
explore
LUNA®
HILIC
Discover
HPLC
Polar Retention
phenomenex®
. . . breaking with tradition℠

explore LUNA® HILIC

Leave Your Footprint!

Luna HILIC

Luna HILIC columns retain a water-enriched layer on the surface of the silica.

This water layer facilitates the transfer of polar compounds into the stationary phase for increased retention.

Separation is achieved through the partitioning of polar solutes from the high concentration, water-miscible, organic mobile phase into the hydrophilic surface environment. Polar solutes exhibit increased retention, and elute in the order of **increasing hydrophilicity.**

The Luna Legacy

Luna C18
Luna C18(2)
Luna C8
Luna C8(2)
Luna C5
Luna Phenyl-Hexyl
Luna CN
Luna NH_2
Luna SCX
Luna Silica(2)
NEW Luna HILIC

Luna HILIC lives up to the exacting standards of quality and customer satisfaction that has been exemplified in the 10 years of the Luna line.

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Luna HILIC delivers

- Superior retention of polar compounds
- Increased mass spec sensitivity
- Increased laboratory throughput and productivity

Finally, reproducible, robust HILIC separations

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Benefits of Luna HILIC

HILIC Method Development Strategy

explore LUNA® HILIC

Benefits of HILIC Chromatography

1 Polar Compound Retention

Polar Compounds on Luna HILIC

A note on analyte retention and solubility characteristics in HPLC:

An analyte must, to some degree, be soluble in or have an affinity for, both the column stationary phase and the mobile phase.

This principle is illustrated in the General Chromatographic Performance equation as retention factor or (*k*), a measure of the degree to which the compound is retained by the column. This critical parameter may be optimized to influence the overall chromatographic separation.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

Although the majority of HPLC separations today are performed under reversed phase conditions using hydrophobic stationary phases (for example, C18, C8, and Phenyl), hydrophilic (polar) compounds do not retain well under reversed phase conditions. The stagnant organic solvent present near the hydrophobic stationary phase is not conducive for interaction with the polar compounds; as a result these compounds are not well retained. They may elute near the void volume making quantitation difficult.

Luna HILIC allows increased retention of polar compounds in a highly organic mobile phase. The functionalized silica surface draws and retains water on the surface of the silica. This stagnant water-enriched layer facilitates the transfer of polar compounds into the stationary phase where analyte retention occurs. Under HILIC conditions, the more polar compounds will have a stronger interaction with this polar stationary phase and the retention factors for these compounds will increase.

Request the Technical Note!

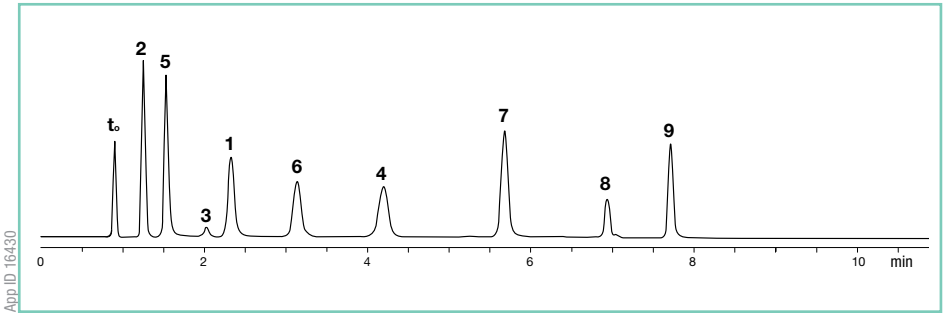
With HILIC mode techniques, polar compounds receive the highest degree of retention! As such, Luna HILIC will retain the polar compounds that elute near the void in reversed phase chromatography.

To effectively illustrate the competencies of HILIC mode chromatography, we have selected nine water-soluble vitamins, demonstrating diverse chemistry and selectivity, for use as probes.

Wide Variety of Functionalities and pK_a's
- Carboxylic Acids (pK_a 2.7 – 4.7)
- Amines (pK_a 3.4 – 5.6)
- Phosphate (pK_a 1.5)
- Quaternary Amine (pK_a 5.5)

Wide logP Range (5.4 Units)
- Minimum logP = –4.6 (Thiamine)
- Maximum logP = 0.83 (p-Aminobenzoic Acid)

Vitamin Mix on Luna HILIC



Column: Luna 5 µm HILIC
Dimension: 150 x 4.6 mm
Part No.: 00F-4450-E0
Mobile Phase: A: Acetonitrile
B: Water
C: 100 mM Ammonium Acetate, pH 5.8
Gradient: A/B/C (90:5:5) for 2.5 min to A/B/C (50:45:5) in 7.5 min, hold for 2.5 min. Re-equilibrate @ A/B/C (90:5:5) for 7.5 min.
Flow Rate: 2.0 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample: 1. p-Aminobenzoic Acid pK_a 4.7, H^+pK_a 2.7 logP 0.83
2. Nicotinamide H^+pK_a 3.35 logP –0.37
3. Riboflavin pK_a 10.2 logP –1.46
4. Nicotinic Acid pK_a 4.7, H+pK_a 3.0 logP 0.36
5. Pyridoxine H^+pK_a 5.6, p*K*a 8.6 logP –0.77
6. Thiamine H^+pK_a 5.5 logP –4.6
7. Ascorbic Acid pK_a 4.1, 11.2 logP –1.85
8. Cyanocobalamin pK_a 1.59 logP –0.90
9. Folic Acid pK_a 2.7, 4.1, 8.9 logP –0.02

Why Vitamins?

Vitamins provide an ideal platform to demonstrate the benefits of HILIC chromatography.

The chosen vitamins used in our testing incorporate the following:

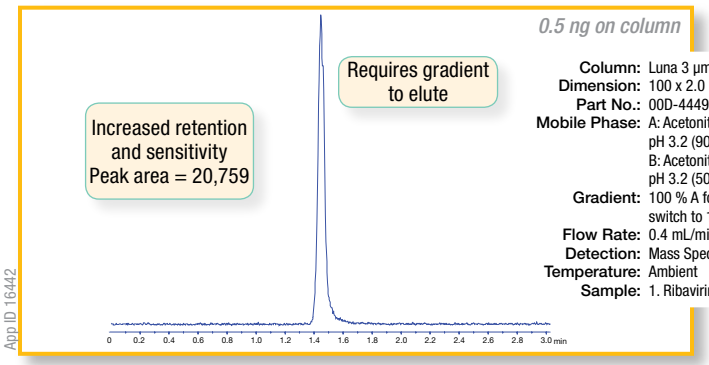
- Broad range of pK_a values
- Variety of functional groups
- Wide logP range (5.4 units)

The effect of increased polar compound retention can be easily seen through this group of vitamins.

Benefits of HILIC Chromatography

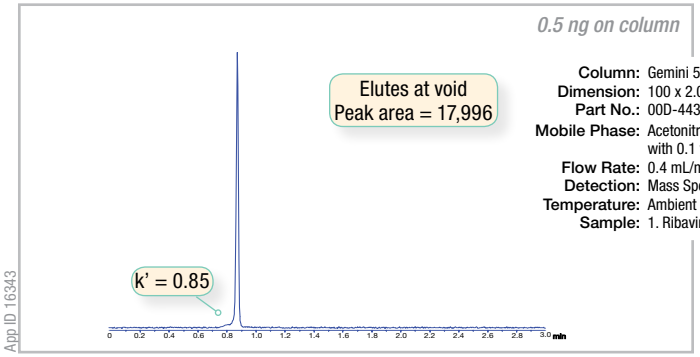
1 Polar Compound Retention

Ribavirin on Luna HILIC



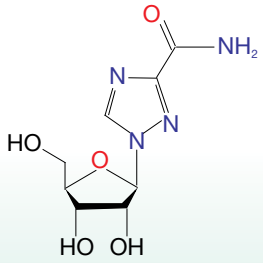
Column: Luna 3 µm HILIC
Dimension: 100 x 2.0 mm
Part No.: 00D-4449-B0
Mobile Phase: A: Acetonitrile/100 mM Ammonium Formate, pH 3.2 (90:10)
B: Acetonitrile/20 mM Ammonium Formate, pH 3.2 (50:50)
Gradient: 100 % A for 3 min, then 100 % B to 4.5 min, switch to 100 % A for 10 min
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS)
Temperature: Ambient
Sample: 1. Ribavirin (MRM: 245.2/113.2)

Ribavirin on C18



Column: Gemini 5 µm C18
Dimension: 100 x 2.0 mm
Part No.: 00D-4435-B0
Mobile Phase: Acetonitrile with 0.1 % v/v Formic Acid/Water with 0.1 v/v % Formic Acid (3:97)
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS) (ambient)
Temperature: Ambient
Sample: 1. Ribavirin (MRM: 245.2/113.2)

