The Benefits of 2.1 mm Internal Diameter Analytical Columns for the Analysis of Drugs of Abuse by LC-MS/MS

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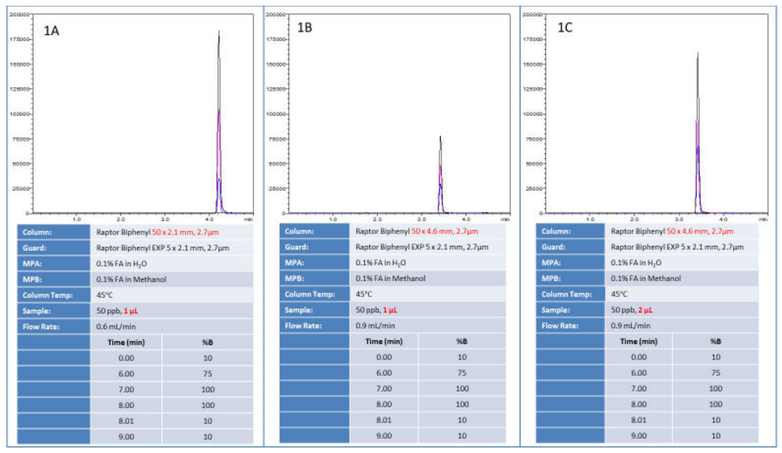
Introduction

The Biphenyl stationary phase lends superior selectivity over a C18 column for drugs of abuse panels, but choosing the right column dimension for analysis is paramount to obtain robust and accurate data. Each column dimension can be advantageous in different scenarios, but generally clinical labs are all working towards the same goals of high throughput, low sample volume, good sensitivity, and low cost. In this work, the advantage of narrow-bore columns is discussed and demonstrated for the analysis of drugs of abuse.

Efficiency

To demonstrate the superior sensitivity of narrow-bore columns, buprenorphine was tested on Raptor Biphenyl 50 mm columns. In the following example, 1 µL of 50 ppb buprenorphine was analyzed by the outlined method using a 2.1 mm internal diameter column (Figure 1A). Next, the same method was used on a 4.6 mm internal diameter column with the exception of adjusting the flow rate from 0.6 mL/min to 0.9 mL/min (Figure 1B). When the same amount of sample is injected on both columns, the larger-bore column produces approximately half of the peak height/sensitivity as the smaller-bore column. The sensitivity of analytes can be increased by injecting more on the larger ID column (Figure 1C) but that can adversely affect chromatography, decrease column lifetime, and enhance matrix interferences.

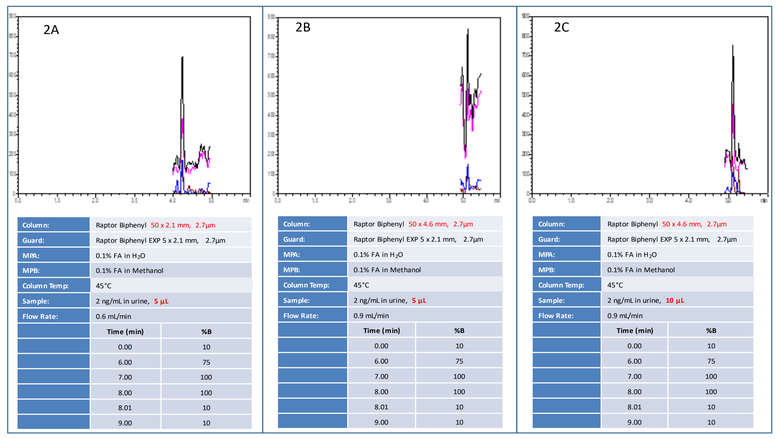


	1A	1B	1C
Column:	Raptor Biphenyl 50 x 2.1 mm, 2.7µm	Raptor Biphenyl 50 x 4.6 mm, 2.7µm	Raptor Biphenyl 50 x 4.6 mm, 2.7µm
Guard:	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O	0.1% FA in H_2O	0.1% FA in H_2O
MPB:	0.1% FA in Methanol	0.1% FA in Methanol	0.1% FA in Methanol
Column Temp:	45°C	45°C	45°C
Sample:	50 ppb, 1 µL	50 ppb, 1 µL	50 ppb, 2 µL
Flow Rate:	0.6 mL/min	0.9 mL/min	0.9 mL/min

Time (min)	%B
0.00	10
6.00	75
7.00	100
8.00	100
8.01	10
9.00	10

Figure 1: Buprenorphine analyzed on a 50 mm Biphenyl column and injecting A: 1 µL on 2.1 mm ID column, B: 1 µL on a 4.6 mm ID column, and C: 2 µL on a 4.6 mm ID column.

