

Advantages of CORTECS C₁₈ 2.7 µm and XBridge BEH Phenyl XP 2.5 µm Columns for the Analysis of a Comprehensive Panel of Pain Management Drugs for Forensic Toxicology

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Abstract

This application note highlights the analysis of a comprehensive panel of opiates, benzodiazepines, and other drugs of abuse. Using either Waters' 2.7 μm CORTECS C₁₈ Column, or a 2.5 μm XBridge BEH Phenyl XP Column, all compounds were analyzed within 4 minutes with excellent peak shape and narrow peak widths. Whether laboratories prefer the performance and efficiency of the solidcore/superficially porous CORTECS C₁₈ Column, or the unique selectivity of the XBridge BEH Phenyl XP Column, each can be used to rapidly analyze this important group of compounds.

Benefits

- Rapid analysis of 35 forensic toxicology drugs
 - Enhanced retention of polar compounds
 - Improved resolution vs. competitive biphenyl columns
 - Low backpressures compatible with traditional HPLC systems
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Introduction

In forensic toxicology, drug screening panels often include such commonly used substances such as opiates, benzodiazepines and stimulants. These panels are often analyzed by LC-MS using traditional C₁₈ column technologies. Key considerations include the ability to chromatographically resolve the various pairs of isobaric compounds included in these panels, while maintaining good peak shape for a variety of compounds. In addition, when using traditional HPLC systems, the ability to analyze samples as rapidly as possible without exceeding the pressure limitations of the system is very important. This application note highlights the capabilities of Waters' new CORTECS C₁₈ 2.7 μm Columns and XBridge BEH Phenyl XP 2.5 μm Columns for this type of application. In the case of the CORTECS C₁₈ Column, the high efficiency packing of solid core 2.7 μm particles yields excellent performance that equals or exceeds competitive columns at lower operating backpressures. If alternative selectivity is desired, the phenyl functionality of the BEH phenyl column enhances the retention of opiate compounds. This enhanced retention can potentially result in reducing ion suppression from urinary matrix components. Both columns achieve excellent baseline

separation between isomers, and the entire panel of 35 compounds, including opioids, benzodiazepines, stimulants, and other drugs of abuse can be analyzed in under 4 minutes at backpressures compatible with any HPLC system.

Experimental

Stock solutions were obtained from Cerilliant Corporation, Round Rock, TX. Stock solutions were prepared in methanol. Working solutions were prepared in 5% acetonitrile containing 0.1% formic acid.

LC conditions

LC system:	ACQUITY UPLC I-Class, Fixed Loop (FL) with Column Manager (CMA)
Columns:	CORTECS C ₁₈ 2.7 µm, 3.0 x 50 mm (p/n 186007370) XBridge BEH Phenyl <i>XP</i> 2.5 µm, 3.0 x 50 mm (p/n 186006069)
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Mobile phase A:	MilliQ water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid

The mobile phase gradient is listed in Table 1.

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI Positive
Capillary voltage:	0.5 V
Collision energy:	Optimized for individual components
Cone voltage:	Optimized for individual components
Data management:	MassLynx v 4.1 scn 855 Software

Results and Discussion

Waters CORTECS C₁₈ 2.7 μm Column, an XBridge BEH Phenyl *XP* 2.5 μm Column, and a competitor's biphenyl core shell column (2.6 μm) were used to analyze a panel of 35 common pain management compounds (Figure 1), including opioids, benzodiazepines, stimulants, benzoylecgonine (BZE), and phencyclidine (PCP). All columns had the same dimensions (3.0 x 50 mm). The solvent gradient is listed in Table 1. The entire gradient cycle was 5 minutes.

1) Amphetamine	}	amines	17) Morphine	}	opioids
2) MDA			18) Oxymorphone		
3) Methamphetamine			19) Hydromorphone		
4) MDMA			20) Dihydrocodeine		
5) Phentermine			21) Codeine		
6) MDEA			22) Oxycodone		
7) BZE	}	benzodiazepines	23) 6-AM		
8) PCP			24) O-desmethyl Tramadol		
9) Nitrazepam			25) Hydrocodone		
10) Oxazepam			26) Norfentanyl		
11) Alprazolam			27) Tramadol		
12) Lorazepam			28) Normeperidine		
13) Clonazepam			29) Meperidine		
14) Flunitrazepam			30) Norbuprenorphine		
15) Temazepam			31) Fentanyl		
16) Diazepam			32) Buprenorphine		
			33) EDDP		
			34) Propoxyphene		
			35) Methadone		

Figure 1. Compound key.

Time (min)	Flow (mL/min)	% MPA	% MPB
0.0	0.6	95	5
4.0	0.6	40	60
4.1	0.6	95	5
5.0	0.6	95	5

Table 1. LC Gradient.

