HILIC Chromatography: When and How?

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January 25, 2022

Agilent InfinityLab

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Agenda

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1 What is HILIC and when should you consider it?

2 HILIC method development

- Agilent HILIC column options
- Mobile phase considerations

3 Tips and tricks for successful HILIC column use and care

- Column equilibration
- Sample solvent compatibility
- Inert HILIC solution for metal-sensitive compounds

4 Summary

What Is HILIC and When Should You Consider It?

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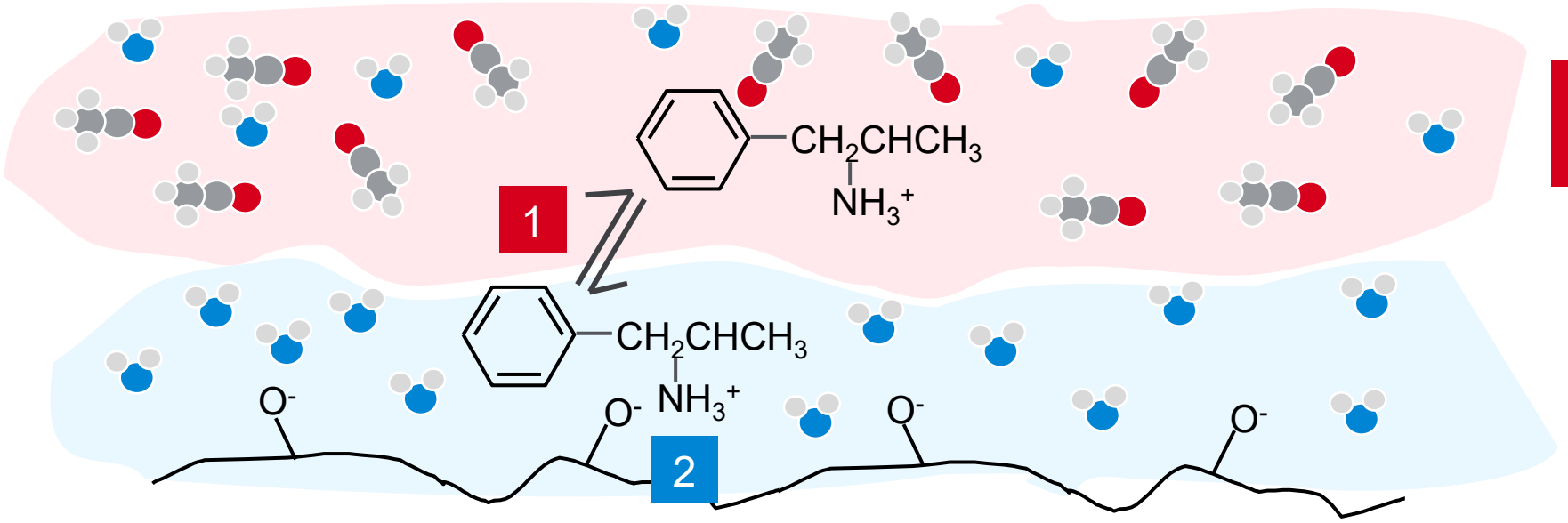
HILIC retention mechanism

A water layer is adsorbed onto the polar silica surface, creating a liquid/liquid extraction system

1 Polar analytes can partition into and out of the water layer, with more polar analytes having a stronger interaction.

2 Charged polar analytes can also undergo ion exchange with the silica surface.



Elution is typically from least to most polar, which is the opposite of RPLC

Solvent strengths in HILIC mode are: THF < acetone < acetonitrile < isopropanol < ethanol < methanol < water

What Is HILIC and When Should You Consider It?

HILIC compared to RPLC

What Is HILIC and When Should You Consider It?

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HILIC complements RPLC

Reversed-Phase LC		Hydrophilic Interaction LC (HILIC)
Nonpolar stationary phase (for example, C18)	Polarity	Polar stationary phase (for example, silica)
Polar mobile phase H_2O/CH_3OH, H_2O/CH_3CN	Mobile phase	Polar mobile phase H_2O/CH_3CN
Decrease retention by decreasing polarity of mobile phase H2O ↓ = retention ↑ CH_3CN ↑ = retention ↓	Gradient	Decrease retention by increasing polarity of mobile phase H2O ↑ = retention ↓ CH_3CN ↓ = retention ↑
Polar to nonpolar	Elution order	Nonpolar to polar

Find the Best Column to Retain and Separate All Analytes

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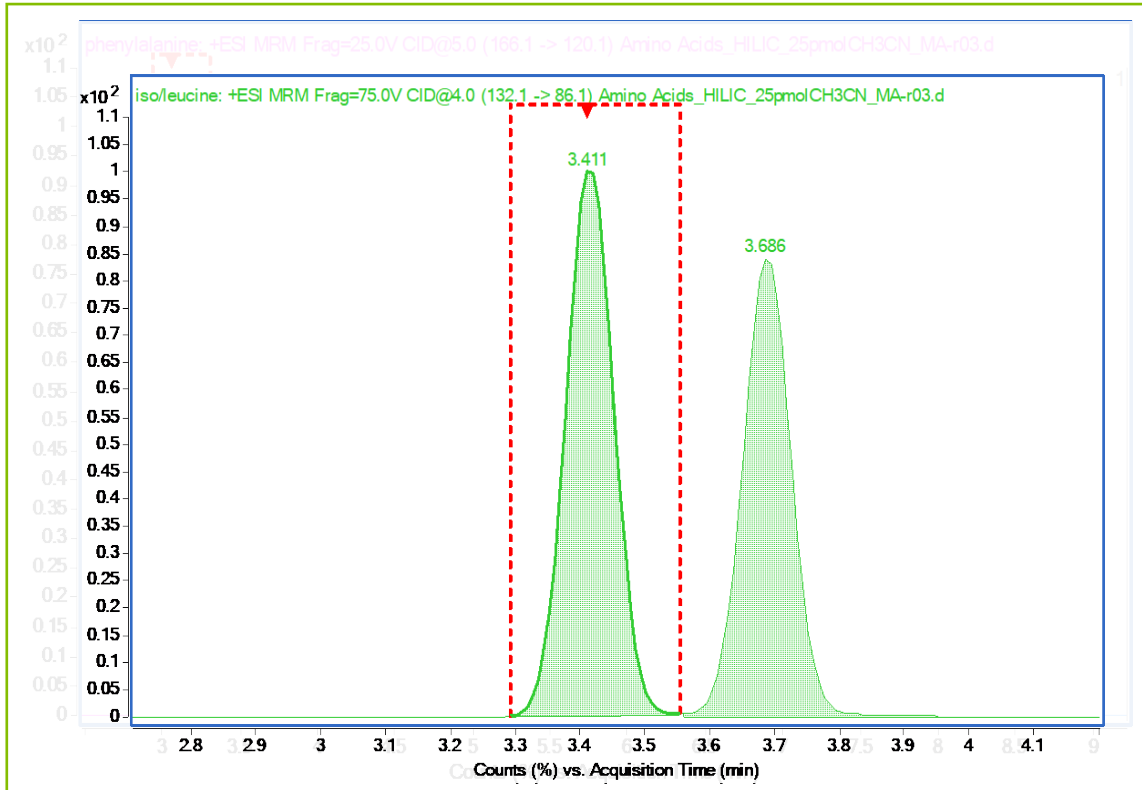
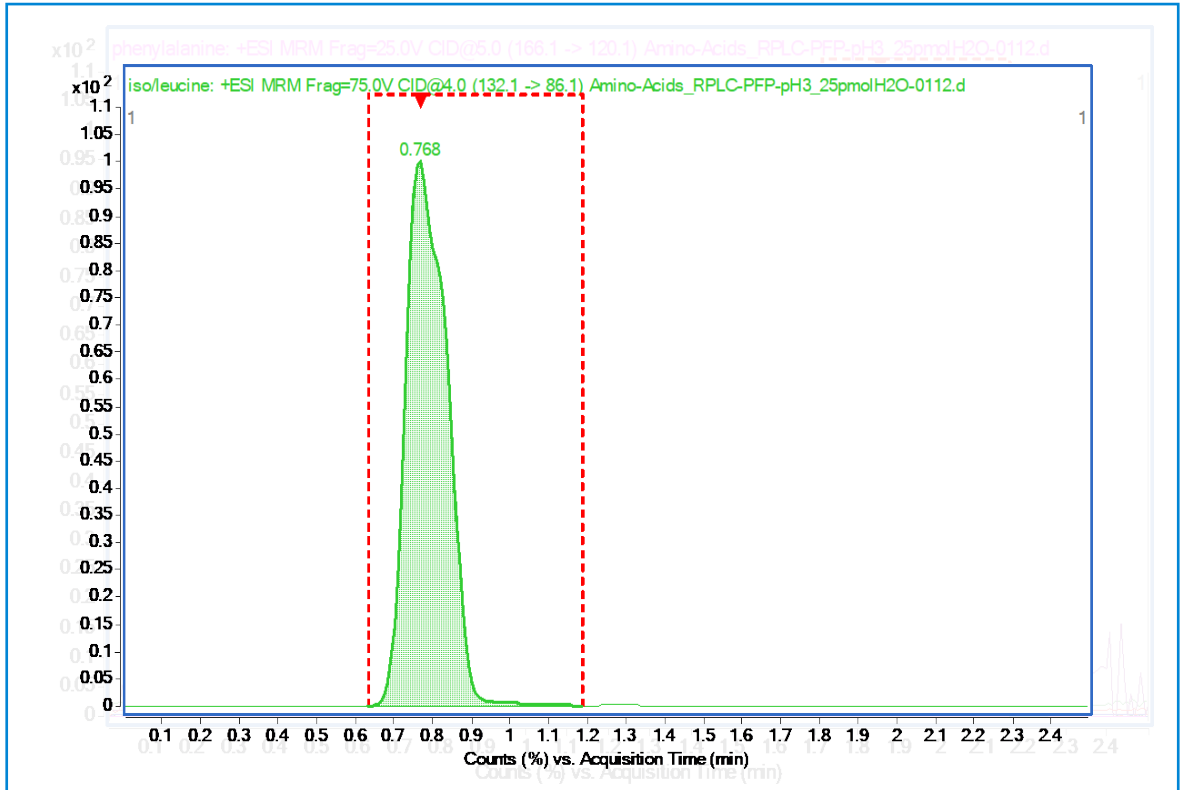
HILIC retains amino acids and separates isobars, while RPLC can't

HILIC: Poroshell 120 HILIC-Z



RPLC: Poroshell 120 PFP



Separation of isobars leucine/isoleucine

Find the Best Column to Retain and Separate All Analytes

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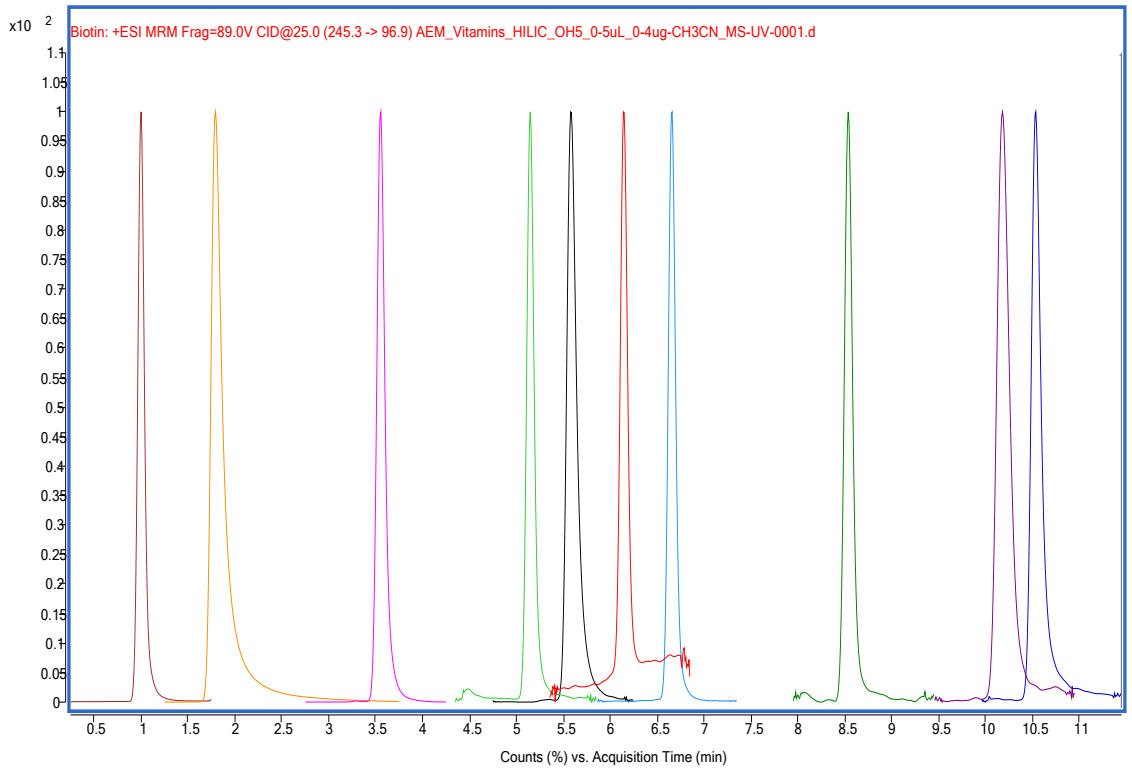
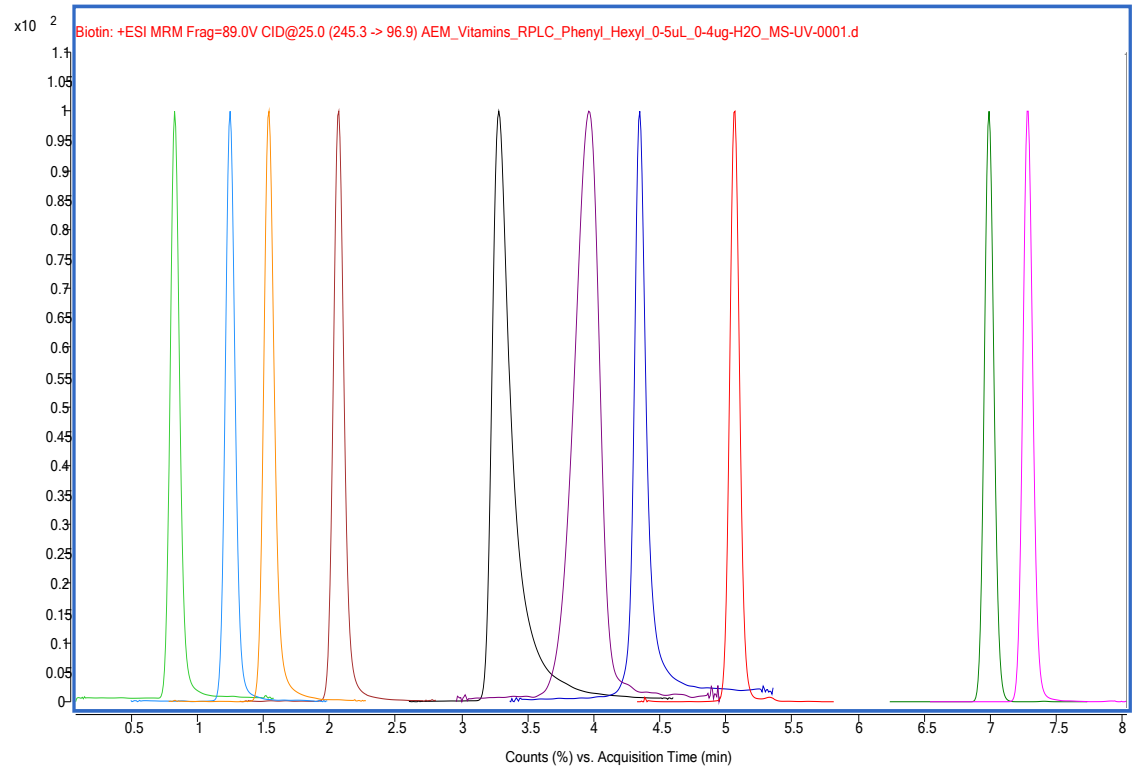
Both RPLC and HILIC are able to retain and separate water-soluble vitamins

HILIC: Poroshell 120 HILIC-OH5



Mobile phase A: H_2O; Mobile phase B: CH_3CN; Mobile phase D: 200 mM ammonium acetate (no pH adjustment), pH ~6.7; Flow rate: 0.5 mL/min; Gradient: 95-65% B in 10 min, hold 5% D constant throughout analysis, 5 min post run; Injection: 0.5 µL injection of 0.4 µg/mL vitamin standard in CH_3CN; Column: 25 °C, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-OH5; Detection: Ultivo TQ/MS ESI+ dMRM (parameters above), DAD Sig = 260 nm, 80 Hz

RPLC: Poroshell 120 Phenyl-Hexyl



Mobile phase A: H_2O; Mobile phase B: CH_3CN; Mobile phase D: 200 mM ammonium acetate + 0.2% acetic acid, pH ~5.3; Flow rate: 0.5 mL/min; Gradient: 0% B for 1 min, 0-25% B in 8 min, hold 5% D constant throughout analysis, 3 min post run; Injection: 0.5 µL injection of 0.4 µg/mL vitamin standard in H_2O; Column: 25 °C, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 Phenyl-Hexyl; Detection: Ultivo TQ/MS ESI+ dMRM, DAD Sig = 260 nm, 80 Hz

What Is HILIC and When Should You Consider It?