Sensitivity can become even more challenging when applied to matrix. In the next example buprenorphine is spiked at 2 ng/mL into urine, hydrolyzed, diluted 30-fold, centrifuged, and analyzed.



	2A	2B	2C
Column:	Raptor Biphenyl 50 x 2.1 mm, 2.7µm	Raptor Biphenyl 50 x 4.6 mm, 2.7µm	Raptor Biphenyl 50 x 4.6 mm, 2.7µm
Guard:	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O	0.1% FA in H_2O	0.1% FA in H_2O
MPB:	0.1% FA in Methanol	0.1% FA in Methanol	0.1% FA in Methanol
Column Temp:	45°C	45°C	45°C
Sample:	2 ng/mL in urine, 5 µL	2 ng/mL in urine, 5 µL	2 ng/mL in urine, 10 µL
Flow Rate:	0.6 mL/min	0.9 mL/min	0.9 mL/min

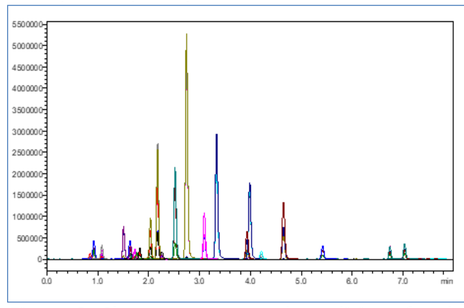
Time (min)	%B
0.00	10
6.00	75
7.00	100
8.00	100
8.01	10
9.00	10

Figure 2: Buprenorphine spiked into urine at 2 ng/mL, analyzed on a 50 mm Biphenyl column, and injected A: 5 µL on 2.1 mm ID column, B: 5 µL on a 4.6 mm ID column, and C: 10 µL on a 4.6 mm ID column.

Figure 2 outlines the importance of sensitivity when analyzing samples in matrix. The injection volume must be increased to 5 µL in order to be able to detect 2 ng/mL in urine on a 2.1 mm ID column (2A). When analyzing the same amount (5 µL) on a 4.6 mm ID column, buprenorphine could not be detected (2B). In order to achieve approximately the same amount of sensitivity, the injection volume had to be increased to 10 µL (2C). The increase in sample volume also means an increase in matrix being injected onto column. In order to conserve sample and improve sensitivity, narrow-bore columns are favored.

Resolution of Isobars

The Biphenyl stationary phase provides aromatic selectivity and greater retention of dipolar, conjugated, and strong electron withdrawing group compounds than a C18 phase. This means that Biphenyl can separate compounds that are hard to resolve or early eluting on C18 or other phases, without the need for longer or wider column dimensions. Oftentimes, drugs of abuse assays contain many compounds that share molecular weights, or isobars, that must be resolved in order to accurately quantitate each analyte. To produce precise and rugged quantitative methods, a resolution of 1.5 or greater should be achieved. In the next example, 9 groups of drugs of abuse isobars were analyzed on a Raptor Biphenyl 50 x 2.1 mm column and their resolutions calculated.



Column:	Raptor Biphenyl 50 x 2.1 mm, 2.7µm
Guard:	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm
MPA:	0.1% FA in H_2O
MPB:	0.1% FA in Methanol
Column Temp:	45°C
Sample:	50 ppb
Injection Volume:	1 µL
Flow Rate:	0.6 mL/min

Time (min)	%B
0.00	10
6.00	75
7.00	100
8.00	100
8.01	10
9.00	10

Figure 3: Chromatogram and conditions for the analysis of isobar panel.