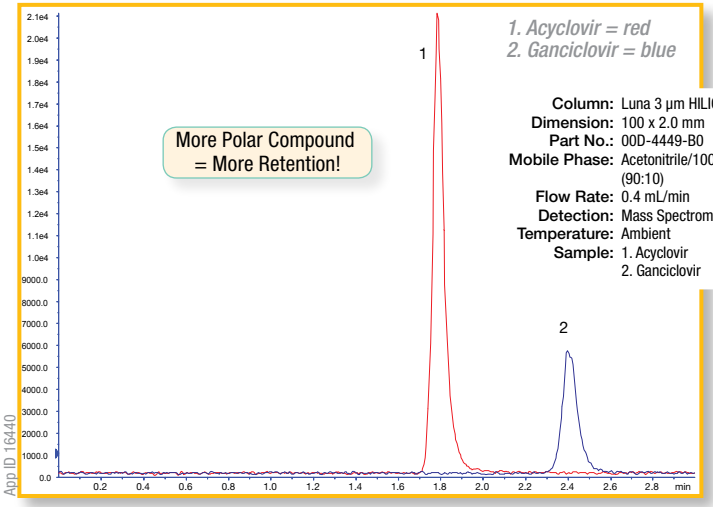
Ribavirin



- Highly polar compounds such as Ribavirin may be poorly retained on reversed phase columns
- HILIC techniques will increase polar compound retention and sensitivity

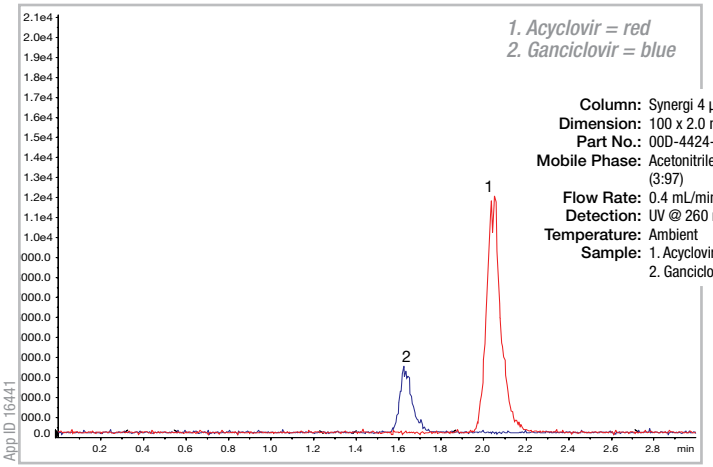
- Because HILIC uses a solvent system that is the reverse of what is common in reversed phase, the peak elution order is reversed on Luna HILIC.
- Polar/ Hydrophilic compounds that are particularly difficult to retain even on polar-embedded reversed phase columns will enjoy maximum retention on HILIC.

Ganciclovir & Acyclovir on Luna HILIC



Column: Luna 3 µm HILIC
Dimension: 100 x 2.0 mm
Part No.: 00D-4449-B0
Mobile Phase: Acetonitrile/100 mM Ammonium Formate, pH 3.2 (90:10)
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS)
Temperature: Ambient
Sample: 1. Acyclovir
2. Ganciclovir

Ganciclovir & Acyclovir on Polar-Embedded C18



Column: Synergi 4 µm Fusion-RP 80 Å
Dimension: 100 x 2.0 mm
Part No.: 00D-4424-B0
Mobile Phase: Acetonitrile/10 mM Ammonium Formate, pH 3.2 (3:97)
Flow Rate: 0.4 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample: 1. Acyclovir
2. Ganciclovir

Benefits of HILIC Chromatography

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2 Improved Mass Spec Sensitivity

Increased Sensitivity in LC-MS/MS with Luna HILIC

The analysis of bioanalytical samples in support of clinical and preclinical pharmacokinetic studies requires a highly sensitive analytical methodology, capable of achieving low detection and quantitation limits.

Obtaining very low LOD and LOQ levels can be achieved by an increase in the analyte signal, a decrease in the noise, or both.

Poor sensitivity is a problem observed for a number of analytes. The two most critical factors of sensitivity in LC-MS/MS are:

1. The chemical and physical properties of the sample
2. The composition of the mobile phase

We have illustrated an undesirable combination of these two factors coming together in the reversed phase application to the right.

1. Difficult to retain polar compounds
2. The biological matrix creates an ion suppression zone, or the area in which many of the endogenous compounds in the biological matrix elute - the result is a shortcoming of LC-MS/MS commonly referred to as the Matrix Effect.

Using the Luna HILIC column, we observe an increase in analyte retention away from the suppression zone - effectively decreasing noise. The high organic mobile phase offers more favorable desolvation and ionization conditions, resulting in an increase in analyte signal.

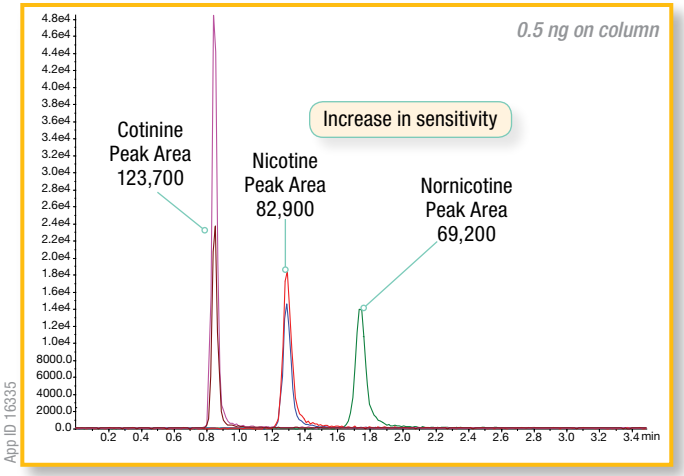
By creating these more favorable conditions for both retention and ionization, Luna HILIC columns allow for improved sensitivity in the analysis of bioanalytical samples.

Request the Technical Note!

The increased retention in HILIC allows elution of the analytes outside the suppression region and thus increased detector sensitivity. In addition to the increased retention and sensitivity, HILIC also resolved the compounds with the reverse order of that seen in RPLC.

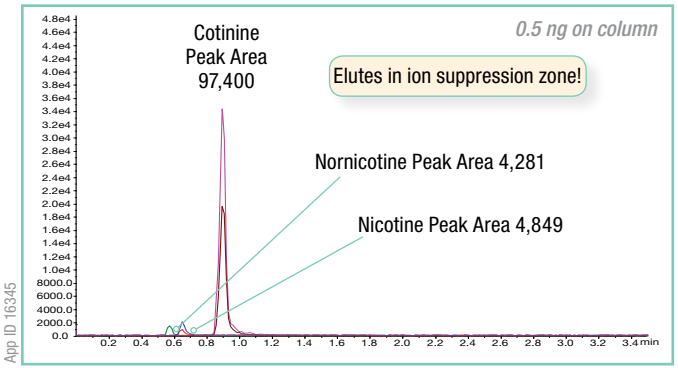
Elution reversal in HILIC can be a benefit when interferences are observed in RPLC.

Nicotine & Metabolites on Luna HILIC



Column: Luna 5 µm HILIC
Dimension: 100 x 2.0 mm
Part No.: 00D-4450-B0
Mobile Phase: Acetonitrile /100 mM Ammonium Formate, pH 3.2 (90:10)
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS)
Temperature: Ambient
Sample: 1. Cotinine (MRM: 177/80)
2. Nicotine (MRM: 163/80)
3. Nornicotine (MRM: 149/80)

Nicotine & Metabolites on C18



Column: Gemini 5 µm C18
Dimension: 100 x 2.0 mm
Part No.: 00D-4435-B0
Mobile Phase: A: Water with 0.1 % v/v Formic Acid
B: Acetonitrile with 0.1 % v/v Formic Acid
Gradient: A/B (97:3) hold 1 min, to (70:30) in 2 min, to (97:3) in 0.01 min, hold for 2.5 min
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS)
Temperature: Ambient
Sample: 1. Nornicotine (MRM: 149.1/80.1)
2. Nicotine (MRM: 163.3/105.9)
3. Cotinine (MRM: 177.3/80.1)

