Evaluation of pH Stability for an Organosilica-based HPLC Stationary Phase

Ilio Durandis, Jeffrey A. Kakaley, Laura Pankoe, J. Preston, PhD
YMC America, Inc., MA, USA

Abstract

Silica-based stationary phases are the workhorse for most HPLC methods. Mechanical strength, availability, and options for chemically bonding the stationary phase are a few of the many reasons for this. However, one significant limitation for pure silica base particles is the operating pH range. Typical silica particles are susceptible to dissolution above pH 8. There are many compounds, such as oligonucleotides, that need the eluent to be above pH 8 for suitable interaction with the stationary phase or for compound stability reasons. There are also many biomolecule methodologies that need to be cleaned with caustic NaOH as part of the process. Situations like these have disastrous effects on traditional silica-based media. Organosilanes have been developed to extend the working pH range for silica-based HPLC media and are well-suited to these high pH applications. The work presented here will describe a process to evaluate the pH stability of an organosilane HPLC media with an extended pH range and report the obtained results.

Objectives

Previous studies have explored the stability of bonded-phase silica columns, which is an important determinant for any successful HPLC method. There are various factors that can affect a column during method development, such as concentration of buffers, temperature, and organic modifiers to cite just these few. In this study, we set out to investigate the impact of low pH (<2.0) by using water with TFA, and high pH (>11.4) by using ammonium bicarbonate buffer on our YMC-Triart C18 column.

Table 1 Chromatographic method

Column	YMC-Triart C18 50 x 2.1mm, 5µm, 12nm	
UV Detector	254nm	
Mobile Phase A	0.1% TFA in water (pH ~ 2.1) low pH or 10mM ammonium bicarbonate (pH: 11.5) high pH	
Mobile Phase B	Acetonitrile	
Column Temp	25ºC	
Gradient	%A	%B
Initial (min)	95	5
5.00	5	95
5.1	95	5
9.0	95	5

For the blank injection, instead of stopping the gradient run at 9 minutes, we allowed it to run for 90 minutes (210 CV) with the gradient being held at 5%B.

The column stability will be evaluated against a few performance parameters such as theoretical plates (N), peak area, and resolution. We will also monitor change in column back pressure, since this can be impacted by mobile phase saturation.

Materials and Methods

Trifluoroacetic acid (Alfa Aesar)
Ammonium bicarbonate (MP Biomedicals)
DI water (In-house)
Ammonium hydroxide (Sigma Aldrich)
Acetonitrile (J.T. Baker)
HPLC Agilent 1260 Infinity
Agilent 1200 Infinity Diode Array Detector (DAD)
Agilent OpenLAB software was used to control HPLC and data processing
YMC-Triart C18 50x2.1 mm I.D, S-5µm, 12nm (P/N TA12S05-05Q1PTH)

The sample mix contained the following solutes:
Uracil, amitriptyline, hexanophenone, butyl paraben, toluene, and naphthalene.

A stock solution of each solute was prepared at 1mg/mL. The solutes were mixed at different concentrations in the final sample used for the experiment. The test mixture contained acidic, basic and neutral compounds to reflect some of the most common compounds used with this type of column (Table 2).

Table 2 Solutes and diluent used for test mix

Test compounds	Diluent for stock (1mg/mL)	Amount in test mix (µL)
Uracil (1)	50/50 Water/Acetonitrile	25
Butyl Paraben (2)		100
Toluene (3)		500
Naphthalene (4)		100
Hexanophenone (5)		25
Amitriptyline (6)		200

For the mobile phases, we used 0.1% TFA on water for the low pH experiment and a 10mM buffer of ammonium bicarbonate pH adjusted to 11.5 with ammonium hydroxide.

A new Triart C18 column was selected and equilibrated with the eluent for neutralization.

Table 3 Injections sequence

High pH Study	10 injections of test mixture, followed by 90 minutes of blank injections. This cycle was repeated 6 times for a total of 60 injections of sample mix and 5 blank injections, where 1160 CV of high pH mobile phase ran through the columns.
Low pH Study	10 injections of test mixture, followed by 90 minutes of blank injections. This cycle was repeated 6 times for a total of 60 injections of sample mix and five blank injections, where 1160 CV of low pH mobile phase ran through the columns.