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When to chose which separation mode for your sample

01 Find the best column to retain and separate all analytes.

02 Consider the sample: analyte solubility and sample solvent

03 Ensure reliable detection of your sample

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What Is HILIC and When Should You Consider It?

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InfinityLab Poroshell 120 offers a broad portfolio to suit your needs

Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for more polar analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	S 1.9 µm,	RP chemistries for polar analytes	Bonus-RP 2.7 µm	SB-Aq 1.9 µm, 2.7 µm, 4 µm	Chiral-V 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	S 2		PFP 1.9 µm, 2.7 µm, 4 µm	EC-CN 2.7 µm	Chiral-T 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm				HILIC 1,9 µm, 2.7 µm, 4 µm, pH range 0.0-8.0	Chiral- CD 2.7 µm
		HILIC chemistries		HILIC-Z 1.9 µm, 2.7 µm, 4 µm, pH range 2.0-12.0	Chiral-CF 2.7 µm
				HILIC- OH5 2.7 µm, pH range 1.0-7.0	

What Is HILIC and When Should You Consider It?

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When to chose which separation mode for your sample

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Analyte Solubility and Sample Solvent

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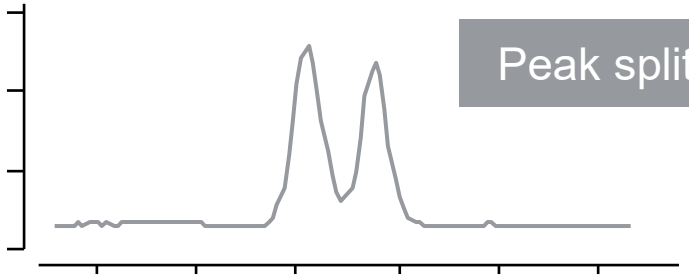
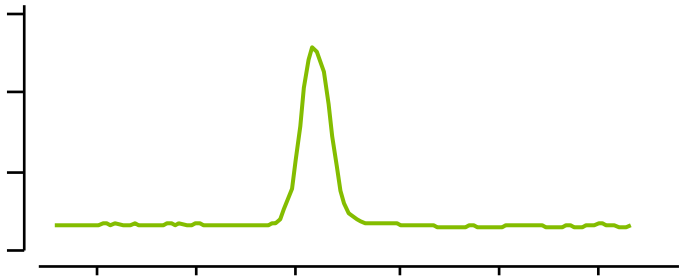
Strong injection solvents distort peak shapes for HILIC and RPLC

HILIC: Glufosinate

in **100% H_2O**



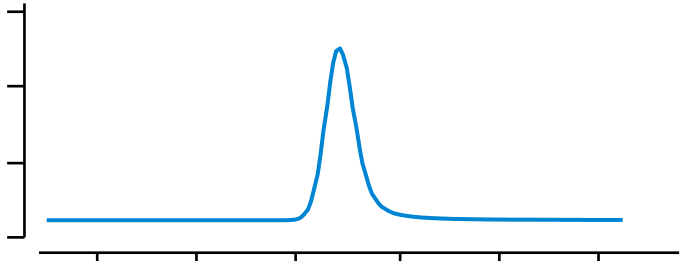
in **1:1 CH_3CN/H_2O**



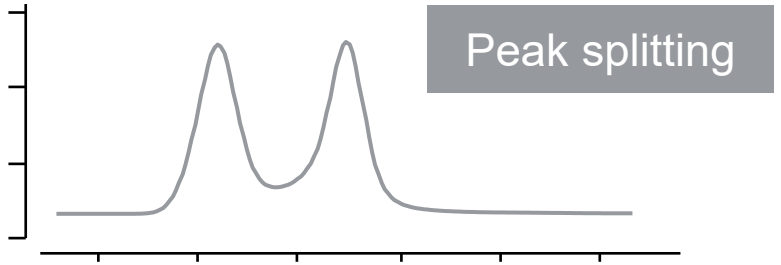
4 µL injection of Glufosinate, 100 ppb, InfinityLab Poroshell HILIC-Z, 2.1x100 mm; Temperature: 30 ºC; Flowrate: 0.6 mL/min, Mobile phase A: 10 mM ammonium acetate, pH 9, Mobile phase B: 100 mM ammonium acetate, pH 9 in 90% ACN (final concentration: 10 mM), Gradient: 90% B => 60% B in 10 minutes, System: Agilent 6490 LC/QQQ

RPLC: Pyridoxine (vitamin B6)

in **100% H_2O**



in **9:1 CH_3CN/H_2O**



0.5 µL injection of 13 µg/mL pyridoxine, A: H_2O; B: CH_3CN; D: 200 mM ammonium acetate + 0.2% acetic acid, pH ~5.3; 0.5 mL/min; Gradient: 0% B for 1 min, 0-25% B in 8 min, hold 5% D constant throughout analysis; Column: 25 ºC, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 Phenyl-Hexyl; Detection: Ultivo TQ/MS ESI+ dMRM

Analyte Solubility and Sample Solvent

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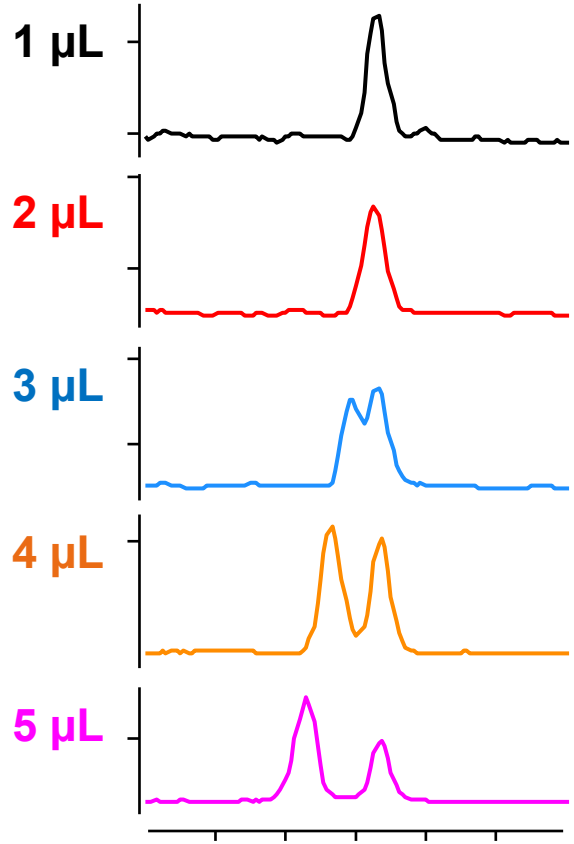
Strong solvent effects are greater with larger injection volumes

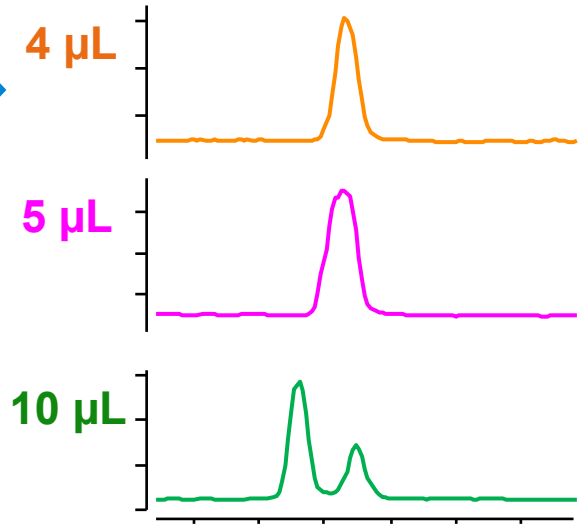
Sample solvent: **100% water**



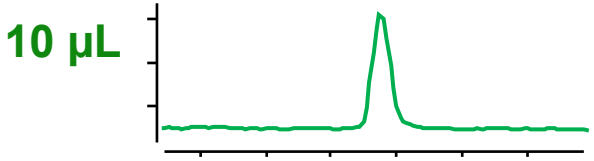
Sample: Glufosinate, 100 ppb
Column: InfinityLab Poroshell HILIC-Z, 2.1 x 100 mm
Temperature: 30 ºC
Flowrate: 0.6 mL/min
Mobile phase A: 10 mM ammonium acetate, pH 9
Mobile phase B: 100 mM ammonium acetate, pH 9 in 90% ACN (final concentration: 10 mM)
Gradient: 90% B => 60% B in 10 minutes
System: Agilent 6490 LC/QQQ

Note: System required phosphoric acid wash

Sample solvent: **50:50 CH_3CN/H_2O**



Sample solvent: **80:20 CH_3CN/H_2O**



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Ensure Analytes are Completely Soluble

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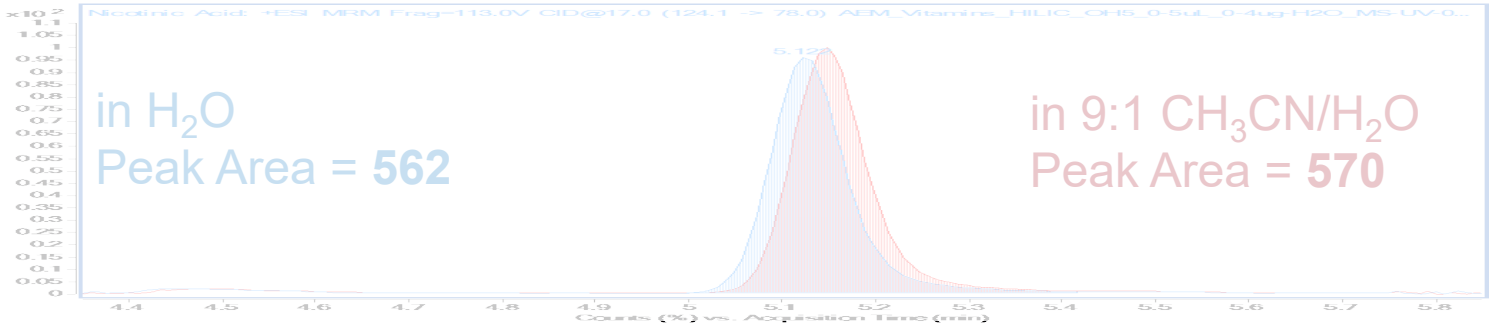
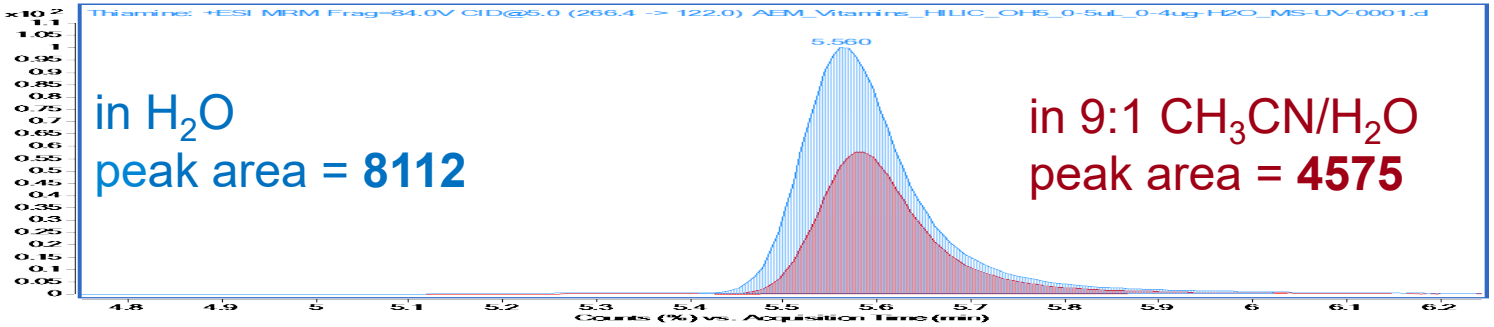
Ensure samples are completely soluble

HILIC

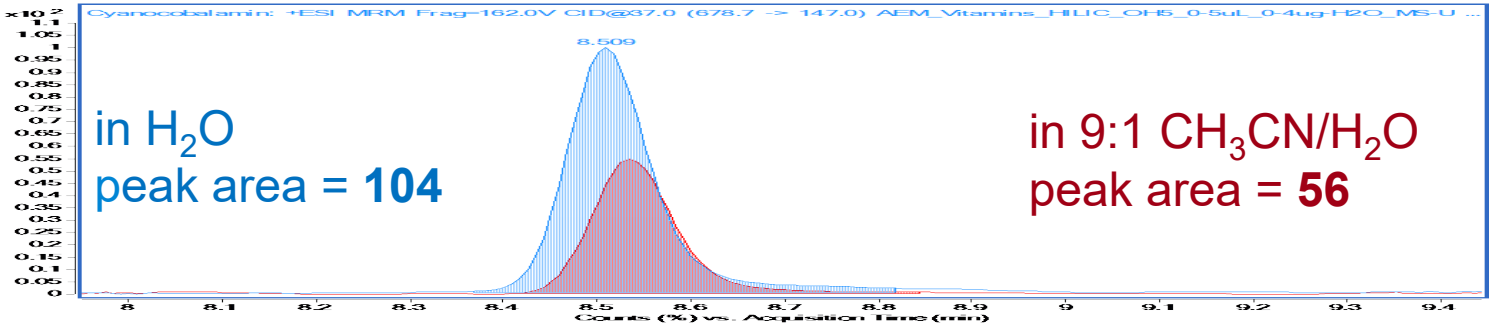
Nicotinic Acid
0.4 µg/mL



Thiamine
0.4 µg/mL



Cyanocobalamin
0.4 µg/mL



~50% sample lost due to poor solubility in CH_3CN

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What Is HILIC and When Should You Consider It?

