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Routine Switching between High and Low pH on XBridge HPLC Columns

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The effect of switching between high and low pH mobile phases on a single analytical HPLC column was investigated. The ability to rapidly switch between pH extremes on XBridge™ columns without special washing/re-equilibration steps dramatically reduces the time for separation of pharmaceutical compounds.

Introduction

The time required for drug screening, method development, and compound purification using chromatographic methods is a key factor in meeting the demands of the pharmaceutical industry. It is desirable to have technology that is robust and stable under many different operating conditions to maximize HPLC method development efficiency. XBridge columns have the flexibility to operate under a variety of mobile phase and pH conditions without deterioration in performance over several hundred hours of operation.

XBridge columns are the second generation of hybrid particle technology first introduced by Waters in 1999. They offer improved chromatographic lifetime in both low and high pH conditions while still maintaining the efficiencies associated with traditional silica-based HPLC columns. This makes XBridge suitable for fast drug screening and purification of pharmaceutical compounds.

The current work investigates the use of a single XBridge column at both low and high pH for the separation of acidic, basic, and neutral compounds. The mobile phase pH was varied at specific intervals to evaluate its effect on column performance. To date, few commercially available columns have the ability to switch between high and low pH without severe loss in chromatographic performance. Having the ability to switch between pH extremes on a single HPLC column can reduce instrument downtime by at least 50%, and allows for a broader range of operating conditions.

