Application Note 20685

Fast, Reproducible LC-MS/MS Analysis of Dextromethorphan and Dextrorphan

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Key Words

Accucore C18, dextromethorphan, dextrorphan, SOLA CX

Abstract

A liquid chromatography–tandem mass spectrometry method for the analysis of dextromethorphan and dextrorphan from rat plasma has been developed. A limit of quantification of 0.1 ng/mL is readily achieved. When using Thermo Scientific™ SOLA™ CX cartridges or plates, sample preparation is fast and reproducible giving excellent precision, low matrix suppression, and good recovery. The analysis was carried out on a Thermo Scientific™ Accucore™ C18 50 × 2.1 mm HPLC column for a fast separation with a cycle time of 2 minutes and good peak shape.

Introduction

Dextromethorphan (Figure 1) is used as a cough suppressant and can be found in many over-the-counter cough and cold remedies. Dextromethorphan is quickly metabolized to dextrorphan (Figure 2) in the liver. This process can be used to differentiate fast and slow metabolizers, which can be useful information when starting metabolism drug research. The extraction from plasma using SOLA solid phase extraction (SPE) products and LC analysis of dextromethorphan and dextrorphan are demonstrated in this application.

SOLA SPE products introduce next-generation, innovative technological advancements, which give unparalleled performance characteristics compared to conventional SPE, phospholipid, and protein precipitation products.

These include:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA SPE plates or cartridges have significant advantages when analyzing compounds in complex matrices, particularly in high-throughput bioanalytical and clinical research laboratories where reduced failure rates, higher analysis speed, and lower solvent requirements are critical. SOLA products' superior performance gives higher confidence in analytical results and lowers cost without compromising ease-of-use or requiring complex method development.

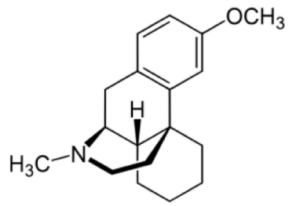

Figure 1. Dextromethorphan

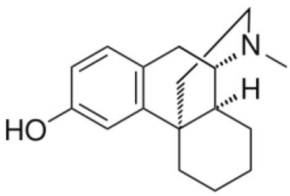

Figure 2. Dextrorphan

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Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore C18 columns provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Experimental Details

Sample Handling	Part Number
Fisher Scientific™ LC-MS grade water	W/011217
Fisher Scientific LC-MS grade methanol	M/4062/17
Fisher Scientific LC-MS grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
Fisher Scientific HPLC grade ammonia solution	A/3295/PB05
Thermo Scientific™ National™ Mass Spec Target DP Certified 2 mL clear vial with ID patch, blue DP cap with bonded PTFE/silicone septum	MSCERT4000-34W

Sample Handling Equipment	Part Number
Thermo Scientific 96 well plate vacuum manifold	60103-351
Thermo Scientific™ UltraVap™	CLS-229070

Sample Pretreatment

Standard spiking solutions of dextromethorphan and dextrorphan were prepared in acetonitrile.
A working internal standard solution (dextromethorphan-d_3) was prepared in acetonitrile. Spiking solutions for each level of calibrant were made in acetonitrile.
180 µL of blank plasma or sample was taken and added to a clean vial.
For standards and quality control (QC) samples, 10 µL of standard spiking solution was added, with 10 µL of acetonitrile added for blanks. For standards and QCs, 10 µL of working internal standard solution was added. For blanks, 10 µL of acetonitrile was added.
All samples were vortexed for 30 seconds and 200 µL of 0.1% formic acid in water was added. All samples were vortexed for 30 seconds and centrifuged at 14,000 rpm for 5 min.

