STABILITY OF HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) STATIONARY PHASES UNDER LOW AND HIGH PH TESTING CONDTIONS

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INTRODUCTION

HILIC stationary phase stability has been studied sporadically and under specific assay conditions. While this tests the stability of a given column for a given assay, direct comparisons between HILIC stationary phases can not be accurately drawn. However, by using accelerated pH stability tests with generic conditions across all columns, direct comparisons between columns can be investigated.

Accelerated low pH stability testing was performed on six stationary phases using 0.5% Trifluoroacetic acid (TFA) at 70° C. Additionally, an accelerated high pH stability test was performed on eight stationary phases in triplicate using a challenge mobile phase with ammonium bicarbonate at pH 11.3 at 70° C. Direct comparisons between similar stationary phases will be made.

METHODS

Low pH Stability Testing

System: ACQUITY® UPLC™ H-Class
Columns: 2.1 x 50 mm
Column Temp: 70 °C
Mobile Phase A: 0.5% TFA in Water
Mobile Phase B: 0.5% TFA in Acetonitrile
Flow Rate: 0.4 mL/min
Gradient: Start at 5% A, hold for 0.25 min. Linear gradient to 50% A in 2.75 minutes. Hold at 50% A for 0.5 minutes. Return to 5% A and hold for 2.5 minutes.
Sample: (25µg/mL) Trimethylphenyl Ammonium Chloride (25µg/mL) (TMPA), Sodium p-toluenesulfonate (Tosylate), and Uridine (7µg/mL).
Detection: UV @ 254 nm

High pH Stability Testing

System: ACQUITY® ARC™
Columns: 2.1 x 50 mm
Column Temp: 70 °C
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM ammonium formate pH 3.0
Mobile Phase D: 60:40 Acetonitrile:Water with 10mM ammonium bicarbonate pH 11.3
Flow Rate: 0.4 mL/min
Gradient: See **Table 1**.
Sample: mL), mL) (HILIC Acenaphthene (19µg/mL), Thymine (3.7µg/mL) Adenine (3.7 µg/mL) and Cytosine (7.7µg/mL) QCRM PN:186007226)
Detection: UV @ 260 nm

Table 1. Gradient Method for High pH stability Testing

Time (min)	%A	%B	%C	%D	Curve
0.00	0	95	5	0	-
13.73	0	0	0	100	11
34.30	0	0	0	100	11
35.97	50	50	0	0	6
39.27	50	50	0	0	6
40.93	10	90	0	0	6
44.23	10	90	0	0	6
45.90	0	95	5	0	11
68.13	0	95	5	0	11

RESULTS AND DISCUSSION

Low pH Stability Testing

Under low pH conditions, attached ligands are susceptible to being removed. The acidic additives catalyze the hydrolysis of the siloxane bonds[1] which typically hold the ligand to the base particle. This principle is well documented for reversed-phase stationary phases[2] but has not been studied for HILIC phases. Six columns were tested for low pH stability by first equilibrating each column to analysis conditions for 10 min (~35 CV). Next, replicate injections (n=61) of the test sample were performed (610 minutes total). Retention times for each probe were recorded and the % Change was calculated between injections 1 and 61. **Table 2** shows the results of accelerated low pH stability testing.

Table 2. *% Change in retention time for three probe analytes on 6 columns subjected to accelerated low pH stability testing.*

	% Change in RT		
Column	Uridine	TMPA	Tosylate
BEH HILIC	0.00	-4.55	2.03
BEH Amide	-7.03	0.00	-1.62
CORTECS HILIC	0.00	-6.35	-0.01
Ascentis Si	4.41	10.95	-8.18
Accucore HILIC	0.00	-1.60	0.93
ZIC-HILIC	0.00	0.00	-0.71

For most of the phases, the changes in retention were <10%, except for the Ascentis Si column which showed a 10.95% increase in retention for the basic probe trimethylphenyl ammonium chloride (TMPA). This would indicate that all tested phases are relatively stable at low pH, at 70° C. For some phases, such as the BEH HILIC, CORTECS HILIC, Ascentis Si, and Accucore HILIC, there is no attached ligand, which would account for the stability of these phases. For the two phases which have attached ligands, the columns appear to be designed for low pH stability as only minor changes in retention are detected.

