RESTEK
Chromatography Products

Fast Analysis of Pain Management Drugs in Urine by LC/MS/MS: A Painless Task

The analysis of pain management medications, such as opiates and opioids, can be problematic due to poor retention and coelution with matrix interferences. Ultra II® Biphenyl columns have greater retention than other phenyl columns and provide the fast, reliable separations needed for routine analysis.

Introduction

Chronic pain is estimated to affect over 50 million Americans, and is the leading cause of adult disability in the United States. Much of the time NSAID painkillers are used to combat pain, but in more severe cases, opiate and opioid drugs are prescribed, often in conjunction with NSAIDs and benzodiazepines. Over the past several years, LC/MS/MS instrumentation has gained popularity in toxicology laboratories, and many analysts are attempting to transfer GC/MS methods for pain management drugs to LC/MS/MS techniques. Analyzing pain management drugs by LC/MS/MS has several advantages in that sample preparation is often simplified and derivatization is not required.

Although analysis by LC can be more straightforward than GC analysis, some compounds can be problematic because they are not retained well using conventional reversed phase stationary phases. To increase retention of these compounds, analysts have begun to adopt phenyl-based stationary phases, which use an alternate mode of selectivity based on π–π (pi-pi) interactions. Phenyl phases can provide greater retention for hydrophilic compounds, but not all phenyl columns offer equivalent performance. Here biphenyl (Ultra II® Biphenyl, Restek) and phenyl hexyl (Gemini® C6-Pheny, Phenomenex) columns were compared based on retention and resolution of target compounds.

Procedure

Samples were prepared by fortifying control urine at 50 ng/mL with 10 commonly administered pain medications, including NSAIDs, opiates, and benzodiazepines. Fortified samples were then diluted 10x in mobile phase for a final concentration of 5 ng/mL. 5 µL injections of prepared samples were analyzed on 2 phenyl-based columns: a Restek 3 µm Ultra II® Biphenyl (50 mm x 2.1 mm) and a Phenomenex 3 µm Gemini® C6-Phenyl (50 mm x 2.0 mm). The chromatographic system and instrument conditions are described below.

Mobile Phase
A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

Time (min.)	Flow (mL/min.)	%B
0	0.5	10
10	0.5	100
10.1	0.5	10
12	0.5	stop

Detector: Applied Biosystems/MDS Sciex LC/MS/MS
Model #: API-5000™

Ion Source:	TurbolonSpray®
Ion Mode:	ESI+
Curtain Gas:	25 psi (172.4 kPa)
Gas 1:	60 psi (413.7 kPa)
Gas 2:	40 psi (275.8 kPa)
Source Temp.:	550 °C
Source Voltage:	2000 V
Mode:	MRM
Dwell Time:	50 ms
Instrument:	Applied Biosystems/MDS Sciex LC/MS/MS System

Results

The Ultra II® Biphenyl column provided greater retention than the Gemini® C6-Phenyl column, resulting in excellent resolution of test compounds from each other. As shown in Figure 1, peaks 1-4 elute very early on the Gemini® C6-Phenyl column; peak 1 (morphine) elutes in the column void volume and the k' value of peak 4 is approximately 1.3. While the elution order on the Biphenyl column is different, the k' value of the first eluting peak (acetaminophen) is 3.8, and the k' value for morphine is 6.41. Although the earlier-eluting compounds are retained much longer on the Ultra II® Biphenyl column, the overall elution time for all compounds, including more strongly retained benzodiazepines, is only about one minute longer than on the Gemini® C6-Phenyl column with the same gradient profile. In order to achieve acceptable retention for the early-eluting compounds on the Gemini® C6-Phenyl column, a much longer run would be necessary.

