New Generation of HPLC Silica-Based C18 Column for Both Highly Acidic and Basic pH: The Silia*Chrom*® SB C18 and Silia*Chrom*® XT C18 Fidelity

Charles Lévesque, Vincent Bédard and Francois Béland
SiliCycle® Inc. (www.silicycle.com) 2500 Parc-Technologique Blvd, Quebec City, Quebec, G1P 4S6, Canada
*(charleslevesque@silicycle.com)

Introduction

Acidic mobile phases have found widespread applications in the reversed-phase HPLC separation of many important pharmaceutical and environmental compounds. Analytes such as pharmaceuticals and biomolecules often show peak shape, retention and selectivity changes when the mobile phase pH is changed from neutral to acidic pH (pH=1.0). In fact, lowering the pH helps to suppress silanol interactions between basic solutes and the residual surface silanols, thus resulting in less tailing and better retention for acidic compounds (pKa lower than 2), thus increasing retention and altering selectivity (figure 1). For example, hydrophobic peptides and protein separations use TFA for solubility. Additionally, basic analytes are preferably positively charged in the HPLC eluent for positive-mode electrospray LC-MS applications thus requiring low pH mobile phases and high column robustness.

Also is well known in reversed phase chromatography that the uncharged form of a compound is best suited for troubleless HPLC (figure 1). Most basic drugs have pK_a values of around 9.5. To keep basic substances uncharged they must be kept in an environment with pH higher than its pK_a value of the analyte. Today, HPLC columns with silica based stationary phase for acidic and basic mobile phases are important solution for end-users in HPLC.

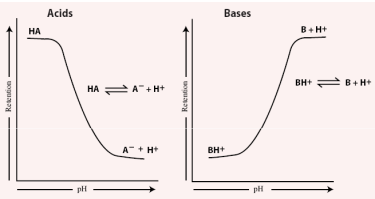


Figure 1: Effect of pH on the retention acids and bases in reversed phase HPLC

HPLC Column Evaluation

Silia*Chrom* SB C18 and Silia*Chrom* XT C18 Fidelity

Silia*Chrom* SB C18

The surface of Silia*Chrom* SB C18 is treated with an organic form of silicon to increase the number of silanol groups on the surface. After this, the surface is bonded with two types of octadecyl silanes. One of them has a protecting group that shields the area under it and protects the surface from an acid attack from the mobile phase (figure 2).

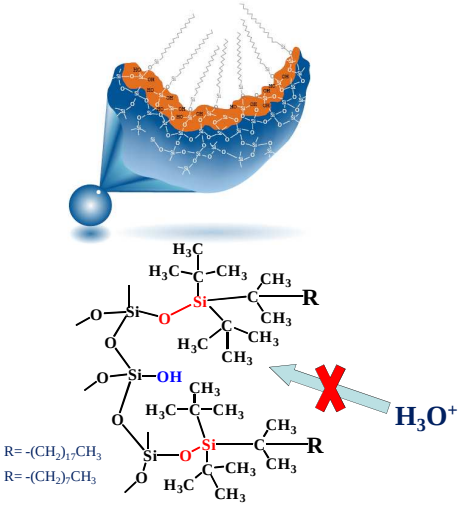


Figure 2 Silia*Chrom* SB C18 phase

Silia*Chrom* XT C18 Fidelity

The key is to have a hybrid particle or hydrid surface to reduce the solubility of silica at high pH. In fact, the Silia*Chrom* XT is silica coated with a prepolymer of ethyltriethoxysilane/tetraethoxysilane, followed by special thermo treatment to get a rigid surface that is less soluble than silica itself at high pH (figure 3)