Individual injections of each solute will be made to establish retention for peak identity and column back pressure (data not shown).

One column volume is equivalent to 0.17mL. One injection of our sample mix ran for 9 minutes at a flow rate of 0.4mL/min, which is approximately 21 column volumes. Thus, one cycle consists of 10 injections of the test mix and a 90-minute wash/equilibration with corresponding eluent of 232 CV.

Results

The pH stability evaluation was done by using 0.1% TFA in water as the low pH mobile phase, and 10mM ammonium bicarbonate adjusted to pH 11.5 with ammonium hydroxide.

The experimental design was to test the column stability with these low and high pH eluents in a gradient mode with acetonitrile (Table 1). A new YMC-Triart C18 column was equilibrated with the high buffer mobile phase and an individual injection of each solute was made to establish peak identity and retention time. Once all peaks were properly identified, a sample mix containing all solutes at various concentrations was injected for 10 injections, followed by a 90-minute equilibration step before the next 10 injections. This cycle was repeated five more times to establish the stability of the column under the different pH conditions. This would allow each set of pH eluent to run for a total of 1160 column volumes (Table 3).

Figure 1 YMC-Triart C18 column stability in 0.1% TFA in water (representative of sample mix chromatogram)

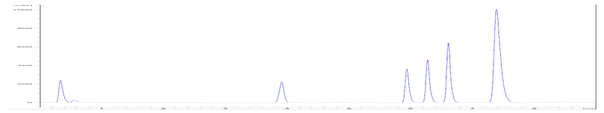


Figure 2 YMC-Triart C18 column stability in 0.1% TFA in water (acidic condition); overlay of injections after cycle 1, cycle 3, and cycle 6

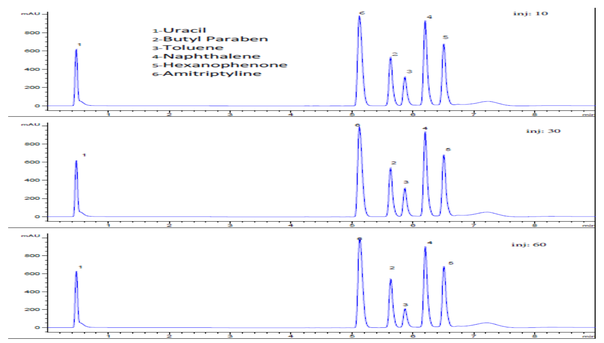
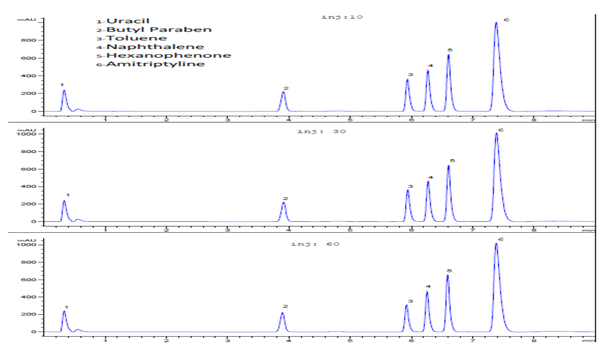


Figure 3 YMC-Triart C18 column stability in 10mM ammonium bicarbonate (basic condition); overlay of injections after 20 CV, 70 CV, and 100 CV



Results Cont'd

Table 4 Average theoretical plate using 10mM ammonium carbonate pH 11.5

Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	132	128	131	1.6
Propyl Paraben	13,337	13,610	13,582	1.1
Toluene	44,848	44,908	45,834	1.2
Naphthalene	51,893	51,758	53,084	1.3
Hexanophenone	54,456	55,003	56,538	2.0
Amitriptyline	25,074	24,416	25,848	2.9

Table 4a Average theoretical plate using 0.1%TFA in water

Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	550	552	555	0.46
Propyl Paraben	41,860	42,020	41,823	0.25
Toluene	43,282	44,023	42,896	1.31
Naphthalene	50,440	49,966	49,661	0.78
Hexanophenone	54,138	54,148	54,110	0.03
Amitriptyline	20,923	20,832	21,266	1.09