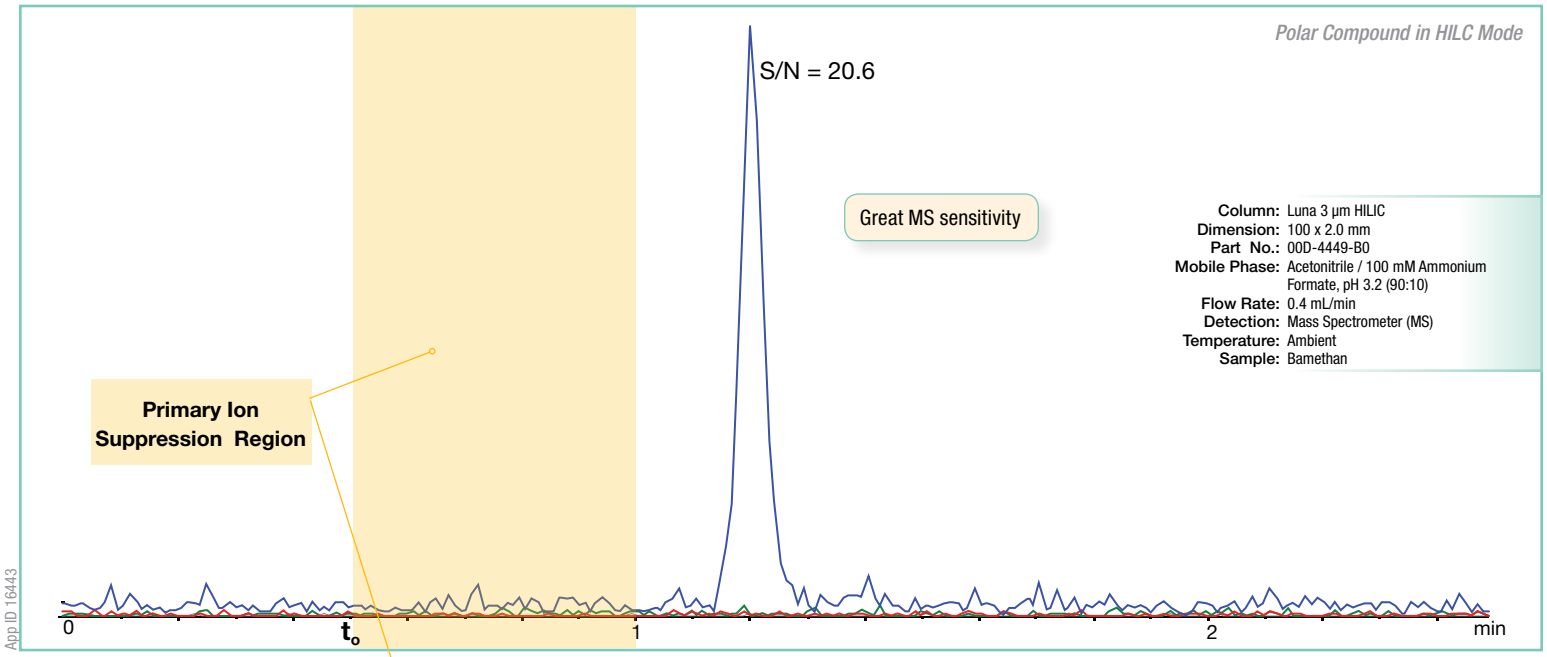
Benefits of HILIC Chromatography

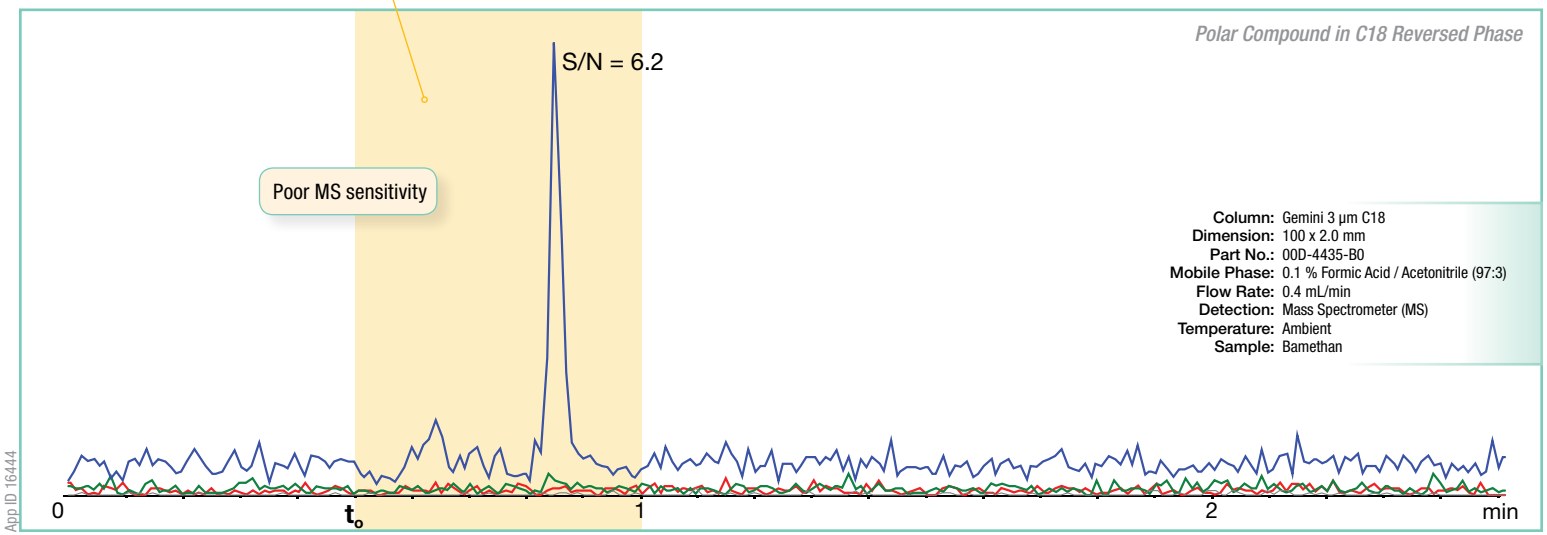
Improved Mass Spec Sensitivity

2

Luna HILIC allows low level polar metabolites to be retained on column past the critical ion suppression zone, allowing:

- Increased MS sensitivity
- Higher signal-to-noise ratio (S/N)





Ion Suppression Region is from 0.5-1.0 min

$t_o \cong 0.5$ min

$$k'=1=\frac{t_R-t_o}{t_o}=\frac{1-0.5}{0.5}$$

Benefits of HILIC Chromatography

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Increased Sample Throughput

3

Increased Sample Throughput with Luna HILIC

Rational method development today includes optimization of chromatographic conditions, detector conditions, and sample preparation design. Improving the interface between these three parameters can increase productivity and decrease analysis cost.

ADME and DMPK departments in pharmaceutical companies, as well as contract research organizations (CROs), provide an excellent example of the need for a more elegant chromatographic/ sample preparation(s) strategy. In this arena, the two most commonly used sample preparation techniques remain protein precipitation (PPT) and solid phase extraction (SPE).

The commonly used elution solvents for these techniques have stronger organic strength than the starting mobile phase used in typical reversed phase (RP) column gradient methods. This disparity will generally lead to significant band broadening or very early elution in subsequent chromatographic injections. Therefore, eluent evaporation, followed by reconstitution with a mobile phase compatible solvent is necessary. The elimination of these time-consuming steps would greatly enhance sample throughput in the high volume analysis lab.

Luna HILIC columns increase laboratory throughput by improving the interface between chromatography and sample cleanup. Luna HILIC allows for the direct injection of the high organic containing elution solvents of both SPE and protein precipitation. In the case of HILIC, these are weaker elution solvents than the HILIC mobile phase and will not contribute to band broadening or early elution. Elimination of the final evaporation and reconstitution steps can significantly decrease (up to 50 %) the total time spent on sample preparation.

Request the Technical Note!

HILIC mode allows you to directly inject after sample preparation in high organic eluents and therefore skip the time-consuming evaporation and reconstitution step.

HILIC Workflow

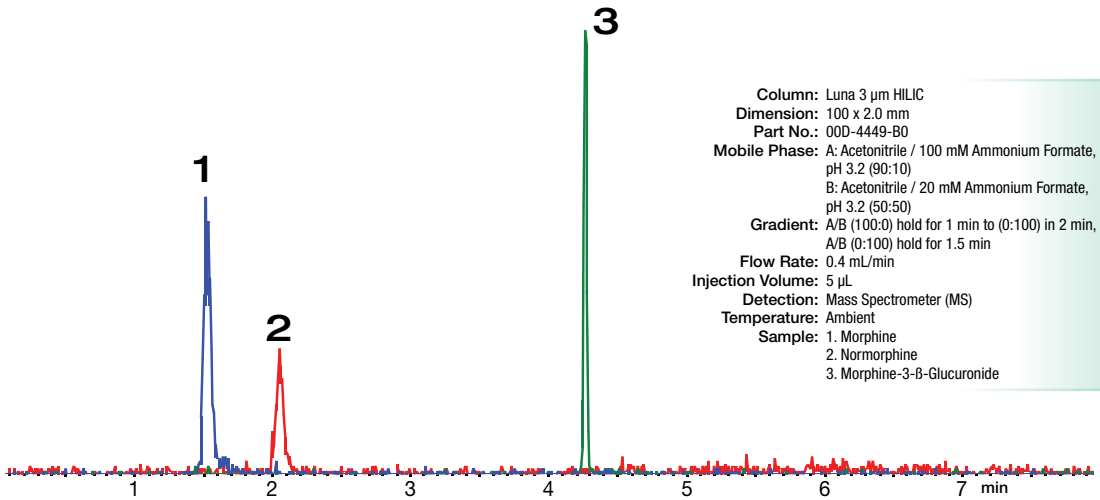
RP Workflow

Benefits of HILIC Chromatography

Increased Sample Throughput

Injected after Sample Preparation (5:1 Acetonitrile/Water)

In HILIC chromatography, a 5:1 acetonitrile : water injection solvent constitutes a 'weak' injection solvent. Morphine and metabolites were injected on the Luna HILIC column in a typical sample preparation eluent 5:1 acetonitrile : water and the compounds are well retained and well resolved.

