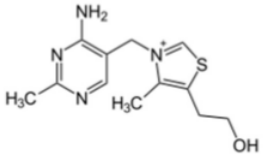
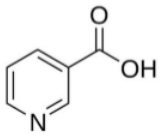
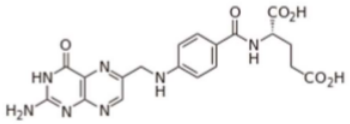


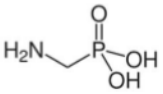
B1: Thiamine



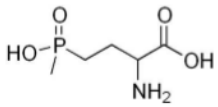
B3: Nicotinic Acid



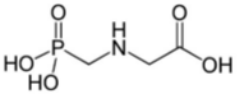
B9: Folic Acid



AMPA

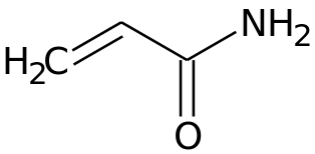


Glufosinate

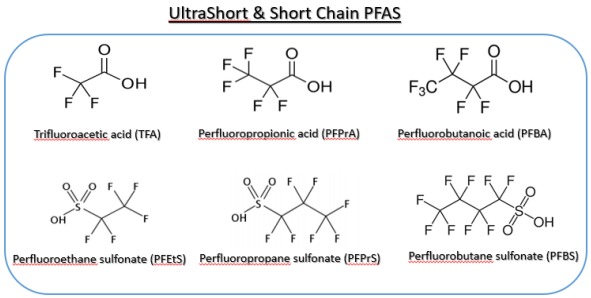


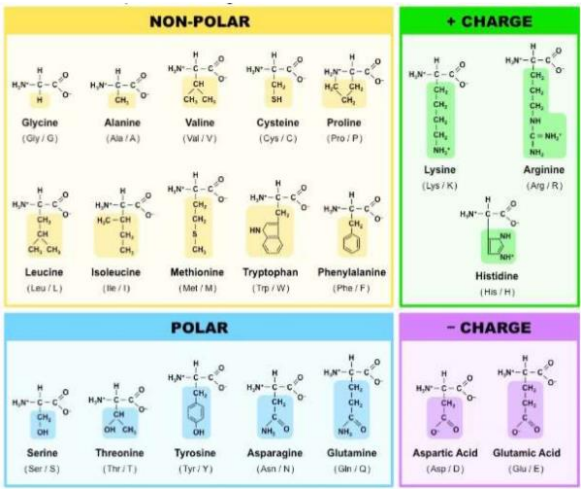
Glyphosate

Update on HPLC analysis of Polar Compounds



Acrylamide





Summary

- What is a polar molecule ?
- Acrylamide Analysis
  - Who is analysing it & Why ?
  - Acrylamide in Food Matrices – new Allure Acrylamide LC column for Food
  - Acrylamide in Water – new Allure Acrylamide LC column for Water
- New Raptor Polar X LC Column
  - Chemistry of the stationary phase
  - Applications examples
    - Glyphosate & Co.
    - QuPPe – Quick Polar Pesticides
    - PFAS (& TFA)
    - Underivatized Amino Acids
    - Water Soluble Vitamins