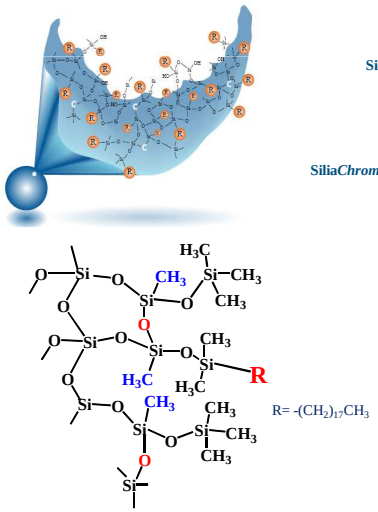


Figure 3 Silia*Chrom* XT C18 Fidelity phase

Stability Study

The new Silia*Chrom* SB and XT C18 5µm phase were packed in-house. The column configuration was 4.6mm x 150mm. The low and high pH stability test were performed using a Thermo Surveyor HPLC with variable UV detector monitoring absorbance at 270 nm, using a flow rate of 1.00 mL/min with the column heated at 35°C. A sample containing uracil (void marker), phenol, toluene and ethylbenzene was injected to get K' between 1.0 and 1.5 in order to be very sensitive to minute variations of the stationary phase. We have monitored all injections every 6 hours for a total of 120 hours. The flow was kept constant during 120 hours (1.00 mL/min, 35°C) with appropriate aggressive mobile phase:

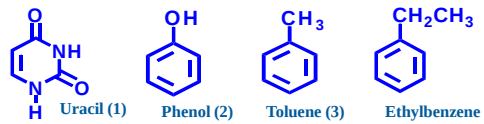
Low pH conditions

ACN:Water:TFA (59:39:2) **pH=1.00**
UV 270 nm
T=23.0°C
1.00 mL/min
Silia*Chrom* SB C18 , 5µm, 4.6x150mm (SiliCycle P/N: **HPC- 1105-15N**)

High pH conditions

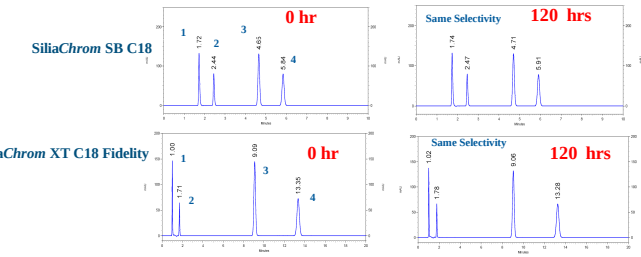
ACN:Water:TEA (54.9:44.9:0.2) **pH=11.50**
UV 270 nm
T=23.0°C
1.00 mL/min
Silia*Chrom* XT C18 Fidelity, 5µm, 4.6x150mm (SiliCycle P/N: **HPC-11005-15N**)

Test Mix:



Results

The chromatograms illustrate separations where the retention time for each analyte is constant. The Figure 2 shows no modification of retention time from the beginning (0 hour) and 120 hours later with the flow kept constant with the low or high pH mobile phase



Time/h	Retention time (Ethylbenzene) (min)	Tailing Factor (Ethylbenzene) (USP)	Theoretical Plates (Ethylbenzene) (USP)
0	5.91	1.01	14014
24	5.89	1.02	14085
48	5.77	1.02	14023
72	5.89	1.02	14076
96	5.85	1.01	14087
120	5.84	1.02	14050
SD	0.05	0.01	32
Mean	5.85	1.02	14056
RSD (%)	0.84	0.51	0.23

Time/h	Retention time (Ethylbenzene) (min)	Tailing Factor (Ethylbenzene) (USP)	Theoretical Plates (Ethylbenzene) (USP)
0	13.35	1.01	13623
24	13.39	1.01	13648
48	13.27	1.01	13669
72	13.25	1.00	13604
96	13.24	1.00	13649
120	13.28	1.00	13582
SD	0.04	0.01	38
Mean	13.28	1.01	13633
RSD (%)	0.29	0.54	0.28

Conclusion

This poster demonstrates clearly that the Silia*Chrom* SB C18 and Silia*Chrom* XT C18 Fidelity are very stable for aggressive mobile phases (low and high pH). The beauty is the fact that material for each one still to be silica based in order to keep the powerful efficiency in HPLC. We decided to stop the acquisition after 120 hours but the stability limit of those HPLC columns is not well known. We presume no degradation at all, for at least many months as typical HPLC column using buffer with pH range 2-8. Also we did not observed any tailing effect for toluene as XTerra® shown. It is well know that small molecules have some tailing with hybrid packing (hole trap). Silia*Chrom* SB C18 and XT C18 Fidelity are the best HPLC columns for method development especially using pH as a tool to have best HPLC analysis.

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