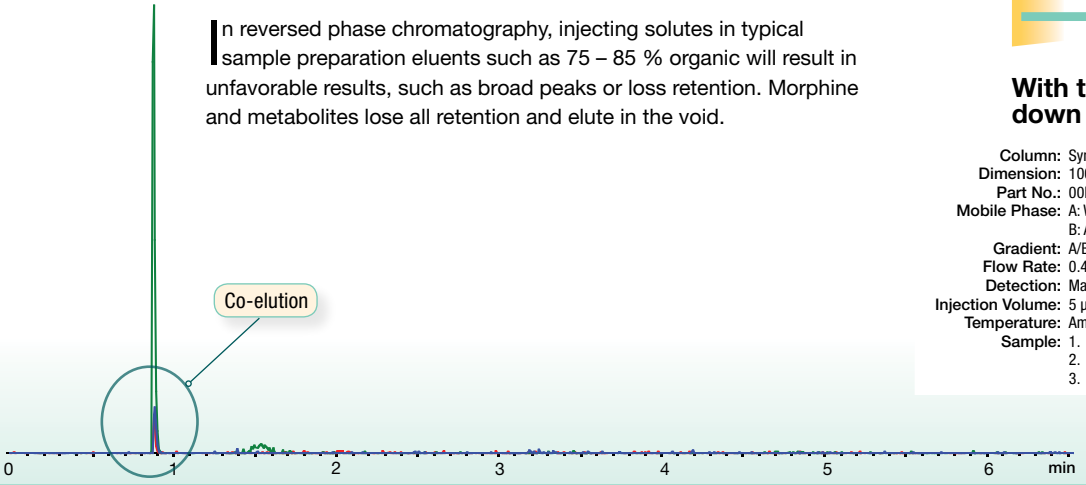


App ID 16445

Column: Luna 3 µm HILIC
Dimension: 100 x 2.0 mm
Part No.: 00D-4449-B0
Mobile Phase: A: Acetonitrile / 100 mM Ammonium Formate, pH 3.2 (90:10)
B: Acetonitrile / 20 mM Ammonium Formate, pH 3.2 (50:50)
Gradient: A/B (100:0) hold for 1 min to (0:100) in 2 min, A/B (0:100) hold for 1.5 min
Flow Rate: 0.4 mL/min
Injection Volume: 5 µL
Detection: Mass Spectrometer (MS)
Temperature: Ambient
Sample: 1. Morphine
2. Normorphine
3. Morphine-3-ß-Glucuronide

Injected after Sample Preparation (5:1 Acetonitrile/Water)

In reversed phase chromatography, injecting solutes in typical sample preparation eluents such as 75 – 85 % organic will result in unfavorable results, such as broad peaks or loss retention. Morphine and metabolites lose all retention and elute in the void.



App ID 16446

Injected after Evaporation and Reconstitution

With time consuming dry down / reconstitution step.

Column: Synergi 4 µm Polar-RP
Dimension: 100 x 2.0 mm
Part No.: 00D-4336-B0
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid
Gradient: A/B (97:3) hold for 2 min, to (50:50) in 2.8 min
Flow Rate: 0.4 mL/min
Detection: Mass Spectrometer (MS)
Injection Volume: 5 µL
Temperature: Ambient
Sample: 1. Morphine
2. Normorphine
3. Morphine-3-ß-Glucuronide

App ID 16447

Benefits of LUNA HILIC

explore LUNA® HILIC

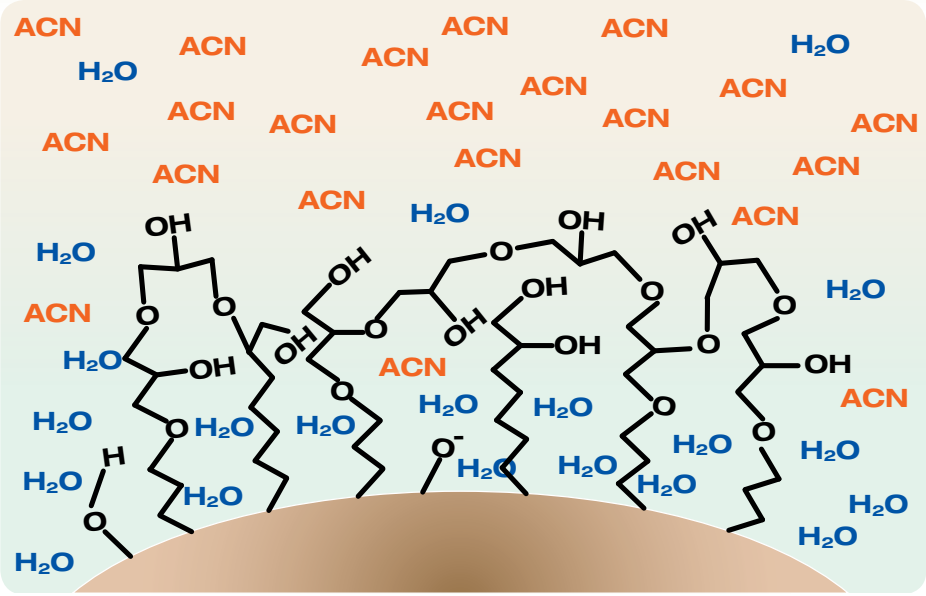
Stability

Luna HILIC Columns Utilize a Stable Cross-linked DIOL Phase

The stabilizing effects of our formulated ethylene bridges provide flexible covalent bonds that are robust enough to withstand harsh conditions that would cause most other Diol phases to bleed.

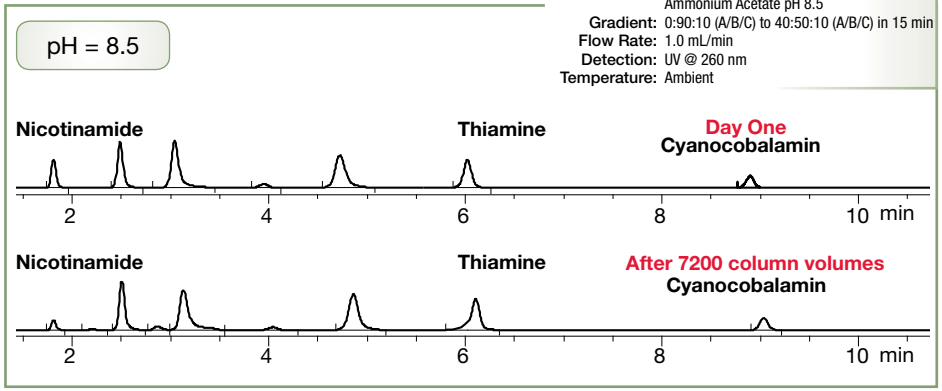
Diol phases provide:

- Attractive water layer retention
- Non pH dependent hydrogen bonding that is important to the HILIC retention mechanism
- Dipole-dipole interactions
- Absence of dissociable moieties



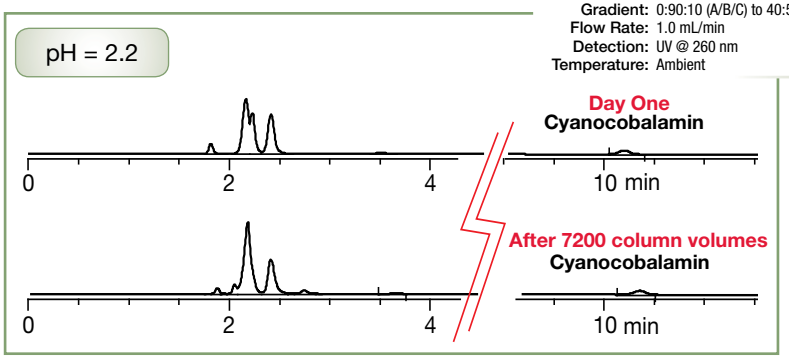
High pH Stability Test

Column: Luna 5 µm HILIC
Dimension: 150 x 4.6 mm
Part No.: 00F-4450-E0
Mobile Phase: A: Water, B: Acetonitrile, C: 100 mM Ammonium Acetate pH 8.5
Gradient: 0:90:10 (A/B/C) to 40:50:10 (A/B/C) in 15 min
Flow Rate: 1.0 mL/min
Detection: UV @ 260 nm
Temperature: Ambient



Low pH Stability Test

Column: Luna 5 µm HILIC
Dimension: 150 x 4.6 mm
Part No.: 00F-4450-E0
Mobile Phase: A: Water, B: Acetonitrile, C: 0.1 %TFA in Water, pH 2.2
Gradient: 0:90:10 (A/B/C) to 40:50:10
Flow Rate: 1.0 mL/min
Detection: UV @ 260 nm
Temperature: Ambient