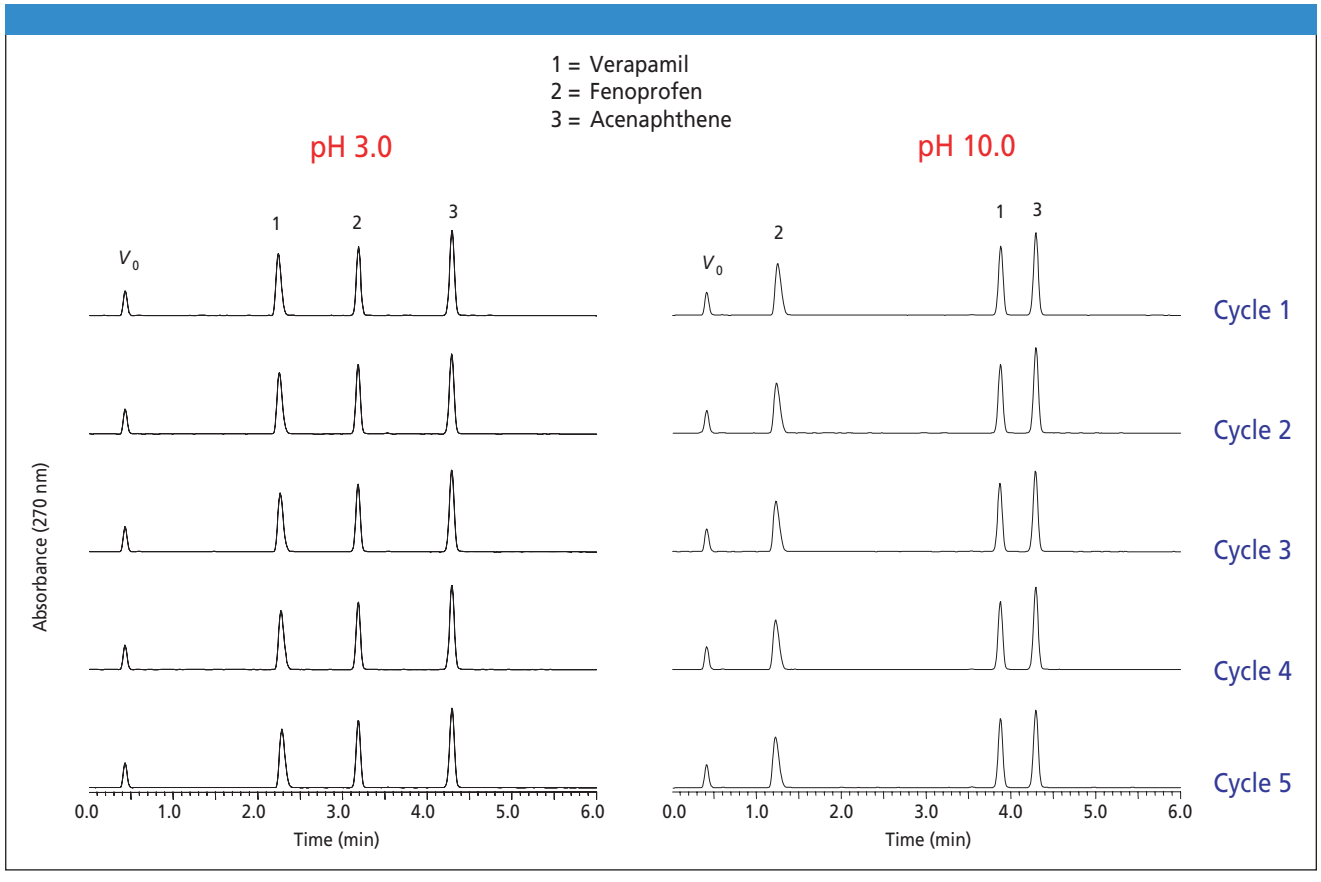

Figure 1: XBridge separation of verapamil, fenoprofen, and acenaphthene after successive cycles of switching between pH 3.0 and 10.0.

Table I: System suitability results for 3 compounds (100 injections at each pH)

	Verapamil				Fenoprofen				Acenaphthene			
	pH 10.0		pH 3.0		pH 10.0		pH 3.0		pH 10.0		pH 3.0	
	AVG	%RSD	AVG	%RSD	AVG	%RSD	AVG	%	AVG	%	AVG	%
RT (min)	3.88	0.0	2.26	0.7	1.23	0.7	3.18	0.0	4.30	0.0	4.29	0.0
Area (µV*sec)	646533	0.7	661156	0.9	620443	0.8	612723	1.1	781319	4.8	793385	2.8
k′	8.32	0.0	4.21	0.9	1.95	1.1	6.35	0.1	9.32	0.0	8.91	0.0
Selectivity	4.26	1.1	N/A*	N/A*	N/A*	N/A*	1.51	0.9	1.12	0.0	1.40	0.0
Resolution	21.63	0.4	17.17	0.4	7.02	1.4	8.36	2.6	3.94	0.4	10.70	0.6
USP Tailing	1.00	0.3	1.22	0.9	1.25	1.2	1.00	0.2	0.98	0.3	0.98	0.3
Width @ 4.4% (min)	0.129	0.8	0.148	1.5	0.164	0.6	0.121	1.1	0.133	0.4	0.133	0.5
Width @ 10% (min)	0.110	0.8	0.128	1.6	0.144	0.6	0.104	1.1	0.113	0.4	0.114	0.4

* No selectivity data is available for verapamil at pH 3.0 or fenoprofen at pH 10.0 because they are the first peaks to elute.

Experimental Conditions

LC System:	Alliance® 2695 with a 2996 PDA detector
Column:	XBridge C_{18}, 4.6 × 50 mm, 5 µm (Part # 186003113)
Mobile Phase:	A: 10 mM NH_4COOH, pH 3.0 OR 10 mM NH_4HCO_3, pH 10.0 B: Acetonitrile (ACN)
Gradient:	25–90% B in 5 minutes, hold at 100% B for 2 minutes, reset (10 minute total run time)
Flow Rate:	1.5 mL/min
Injection:	10 µL
Temperature:	30 °C
Detection:	UV @ 270 nm
Sampling rate:	2 Hz
Time Constant:	1.0
Test mixture:	10 µg/mL uracil (void marker), 100 µg/mL acenaphthene, and 200 µg/mL each of fenoprofen and verapamil in ACN:H_2O (1:1)

pH Switching Procedure

The column was first equilibrated at pH 3.0 for 10 minutes, followed by 20 injections of the test mixture. The column was then equilibrated at pH 10.0 for 10 minutes, followed by another 20 injections of the standard mixture. This cycle was repeated 4 more times (100 injections total at each pH).

Results and Discussion

Figure 1 demonstrates the separation performance of the XBridge C18 column after five cycles of switching between pH 3.0 and pH 10.0. It is clear that exposure to pH extremes does not compromise chromatographic performance on XBridge in subsequent pH cycles. In addition, reproducibility of the separation at each pH is exceptional (Table 1). These experiments clearly demonstrate the ability of XBridge columns to operate at high and low pH without loss of chromatographic performance. As a result, routine drug screening, method development, and purification of pharmaceutical compounds can be performed on one column.

Conclusions

The effect of switching mobile phase pH on a single HPLC column was investigated for a mixture of acidic, basic, and neutral compounds. After several cycles of exposure to high and low pH, no deterioration was seen with XBridge columns, which demonstrates the flexibility, strength, and reproducibility of hybrid particle technology. This makes XBridge an ideal choice for the routine screening, method development, and purification of pharmaceutical compounds.

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