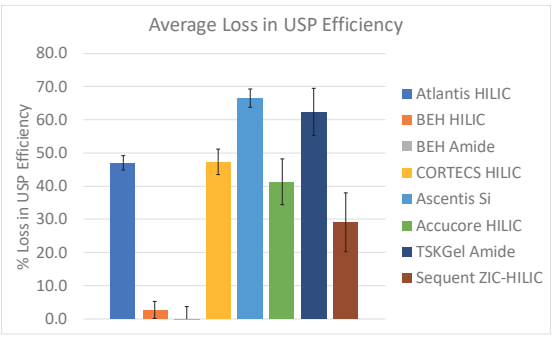
With good low pH stability, any of these columns can be used in assays with acidic mobile phase additives with confidence.

High pH Stability Testing

In reversed-phase liquid chromatography, the high pH stability of silica phases has been extensively studied.[3-5] It has well been established that in high pH mobile phases, the silica backbone of the particle is hydrolyzed leading to particle dissolution, and loss of efficiency. Theoretically, the same phenomenon should happen for HILIC columns, however no test has been performed to compare the stability of HILIC phases under such conditions.

Triplicate columns of eight HILIC stationary phases were evaluated for high pH stability using the conditions indicated. Each column was equilibrated to starting mobile phase conditions (95:5 Acetonitrile:Water with 5 mM ammonium formate pH 3.0) for 20 minutes (~80CV). Then 10 replicate injections of the test sample were performed using the gradient outlined in Table 1. The USP efficiency was recorded and the % Loss was determined. The average loss for each stationary phase was calculated (n=3) and compared. **Figure 1** shows the average %loss of USP plate count for each stationary phase using the most retained probe, cytosine.

Figure 1. Average %Loss in efficiency for Cytosine. Standard deviation represented by error bars.



The hybrid columns (BEH Amide and BEH HILIC) are very stable during this testing showing <5% loss in efficiency. Interestingly, the unbonded BEH particle is slightly less stable than the bonded BEH Amide. The attached amide ligand appears to help further protect the hybrid particle from being dissolved. This is not the case for TSKGel Amide, which shows poor stability. For that column, the amide group is not able to protect the silica particle from being dissolved, leading to a significant loss in efficiency.

The silica particles, as expected, are less stable under these test conditions. However, not all silica particles have the same stability. The Atlantis HILIC column, which shows a loss of ~50% appears more stable than the Ascentis Si column, which showed ~65% loss. This could be due to the difference in surface areas of the silica particle. The CORTECS and Accucore HILIC solid-core silica particles show a smaller difference in stability, which is not statistically significant.

After testing, select columns were opened to determine if bed loss occurred. **Figure 2** shows the packed beds of a BEH HILIC and CORTECS HILIC column after testing. As the images show, the hybrid particles show no signs of bed loss while the CORTECS HILIC bed is obviously degraded and a void has formed.

Figure 2. Pictures of the packed bed of CORTECS HILIC (left) and BEH HILIC (right) columns after high pH stability testing.

It would appear that silica particles are unstable at high pH both in reversed-phase and HILIC columns. While this is not unexpected, an in-depth look at HILIC columns had yet to be performed. Individual column stability has been examined for given assays, but columns have never been compared under standardized accelerated conditions. Based on these results, if an assay requires high pH mobile phase additives, it would be prudent to use a hybrid particle column, like the BEH HILIC or BEH Amide.

CONCLUSION

- **Six HILIC stationary phases showed good stability during the low pH stability testing, indicating all are appropriate for low pH assays.**
- **Eight HILIC stationary phases were tested in triplicate for high pH stability. Most of the columns showed >20% loss in USP efficiency during the test. The only phases found to be stable under the described test conditions were hybrid particle phases.**

References

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