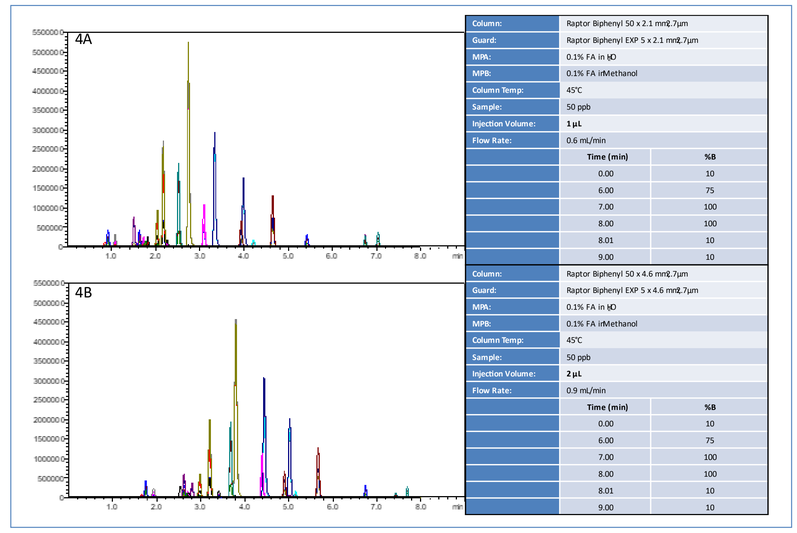
Isobar Group	Name	Molecular Weight (g/mol)	Retention Time (min)	Peak Width	Resolution
1	*Methamphetamine*	149.23	1.51	0.091	1.5
	Phentermine		1.65	0.096	
2	*Oxymorphone*	301.34	0.92	0.094	7.5
	Noroxycodone		1.63	0.094	
3	*Citalopram*	324.39	3.93	0.108	13.7
	Alpha-hydroxyalprazolam		5.42	0.109	
4	*Naloxone*	327.27	1.64	0.099	2.0
	6-acetylmorphine		1.82	0.082	
5	*Morphine*	285.34	0.85	0.102	2.3
	Hydromorphone		1.08	0.097	
	Norhydrocodone		1.73	0.085	7.1
	7-aminoclonazepam		3.09	0.099	14.9
6	*Lamotrigine*	256.09	2.26	0.093	2.7
	Hydroxybupropion	255.74	2.52	0.095	
7	*Codeine*	299.36	1.76	0.088	3.1
	Hydrocodone		2.03	0.089	
8	*O-desmethylvenlafaxine*	263.37	2.17	0.091	6.3
	Tramadol		2.74	0.091	
	Mirtazapine		3.33	0.097	6.3
	Nortriptyline		4.65	0.110	12.7
9	*CBD*	314.47	6.74	0.102	2.8
	9-THC		7.03	0.104	

Table 1: Compound name, shared molecular weight, analyte retention time (min), peak width, and calculated resolution between isobar groups

In this example, all pairs of isobars return a resolution value of 1.5 or greater, and are retained well on the 2.1 mm ID. Several contributing factors are at play to achieve acceptable resolution including the selectivity of the biphenyl phase, superficially porous particles lending a hand to increase efficiency, and the narrow-bore column mitigating band broadening contributions.

The Green Advantage

The use of narrow-bore column dimensions oftentimes means greener solutions, for your lab and your budget. Smaller ID columns can be advantageous over larger diameter columns when considering the consumption of solvent, as typically the larger the column internal diameter the higher the flow rate. Higher flow rates also hinder ionization efficiency for MS detection and can reduce sensitivity. In the following example, DOA isobars are compared using two different ID columns, 2.1 and 4.6 mm. Although in this example (Figure 4), the run time does not increase when transferring to a larger-bore column, which can sometimes be the case, the flow rate was adjusted from 0.6 mL/min to 0.9 mL/min. This means that the consumption of solvent has increased by one third per minute, costing more money than the smaller bore column, and generating more waste. Since acceptable resolution is already achieved on the 2.1 mm ID column, there is minimal advantage to moving to larger column IDs that will only consume more resources