All compounds eluted within 4 minutes and showed good, symmetrical peak shape. Average peak width and maximum backpressure for all columns are shown in Table 2.

Column	Particle size (µm)	Backpressure	Mean peaks width (s)
CORTECS C ₁₈	2.7	2206	2.52
XBridge BEH Phenyl	2.5	3274	2.94
Competitor biphenyl	2.6	2492	2.71

Table 2. Performance summary.

The columns operated at backpressures well within the limit of traditional HPLC systems and, predictably, backpressure increased with decreasing particle size. Interestingly, the CORTECS C₁₈ Column, despite its larger particle size, demonstrated the best resolution, as measured by average peak width (see Table 2). The chromatography of all opioid compounds is shown in Figure 2a and the separation of key isobaric opiates can be seen in Figure 2b. All opioid drugs elute within 3.5 minutes and demonstrate good peak shape. As Figure 2b shows, the isobaric pairs of morphine and hydromorphone (peaks 17 and 19), and codeine and hydrocodone (peaks 21 and 25) are well separated on all columns. This is an important feature as these compounds must be resolved from each other for accurate identification and quantification. While the BEH phenyl and biphenyl column both show increased retention of these compounds, which is most likely a result of their phenyl functionality, excellent resolution is easily achieved on the CORTECS C₁₈ Column.

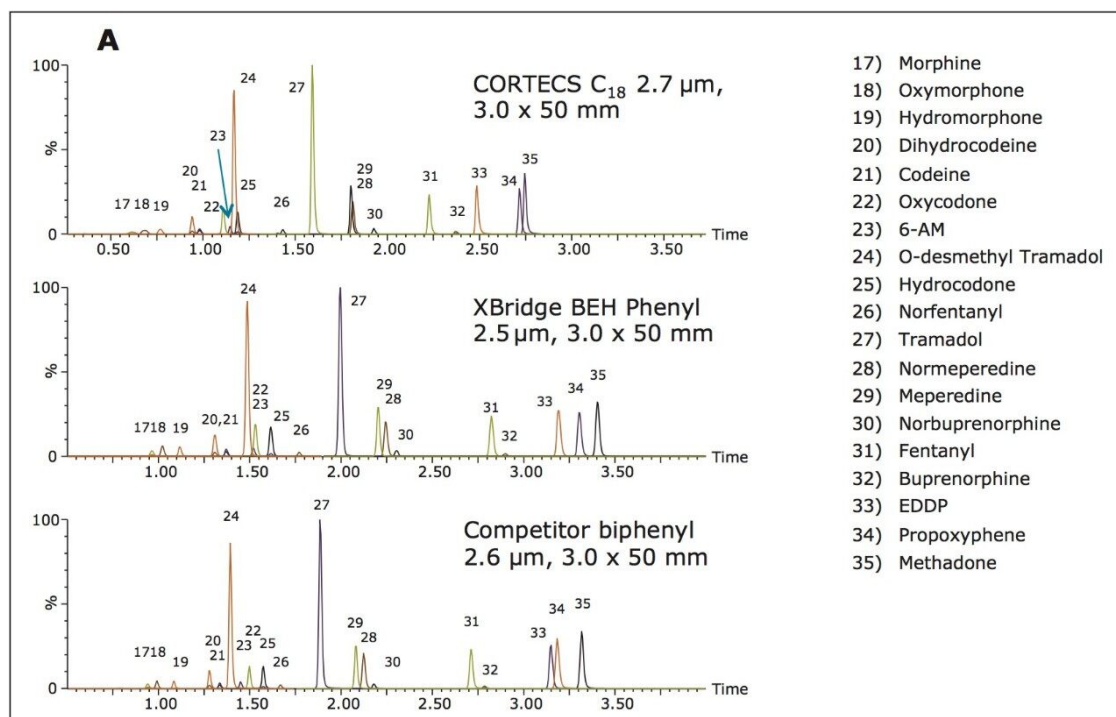
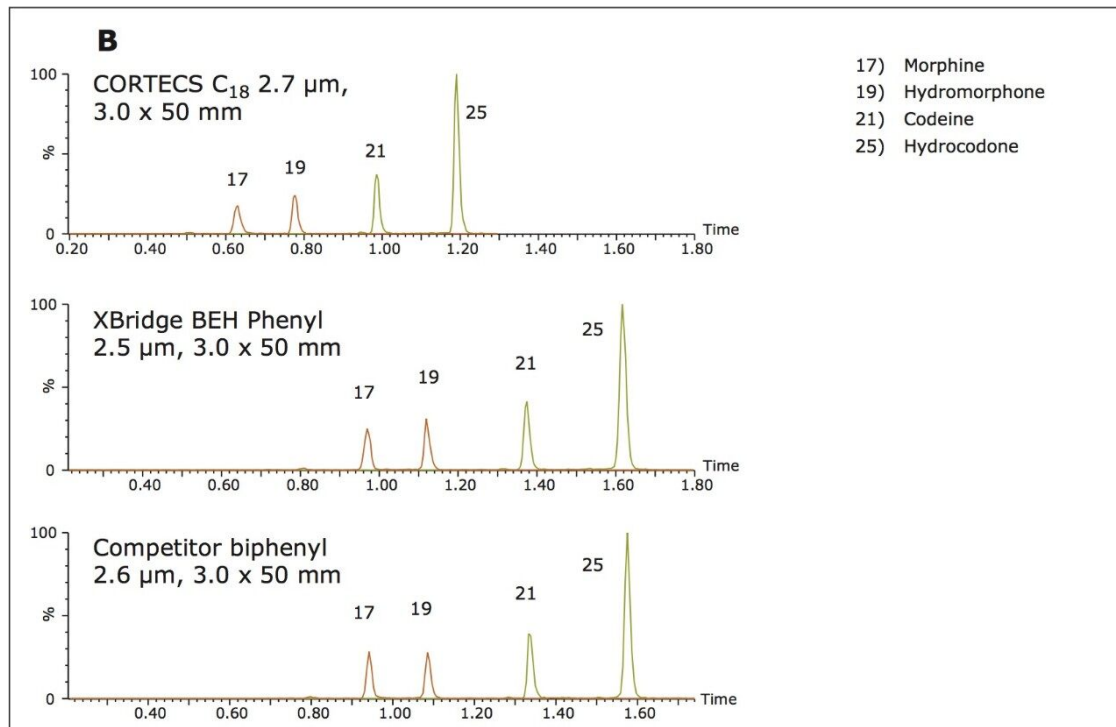