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When to chose which separation mode for your sample

01 Find the best column to retain and separate all analytes.

02 Consider the sample: analyte solubility and sample solvent

03 Ensure reliable detection of your sample

Ensure Reliable Detection of Your Sample

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Choose a Detector that Can Analyze Compounds of Interest

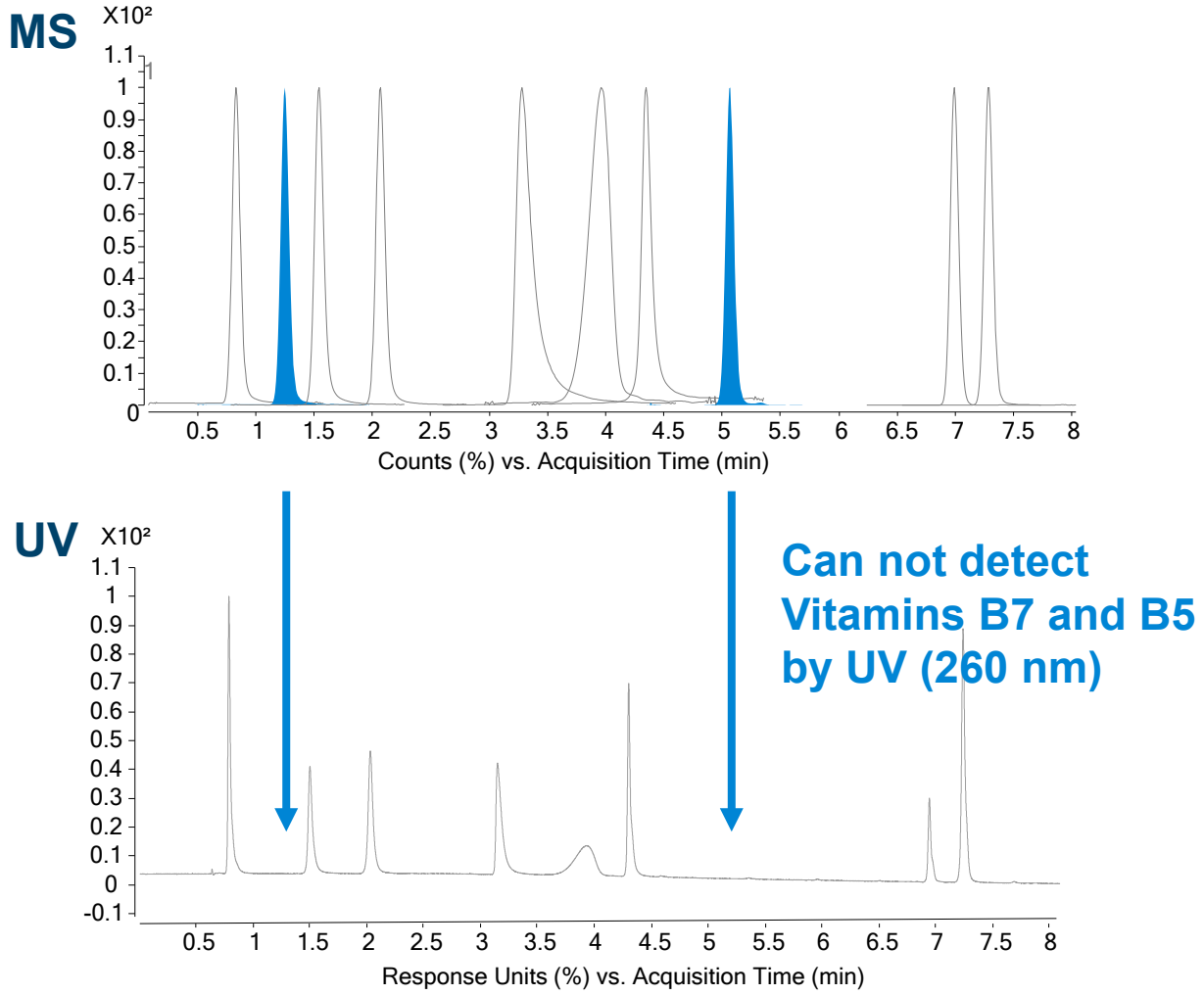
UV, VIS absorbance
- For light-absorbing compounds

Refractive index
- Universal detection, but poor sensitivity; can only run isocratic

Evaporative light scattering
- For nonvolatile analytes

Mass spectrometer
- Low limits of detection based on molecular weight

Fluorescence
- For compounds that fluoresce or can be derivatized to do so



Ensure Reliable Detection of Your Sample

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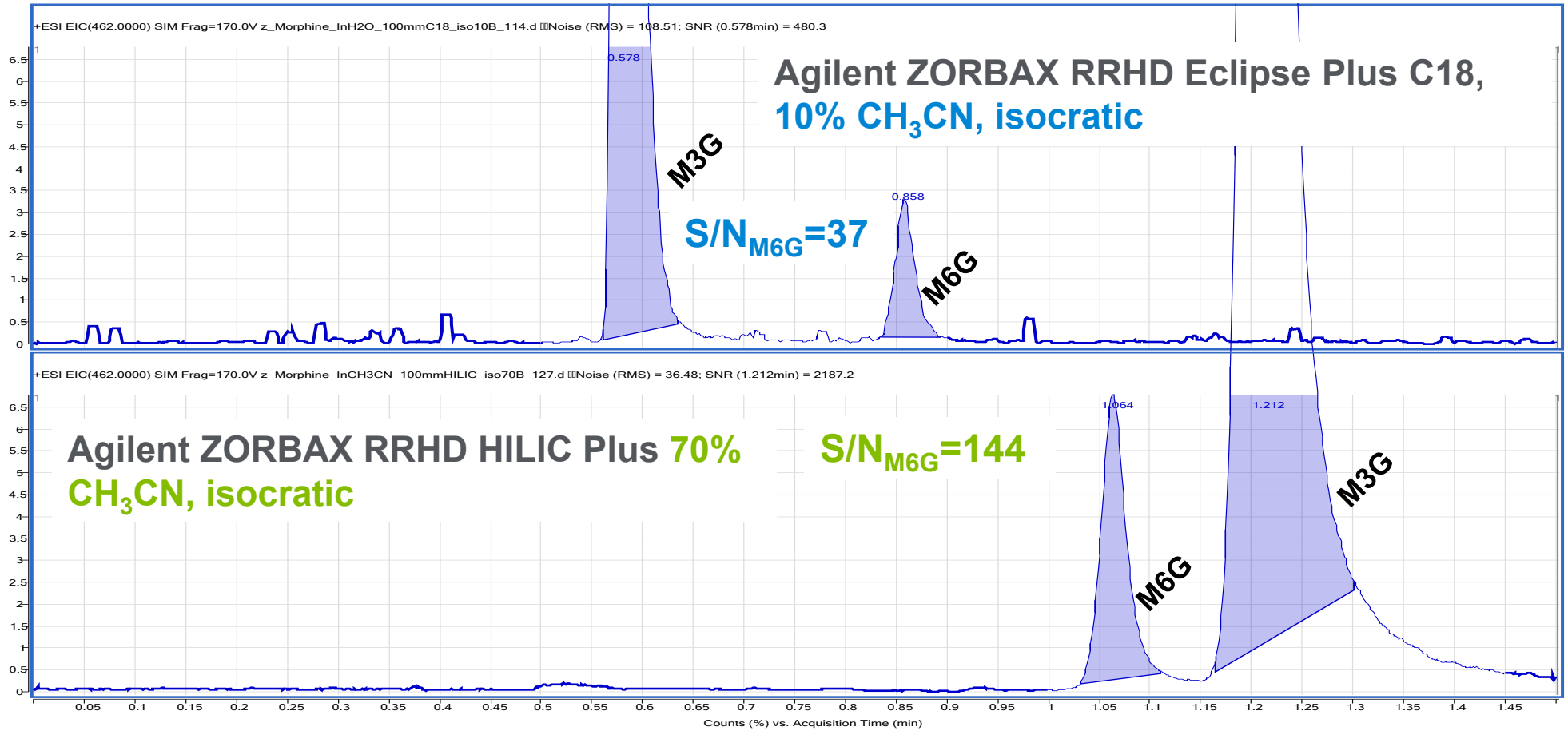
HILIC pairs well with LC/MS and can improve sensitivity compared to RPLC for opioid metabolites



Columns used were 2.1 x 100 mm, 1.8 µm; A: 10 mM ammonium formate pH 3.2 in water, B: acetonitrile/ 100 mM ammonium formate pH 3.2 in water (9:1), isocratic elution, 0.4 mL/min, 2 µL injection of 1 µg/mL each of morphine-3-β-D-glucuronide, and morphine-6-β-Dglucuronide; 25 °C, MS Source: ESI+, 200 V, 250 °C, 11 L/min,, 30 psi, 4000 V; SIM: 462, Frag 170 V, Agilent publication: 5991-0245

Reversed-Phase LC and UV Detection are Compatible with a Wider Range of Mobile Phases, Especially at Low pH

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Mobile Phase	Useable pH/Range	Recommended for HILIC?	Recommended for MS?	Recommended for RPLC and UV?
TFA	<1.5	No	No	Yes
Phosphate	1.1-3.1	No	No	Yes
Formic Acid	<2.8	No	Yes	Yes
Acetic Acid	<3.8	No	Yes	Yes
Formate	2-8-4.8	Yes	Yes	Yes
Acetate	3.8-5.8	Yes	Yes	Yes
Carbonate	5.4-7.4	Yes	Yes	Yes
Phosphate	6.2-8.2	No	No	Yes
Bicarbonate	6.6-8.6	Yes	Yes	Yes
Ammonia	8.2-10.2	Yes	Yes	Yes
Phosphate	11.3-13.3	No	No	Yes

What Is HILIC and When Should You Consider It?

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Summary of when to use which separation mode

01

Find the best column to retain and separate all analytes.

- RPLC cannot retain all polar/ionized analytes, HILIC may work for these
- Some analytes can be retained and separated equally well in both modes of LC

02

Consider the sample: analyte solubility and sample solvent

- Injecting strong solvent in both RPLC and HILIC will negatively affect chromatographic quality
- Strong solvent effects get worse with larger injection volumes
- Polar compounds are generally more soluble in water than acetonitrile, which is good for RPLC

It's a balancing act

03

Ensure reliable detection of your sample

- Ensure analytes are compatible with detector choice
- HILIC can improve LCMS analyses due to more volatile mobile phases
- UV and RPLC are compatible with a wider variety of mobile phases, which may improve analyte retention and separation

Other Techniques for Polar Compounds

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Advantages and disadvantages of each technique

	Advantage	Disadvantage
Ion Pairing	Fast. Uses standard system and reverse phase columns.	Often contaminates system, reagents can cause ion suppression, restricted to only positive or only negative mode MS.
Ion Chromatography	Well understood mechanism, established for over 40 years.	Slower than modern HPLC, expensive systems and consumables, cannot resolve cations and anions simultaneously, difficult to make MS-compatible.
Ion Exchange	Strong retention and separation	Slower than HPLC, cannot resolve cations and anions simultaneously, difficult to make MS-compatible.
Normal Phase	Fast. Uses standard HPLC and common columns.	Safety and compatibility of organic solvents, smaller selection of stationary phases, sample solubility issues.
Derivatization	Tailored selectivity, adds chromophore or fluorophore	Lengthy sample preparation, repeatability issues.

What Is HILIC and When Should You Consider It?

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Why we choose HILIC

Advantages

- Uses a standard system and solvent, just swap columns
  - Easily adopted by labs currently performing reverse phase analysis
- Retains cations, anions, and polar neutrals
  - Widely applicable across all major polar samples
- Fully MS compatible
  - Operate in positive or negative mode with high sensitivity

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HILIC Method Development

And common application areas

HILIC Method Development

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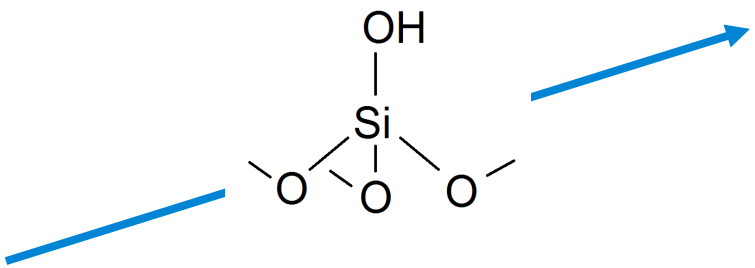
InfinityLab Poroshell 120 HILIC column options

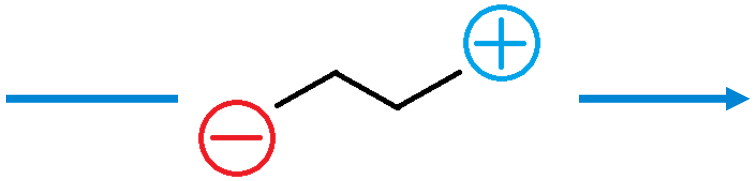
Best for polar analytes

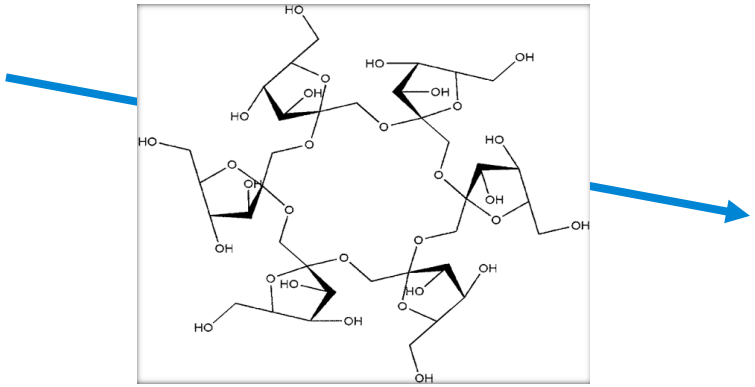
InfinityLab Poroshell
HILIC
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell
HILIC-Z
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell
HILIC-OH5
2.7 µm







HILIC

- Bare silica chemistry
- For very simple mixtures, low column bleed

HILIC-Z

- Proprietary zwitterionic chemistry, high pH stable
- **The most modern and robust column – start method development here**
- PEEK-lined version available

HILIC-OH5

- Brushed fructan chemistry
- Alternative selectivity

HILIC Method Development

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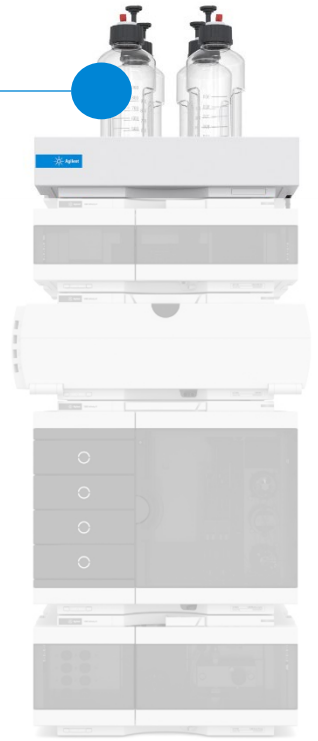
Mobile phase considerations

	Recommendations
Organic solvent concentration	• Solvent strength in HILIC mode: *THF < Acetone < CH_3CN < IPA < EtOH < MeOH < H_2O* • H_2O must be present — *need >3% H_2O* for hydration of silica • Mobile phase will typically be >50% acetonitrile
Ionic strength of buffer	• Concentration of (salt) buffer increases strength • Different anions and cations may can also affect analyte retention
Type of buffer	• Acetates and formates are good, soluble in CH3CN—also MS friendly • Phosphate salts are bad due to low CH_3CN solubility



