mL for urine (**Table 2**). Accuracy ranged from 79 % to 121 % for all calibrators, and curves were linear with $r^2 \geq 0.9982$ (**Figures 3 and 4**). Precision ranged from 2 % to 5 % CV, and accuracy ranged from 91 % to 113 % for urine quality controls (**Table 3**).

Recoveries and matrix effects were evaluated for both urine and serum extracts (**Table 4**) and calculated according to Matuszewski et al. For urine, recoveries ranged from 72 % to 78 % for all analytes. For serum, recoveries were comparable to urine for dextrophan and levorphanol at 75 % and 77 %, but lower for dextromethorphan and levomethorphan at 54 % and 51 % respectively. Matrix effects ranged from 96 % to 103 % for all analytes in both matrices.

Figure 1.
Representative Chromatogram, 25 ng/mL Urine Extract



Analyte	Retention Time (min)
Dextrophan-D3	3.79
Dextrophan	3.85
Levorphanol	4.17
Dextromethorphan-D3	7.21
Dextromethorphan	7.24
Levomethorphan	7.69

Figure 2.
Low End Chromatogram, 0.1 ng/mL Serum Extract

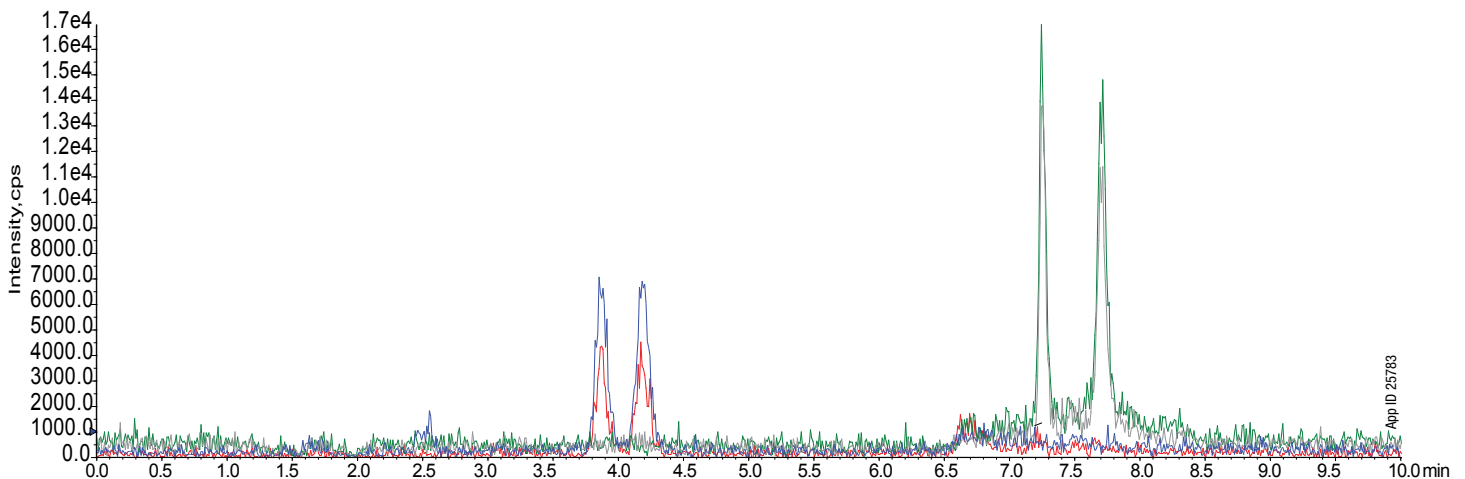


Table 1.
Serum Calibration Curves (0.1 to 50 ng/mL)

Calibrator Concentration (ng/mL)	Dextromethorphan Accuracy (%)	Dextrorphan Accuracy (%)	Levomethorphan Accuracy (%)	Levorphanol Accuracy (%)
0.1	118	122	81	86
0.5	89	90	94	93
1	94	98	113	109
5	97	92	113	112
10	102	97	101	102
50	100	101	98	98

Figure 3.
Serum Calibration Curves (0.1 to 50 ng/mL)