Figure 2a. Chromatographic separation of opioids.



2b. Chromatographic separation of key isobaric opiates.

Figure 3 shows the chromatography of the amines, PCP, and BZE. While all peaks demonstrate good peak shape, the CORTECS C₁₈ Column and XBridge BEH Phenyl XP Column both show excellent separation of these compounds. Of particular note are methamphetamine and phentermine (peaks 3 and 5) which demonstrate baseline separation on these two columns, but co-elute on the biphenyl column. This is an important feature as these compounds have identical molecular formulas and both have a major fragment ion at m/z 91. The ability to separate these compounds eliminates the risk of cross talk between these two stimulants and can be crucial to unambiguous identification. Figure 3 also demonstrates that MDEA and benzoylecgonine (peaks 6 and 7), which coelute on the biphenyl column, are separated on both the C₁₈ and BEH phenyl columns.

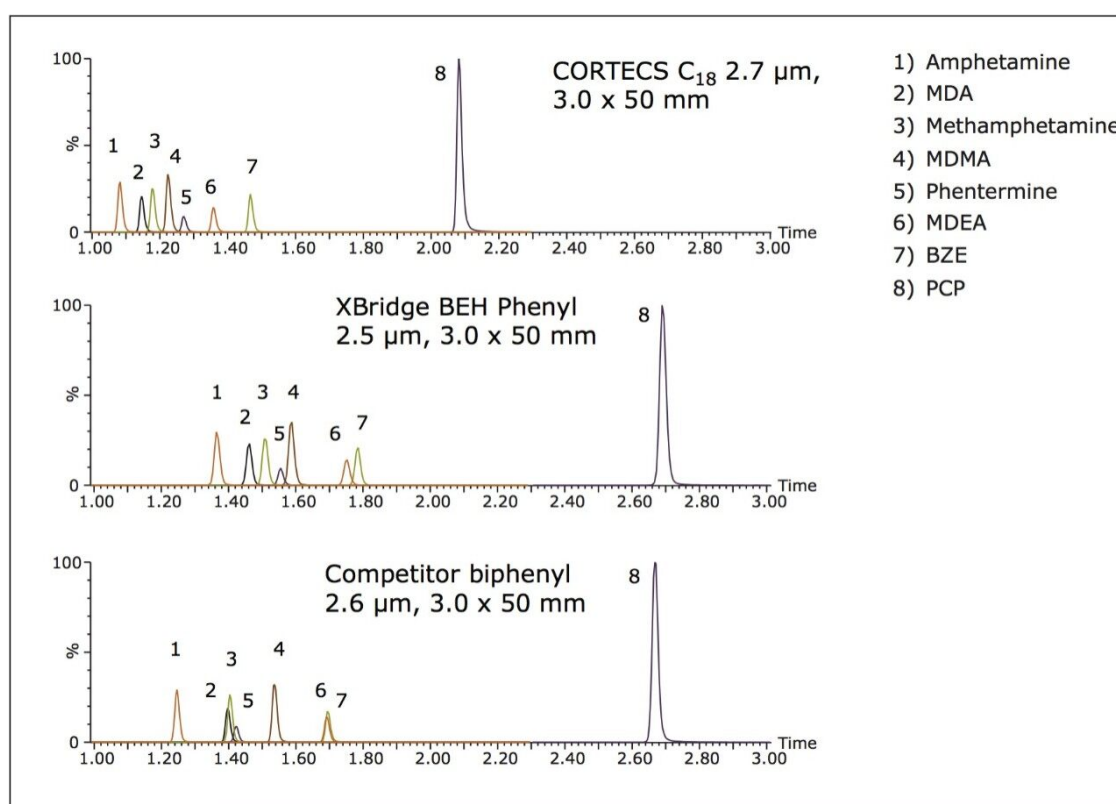


Figure 3. Chromatographic separation of amines, BZE & PCP.

