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SiliaChrom® Generic approach for HPLC analysis: The modern silica-based HPLC columns; SiliaChrom Series.

Introduction

HPLC method development for the analysis of mixtures is a task that usually requires much expertise and is also extremely time-consuming. In spite of advances in chromatographic theory, HPLC method development is still based mainly on "trial and error". Consequently, many attempts have been made to use computer programs to facilitate this process. In general, these are stand-alone programs, such as ChromSword® (Merck KGaA), DryLab® (Molnár-Institute) and others. But these approaches are very limited due to the fact that they require the use of an universal HPLC column.

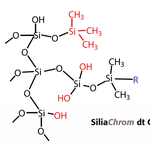
Based on the United States Pharmacopia (USP) website, there are about 380 different HPLC C18 columns on the market and this number increases each year. It's very challenging to find the best HPLC column to suit specific applications. The chromatographer needs a lot of experience to elaborate a quick and efficient HPLC method. To address this, SiliCycle has developped several C18 chemistries covering a very wide range of HPLC applications in combination with a generic approach in order to determine the best **Silia*Chrom*** column.

A Generic Approach to determine the best HPLC column to use

A Universal HPLC Column - Silia*Chrom* AQ C18 (or C8) and Silia*Chrom* dt C18

Features

- Rapid equilibration
- Unique reversed-phase selectivity (universal HPLC column)
- Exceptional stability from pH 1.5 to 9.0
- Reduce need for mobile-phase modifiers
- Compatible with 100% aqueous mobile phase (buffer)
- Long column lifetime
- Surface area: 380 m^2/g
- Carbon load: 18 %
- Endcapped: Partially endcapped (unique feature)
- USP L-code: L1



R = C18 or C8

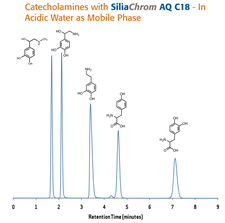
Silia*Chrom* AQ C18: Purity of 99.999%
Silia*Chrom* dt C18: Purity of 99.9999% (no metal content)

Optimum Ratio of TMS-Silanol-C18 (or C8)

Silia*Chrom* AQ (or **dt**) adsorbent employs an optimum ratio of C18 (or C8), short TMS chains and some free silanol groups. This new technology shows good peak shapes for any type of molecule (acid, neutral and basic).

Silia*Chrom* AQ Applications

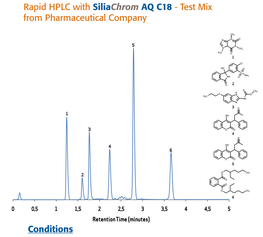
Catecholamines with **Silia*Chrom* AQ C18** - In Acidic Water as Mobile Phase



Conditions

Column: Silia*Chrom* AQ C18, 5 µm
Column Size: 4.6 x 150 mm
SiliCycle P/N: H151805E-N150
Mobile Phase: 1 % Acetic Acid in Water
Temperature: 23 °C
Flow Rate: 1.00 mL/min
Detector: UV at 265 nm
Injection Volume: 5 µL

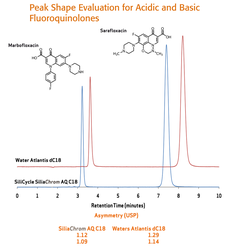
Rapid HPLC with **Silia*Chrom* AQ C18** - Test Mix from Pharmaceutical Company



Conditions

Column: Silia*Chrom* AQ C18, 5 µm
Column Size: 3.0 x 30 mm
SiliCycle P/N: H151805E-H030
Mobile Phase: MPA: 0.1 % TFA in ACN/Water (5/95)
MPB: 0.1 % TFA in ACN/Water (95/5)
Linear Gradient: MPA to MPB, 2.25 min.
Temperature: 23 °C
Flow Rate: 1.75 mL/min
Detector: UV at 254 nm
Injection Volume: 5 µL

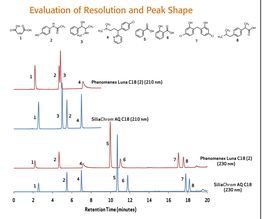
Peak Shape Evaluation for Acidic and Basic Fluoroquinolones



Conditions

Column: Silia*Chrom* AQ C18, 5 µm
Waters Atlantis dC18, 5 µm
Column Size: 4.6 x 150 mm
SiliCycle P/N: H151805E-N150
Mobile Phase: 2.5 mM potassium phosphate monobasic (adjust to pH 2.50 with H_3PO_4)/MeOH (68/32)
Temperature: 23 °C
Flow Rate: 1.00 mL/min
Detector: UV at 275 nm
Injection Volume: 10 µL

Evaluation of Resolution and Peak Shape

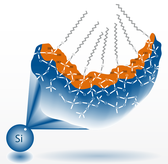


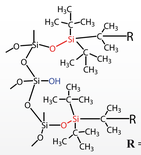
Conditions

Column: Silia*Chrom* AQ C18, 5 µm
Phenomenex Luna C18 (2), 5 µm
Column Size: 4.6 x 150 mm
SiliCycle P/N: H151805E-N150
Mobile Phase:
MPA: 5 mM potassium phosphate monobasic (adjust to pH 2.50 with H_3PO_4)/ACN (90/10)
MPB: 5 mM potassium phosphate monobasic (adjust to pH 2.50 with H_3PO_4)/ACN (10/90)
Linear Gradient: MPA to MPB, 20 min.
Temperature: 23 °C
Flow Rate: 1.00 mL/min
Detector: UV at 210 and 230 nm
Injection Volume: 20 µL

B HPLC Column for Low pH (0.5-7.5) Silia*Chrom* SB C18 or C8

Silia*Chrom* SB C18 surface is treated with an organic form of silicon to increase the number of silanol groups on the surface. After this step, the surface is bonded with a silane containing two fonctions. One of them has a protecting group that shields the area and protects the surface from an acid attack from the mobile phase.