Sample Preparation		Part Number
Compound(s):	Dextromethorphan, dextromethorphan-d_3, and dextrorphan	
Matrix:	Plasma	
Plate type: Conditioning stage:	Thermo Scientific SOLA CX Apply 500 µL of methanol, then 500 µL 0.1% formic acid in water to the SPE plate	60309-002
Application stage:	Apply all supernatant to the SPE plate at a flow rate of 0.5 mL/min	
Washing stage:	Apply 500 µL of methanol / water (40:60 v/v) to the SPE plate	
Elution stage:	Apply 4 × 250 µL 5% ammonia in methanol to the SPE plate and dry well	
Additional stage:	Dry down under nitrogen and reconstitute in 200 µL acetonitrile / water (50:50 v/v). Mix well.	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC HPLC System	
Column:	Accucore C18 2.6 µm, 50 × 2.1 mm	17126-052130
Mobile phase A:	Water + 0.1% formic acid	
Mobile phase B:	Acetonitrile + 0.1% formic acid	
Gradient:	Time (min)	%B
	0	5
	1	95
	1.01	5
	2	5
Flow rate:	1.4 mL/min	
Column temperature:	40 °C	
Pressure:	360 Bar	
Injection details:	2 µL	

MS Conditions	
Instrumentation:	Thermo Scientific™ TSQ Vantage™ MS
Ionization conditions:	HESI
Polarity:	Positive
Spray voltage (V):	5000
Vaporizer temperature (°C):	450
Sheath gas pressure (Arb):	60
Aux gas pressure (Arb):	40
Capillary temp (°C):	300
Collision pressure (m Torr):	1.5
Q1 (FWHM):	0.7
Q3 (FWHM):	0.7
Compound transition details are provided in Table 1.	

Compound	Dextromethorphan	Dextrorphan	Dextromethorphan-d_3
Parent (*m/z*)	272.2	258.2	275.2
Products (*m/z*)	215.2	157.1	215.1
Collision energy (V)	22	37	22
S Lens (Arb)	76	84	77

Table 1. Compound transition details

Data Processing	
Software:	Thermo Scientific™ LC QUAN™ 2.6

Results

Chromatography

The Accucore C18 HPLC column gave excellent peak shape. The chromatography of the QCM at 2.5 ng/mL is shown in Figure 3.

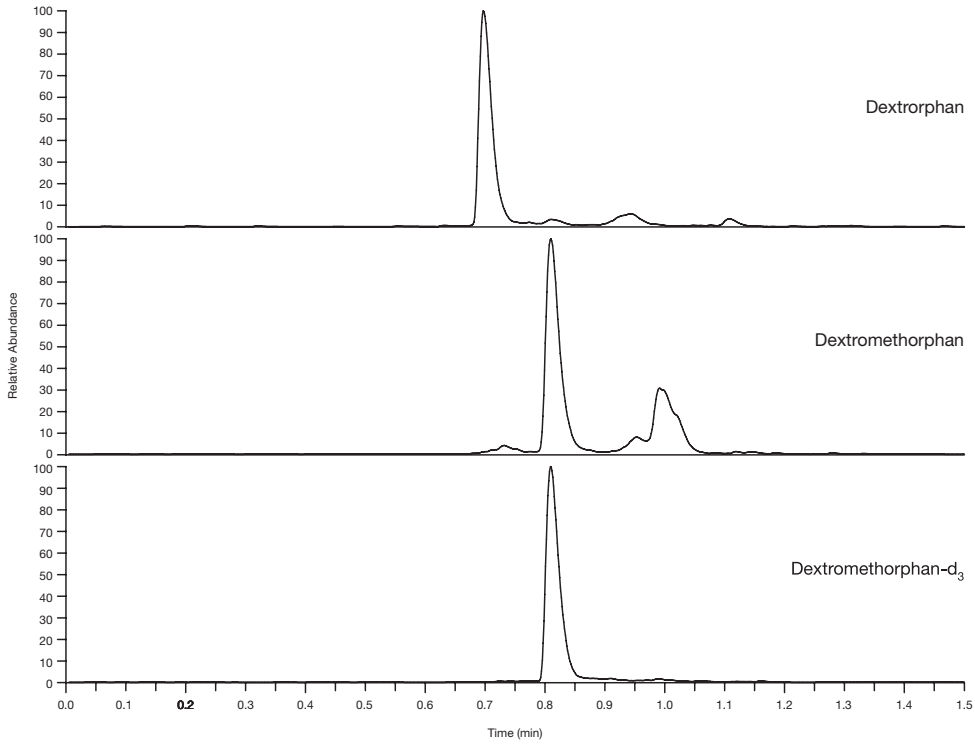

Figure 3: Representative chromatogram of dextromethorphan and dextrorphan SRM, extracted from plasma at 2.5 ng/mL