The benzodiazepine chromatography is shown in Figure 4. Good peak shape can be achieved on all columns. Once again, the CORTECS C₁₈ Column, despite its larger particle size, demonstrates the highest resolution for this group of compounds (average peak width of 2.89 s).

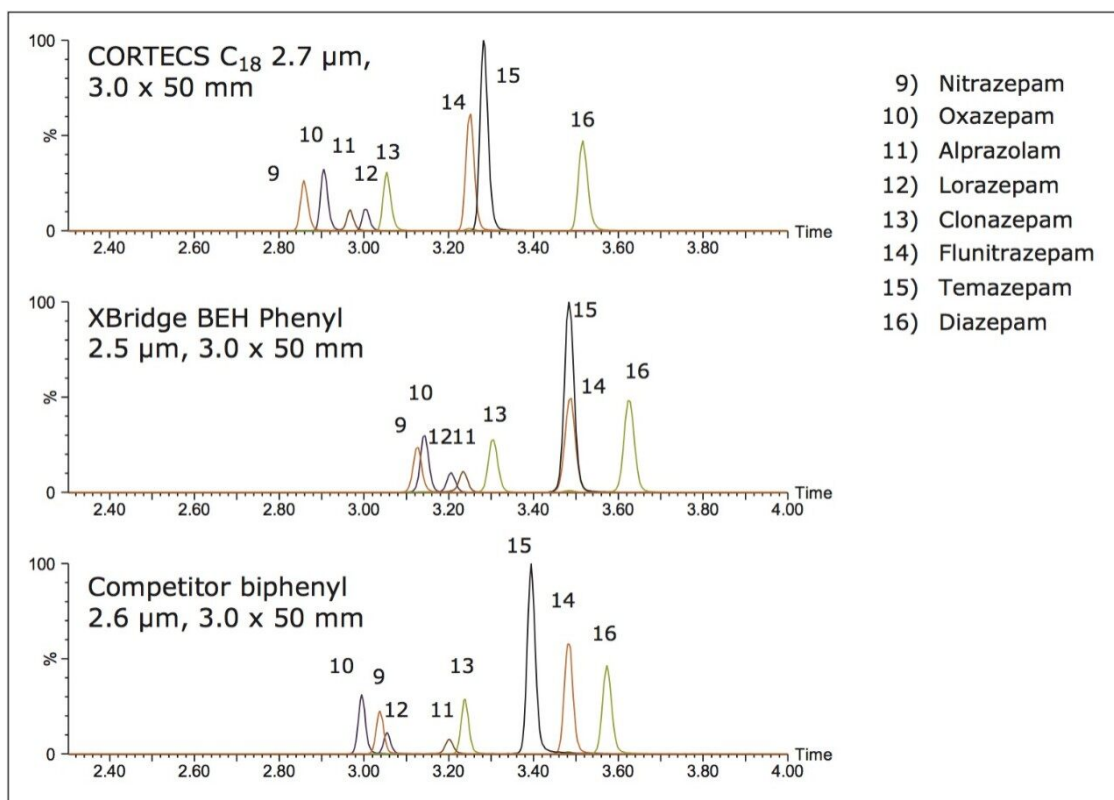


Figure 4. Chromatographic separation of benzodiazepines.

Conclusion

This application note highlights the analysis of a comprehensive panel of opiates, benzodiazepines, and other drugs of abuse. Using either Waters' 2.7 μm CORTECS C₁₈ Column, or a 2.5 μm XBridge BEH Phenyl XP Column, all compounds were analyzed within 4 minutes with excellent peak shape and narrow peak widths. Maximum backpressures were respectively 2206 and 3274 psi, enabling the use of these columns on traditional HPLC systems. Perhaps most importantly, baseline separation was achieved between isobaric compounds, allowing for their unambiguous identification and quantification. Whether laboratories prefer the performance and efficiency of the solidcore/superficially porous CORTECS C₁₈ Column, or the unique selectivity of the XBridge BEH Phenyl XP Column, each can be used to rapidly analyze this important group of compounds.

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