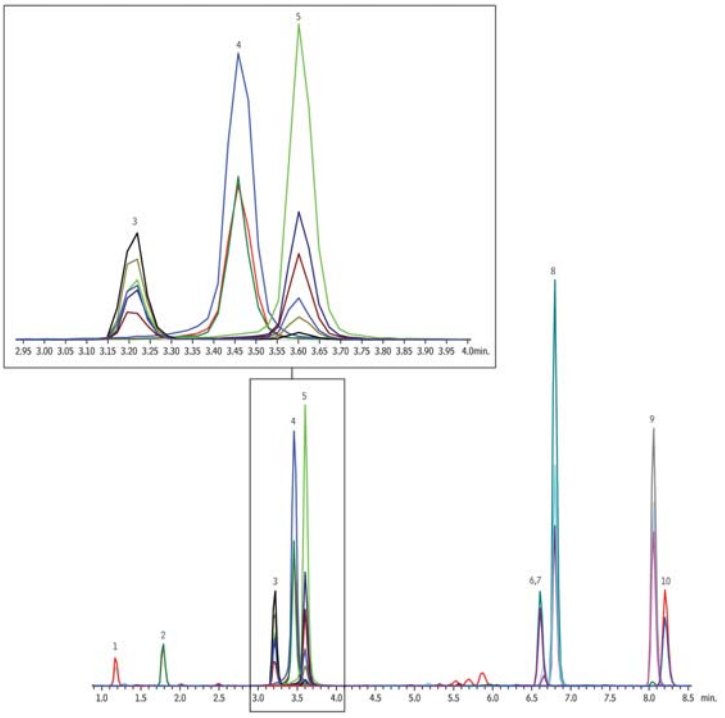
Conclusion

The analysis of pain management drugs by LC/MS/MS can be challenging due to the poor retention often obtained on conventional phases. Phenyl phases are a good alternative, but not all provide adequate resolution. This application shows how the balanced retention and selectivity of the Ultra II® Biphenyl column can help analysts reliably separate a wide variety of pain management medications in one fast run.

Figure 1 Target drug compounds can be quickly resolved on Ultra II® Biphenyl columns.

A: Ultra II® Biphenyl, Restek

- **Fast, reliable resolution of target compounds.**
- **Improved retention reduces risk of matrix effects.**



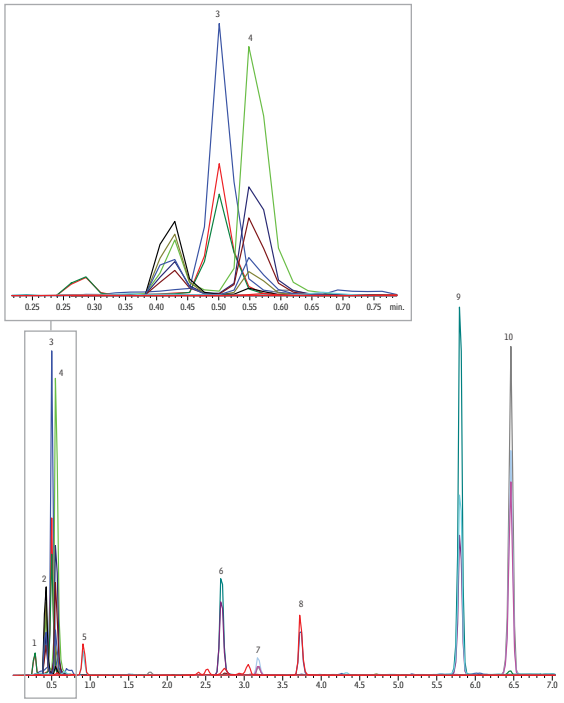
LC_CF0516

Peaks	RT (min.)	MRM1	MRM2
1. Acetaminophen	1.18	152/110	152/65
2. Morphine	1.79	286/157	286/181
3. Codeine	3.21	300/165	300/215
4. Oxycodone	3.46	316/298	316/241
5. Hydrocodone	3.60	300/199	300/171
6. Fentanyl	6.61	337/132	337/216
7. Buprenorphine	6.66	468/187	468/84
8. Lorazepam	6.79	321/275	321/229
9. Diazepam	8.06	285/154	285/193
10. Methadone	8.21	310/77	310/223

Column	Ultra II® Biphenyl (cat.# 9609352)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Temp.:	40 °C
Sample	
Conc.:	5 ng/mL in 1:10 urine:mobile phase
Inj. Vol.:	5 µL

B: Gemini® C6 Phenyl, Phenomenex

Poor retention, inadequate resolution from matrix.



LC_CF0517

Peaks	RT (min.)	MRM1	MRM2
1. Morphine	0.28	286/157	286/181
2. Codeine	0.43	300/165	300/215
3. Oxycodone	0.50	316/298	316/241
4. Hydrocodone	0.55	300/199	300/171
5. Acetaminophen	0.91	152/110	152/65
6. Fentanyl	2.70	337/132	337/216
7. Buprenorphine	3.18	468/187	468/84
8. Methadone	3.73	310/77	310/223
9. Lorazepam	5.80	321/275	321/229
10. Diazepam	6.46	285/154	285/193

Column	Gemini® C6-Phenyl (phenyl hexyl)
Dimensions:	50 mm x 2.0 mm ID
Particle Size:	3 µm
Pore Size:	110 Å
Temp.:	40 °C
Sample	
Conc.:	5 ng/mL in 1:10 urine:mobile phase
Inj. Vol.:	5 µL

Acknowledgement Special thanks to Applied Biosystems for providing instrument time.

Ultra II® Biphenyl Columns (USP L11)

Physical Characteristics:

particle size: 1.9µm, 2.2µm, 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

3µm Columns	cat. #
50mm, 2.1mm ID	9609352
50mm, 2.1mm ID (with Trident Inlet Fitting)	9609352-700

Visit our website at **www.restek.com** for additional dimensions and product information.

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ISO 9001:2008 cert.# FM80397

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