More information

! For more HILIC method development tips, see this publication: 5991-9271EN

HILIC Method Development

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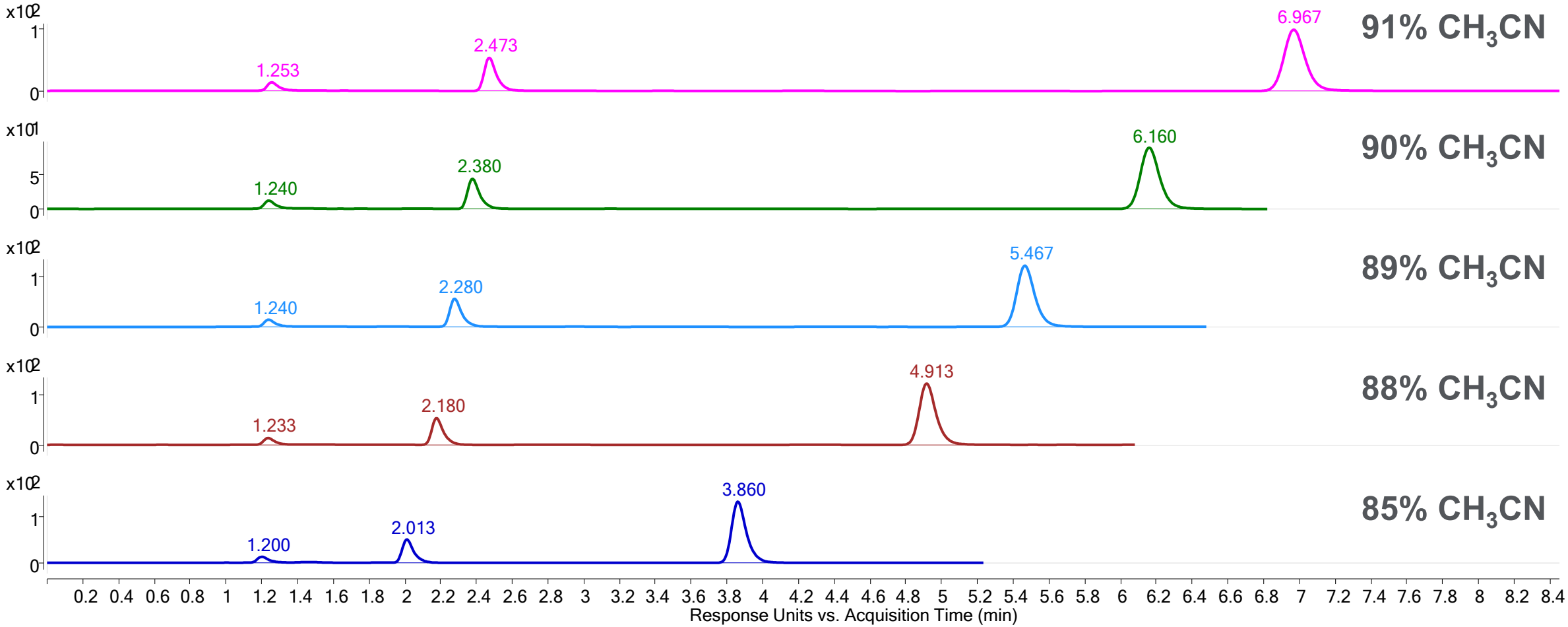
Less CH_3CN makes a HILIC mobile phase stronger, causing less retention



Column used was 2.1 x 150 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (PEEK lined); A: 100 mM pH 3 ammonium formate in Water, B: Acetonitrile, x % B, isocratic elution, 0.25 mL/min, 30 °C, 1 µL injection of toluene, cytosine, uracil QC mixture, 254 nm

HILIC Method Development

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Starting mobile phases

Mobile phase A (strong phase, H_2O):

- Typical buffer concentration: 5 to 30 mM
  - 10 to 20 mM is most common

Basic analytes

- Ammonium formate, pH 3
- Ammonium acetate, pH 4-5

Acidic analytes

- Ammonium acetate, pH ~7
  - Ammonium acetate solution is near pH 7, before adjusting with other modifiers
  - Not a true buffer, but still commonly used at mid-pH
- Ammonium acetate or formate, pH 9-10
  - Can be formate or acetate because the ammonium ion is buffering
  - HILIC-Z only

Sugars

- Ammonium hydroxide, pH 10-11
  - HILIC-Z only

- *Phosphate buffers are not recommended **

Mobile phase B (weak phase, CH_3CN):

- Buffer concentration should match mobile phase A for improved reproducibility
- Adding 10% water in ACN is generally recommended for improved solubility and faster re-equilibration
- Pure MeOH is too strong a solvent for most HILIC separations. Mixed with ACN in small quantities (<15%), it can be used to change selectivity slightly

*Note: Phosphates have low solubility in high % ACN (1-30 mM). Always test solubility before running. Never run in >80% ACN to avoid precipitation.

Effect of pH on Retention of Acidic Compounds with HILIC

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Starting mobile phases

In HILIC mode, ionizable compounds are better retained when they are ionized

- Acids at high pH
- Bases at low pH

Once the analyte is fully ionized, retention should stabilize

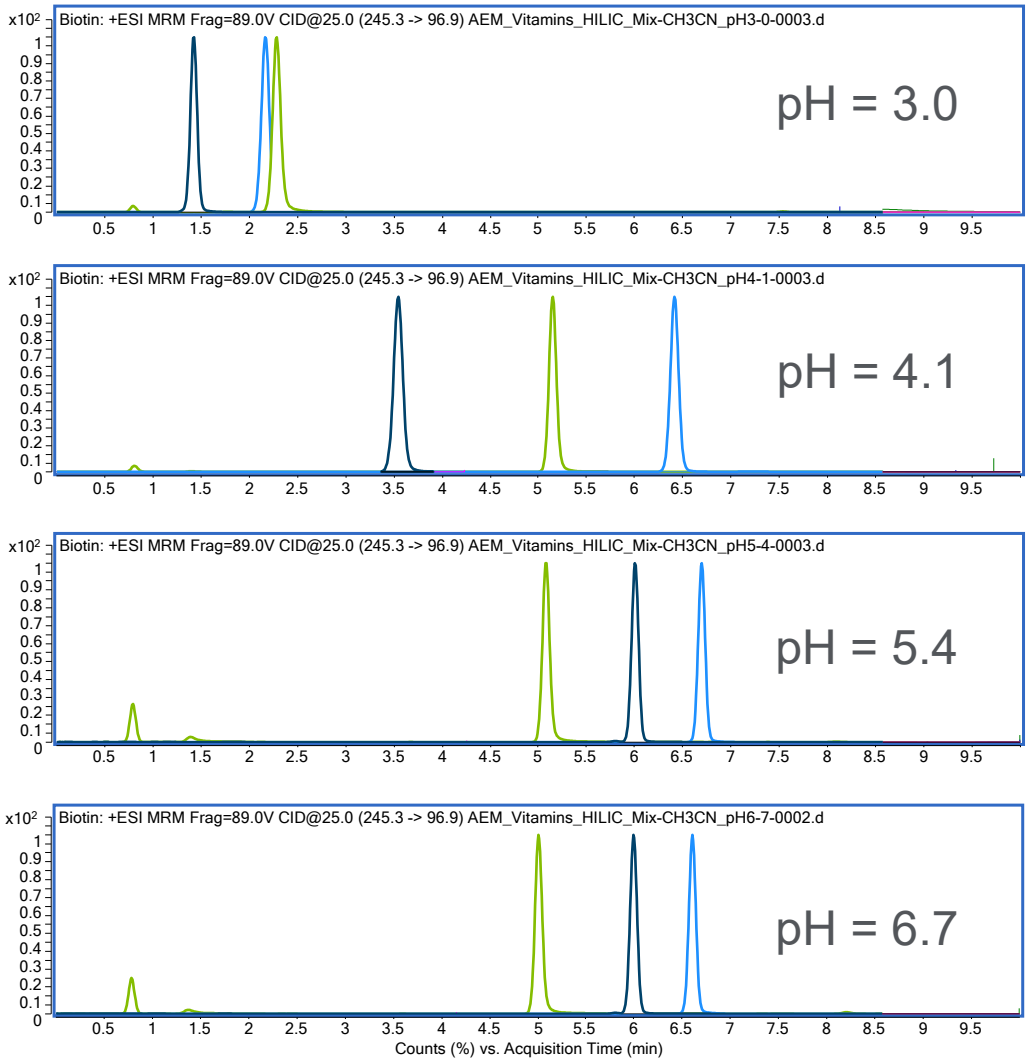
- Note: If other retention mechanisms are occurring, this may not be true

Biotin pKa = 4.5

Nicotinic acid pKa = 4.8

Pantothenic acid pKa = 4.3

Mobile phase A: H_2O, B: CH_3CN, D: varies, 200 mM ammonium formate or acetate; Flow rate: 0.5 mL/min; Gradient: 95% B for 1 min, 95-65% B in 9 min, hold 5% D constant throughout analysis, 5 min post run; Injection: 0.5 µL of 13.3 µg/mL each in CH_3CN/H_2O 19:1; Column: 25 °C, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z; Detection: Ultivo TQ/MS ESI+ dMRM



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HILIC Method Development

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Common starting conditions for HILIC method development

	Method Parameter
Column	Agilent InfinityLab Poroshell 120 HILIC-Z
Buffer	• **Acidic analytes**: mid to high pH (HILIC-Z only) • **Basic analytes**: low to mid pH • **Mixed analytes**: mid pH
Isocratic	Column equilibration is faster as you move from high to low aqueous • **50% ACN** – Column wash (typically no retention) • **70% ACN** – Very polar analytes • **80% ACN** – Polar analytes, mixtures • **90% ACN** – Less polar analytes separation
Gradient	• **90% → 50% ACN** – Scouting gradient • Isocratic holds or shallow gradients (1-3% per min) recommended for critical pair separation

Tips and Tricks

For successful HILIC column use and care

Tips and Tricks for Your HILIC separations

Considerations on solvent and sample handling

	Impact
Add 10% aqueous to your organic	• Buffer solubility increases drastically with addition of 10-20% water • HILIC columns equilibrate faster with more aqueous
Have the same ionic strength in both mobile phases	• Ionic strength gradients have more variability than constant ionic strength • Near 90-100% ACN, many buffers crash out, causing serious clogs
Increasing buffer concentration can improve peak shape and sample loadability	• High buffer concentrations can cause ion suppression when using MS detection
Follow good measurement practices when mixing buffers	• Retention can vary from bottle-to-bottle if eluent is not mixed accurately and consistently
Prepare samples in as much acetonitrile as possible and keep injection volumes small	• Avoid peak shape and retention issues from strong solvent effects
Use inert solution, if needed	• Reduce unwanted interactions of analytes with metal in the flow path

InfinityLab solvent bottles

InfinityLab Stay Safe caps

HILIC Column Equilibration is Faster with Higher Amounts of Aqueous

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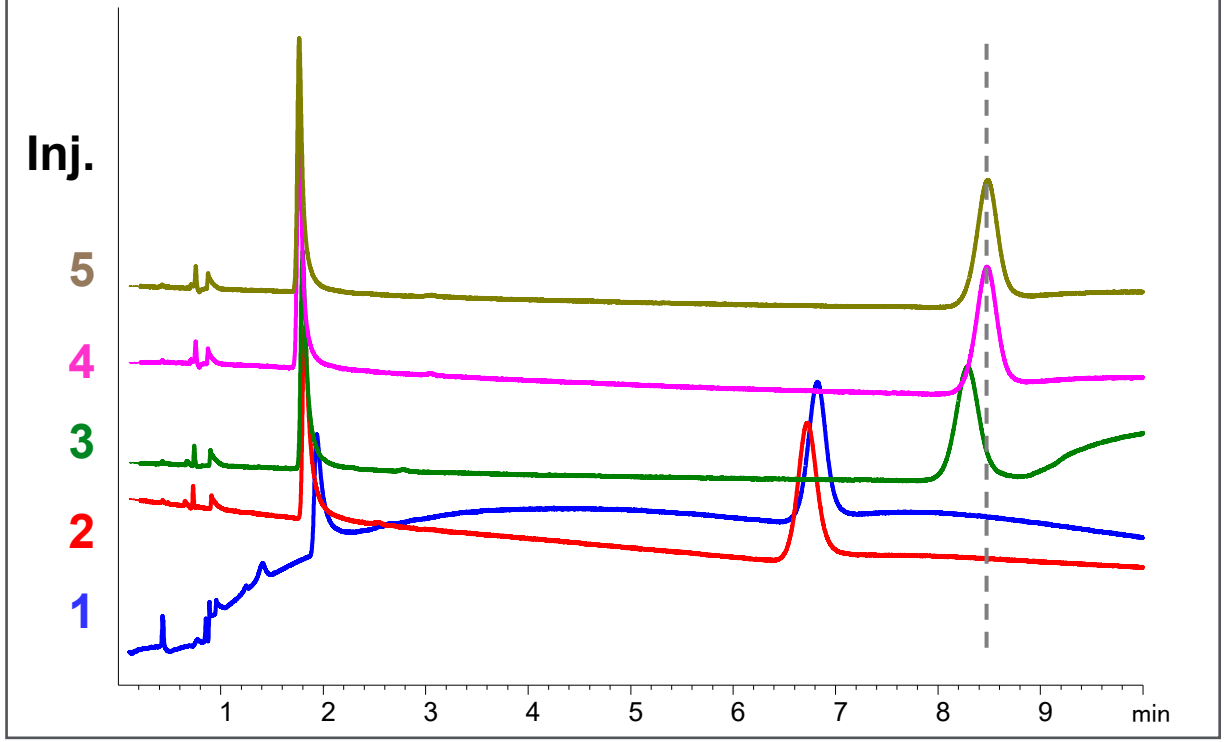
B vitamins on Agilent InfinityLab Poroshell 120 HILIC-OH5 (2.1 x 100 mm, 2.7 µm)

4% aqueous, isocratic

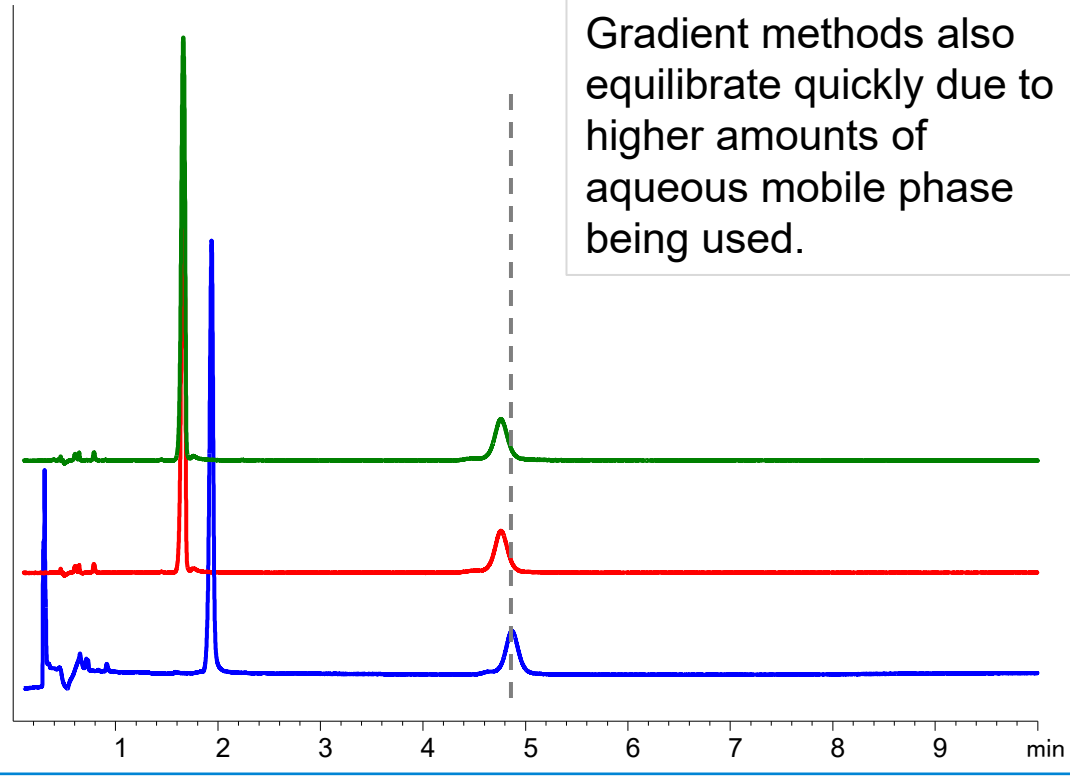
Equilibrated in 30 minutes (75 column volumes)