Linearity

Standards of dextromethorphan and dextrorphan extracted from spiked rat plasma gave a linear calibration curve over the dynamic range of 0.1 to 100 ng/mL with an r^2 of 0.998 and 0.999, respectively (Figures 4 and 5 and Table 2).

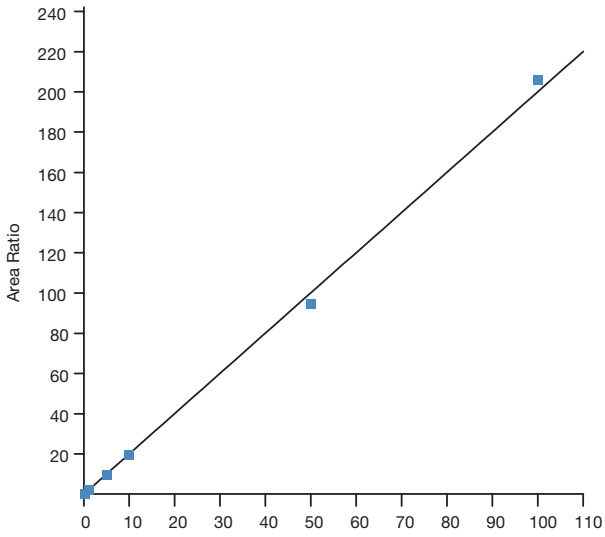

Figure 4: Dextromethorphan linearity over the dynamic range 0.1 to 100 ng/mL

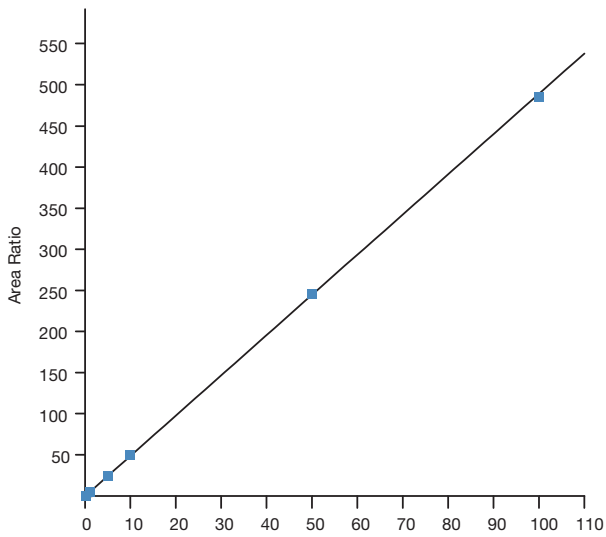

Figure 5: Dextrorphan linearity over the dynamic range 0.1 to 100 ng/mL

Accuracy

Eight standards were run over the linear range 0.1 to 100 ng/mL. The accuracy of the back calculated values was ≤14% in all cases.

Standard	Specified Concentration (ng/mL)	Dextromethorphan Calculated Concentration (ng/mL)	Dextromethorphan % Diff	Dextrorphan Calculated Concentration (ng/mL)	Dextrorphan % Diff
S1	0.1	0.101	1	0.090	-10
S2	0.2	0.204	2	0.228	14
S3	0.5	0.480	-4	0.503	1
S4	1	1.10	10	0.904	-10
S5	5	1.73	-5	5.06	1
S6	10	9.86	-1	10.3	3
S7	50	47.2	-6	50.4	1
S8	100	103	3	99.2	-1

Table 2: Accuracy data for eight extracted dextromethorphan and dextrorphan standards over the linear range 0.1 to 100 ng/mL

Precision

QC samples were run in replicates of six at concentrations of 0.25, 3, and 25 ng/mL. The precision of the QC level was ≤ 8% CV in all cases (Table 3).