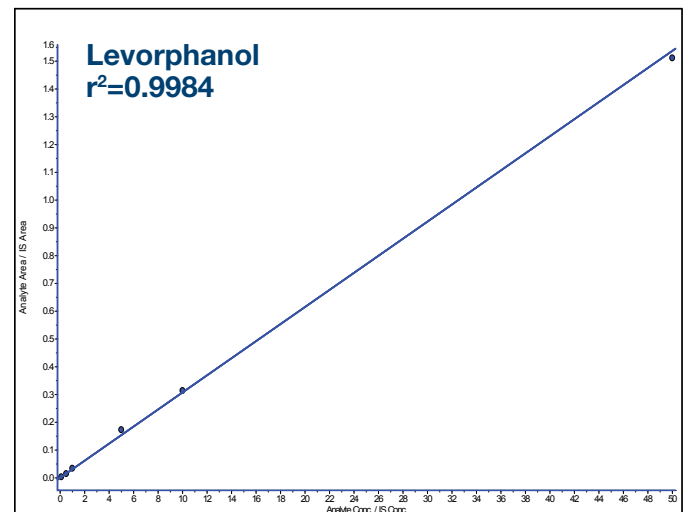
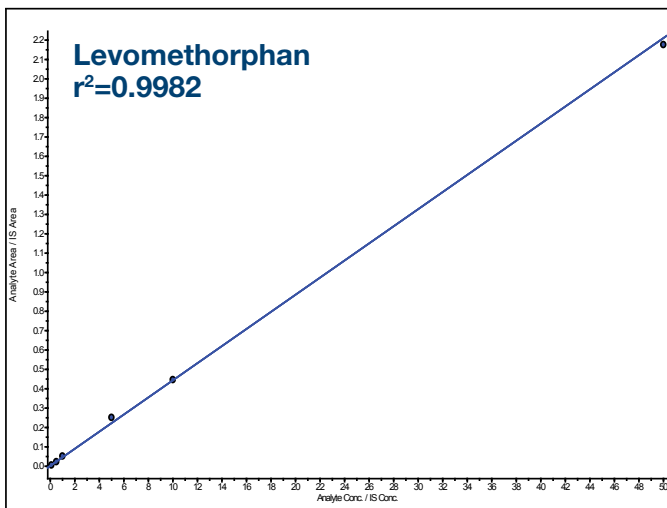
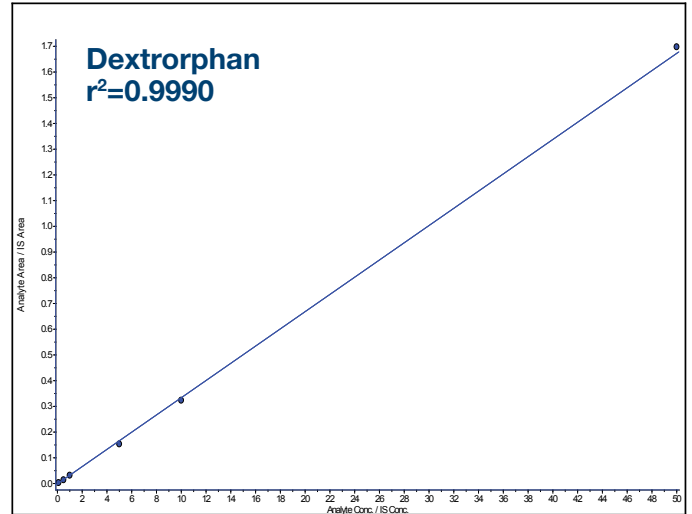
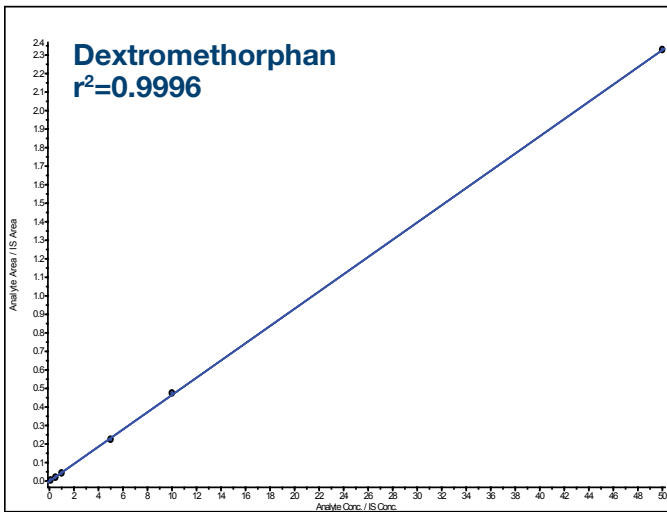