Column stored in 100% CH_3CN before analysis; A: 100 mM ammonium formate pH 3.0, B: CH_3CN, 96% B isocratic, 0.5 mL/min, 1 µL injection of B2+B6, 25 °C, 260 nm, 80 Hz

20% aqueous, isocratic

Equilibrated in <10 minutes (<25 column volumes)



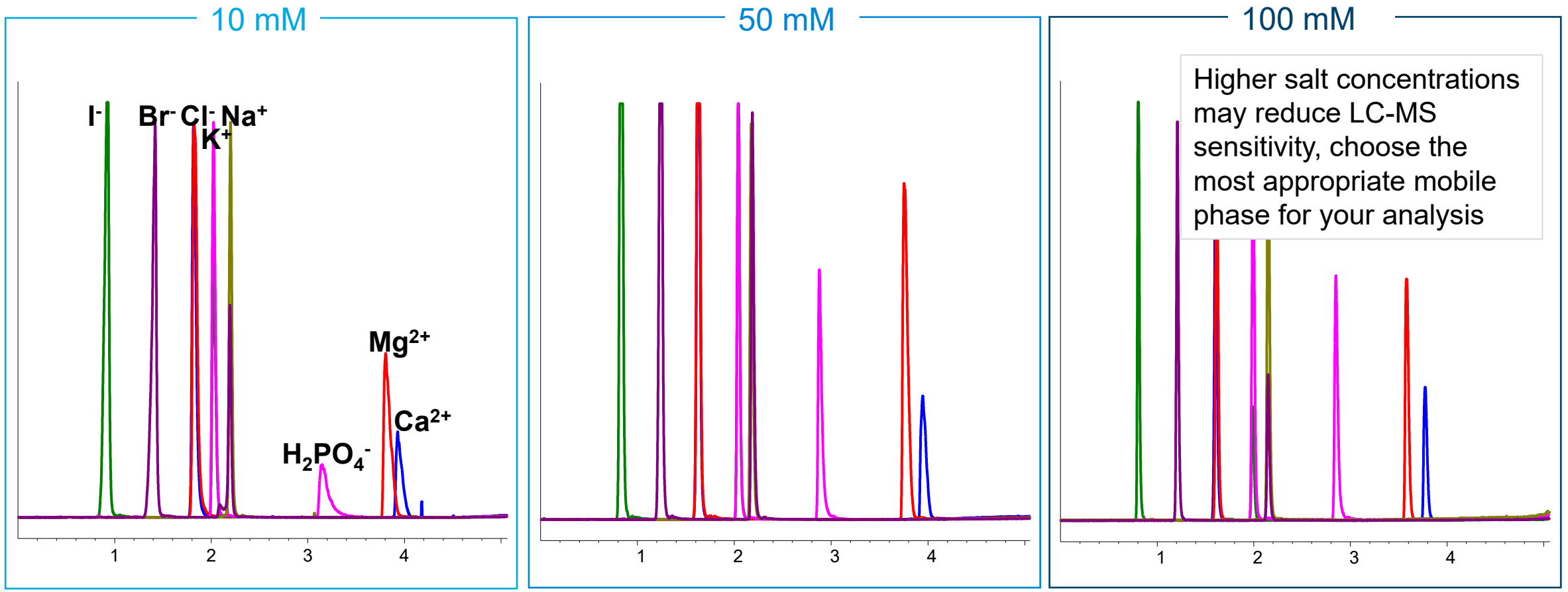
Gradient methods also equilibrate quickly due to higher amounts of aqueous mobile phase being used.

Column stored in 100% CH_3CN before analysis; A: 100 mM ammonium formate pH 3.0, B: CH_3CN, 80% B isocratic, 0.5 mL/min, 1 µL injection of B9+B12, 25 °C, 260 nm, 80 Hz

Higher Salt Concentrations Can Improve Peak Shapes and Resolution

Agilent InfinityLab

Inorganic Ions on Agilent InfinityLab Poroshell 120 HILIC-Z

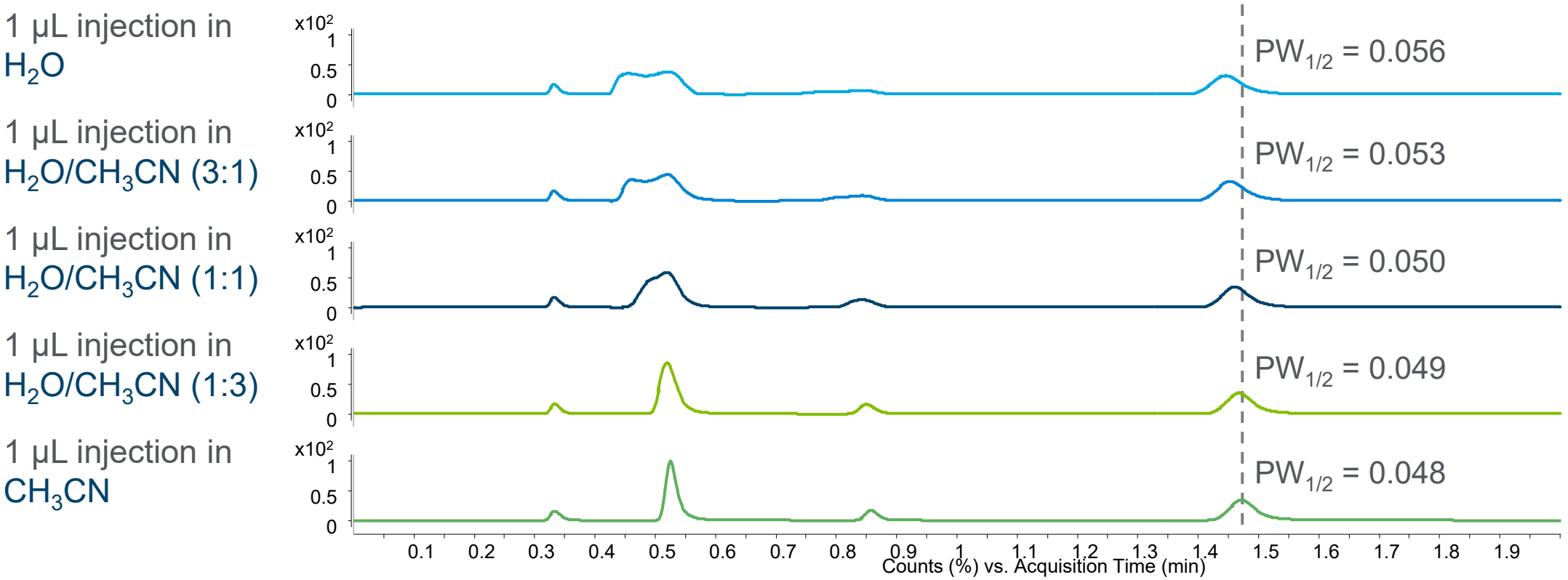


Agilent InfinityLab Poroshell 120 HILIC-Z 2.1 x 100 mm, 2.7 µm; A: 10, 50, or 100 mM pH 3 ammonium formate, B: Acetonitrile, 80-20% B in 5 min, 3 min re-equilibration, 0.4 mL/min, 30 C, 2 µL injection of individual standards (0.3 to 0.5 mg/mL), ELSD 40 °C/3.5 psi/30Hz

HILIC Analyses Perform Best with Weak Injection Solvents

Agilent InfinityLab

B vitamins on HILIC with isocratic elution



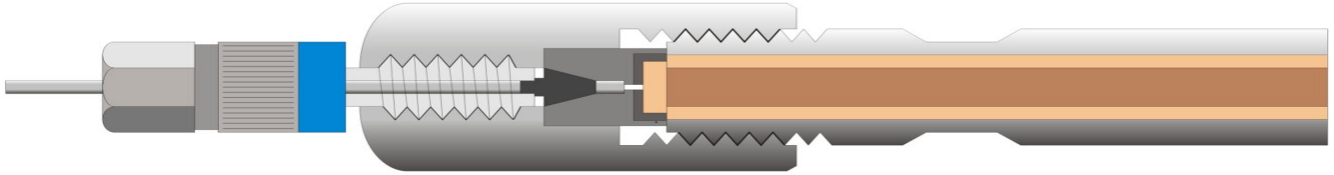
Agilent ZORBAX RRHD HILIC Plus 2.1 x 50 mm, 1.8 µm; Mobile phase: acetonitrile/100 mM ammonium formate pH 3.2 in water (9:1), isocratic elution, 0.4 mL/min, 1 µL injection of 5.7 µg/mL each of 4-aminobenzoic acid, nicotinamide, riboflavin, nicotinic acid ; 25 ˚C, MS source: ESI+, 200 ˚C, 10 L/min, 30 psi, 4000 V; SIM: 138, 123, 377, 124

HILIC Sensitivity Can Be Improved with a PEEK-Lined Column

Agilent InfinityLab

PEEK-lined stainless steel and PEEK-coated titanium frits

- Metal-free flow path minimizes unwanted interactions
- Stainless steel provides strength for UHPLC use



For best results, use the full InfinityLab bio-inert LC Solution:

- InfinityLab bio-inert LC System
- Bio-inert quick connect heat exchanger, p/n: G7116-60009
- All Agilent PEEK/SST Bio-inert capillaries with Quick Turn fitting (5067-5966) or UHP-FF fitting Bio-inert (5067-5695)

Tips and Tricks for Your HILIC Separations

Agilent InfinityLab

InfinityLab deactivator additive pairs well with PEEK-lined HILIC-Z

	Improvement
Reduce Metal-Analyte Interaction	• Chelate-free metals, covers exposed active sites in sample flow path, reducing unwanted metal-analyte interactions and allowing lower detection limits using LC/MS
Amenable to LC/MS use	• Optimized for use at a 5 µM (1:1000 dilution) with minimal ion suppression effects • Does not persist in the LC/MS system after use (unlike traditional ion pairing reagents)
Operational time and cost savings	• Saves time needed to passivate your system • Can avoid derivatization • Can avoid potential system contamination from ion pairing agents • Limits of detection can be lowered for challenging compounds such as phosphorylated metabolites, phosphate pesticides, and organic acids

InfinityLab deactivator additive
50 mL: 5190-4506

Recommended read

More information can be found in the InfinityLab Deactivator Additive user guide 5991-9516EN.

Tips and Tricks for Your HILIC Separations

Agilent InfinityLab

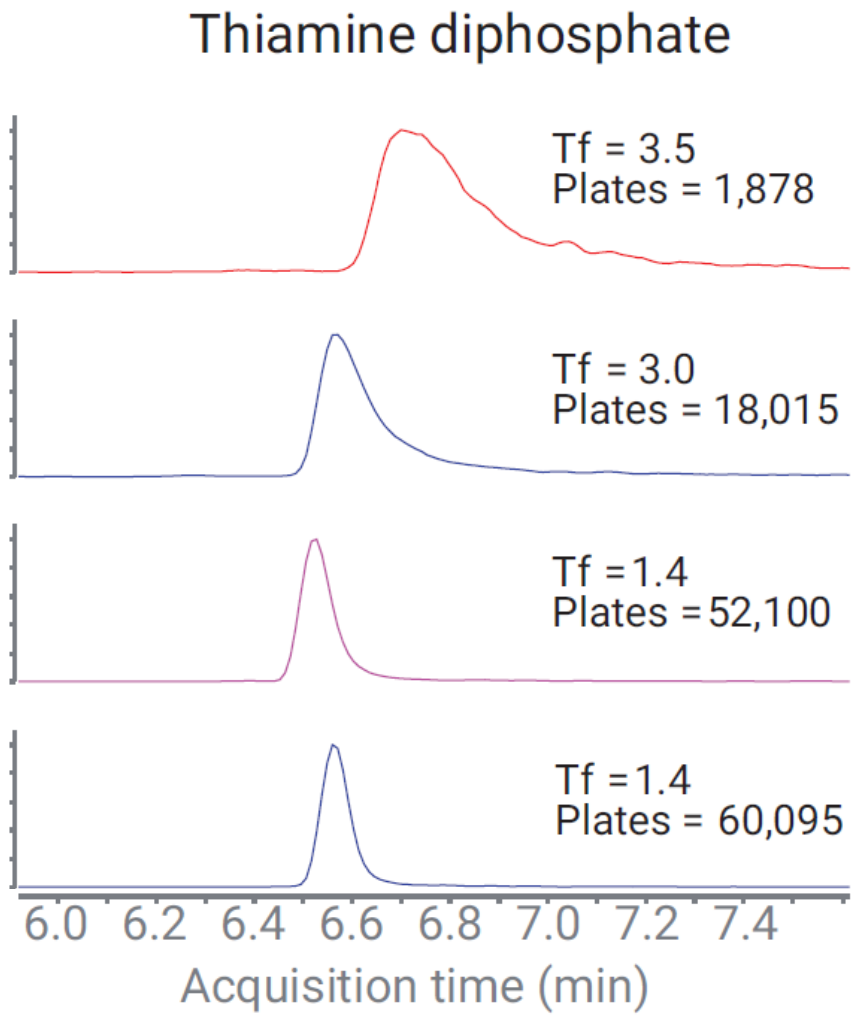
LC passivation procedure to reduce unwanted metal interactions

- LC disconnected from MS and going directly to waste
- IPA at 5 mL/min for 5 min
- Water at 5 mL/min for 5 min
  - Flow at 0.5 mL/min for 1 hour
- 0.5% phosphoric acid in 90% acetonitrile/10% water at 5 mL/min for 5 min
  - Flow at 0.1 mL/min overnight (at a minimum)
- Water at 5 mL/min for 5 min
  - Flow at 0.5 mL/min for 1 hour
- Mobile phase at 5 mL/min for 5 min
  - Flow at 0.25 mL/min for 1 hour
- Reconnect LC to MS and proceed with analysis
  - Flow at 0.25 mL/min for 20 to 30 min

Tips and Tricks for Your HILIC Separations

Agilent InfinityLab

Stepwise improvements for metal sensitive analytes



Before system passivation

↓

After LC passivation with 0.5% phosphoric acid in 9:1 acetonitrile/water

↓

Added InfinityLab deactivator additive to mobile phase

↓

Installed PEEK-lined HILIC-Z column

HILIC Column Care

Agilent InfinityLab

How to clean and store a HILIC Column

Cleaning a HILIC column:

- Use a strong HILIC solvent to clean HILIC columns
- Flush HILIC columns with 100% water
- If that is insufficient, add in 100 to 500 mM salt
  - You can use a strong salt like NaCl or, if you prefer to avoid that, you can use buffer salts like ammonium acetate
- Increasing the temperature to 35 to 55 ˚C can also help with the cleaning efficiency
- Flush with about 30 column volumes per step
- Be sure that once you have finished flushing with high concentration salt, you flush with pure water before reintroducing acetonitrile into the mobile phase

Storing a HILIC Column:

- Flush with acetonitrile/water (20/80) for 30 column volumes
- Flush with acetonitrile/water (80/20) for 30 column volumes
- Store at room temperature

Summary

When to consider a HILIC column:

- Are your analytes unretained with RPLC?
- Are your analytes at least somewhat soluble in acetonitrile?
- Are you using MS detection?
- Do your analytes interact with metals in the LC system?