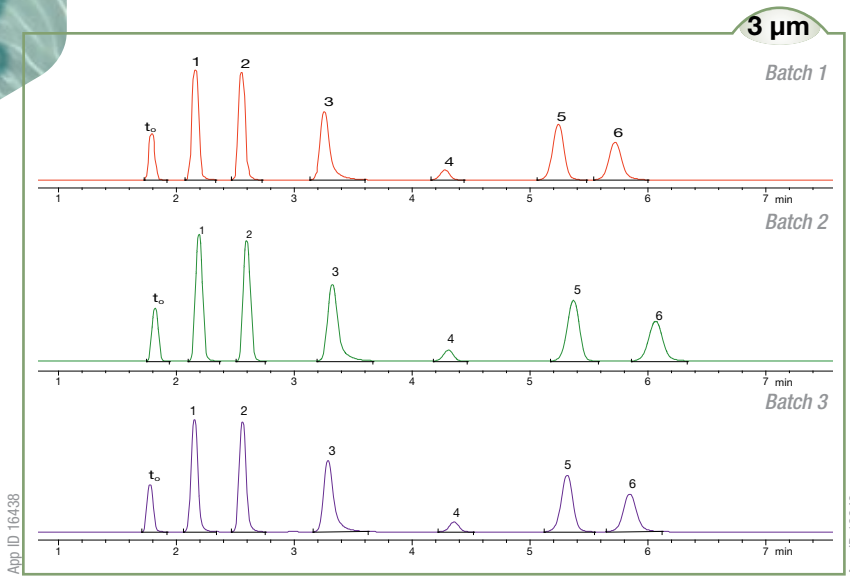
Addition of TFA

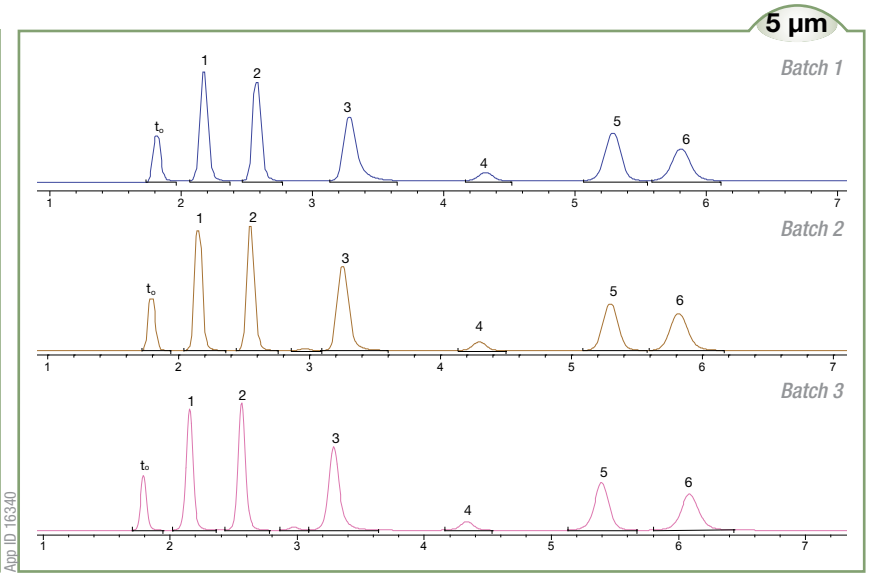
Please note the retention effects caused by the TFA modifier. This weak ion pair reagent associates with the charged groups on the polar vitamins and causes them to become increasingly hydrophobic, REDUCING retention in HILIC mode!

In this example, TFA was used to illustrate low pH stability. For recommended buffers, please see the HILIC LC/MS Method Development Strategy on pp. 12-15.

Benefits of LUNA HILIC

2 Batch-to-Batch Reproducibility

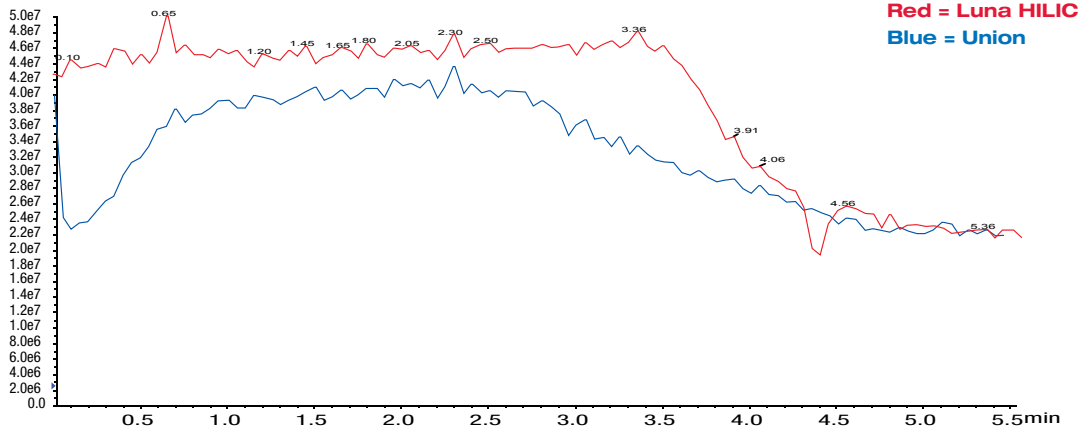




Columns: Luna 3 µm HILIC
Luna 5 µm HILIC
Dimension: 150 x 4.6 mm
Mobile Phase: A: Acetonitrile
B: 100 mM Ammonium Formate, pH 3.2
C: Water
Gradient: A/B/C (90:10:0) to (50:10:40) in 15 min
Flow Rate: 1.0 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample: 1. PABA
2. Nicotinamide
3. Riboflavin
4. Nicotinic Acid
5. Pyridoxine
6. Thiamine

3 Low MS-Bleed

The increased stability of the cross-linked DIOL provides the ultra-low bleed profile required for high sensitivity LC-MS/MS applications.



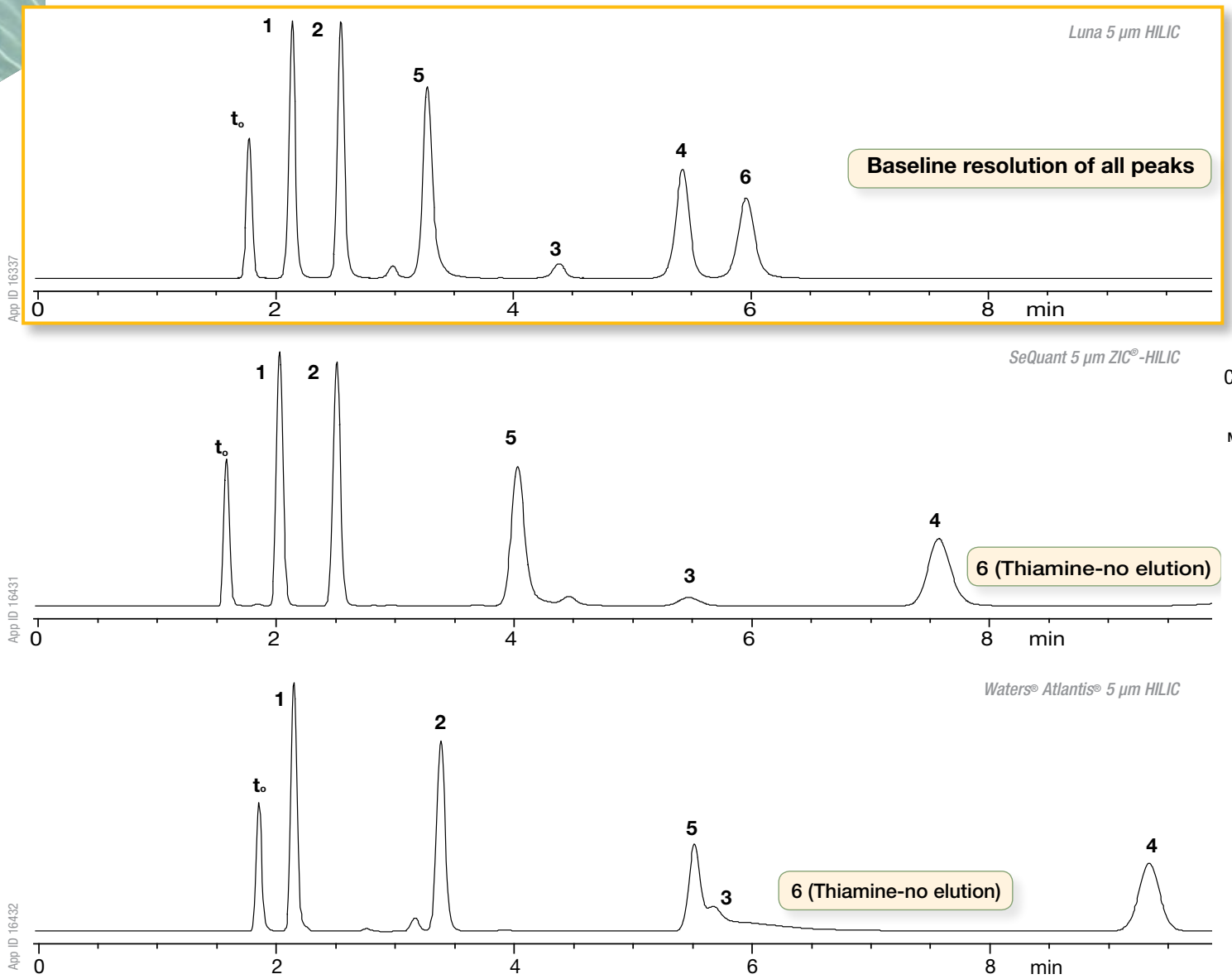
Mobile Phase: A: Water
B: Acetonitrile

Gradient: A/B (10:90) for 1.5 min to A/B (50:50) in 2 min, hold 1.5 min, step to A/B (10:90), hold for 5 min