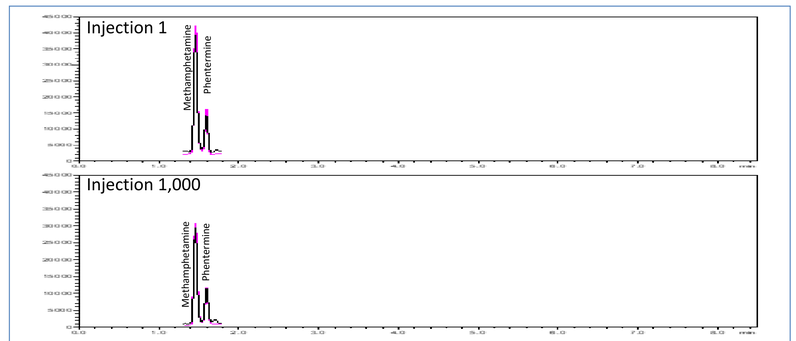
	4A	4B
Column:	Raptor Biphenyl 50 x 2.1 mm, 2.7µm	Raptor Biphenyl 50 x 4.6 mm, 2.7µm
Guard:	Raptor Biphenyl EXP 5 x 2.1 mm, 2.7µm	Raptor Biphenyl EXP 5 x 4.6 mm, 2.7µm
MPA:	0.1% FA in H_2O	0.1% FA in H_2O
MPB:	0.1% FA in Methanol	0.1% FA in Methanol
Column Temp:	45°C	45°C
Sample:	50 ppb	50 ppb
Injection Volume:	1 µL	2 µL
Flow Rate:	0.6 mL/min	0.9 mL/min

Time (min)	%B
0.00	10
6.00	75
7.00	100
8.00	100
8.01	10
9.00	10

Figure 4: 22 isobar drugs of abuse compounds analyzed on a Raptor Biphenyl column using A: 0.6 mL/min flow rate and B: 0.9 mL/min flow rate.

Robustness in Matrix

To demonstrate the narrow-bore column's robustness in matrix over an extended period, 1,000 injections were performed on a 2.1 mm ID column in urine and metrics were obtained for resolution of a critical pair (Figure 5), performance of an early eluting compound (Figure 6), and sensitivity (Figure 6).



Analyte	Resolution Injection 1	Resolution Injection 1
Methamphetamine	1.6	1.5
Phentermine		

Figure 5: Calculated resolution for injection 1 and 1000 of methamphetamine and phentermine, one of the critical pairs, spiked at 2 ng/mL in urine.

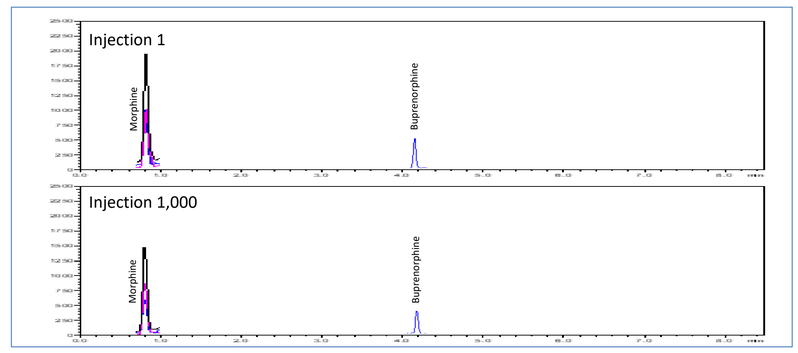


Figure 6: Performance two compounds spiked in urine at 2.5 ng/mL and monitored over 1000 injections. Morphine was used to demonstrate the robustness of an early eluting compound in matrix. Buprenorphine was used to demonstrate sensitivity over a lifetime test. The peak area percent difference was calculated to be 4% for buprenorphine between injection 1 and 1000.

Conclusions

Small ID columns can be beneficial in the reduction of solvent and sample consumption for greener solutions and cost savings. 2.1 mm ID demonstrates superior sensitivity over the 4.6 mm ID columns due to column efficiency, less analyte dilution, and reduced band broadening, while still achieving acceptable resolution for critical pairs. Even in matrix, the narrow-bore columns are reproducible and robust after repeat injections without increasing backpressure or clogging.