Keep sample solvents in mind for HILIC analyses; prepare the sample in as much acetonitrile as possible and keep injection volumes as small as possible

- Most common support issue with HILIC methods

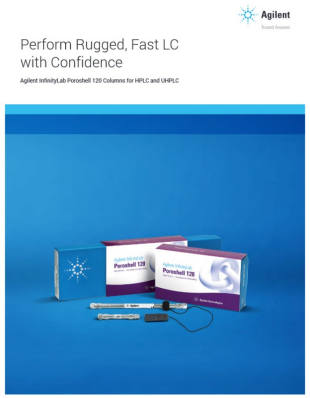
Additional Information

Learn more about Agilent HILIC Column portfolio

More Information on Poroshell 120

Agilent InfinityLab

1. Brochures



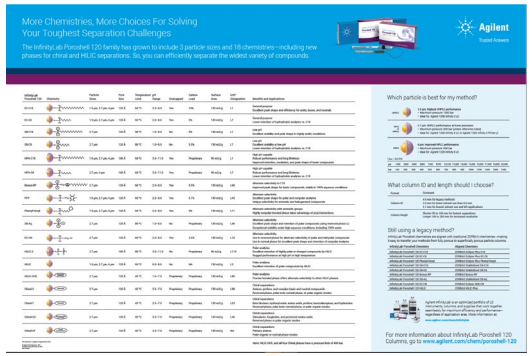
[Poroshell 120 Portfolio brochure]

5991-8750EN

[Poroshell 120 1.9 µm brochure]

5991-7352EN

2. Posters



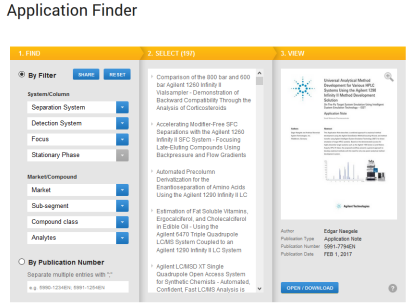
[P120 Selectivity overview]

5991-9013EN

[P120 MethDev poster]

5991-7188EN

3. Application notes



[Agilent Application Finder]

>200 P120 application notes

4. Agilent.com

Videos
Webshop
Collaterals
Specifications
And much more…

Additional Information

Agilent InfinityLab

Application notes on Poroshell 120 HILIC columns

	Application Note Title
Agriculture and Food Testing	• Analysis of Amino Acids in Animal Feed Matrices Using the Ultivo Triple Quadrupole LC/MS System – 5994-0586EN • Analysis of Sugars Using an Agilent InfinityLab Poroshell 120 HILIC-Z Column – 5991-8984EN • Analysis of Organic Acids on an Agilent InfinityLab Poroshell 120 HILIC-Z Column – 5991-8985EN • LC/MS Analysis of Free Amino Acids on Agilent InfinityLab Poroshell 120 HILIC 1.9 µm Columns – 5991-7541EN
Biopharma	• Integrated Transcriptomics and Metabolomics Study of Retinoblastoma Using Agilent Microarrays and LC/MS/GC/MS Platforms – 5991-6215EN • Enhanced Metabolite Profiling from Bark of Alangium Salviifolium Using LC/MS and GC/Q-TOF Techniques – 5991-4663EN • Analysis of Water-Soluble Vitamins and their Metabolites – 5994-1553EN • Methods for the Analysis of Underivatized Amino Acids by LC/MS – 5991-8582EN • HPLC-DAD Analysis of Nucleotides Using a Fully Inert Flowpath – Agilent 1260 Infinity II Bio-inert LC System and a PEEK-Lined Agilent InfinityLab Poroshell 120 HILIC-Z Column – 5994-0680EN • 13C Glucose Qualitative Flux Analysis in HEPG2 Cells Using an Agilent 6546 LC/Q-TOF and VistaFlux – 5994-0713EN • Analysis of Choline Metabolites by Hydrophilic Interaction Chromatography (HILIC) with LC/MS/MS – 5991-9491EN • Monitoring of Mammalian Cell Culture Media with HILIC LC/MS – 5994-0024EN

Filter for Poroshell 120 HILIC phases on https://www.agilent.com/en/applicationfinder/applicationfinder

Additional Information

Agilent InfinityLab

Application notes on Poroshell 120 HILIC columns

	Application Note Title
Small Molecule Pharma	• Impurity Analysis of Aminoglycoside Antibiotic Using the Agilent InfinityLab Poroshell 120 HILIC-S Column with ELSD Detection – 5991-8824EN • Trace Level Quantification of Potential Mutagenic Impurities in Pharmaceuticals Using an Agilent Ultivo LC/TQ with Mixed Mode Detection – 5994-1238EN • How to Catch a Potential Mutagenic Impurity Using Agilent LC/MSD XT and Agilent InfinityLab Poroshell 120 HILIC-Z Column for Sensitive and Reliable Detection of Dalfampridine Impurities – 5994-0864EN • Analysis of Polar Compounds in Plant Material – 5991-8617EN • Analysis of Water-Soluble Vitamins on an Agilent InfinityLab Poroshell 120 HILIC-OH5 Column – 5991-8780EN • Analysis of Aminoglycosides Using the Agilent InfinityLab Poroshell 120 HILIC-Z Column – 5991-8824EN
Environmental	• Paraquat, Diquat, and Mepiquat Analysis in Environmental Water – 5994-1307EN • Modified QuEChERS for HILIC LC/MS/MS Analysis of Nicotine and its Metabolites in Fish – 5991-2408EN • Analysis of Metals, Halides, and Inorganic Ions Using Hydrophilic Interaction Chromatography – 5991-8602EN
General	• Retaining and Separating Polar Molecules – A Detailed Investigation of When to Use HILIC versus a Reversed-Phase LC Column – 5994-1137EN • Hydrophilic Interaction Chromatography (HILIC) Using Agilent Poroshell 120 HILIC – 5991-1242EN • Hydrophilic Interaction Chromatography Method Development and Troubleshooting – 5991-9271EN • The Agilent 1260 Infinity Analytical SFC System with Time-of-Flight Mass Spectrometric Detection - Method Development Using Method Scouting Wizard – 5994-0251EN • Analysis of Highly Polar Compounds by SFC/Q-TOF MS with Identification using Database and Library Searches – Enhanced Fluidity Liquid Chromatography (EFLC) using High Modifier Concentration at Elevated System Pressure – 5994-1096EN

Filter for Poroshell 120 HILIC phases on https://www.agilent.com/en/applicationfinder/applicationfinder

Resources for Support

- LC troubleshooting poster (5994-0709EN)
- Tech support www.agilent.com/chem/techsupport
- Resource page www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
  - Application workflows (such as cannabis, PFAS, and more)
- InfinityLab LC Supplies catalog (5991-8031EN)
- LC handbook (5990-7595EN)
- Best practices for using an Agilent LC system (01200-90090)
- Your local FSE and specialists
- Agilent University www.agilent.com/crosslab/university
- YouTube – Agilent Channel (maintenance videos)
- Agilent service contracts

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for former Prozyme products

Available in the U.S. and Canada, 8–5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Web chat: Product pages of agilent.com

Thank you

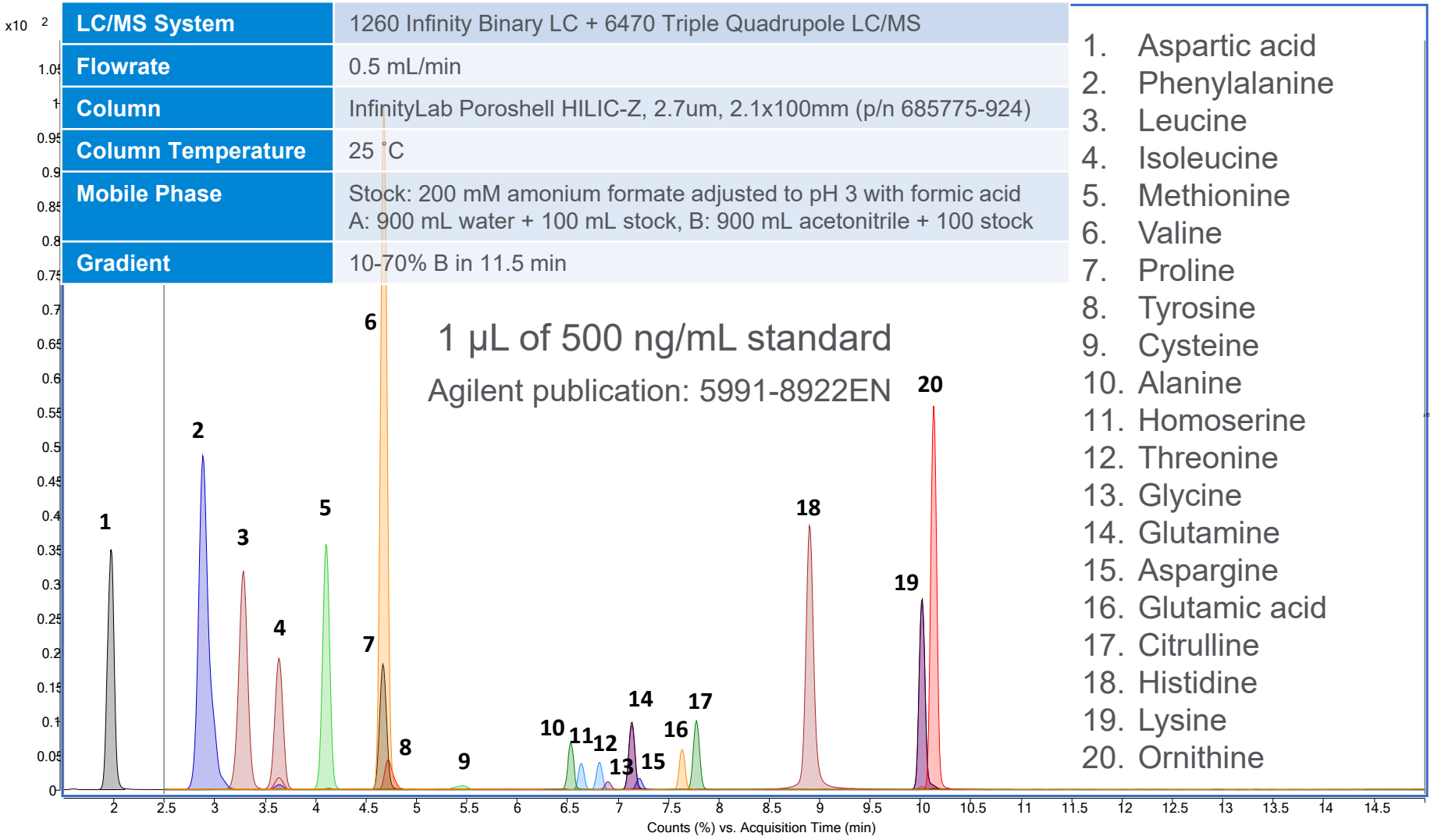
Appendix

Agilent applications

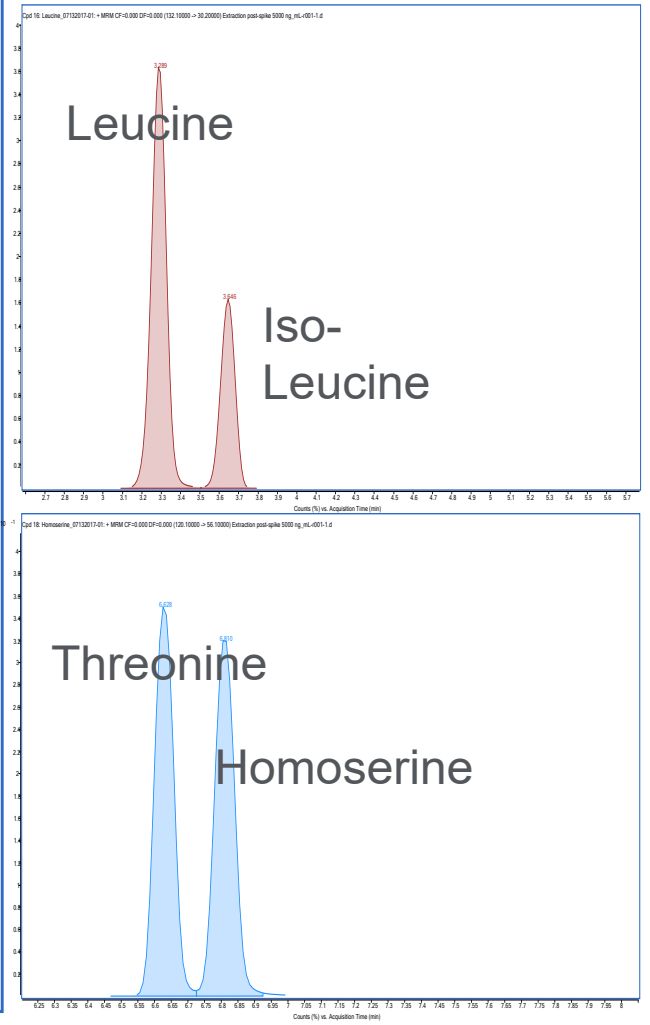
Analysis of Amino Acids (and Isobars) in Plant Tissue with LC-MS/MS

Agilent InfinityLab

LC/MS System	1260 Infinity Binary LC + 6470 Triple Quadrupole LC/MS
Flowrate	0.5 mL/min
Column	InfinityLab Poroshell HILIC-Z, 2.7um, 2.1x100mm (p/n 685775-924)
Column Temperature	25 ˚C
Mobile Phase	Stock: 200 mM amonium formate adjusted to pH 3 with formic acid A: 900 mL water + 100 mL stock, B: 900 mL acetonitrile + 100 stock
Gradient	10-70% B in 11.5 min