Benefits of LUNA HILIC

Selectivity

4



Conditions same for all columns

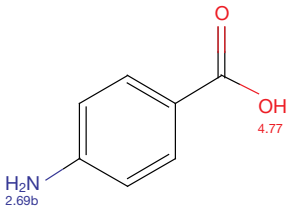
Columns: As noted
Dimension: 150 x 4.6 mm
Mobile Phase: Acetonitrile/100 mM Ammonium Formate, pH 3.2 (90:10)
Flow Rate: 1.0 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample:
1. PABA
2. Nicotinamide
3. Riboflavin
4. Nicotinic Acid
5. Pyridoxine
6. Thiamine

HILIC METHOD DEVELOPMENT STRATEGY

Step-by-Step Approach

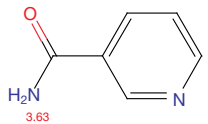
Probes:

1. p-Aminobenzoic Acid



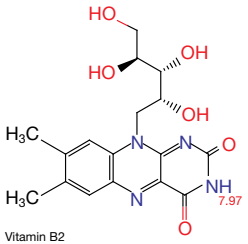
Vitamin B Complex
(logP=0.78, pK_a=4.77a, 2.69b)

2. Nicotinamide



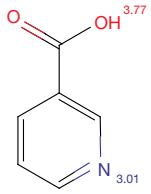
Pro-Vitamin B3
(logP=-0.61, pK_a=3.63b)

3. Riboflavin



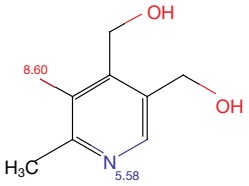
Vitamin B2
(logP=-1.46 , pK_a=7.97a)

4. Nicotinic Acid



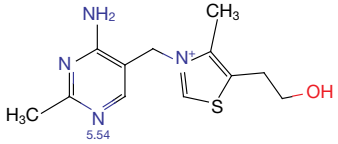
Niacin, B3
(logP=0.25, pK_a=3.77a, 3.01b)

5. Pyridoxine



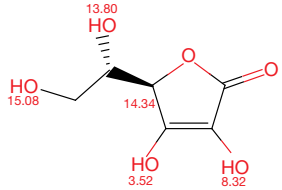
Vitamin B6
(logP=0.45, pK_a=5.58b, 8.60a)

6. Thiamine



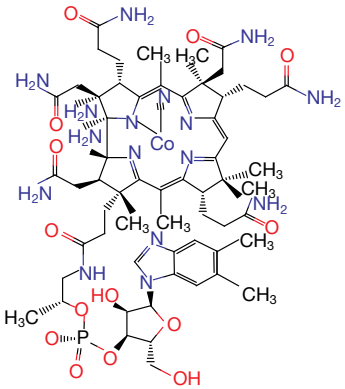
Vitamin B1
(logP=-4.63, pK_a=5.54b)

7. Ascorbic Acid



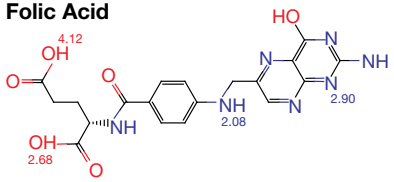
Vitamin C
(logP=-3.13, pK_a=3.52a, 8.32a)

8. Cyanocobalamin



Vitamin B12
(logP=-0.90, pK_a=1.59a, 6.00b, 8.93b)

9. Folic Acid



Vitamin B9
(logP=-0.02, pK_a=2.68a, 4.12a, 8.07a, 2.90b, 2.08b)

Vitamins Profile

The vitamins chosen have a wide scope of functionalities and demonstrate key points of HILIC chromatography well.

The chosen vitamins used in our testing incorporate the following:

- Broad range of pK_a values
- Variety of functional groups
- Wide logP range (5.4 units)

Objective

1. Quickly determine applicability of HILIC for your compounds
2. Approximate “best” separation conditions using set of conditions that provide the most information in the least time
3. Provide the basis for further method optimization, if needed

Part 1:

Method Development

Using set of generic conditions, quickly determine HILIC applicability. Screen for best starting conditions.

Part 2:

Evaluate Method

Evaluate the results of screening process

Part 3:

Method Optimization

Further optimization towards achieving desired criteria

Part 1: Method Development

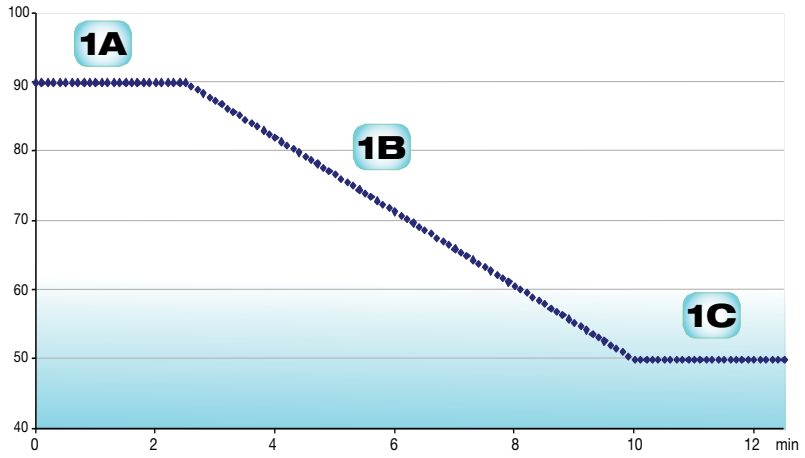
Step 1. Choose mobile phase conditions

Use an inverse gradient beginning at 90 % Acetonitrile

1A **Hold 90 % Acetonitrile isocratically for 2.5 min** - (period is used to determine if a compound is weakly retained in HILIC mode)

1B **The gradient part of the elution profile is the ideal elution area.** If a compound elutes during the gradient then there is maximum flexibility in adjusting selectivity

1C **Hold last 2.5 minutes isocratically at 50 % Acetonitrile.** (This period is used to determine if a compound is strongly retained in HILIC mode)



Step 2. Prepare buffer solutions

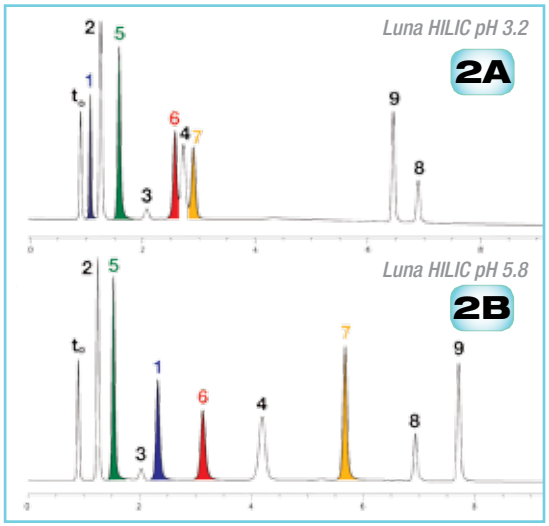
Screening both acidic and basic mobile phase conditions

2A 100 mM Ammonium Formate, pH 3.2: Weigh approx 6.31 g of Ammonium Formate and dissolve in 1 liter (MQ) water. Add 10.5 mL of formic acid and mix well. Take 10 mL of the aliquot and measure the pH of the aliquot.

2B 100 mM Ammonium Acetate, pH 5.8: Weigh approx 7.71 g of Ammonium Acetate and dissolve in 1 liter (MQ) water. Add 0.5 mL of acetic acid and mix well. Take 10 mL of the aliquot and measure the pH of the aliquot.