Table 5 Average resolution using 10mM ammonium carbonate pH 11.5

Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	N/A	N/A	N/A	N/A
Propyl Paraben	28.28	28.47	28.35	0.3
Toluene	16.33	16.37	16.53	0.6
Naphthalene	3.01	3.01	3.06	0.9
Hexanophenone	3.01	3.01	3.05	0.8
Amitriptyline	5.18	5.21	5.38	2.1

Table 5a Average resolution using 0.1%TFA in water

Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	NA	NA	NA	NA
Propyl Paraben	4.1	4.0	4.0	1.43
Toluene	2.11	2.11	2.1	0.27
Naphthalene	2.98	3.0	2.96	0.67
Hexanophenone	2.8	2.7	2.7	2.11
Amitriptyline	41.7	41.7	42.1	0.55

Table 6 Average peak area using 10mM ammonium carbonate pH 11.5

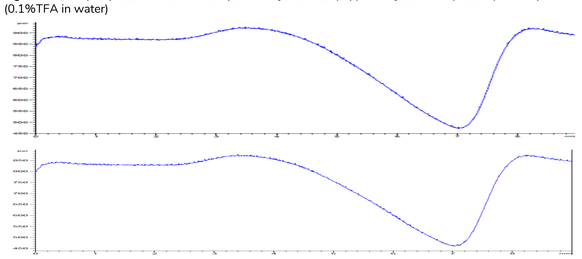
Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	1,094.95	1,110.21	1,115.10	0.9
Propyl Paraben	1,129.20	1,176.65	1,162.68	2.1
Toluene	1,586.16	1,556.30	1,343.68	8.8
Naphthalene	1,980.54	1,983.71	1,960.28	0.6
Hexanophenone	2,860.10	2,867.82	2,878.78	0.3
Amitriptyline	7,326.86	7,373.49	7,369.77	0.4

Table 6a Average peak area using 0.1%TFA in water

Compounds	Injection 10	Injection 30	Injection 60	%RSD
Uracil	2,053.25	2,081.15	2,097.12	1.07
Propyl Paraben	2,248.77	2,269.35	2,274.58	0.60
Toluene	1,351.32	1,151.09	892.41	20.33
Naphthalene	3,953.65	3,908.52	3,844.31	1.41
Hexanophenone	2,943.19	2,957.56	2,921.37	0.61
Amitriptyline	5,180.91	5,225.14	5,233.22	0.54

The chromatographic performances for the low and high pH experiments demonstrated that the YMC-Triart C18 column is very stable under the tested conditions. The percent RSD for the theoretical plates and resolution for all solutes were less than 3% RSD after more than 1160 column volumes (Tables 4-5A). The average peak area for each solute was well under 3% RSD, with the exception of toluene. That is not unusual for this analyte, since toluene can evaporate after multiple injections of the same vial. In Tables 6 and 6A, a significant decrease in area for the toluene peak can be observed.

Figure 4 Overlay of pressure trace for comparison injection 10 (top) and injection 65 (bottom) at low pH (0.1%TFA in water)



Conclusion

Column stability for a wide range of pH is a very critical aspect for chromatographic method development. This experiment seeks to demonstrate the durability of the YMC-Triart C18 column for low pH (~2.0) and high pH (11.5). The YMC-Triart C18 column was able to withstand extended exposure to ammonium bicarbonate buffer at pH 11.5 and 0.1% TFA in water at low pH 2.0, and still performed exceptionally well by maintaining low back pressure.

The data shown in Figures 2 and 3, as well as Tables 4-6A, confirmed that YMC-Triart C18 is very stable for the pH range of 2.0 to 11.5.

References

1- C. Ye, G. Terfloth, Y. Li, A. Kord. A systematic stability evaluation of analytical RP-HPLC columns, J. Pharmaceutical and Biomedical Analysis (2009), 50, 426-431.

2- W. J. Long. Low and High pH Stability of an Agilent Poroshell HPH C18; Agilent Technologies, Inc. Publication number 5591-6525EN