Standard	Concentration (ng/mL)	Dextromethorphan Average Calculated Concentration (n=6)	Dextromethorphan % CV	Dextrorphan Average Calculated Concentration (n=6)	Dextrorphan % CV
QCL	0.25	0.236	7	0.236	8
QCM	3	2.67	3	3.08	7
QCH	25	24.5	8	27.4	8

Table 3: Average precision data for six replicate QCs at three levels for dextromethorphan and dextrorphan

Recovery

Overspikes (post extracted fortified blanks) were run in triplicate at concentrations of 0.25, 3, and 25 ng/mL and used to calculate the percentage recovery level for dextromethorphan and dextrorphan (Table 4). The recovery of detromethorphan was 95.4% and that of dextrorphan was 101.6%. The recovery proved to be consistent throughout the batch for all compounds.

	Standard	Response Ratio	% Recovery	Average % Recovery
Dextromethorphan	Average QCL response ratio	0.596	98.8	95.4
	Average QCL overspike response ratio	0.594		
	Average QCM response ratio	5.45	95.6	
	Average QCM overspike response ratio	5.71		
	Average QCH response ratio	49.8	91.9	
	Average QCH overspike response ratio	54.2		
Dextrorphan	Average QCL response ratio	1.18	102.9	101.6
	Average QCL overspike response ratio	1.15		
	Average QCM response ratio	15.1	102.0	
	Average QCM overspike response ratio	14.8		
	Average QCH response ratio	134	99.8	
	Average QCH overspike response ratio	134		

Table 4: Recovery data for dextromethorphan, dextrorphan and dextromethorphan-d_3

Matrix Suppression

Overspikes and solution standards were run in triplicate at concentrations of 0.25, 3, and 25 ng/mL and used to calculate the percentage matrix suppression for dextromethorphan and dextrorphan (Table 5). There was very little matrix suppression: -0.5% and 7.4% for dextromethorphan and dextrorphan, respectively. The matrix effects were consistently low throughout the batch for all compounds.

	Standard	Response Ratio	% Matrix Suppression	Average % Matrix Suppression
Dextromethorphan	Average QCL overspike response ratio	0.594	-5.9	-0.5
	Average QCL solution response ratio	0.560		
	Average QCM overspike response ratio	5.71	0.2	
	Average QCM solution response ratio	5.72		
	Average QCH overspike response ratio	54.2	4.2	
	Average QCH solution response ratio	56.5		
Dextrorphan	Average QCL overspike response ratio	1.15	14.6	7.4
	Average QCL solution response ratio	1.34		
	Average QCM overspike response ratio	14.8	2.3	
	Average QCM solution response ratio	15.1		
	Average QCH overspike response ratio	134	5.4	
	Average QCH solution response ratio	142		

Table 5: Matrix suppression data for dextromethorphan, dextrorphan and dextromethorphan-d_3

Conclusion

- SOLA CX SPE plates and Accucore C18 HPLC columns allow for simple extraction and rapid quantification of dextromethorphan and dextrorphan from plasma.
- Accucore C18 HPLC columns give a fast runtime for dextromethorphan and dextrorphan of 2 min.
- Accucore C18 HPLC columns give excellent peak shape for dextromethorphan and dextrorphan.
- An LLOQ of 0.1 ng/mL was achieved.
- Extraction recovery was 95.4% and 101.6% for dextromethorphan and dextrorphan, respectively.
- SOLA CX SPE plates achieved excellent precision on the extraction of dextromethorphan and dextrorphan from plasma samples at low concentrations with % CV (n=6) ≤8%
- SOLA CX SPE plates achieved low matrix recovery. This gave excellent matrix suppression effects at 7% and 12% for dextromethorphan and dextrorphan, respectively.

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