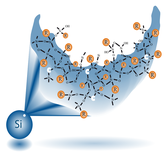


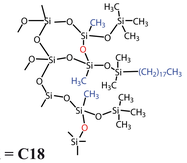
R = C18 or C8

The ion H_3O^+ doesn't have access to the surface to break the O-Si bond (Steric Effect)

C HPLC Column for High pH (1.0-12.0) Silia*Chrom* XT C18 Fidelity

The key is to have a hybrid surface to reduce the solubility of silica at high pH. In fact, **Silia*Chrom* XT C18** and **Silia*Chrom* XT C18 Fidelity** are silica coated with a monomeric ethyltriethoxysilane/tetraethoxysilane prepolymer, followed by a special thermic treatment to get a rigid surface that is less soluble than silica itself at high pH.

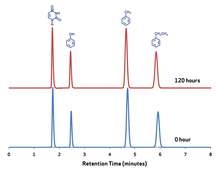




R = C18

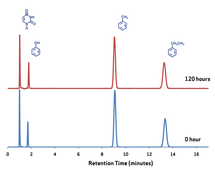
Stability Study of Silia*Chrom* SB C18 and Silia*Chrom* XT C18 Fidelity

Stability studies were performed on both low and high pH conditions using SiliaChrom SB C18 and XT C18 Fidelity HPLC columns respectively. Each HPLC column was used under extreme conditions and even after 5 days of continuous injections, the number of theoretical plates (N), the tailing factor (TF) and the retention times (RT) are comparable. Both sorbents kept their chemical and structural properties as proven by similar chromatograms and scanning electron microscope pictures (SEM) before and after 120 hours of use.



Conditions

Column: Silia*Chrom* SB C18, 5µm
Column Size: 4.6 x 150 mm
SiliCycle P/N: H101805H-N150
Mobile Phase: 2% TFS in ACN/Water (60/40)
Solution pH = 1.00
Temperature: 23 °C
Flow Rate: 1.00 mL/min
Detector: UV at 270 nm



Conditions

Column: Silia*Chrom* XT C18 Fidelity, 5µm
Column Size: 4.6 x 150 mm
SiliCycle P/N: HF171805H-N150
Mobile Phase: 0.2% TEA in ACN/Water (55/45)
Solution pH = 11.50
Temperature: 23 °C
Flow Rate: 1.00 mL/min
Detector: UV at 270 nm

Silia*Chrom* SB C18

Time (hour)	RT (min)	TF (USP)	N (USP)
0	5.91	1.01	14 014
24	5.89	1.02	14 085
48	5.77	1.02	14 023
72	5.83	1.02	14 076
96	5.85	1.01	14 087
120	5.84	1.02	14 050
Mean	5.85	1.02	14 056
RSD (%)	**0.84**	**0.51**	**0.23**

Silia*Chrom* XT C18 Fidelity

Time (hour)	RT (min)	TF (USP)	N (USP)
0	13.35	1.01	13 623
24	13.29	1.01	13 648
48	13.27	1.01	13 689
72	13.25	1.00	13 604
96	13.24	1.00	13 649
120	13.28	1.00	13 582
Mean	13.28	1.01	13 633
RSD (%)	**0.29**	**0.54**	**0.28**

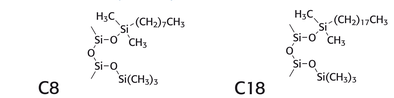
Silia*Chrom* SB C18

Silia*Chrom* SB C18 after 120 hours

Silia*Chrom* XT C18

Silia*Chrom* XT C18 after 120 hours

D Different Hydrophobicity (%C) Silia*Chrom* XDB & XDB1 C18 (or C8) and Silia*Chrom* XDB2 C18

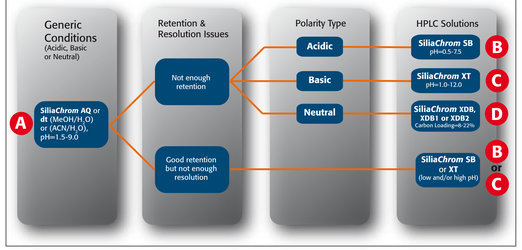


Carbon Loading
Silia*Chrom* XDB C8 (8%) and **Silia*Chrom* C18** (15%)
Silia*Chrom* XDB1 C8 (14%) and **Silia*Chrom* C18** (22%)
Silia*Chrom* XDB2 C18 (18%)

Conclusions

Generic Approach with Silia*Chrom* Series

It is possible, with **Silia*Chrom*** HPLC products, to consider a generic approach for HPLC method development by using just a few HPLC phases. Using only these three phases, we are able to obtain over 90% succes in separating most compounds from different matrices. In this poster, we have shown phases that are effective in low and high pH conditions and a new polar phase that works for practically any type of analyte (acidic, neutral and basic, limited to pH between 1.5-9). We have demonstrated that it is possible to have high efficiency at low cost by using **Silia*Chrom*** generic approach.



The authors would like to thank Ms. Stéphanie Parent for her contribution to this poster.

For further information

Please contact *info@silicycle.com*. More information on this poster and related projects can be obtained at *www.silicycle.com/silica-application-library*. You may as well get this poster in PDF version at *www.silicycle.com/poster*

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