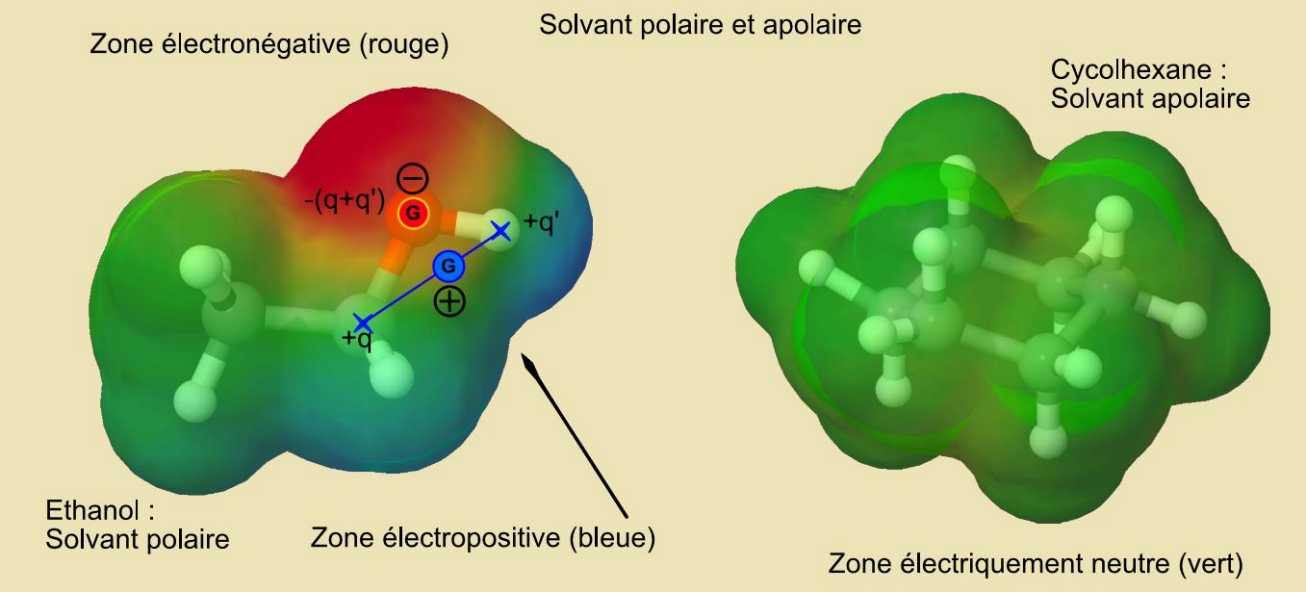
Polar Molecules

Definition

- *** A molecule is polar if :**
  - **It has polarized bonds** = covalent bond between 2 atoms which have a significant difference in electronegativity inducing the creation of partial charges (q) on both sides of the bond.

  AND

  - **The average position of these partial charges is not "confused"**



- * https://www.cours.jlrichter.fr/lycee/premiere-s/1sppc-6-de-la-structure-a-la-polarite-dune-entite/

Polar Molecules

Definition

- Polarity depends on :
  - The difference in electronegativity between molecular atoms
  - Compounds geometry
- The polarity of a molecule is measured by its dipole moment
- Dipole moment = (charge at each end of the dipole) X (distance between charges)

$$\log P = \log\left(\frac{[C]_{octanol}}{[C]_{water}}\right)$$

$$\log D_{\text{acides}} = \log P + \log\left[\frac{1}{(1 + 10^{pH - pK_a})}\right]$$

- LogP = log ($[C]_{Octanol}$ / $[C]_{Eau}$) – Octanol/Water partition coefficient
  - LogP > 0 → molecule more soluble in octanol than water → lipophilic character → non-polar
  - LogP < 0 → molecule more soluble in water than octanol → hydrophilic character → polar
  - LogP < -1 → very polar
- LogD is more precise than LogP because it takes into account the modification of LogP in relation with the pH of the aqueous mobile phase and the pKa of the compound

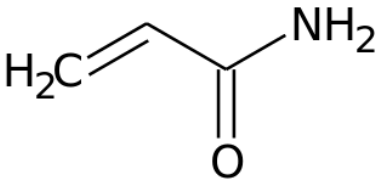
Polar Molecules

Current Solutions & Drawbacks

Technique	Drawbacks
Reverse Phase + Ion Pairing	Equilibration time MS friendly ?
Reverse Phase + Derivatization	Time consuming Cause of variability
*Ionic Exchange	Not ideal for mixtures (acids, bases and neutrals)
*HILIC	Equilibration time Injection solvent match
Chelating agents (in mobile phase, to get rid of metallic ions)	MS friendly ?
*System passivation	Equilibration time Variability

Acrylamide Analysis

Acrylamide is a compound formed when food containing asparagine (amino acid) and glucose is cooked at high temperature. This reaction – which is part of the Maillard reactions – occurs when foods (especially those with high starch content) are roasted of fried. It is highly toxic and the discovery of Acrylamide presence in some cooked foods has made the vigilance around its potential biological effects greatly increasing in the world.



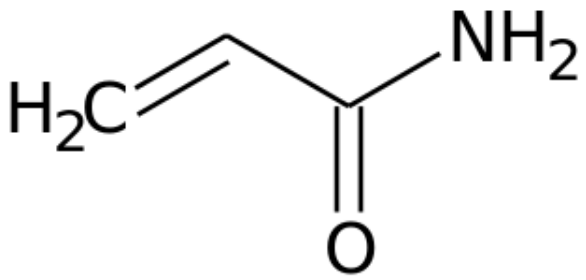
Acrylamide is a white, odourless solid, soluble in water and several organic solvents, which is industrially produced as a precursor for polyacrylamides, these being used as thickeners or flocculating agents.