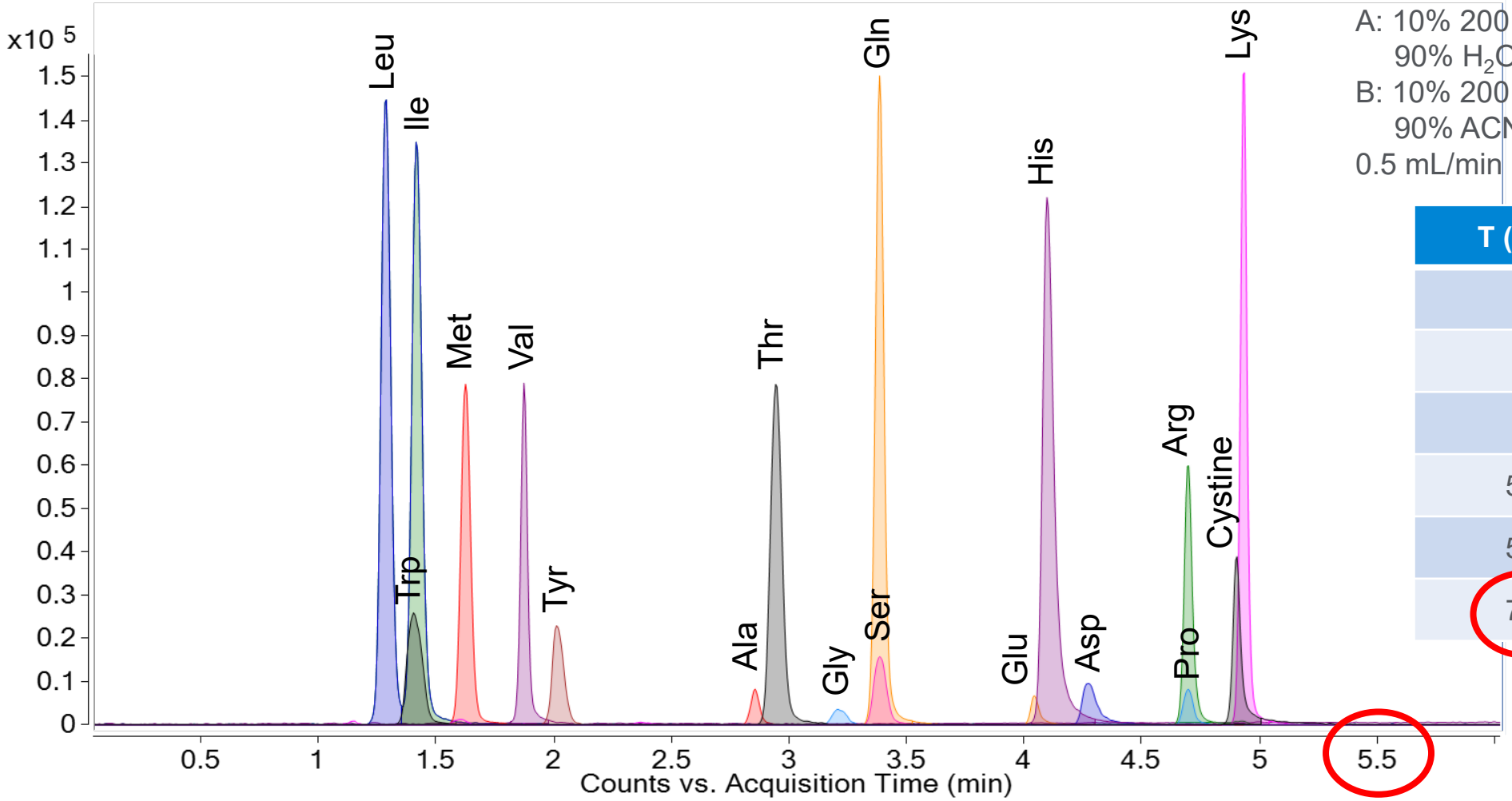


1. Aspartic acid
2. Phenylalanine
3. Leucine
4. Isoleucine
5. Methionine
6. Valine
7. Proline
8. Tyrosine
9. Cysteine
10. Alanine
11. Homoserine
12. Threonine
13. Glycine
14. Glutamine
15. Aspargine
16. Glutamic acid
17. Citrulline
18. Histidine
19. Lysine
20. Ornithine



Agilent

High Throughput LC/MS Analysis of Amino Acids with an Agilent InfinityLab Poroshell 120 HILIC-Z Column



InfinityLab Poroshell 120 HILIC-Z
2.1 x 50 mm
A: 10% 200 mM ammonium formate pH 3.5, 90% H_2O
B: 10% 200 mM ammonium formate pH 3.5, 90% ACN
0.5 mL/min

T (min)	%B
0	97
2	90
5	70
5.5	70
5.6	97
7.5	97

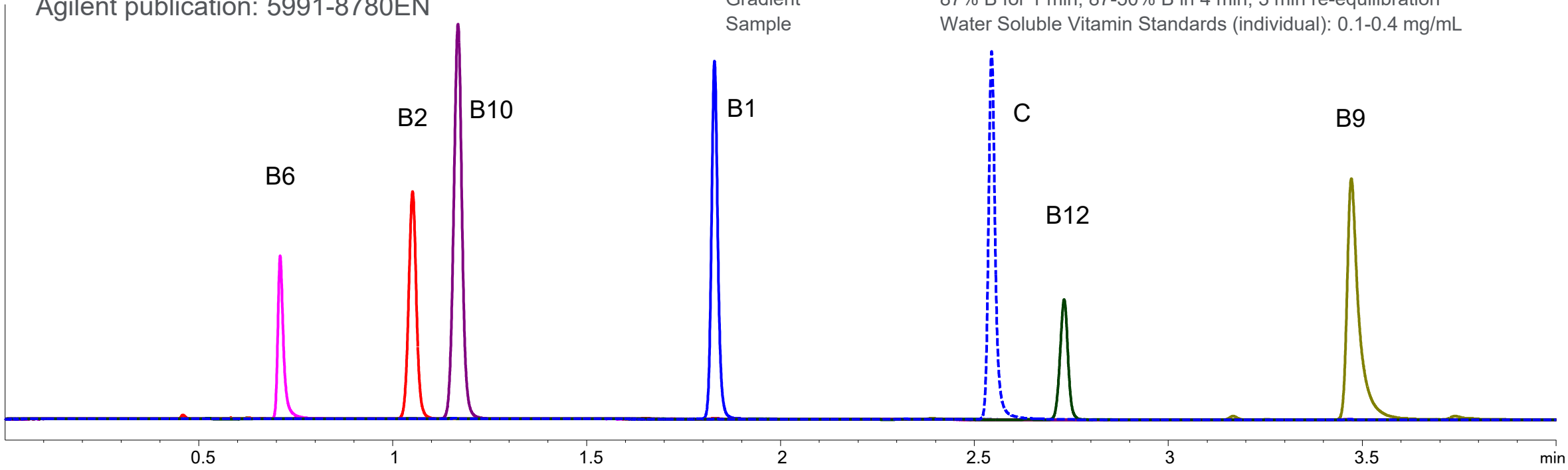
Water Soluble Vitamins on Agilent InfinityLab Poroshell 120 HILIC-Z

Agilent InfinityLab

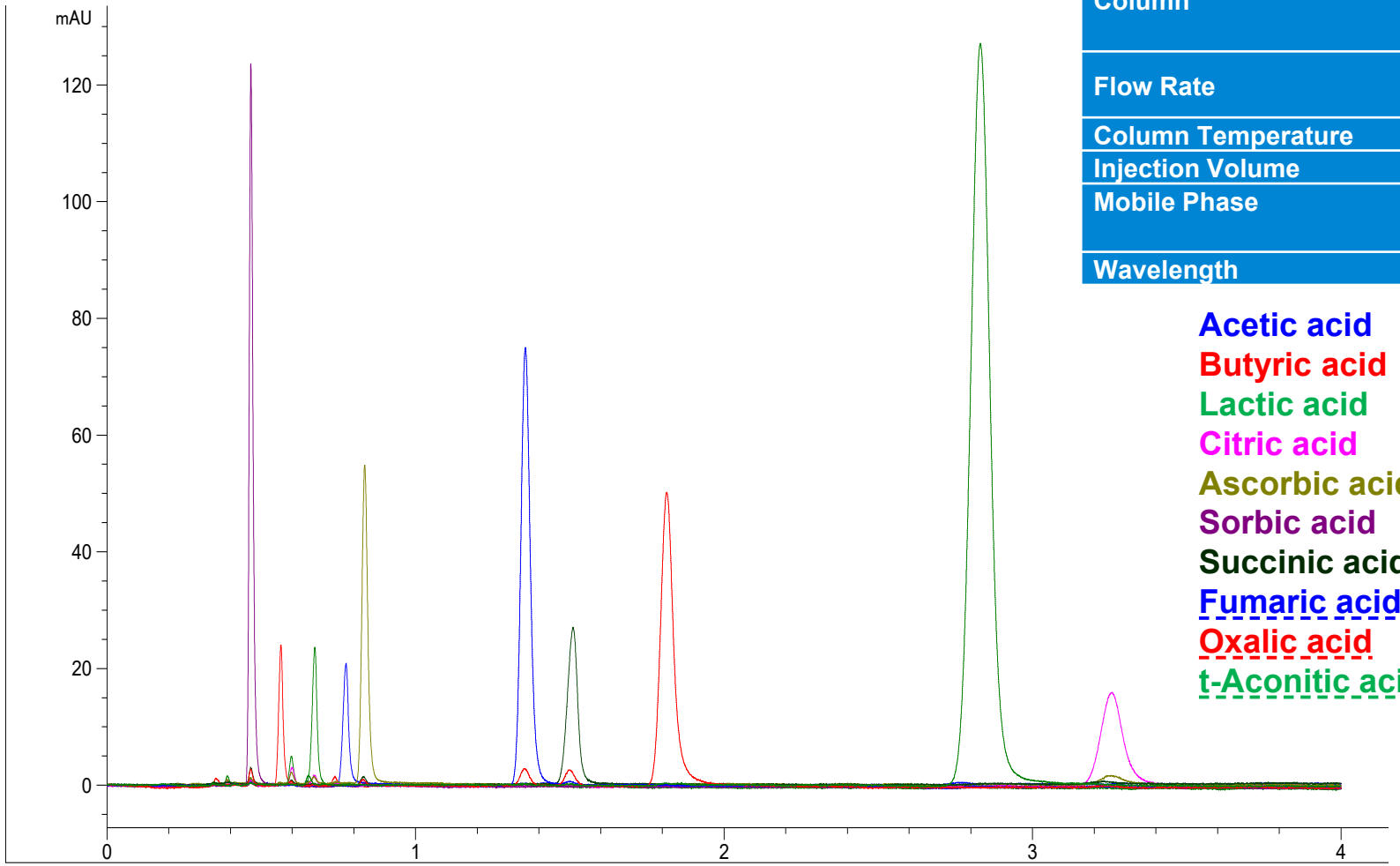
System	Agilent 1260 Infinity Binary HPLC w/ DAD
Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 x 100 mm (p/n: 685775-924)
Flow Rate	0.5 mL/min
Column Temperature	40 ˚C
Injection Volume	1 µL
Mobile Phase A	100 mM ammonium acetate + 0.5% acetic acid in water
Mobile Phase B	Acetonitrile
Wavelength	260 nm
Data Rate	80 Hz
Gradient	87% B for 1 min, 87-50% B in 4 min, 3 min re-equilibration
Sample	Water Soluble Vitamin Standards (individual): 0.1-0.4 mg/mL

InfinityLab Poroshell 120 HILIC-Z

Agilent publication: 5991-8780EN



Organic Acids on Agilent InfinityLab Poroshell 120 HILIC-Z



Agilent 1260 Infinity Binary HPLC with DAD

Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 x 100 mm, 2.7 µm (p/n: 685775-924)
Flow Rate	0.5 mL/min
Column Temperature	30 ˚C
Injection Volume	1 µL
Mobile Phase	30% 30 mM sodium phosphate + 0.075% phosphoric acid, pH ~6.7, 70% ACN*
Wavelength	214 nm

Acetic acid
Butyric acid
Lactic acid
Citric acid
Ascorbic acid
Sorbic acid
Succinic acid
Fumaric acid
Oxalic acid
t-Aconitic acid

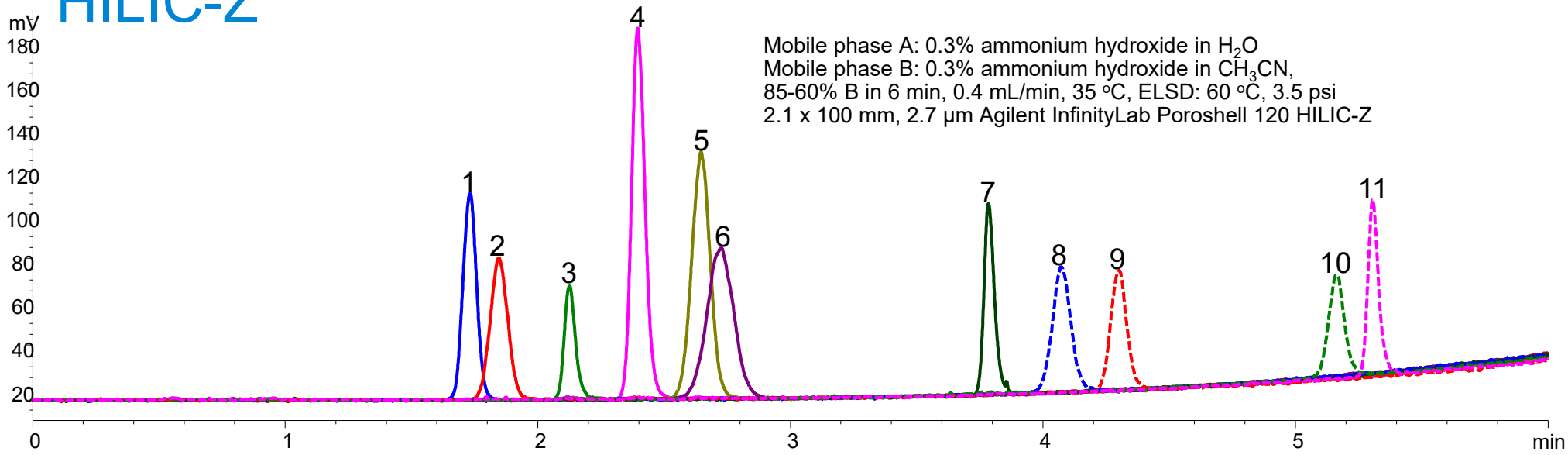
***Sodium phosphate is not soluble in high % ACN.**

- **Do not increase salt concentration in mobile phase A.**
- **Do not increase %B**
- **If using ELSD or MS, use similar pH/concentration ammonium acetate instead**

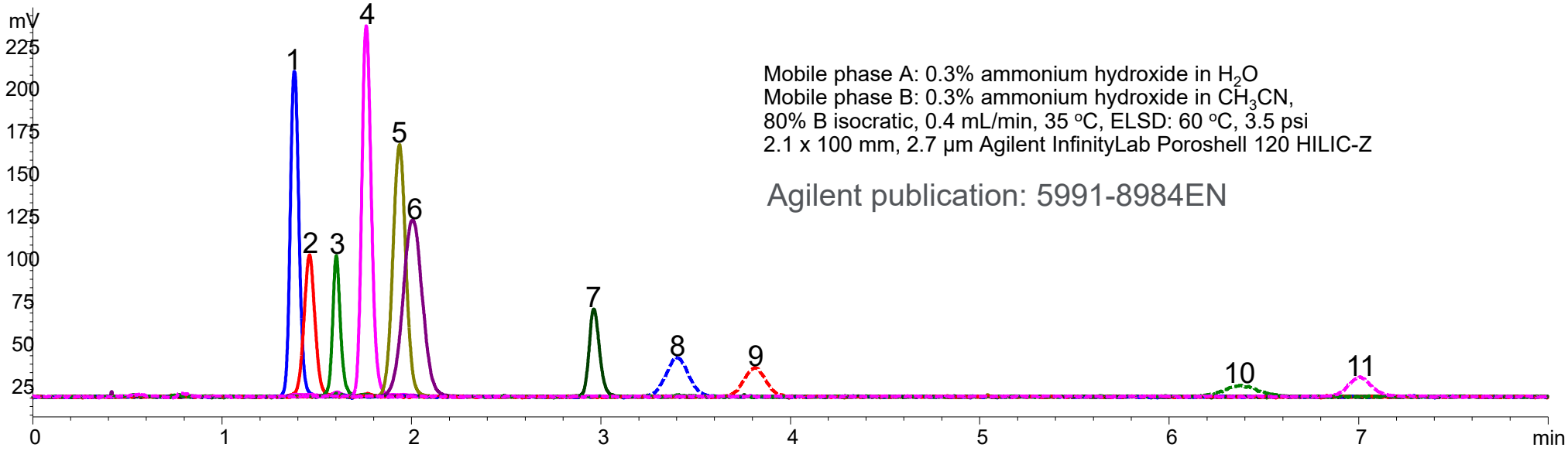
Agilent publication: 5991-8985EN

Separation of 11 Sugars on Agilent InfinityLab Poroshell 120 HILIC-Z

Agilent InfinityLab



Mobile phase A: 0.3% ammonium hydroxide in H_2O
Mobile phase B: 0.3% ammonium hydroxide in CH_3CN,
85-60% B in 6 min, 0.4 mL/min, 35 °C, ELSD: 60 °C, 3.5 psi
2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z