The buffer solutions recommended in 2A and 2B have been selected based upon:

- **The pH desired to attain separations with polar analytes based upon hydrogen bonding**
- **High solubility in eluents with high Acetonitrile content**
- **Compatibility with MS detection**



Practical Considerations for HILIC Gradient:

- Make three (3) injections to test equilibration
- Equilibration ~ 10 Column Volumes at 90 % Acetonitrile

Practical Considerations for HILIC Buffering:

- pH values used here are aqueous pH values
- pK_a's of acidic compounds increase with increasing organic
- pK_a's of basic compounds decrease with increasing organic

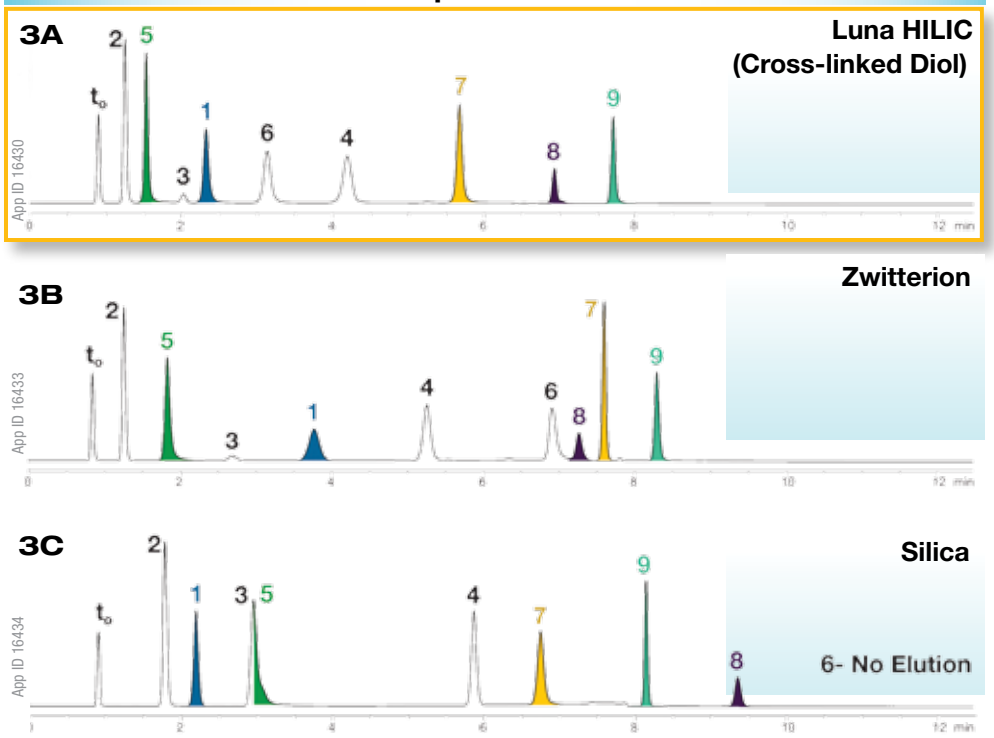
HILIC METHOD DEVELOPMENT STRATEGY

Part 1: Method Development

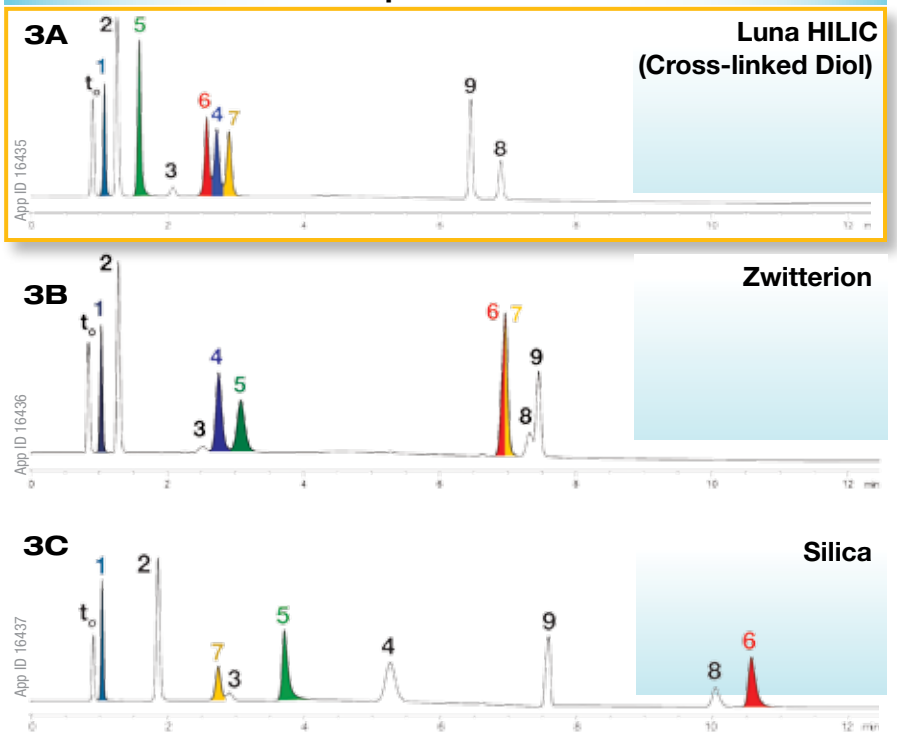
Step 3. Screen alternate column chemistries in both buffer solutions

- There are significant selectivity differences between HILIC columns. Apply generic starting gradient, screen all three columns with both buffer solutions.

pH 5.8



pH 3.2



Columns: As noted
Dimension: 150 x 4.6 mm
Mobile Phase: A: Acetonitrile
B: Water
C: 100 mM Ammonium Acetate, pH 5.8
Gradient: A/B/C (90:5:5) for 2.5 min to A/B/C (50:45:5) in 7.5 min, hold for 2.5 min. Re-equilibrate @ A/B/C (90:5:5) for 7.5 min.
Flow Rate: 2 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample: 1. PABA, 2. Nicotinamide, 3. Riboflavin, 4. Nicotinic Acid, 5. Pyridoxine, 6. Thiamine, 7. Ascorbic Acid, 8. Cyanocobalamin, 9. Folic Acid

Columns:
(3A) Luna 5 µm HILIC
(3B) SeQuant 5 µm ZIC® - HILIC
(3C) Luna 5 µm Silica (2)

Columns: As noted
Dimension: 150 x 4.6 mm
Mobile Phase: A: Acetonitrile
B: Water
C: 100 mM Ammonium Formate, pH 3.2
Gradient: A/B/C (90:5:5) for 2.5 min to A/B/C (50:45:5) in 7.5 min, hold for 2.5 min. Re-equilibrate @ A/B/C (90:5:5) for 7.5 min.
Flow Rate: 2 mL/min
Detection: UV @ 260 nm
Temperature: Ambient
Sample: 1. PABA, 2. Nicotinamide, 3. Riboflavin, 4. Nicotinic Acid, 5. Pyridoxine, 6. Thiamine, 7. Ascorbic Acid, 8. Cyanocobalamin, 9. Folic Acid

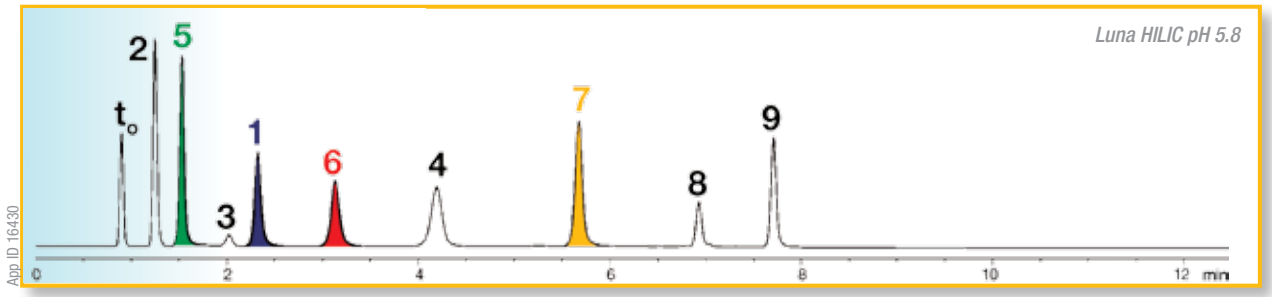
Part 2: Evaluate Method

Rank the best results from your screening process

- Look for the critical compound(s) that matters for your separation goals
- Reproducibility, availability and robustness will matter in the future, keep all your goals in mind