Who is analysing Acrylamide & Why ?

- "Food" laboratories – on Food matrices
  - Private
  - Industrial
  - Public
- "Environment" laboratories – generally to analyse the decomposition of polyacrylamide flocculating agents
  - Private
  - Public
- Why ?
  - An exposure at a high level can cause neurological defects, cancer and reproduction problems.
  - The International Agency for Research on Cancer (IARC) has classified Acrylamide as a potentially carcinogenic substance (group 2A).
  - Requested by Environmental Agencies → France : Concentration of 0,1µg/L in water (Direct Injection)

Acrylamide in Food Matrices

New Restek Allure Acrylamide LC Column, standards & LC-MS/MS method



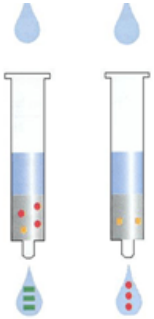
Acrylamide in Food Matrices

Analysis Scheme

Sample

Grinding

Extraction in water

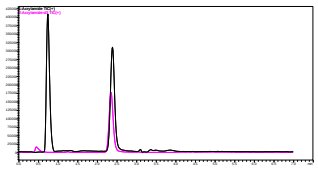


SPE (2 steps)

Sample Preparation

LC Separation & MS Detection

Data Analysis



DIN

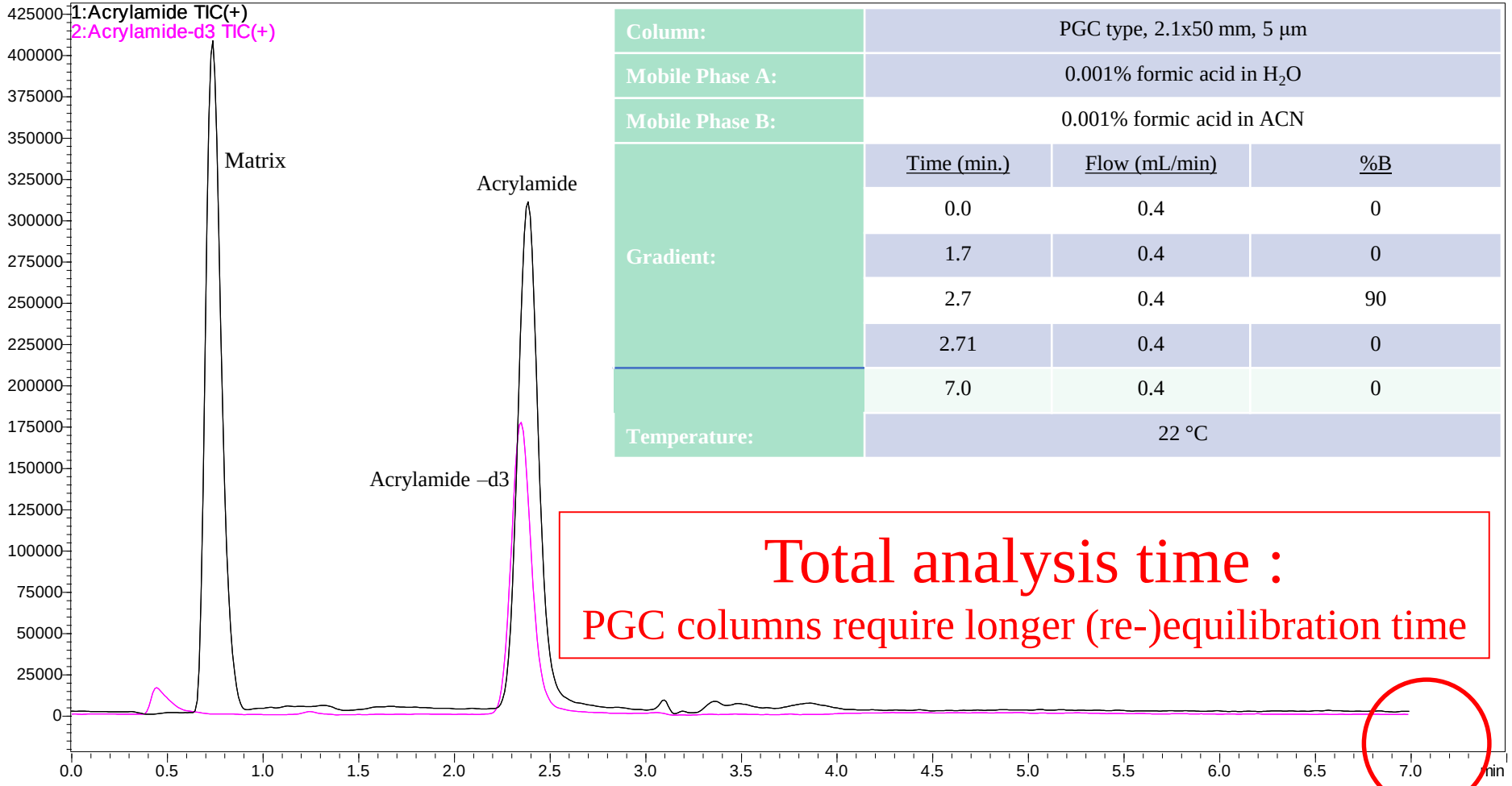
DIN EN 16618

Acrylamide in Food Matrices
Current Methods

- The most “popular” method uses Porous Graphitized Carbon (PGC) columns

- Most of the other methods use reverse phase columns (100% aqueous or not)

Acrylamide in Food Matrices
Analysis on PGC columns

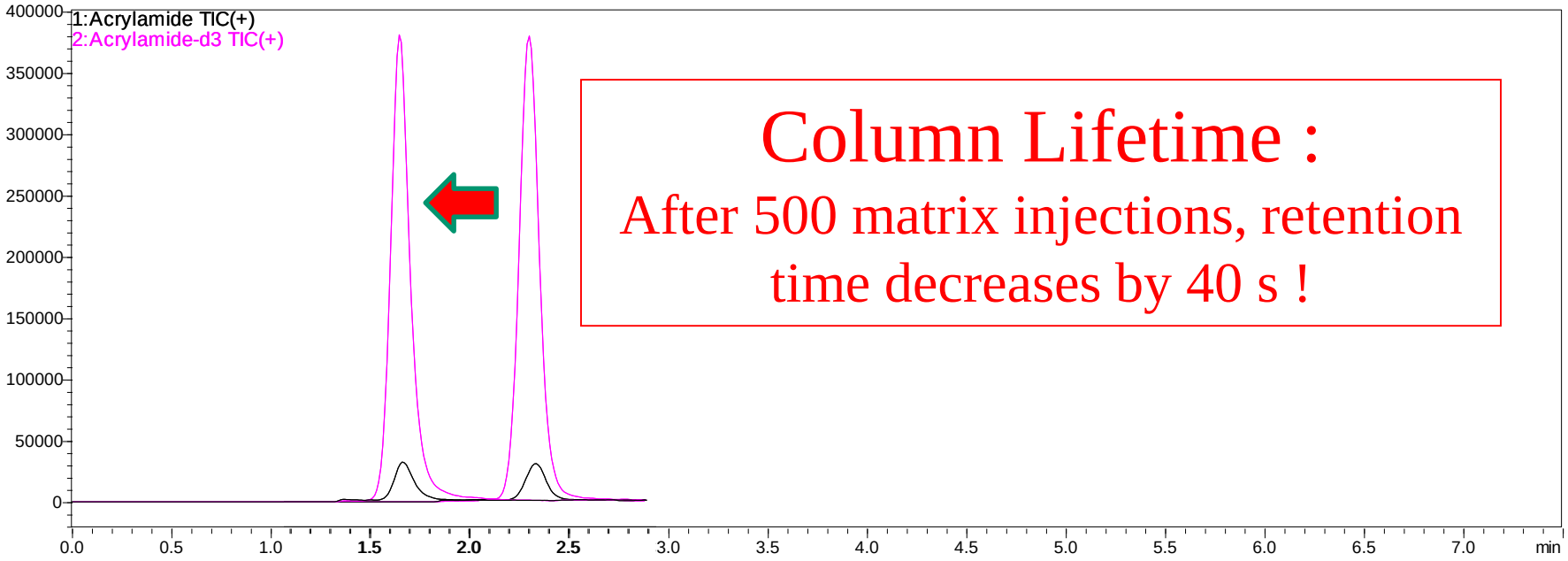


Column:	PGC type, 2.1x50 mm, 5 µm		
Mobile Phase A:	0.001% formic acid in H_2O		
Mobile Phase B:	0.001% formic acid in ACN		
Gradient:	Time (min.)	Flow (mL/min)	%B
	0.0	0.4	0
	1.7	0.4	0
	2.7	0.4	90
	2.71	0.4	0
	7.0	0.4	0
Temperature:	22 °C		