Table 2.
Urine Calibration Curves (5 to 500 ng/mL)

Calibrator Concentration (ng/mL)	Dextromethorphan Accuracy (%)	Dextrophan Accuracy (%)	Levomethorphan Accuracy (%)	Levorphanol Accuracy (%)
5	116	109	119	121
10	79	91	81	79
100	104	99	99	101
250	104	101	101	100
500	98	100	100	100

Figure 4.
Urine Calibration Curves (5 to 500 ng/mL)

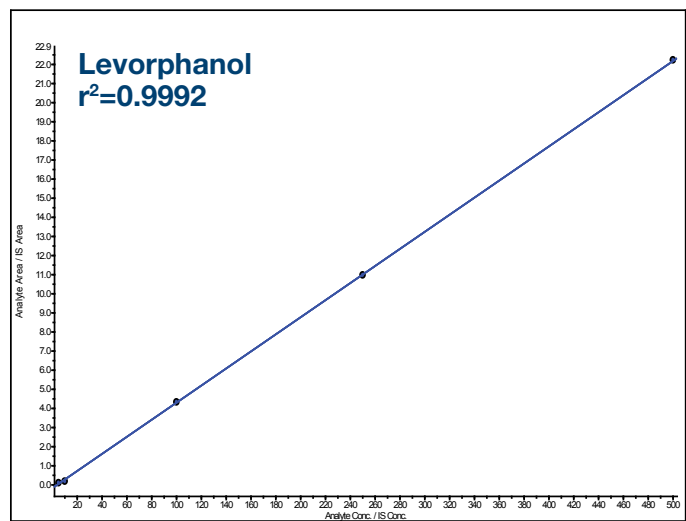
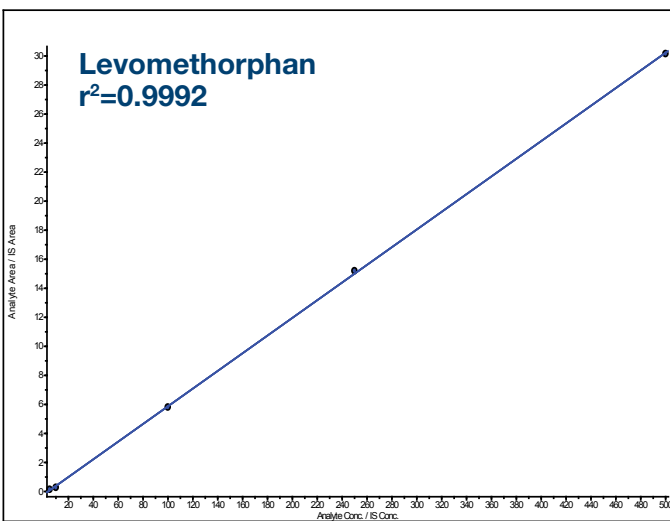
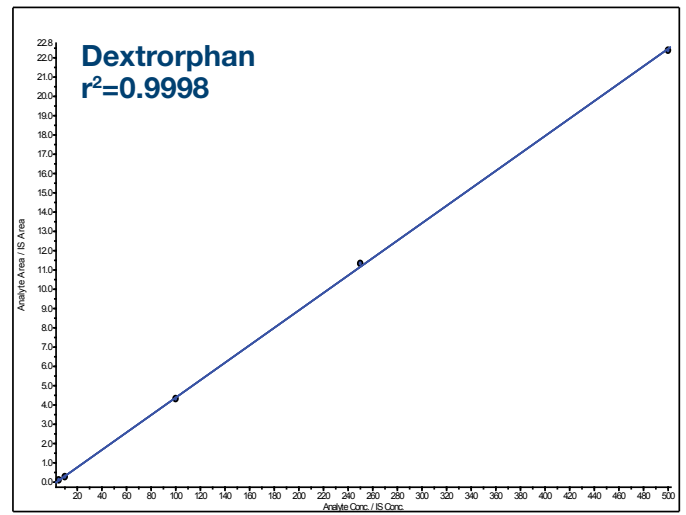
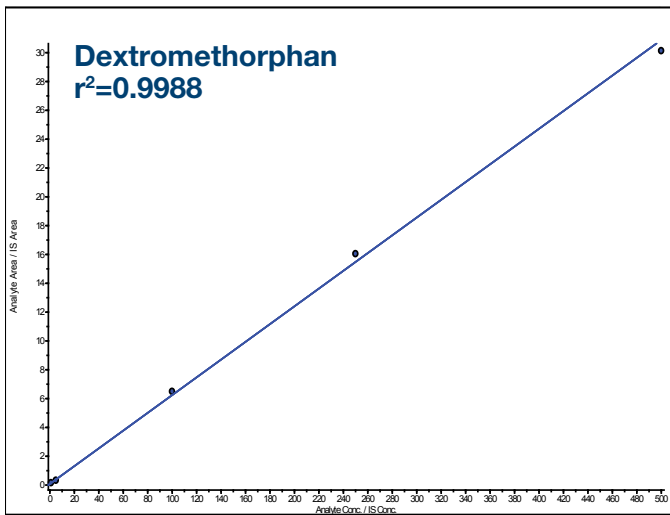