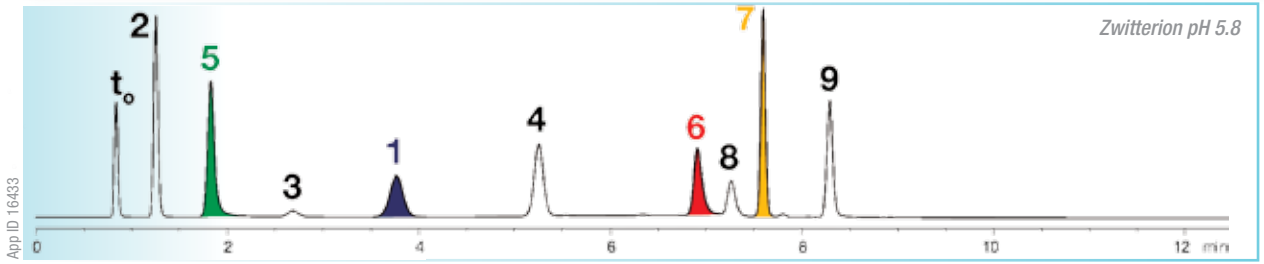
Ranking

#1

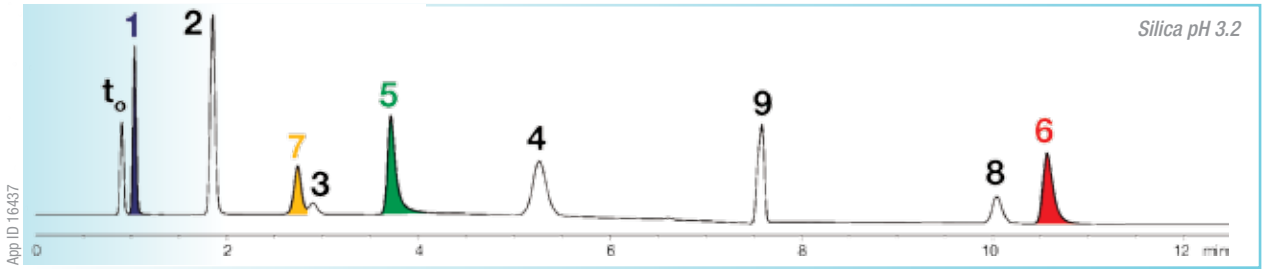


In this example, Luna HILIC gave the best performance in terms of retention, selectivity and peak shape

#2



#3



Practical Considerations for Method Evaluation:

- If results meet your criteria in terms of retention time, selectivity, and efficiency, you may have your final method.
- If adjustments are still needed, see the following section on method optimization.

Part 3: Method Optimization

Important considerations for method optimization

The most suitable column and buffer solution to continue with will have shown favorable signs of retention, selectivity, and peak shape – we now seek to improve them.

Characteristic performance criteria are approximated below.

Retention Time

k'>1 to reduce ion suppression

k'<10 for fast cycle

Selectivity

$1.1 < R_S < 1.5$ optimal for MS

Efficiency/ Peak Shape

Maximize N

0.8 < Asym < 1.5

To achieve these performance criteria in HILIC mode, we suggest:

1. Adding organic modifier

2. Adjusting ionic strength

3. Adjusting initial % organic modifier

Important Considerations for Adding Organic Modifier:

- May produce some unexpected results
- Addition of ethyl acetate increases the retention and selectivity of some compounds
- Addition of THF generally reduces retention and selectivity for all compounds

Important Considerations for Adjusting Ionic Strength:

- Ionic strength can be an effective parameter for adjusting selectivity, retention, and efficiency
- Start with minimum 5 mM buffer for best results

Important Considerations for Initial % Organic Modifier:

- Impact of 5 % increase very noticeable
- Surface equilibration is a relatively slow process

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With over 10 years of proven reproducibility, you can be confident in your choice to develop methods on Luna

3 µm Minibore Columns (mm)				SecurityGuard™ Cartridges
	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0 mm *for ID: 2.0-3.0 mm*
Phases	-	-	-	-/10pk
HILIC	00B-4449-B0	00D-4449-B0	00F-4449-B0	AJ0-8328
Silica(2)	00B-4162-B0	00D-4162-B0	00F-4162-B0	AJ0-4347
C8(2)	00B-4248-B0	00D-4248-B0	00F-4248-B0	AJ0-4289
C18(2)	00B-4251-B0	00D-4251-B0	00F-4251-B0	AJ0-4286
CN	00B-4254-B0	00D-4254-B0	00F-4254-B0	AJ0-4304
Phenyl-Hexyl	00B-4256-B0	00D-4256-B0	00F-4256-B0	AJ0-4350
NH_2	00B-4377-B0	00D-4377-B0	00F-4377-B0	AJ0-4301

3 µm Analytical Columns (mm)					SecurityGuard™ Cartridges	
	50 x 3.0	150 x 3.0	100 x 4.6	150 x 4.6	4 x 2.0 mm *for ID: 2.0-3.0 mm*	4 x 3.0 mm *for ID: 3.2-8.0 mm*
Phases	-	-	-	-	-/10pk	-/10pk
HILIC	00B-4449-Y0	00F-4449-Y0	00D-4449-E0	00F-4449-E0	AJ0-8328	AJ0-8329
Silica(2)	—	00F-4162-Y0	00D-4162-E0	00F-4162-E0	AJ0-4347	AJ0-4348
C8(2)	00B-4248-Y0	00F-4248-Y0	00D-4248-E0	00F-4248-E0	AJ0-4289	AJ0-4290
C18(2)	00B-4251-Y0	00F-4251-Y0	00D-4251-E0	00F-4251-E0	AJ0-4286	AJ0-4287
CN	00B-4254-Y0	00F-4254-Y0	00D-4254-E0	00F-4254-E0	AJ0-4304	AJ0-4305
Phenyl-Hexyl	00B-4256-Y0	00F-4256-Y0	00D-4256-E0	00F-4256-E0	AJ0-4350	AJ0-4351
NH_2	00B-4377-Y0	00F-4377-Y0	00D-4377-E0	00F-4377-E0	AJ0-4301	AJ0-4302

SecurityGuard™ Analytical Cartridges require universal holder Part No.: KJ0-4282

5 µm Minibore Columns (mm)	SecurityGuard™Cartridges
	100 x 2.0	4 x 2.0 mm *for ID: 2.0-3.0 mm*
Phases	-	-/10pk
HILIC	00D-4450-B0	AJ0-8328

SecurityGuard™ Analytical Cartridges require universal holder Part No.: KJ0-4282

5 µm Analytical Columns (mm)					SecurityGuard™ Cartridges	
	150 x 3.0	100 x 4.6	150 x 4.6	250 x 4.6	4 x 2.0 mm *for ID: 2.0-3.0 mm*	4 x 3.0 mm *for ID: 3.2-8.0 mm*
Phases	-	-	-	-	-/10pk	-/10pk
HILIC	00F-4450-Y0	00D-4450-E0	00F-4450-E0	00G-4450-E0	AJ0-8328	AJ0-8329
Silica(2)	—	00D-4274-E0	00F-4274-E0	00G-4274-E0	AJ0-4347	AJ0-4348
C5	00F-4043-Y0	00D-4043-E0	00F-4043-E0	00G-4043-E0	AJ0-4292	AJ0-4293
C8	00F-4040-Y0	00D-4040-E0	00F-4040-E0	00G-4040-E0	AJ0-4289	AJ0-4290
C8(2)	00F-4249-Y0	00D-4249-E0	00F-4249-E0	00G-4249-E0	AJ0-4289	AJ0-4290
C18	00F-4041-Y0	00D-4041-E0	00F-4041-E0	00G-4041-E0	AJ0-4286	AJ0-4287
C18(2)	00F-4252-Y0	00D-4252-E0	00F-4252-E0	00G-4252-E0	AJ0-4286	AJ0-4287
CN	00F-4255-Y0	00D-4255-E0	00F-4255-E0	00G-4255-E0	AJ0-4304	AJ0-4305
Phenyl-Hexyl	00F-4257-Y0	00D-4257-E0	00F-4257-E0	00G-4257-E0	AJ0-4350	AJ0-4351
NH_2	00F-4378-Y0	00D-4378-E0	00F-4378-E0	00G-4378-E0	AJ0-4301	AJ0-4302
SCX	—	00D-4398-E0	00F-4398-E0	00G-4398-E0	AJ0-4307	AJ0-4308

Material Characteristics						
Packing Material	**Particle Shape/ Size (µm)**	**Pore Size (Å)**	**Surface Area (m^2/g)**	**Carbon Load %**	**End Capping**	**pH Range**
Luna HILIC	Spherical 3, 5	200	200	5.7	No	1.5-8.0
Luna C5	Spherical 5, 10	100	440	12.5	Yes	1.5-10
Luna C18(2)-HST	Spherical 2.5	100	400	17.5	Yes	1.5-10
Luna C18(2)	Spherical 3, 5,10,15	100	400	17.5	Yes	1.5-10
Luna C8(2)	Spherical 3, 5, 10, 15	100	400	13.5	Yes	1.5-10
Luna Phenyl-Hexyl	Spherical 3, 5, 10, 15	100	400	17.5	Yes	1.5-10
Luna Silica (2)	Spherical 3, 5, 10, 15	100	400	—	No	—
Luna CN	Spherical 3, 5, 10	100	400	7.0	Yes	1.5-7.0
Luna NH_2	Spherical 3, 5, 10	100	400	9.5	No	1.5-11
Luna SCX	Spherical 5, 10	100	400	0.55 % Sulfur Load	No	2.0-7.0

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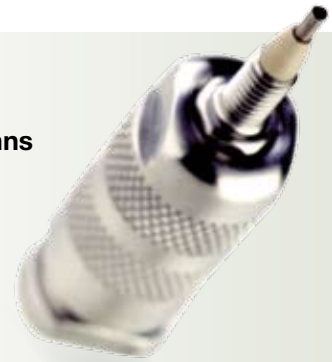

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