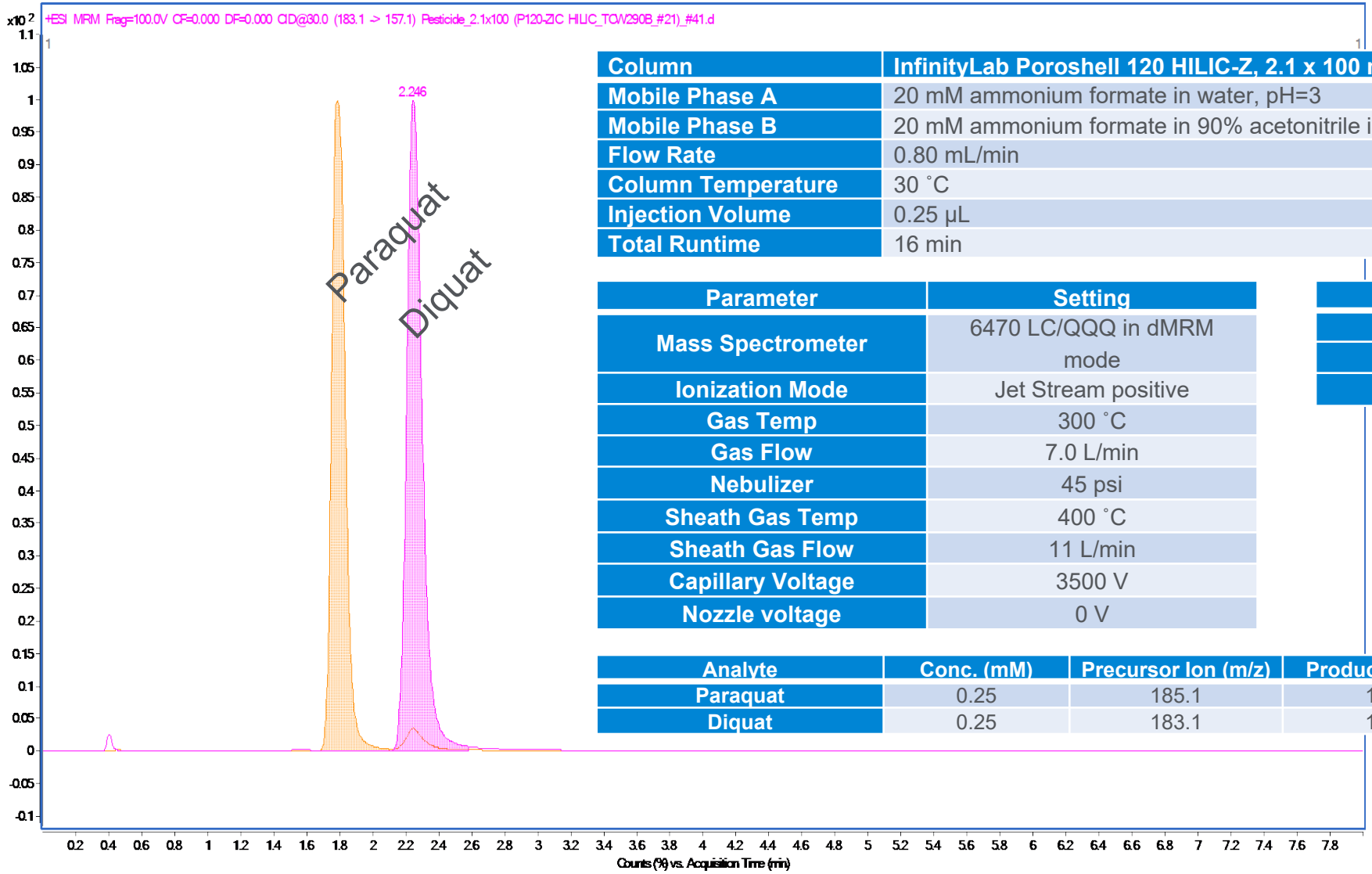
Mobile phase A: 0.3% ammonium hydroxide in H_2O
Mobile phase B: 0.3% ammonium hydroxide in CH_3CN,
80% B isocratic, 0.4 mL/min, 35 °C, ELSD: 60 °C, 3.5 psi
2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z

Agilent publication: 5991-8984EN

1. Xylose
2. Arabinose
3. Fructose
4. Mannose
5. Glucose
6. Galactose
7. Sucrose
8. Maltose
9. Lactose
10. Maltotriose
11. Raffinose

Agilent

InfinityLab Poroshell 120 HILIC-Z Analysis of Paraquat/Diquat



Column	**InfinityLab Poroshell 120 HILIC-Z, 2.1 x 100 mm**
Mobile Phase A	20 mM ammonium formate in water, pH=3
Mobile Phase B	20 mM ammonium formate in 90% acetonitrile in water, pH=3
Flow Rate	0.80 mL/min
Column Temperature	30 ˚C
Injection Volume	0.25 µL
Total Runtime	16 min

Parameter	Setting
Mass Spectrometer	6470 LC/QQQ in dMRM mode
Ionization Mode	Jet Stream positive
Gas Temp	300 ˚C
Gas Flow	7.0 L/min
Nebulizer	45 psi
Sheath Gas Temp	400 ˚C
Sheath Gas Flow	11 L/min
Capillary Voltage	3500 V
Nozzle voltage	0 V

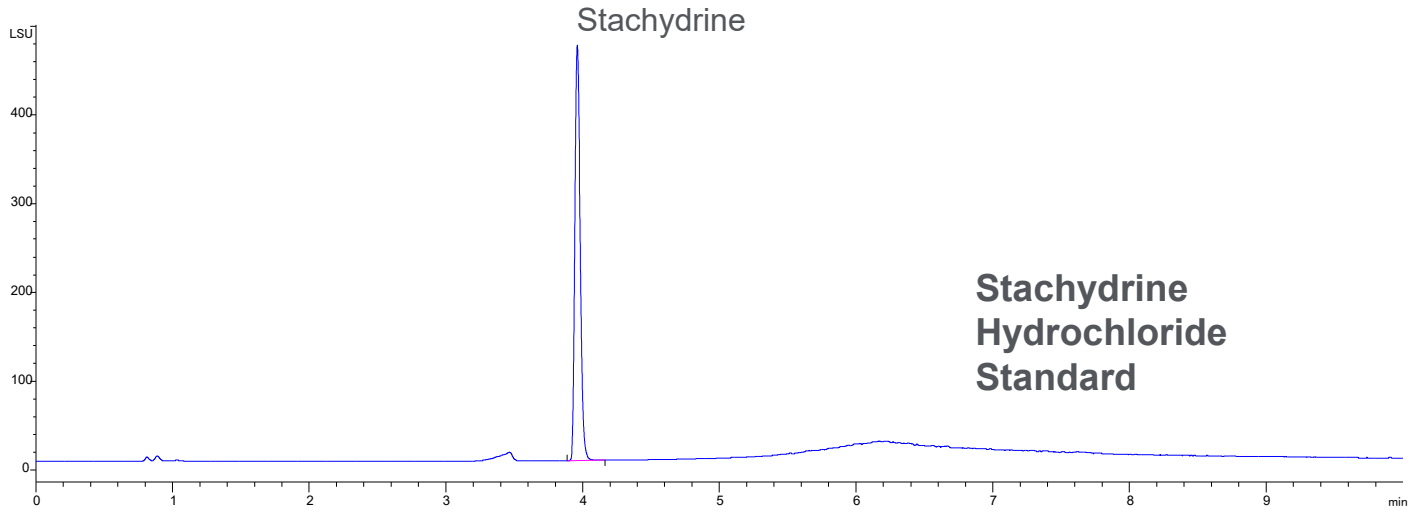
Time (min)	Percentage B
0	100
10	70
11	100

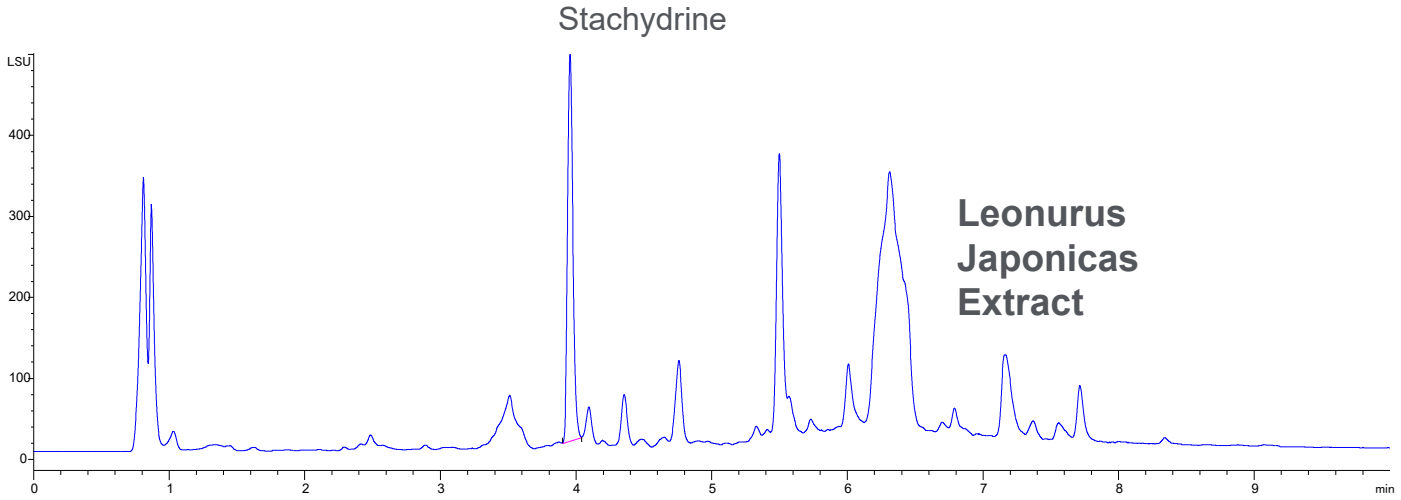
Agilent Pub # 5991-8830EN

Analyte	Conc. (mM)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Dwell Time (ms)
Paraquat	0.25	185.1	170.1	100	10
Diquat	0.25	183.1	157.1	100	10

Analysis of Polar Compounds in Plant Materials: Quantitation of Stachydrine in Chinese Motherwort (Leonurus japonicas) by InfinityLab Poroshell 120 HILIC-Z

Agilent InfinityLab





Agilent 1260 Infinity II HPLC

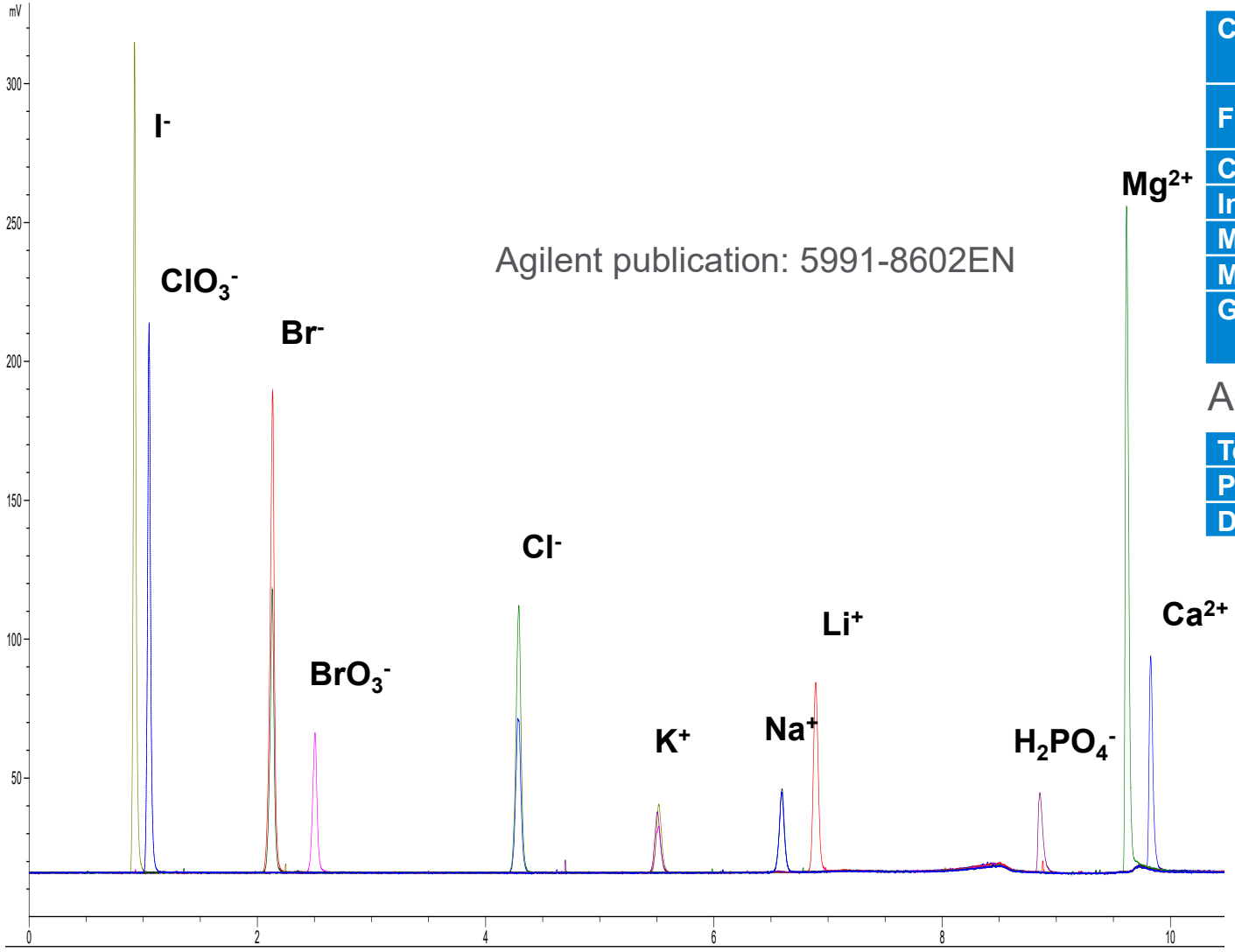
Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 x 100 mm (p/n 685775-924)
Mobile Phase A	10 mmol ammonium acetate
Mobile Phase B	Acetonitrile
Flow Rate	0.30 mL/min
Gradient	95-60% B in 10 min
Column Temperature	30 ˚C
Injection Volume	2 µL

Agilent 1290 Infinity II ELSD

Nebulizer Temperature	40 ˚C
Evaporator Temperature	40 ˚C
Gas flow rate	1.6 SLM
Data Rate	40 Hz

Agilent publication: 5991-8617EN

Analysis of Metals, Halides, and Inorganic Ions on Agilent InfinityLab Poroshell HILIC-Z



Agilent publication: 5991-8602EN

Agilent 1260 Infinity Binary HPLC

Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 x 100 mm, 2.7 µm (p/n 685775-924)
Flow Rate	0.40 mL/min
Column Temperature	30 ˚C
Injection Volume	1 µL
Mobile Phase A	100 mM ammonium formate in water at pH=3
Mobile Phase B	Acetonitrile
Gradient	91% B for 1 min, 91-80% B in 5 min, 80-20% B in 5 min, 3 min re-equilibration

Agilent G4218A ELSD

Temperature	40 ˚C
Pressure	3.5 psi
Data Rate	30 Hz

Samples:

- Calcium chloride
- Lithium bromide
- Magnesium chloride
- Potassium bromate
- Potassium iodide
- Potassium phosphate
- Sodium bromide
- Sodium chlorate