Table 3.
Urine Quality Controls

	QC Low (25 ng/mL)				QC High (300 ng/mL)			
	Accuracy (%)			CV (%)	Accuracy (%)			CV (%)
Dextromethorphan	80	82	83	2.0	96	101	98	2.9
Dextrorphan	97	96	93	2.1	91	95	94	1.8
Levomethorphan	93	100	94	4.1	95	99	98	2.5
Levorphanol	101	98	97	2.1	87	92	94	4.0

Table 4.
Recovery and Matrix Effects at 25 ng/mL

Analyte	Recovery in Urine	Matrix Effects in Urine	Recovery in Serum	Matrix Effects in Serum
Dextromethorphan	72 %	101 %	54 %	96 %
Dextrorphan	78 %	96 %	75 %	98 %
Levomethorphan	72 %	101 %	51 %	100 %
Levorphanol	76 %	103 %	77 %	97 %

Conclusion

This work presents a reproducible and sensitive chiral LC-MS/MS method for identification and analysis of dextromethorphan, levomethorphan, dextrorphan, and levorphanol from urine and serum. Future work may include adjusting the SPE method specifically for serum samples to improve recoveries for dextromethorphan and levomethorphan.

Acknowledgements

Laura would like to acknowledge Waleed Afaq for performing the chiral column screening in our lab that led to this work, and J Preston for his ongoing support of her work in the applications group.




Ordering Information

Lux[®] AMP Chiral LC Column

3µm Analytical Columns (mm)	SecurityGuard [™] Cartridges (mm)			
	150 x 3.0		150 x 4.6	
Phase	150 x 3.0	150 x 4.6	4 x 2.0*	4 x 3.0*
			10/pk	10/pk
AMP	00F-4751-Y0	00F-4751-E0	AJ0-8475	AJ0-8476
			for ID: 2.0 - 3.0mm	3.2 - 8.0mm

*SecurityGuard Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

Strata[®]-X-C Solid Phase Extraction (SPE)

Format	Sorbent Mass	Part Number	Unit
 Tube	30 mg	8B-S029-TAK**	1 mL (100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ**	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)
	 96-Well Plate	10 mg	8E-S029-AGB
30 mg		8E-S029-TGB	2 Plates/Box
60 mg		8E-S029-UGB	2 Plates/Box
 96-Well Microelution Plate	2 mg	8M-S029-4GA	ea

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