Total analysis time :

PGC columns require longer (re-)equilibration time

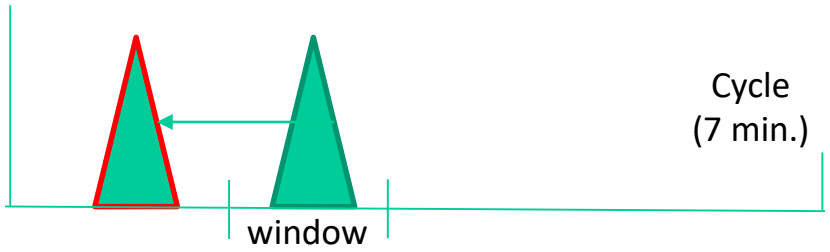
Acrylamide in Food Matrices
Analysis on PGC columns

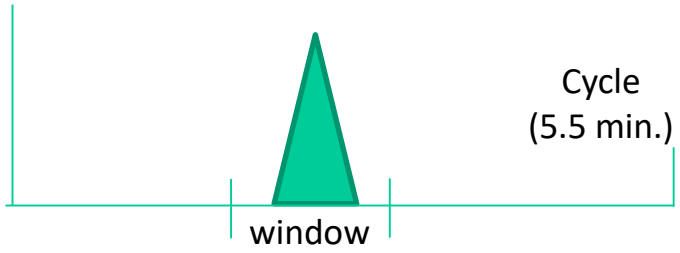


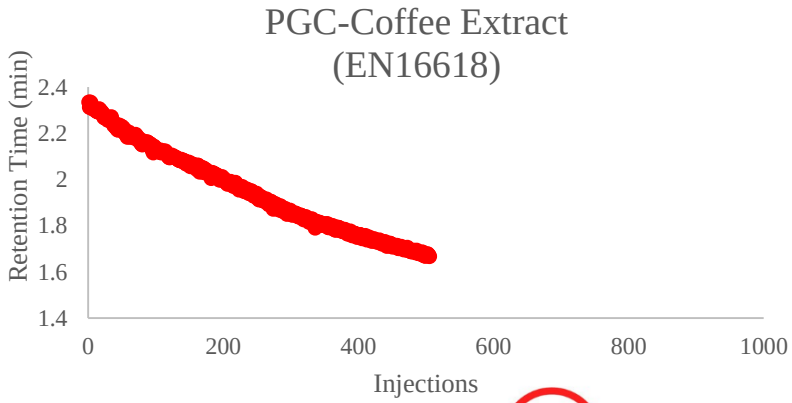
Column Lifetime :

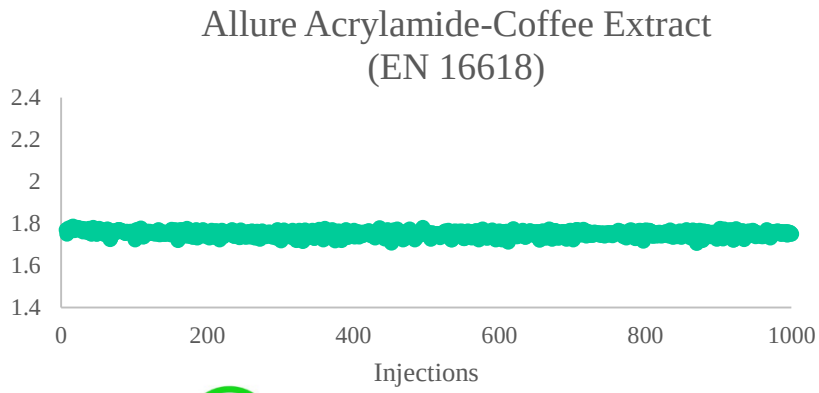
After 500 matrix injections, retention time decreases by 40 s !

Acrylamide in Food Matrices
Analysis on PGC columns









✗	Lifetime	✓
✗	Instrument Uptime	✓
✗	Result Quality	✓

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

Cat.# 9167552

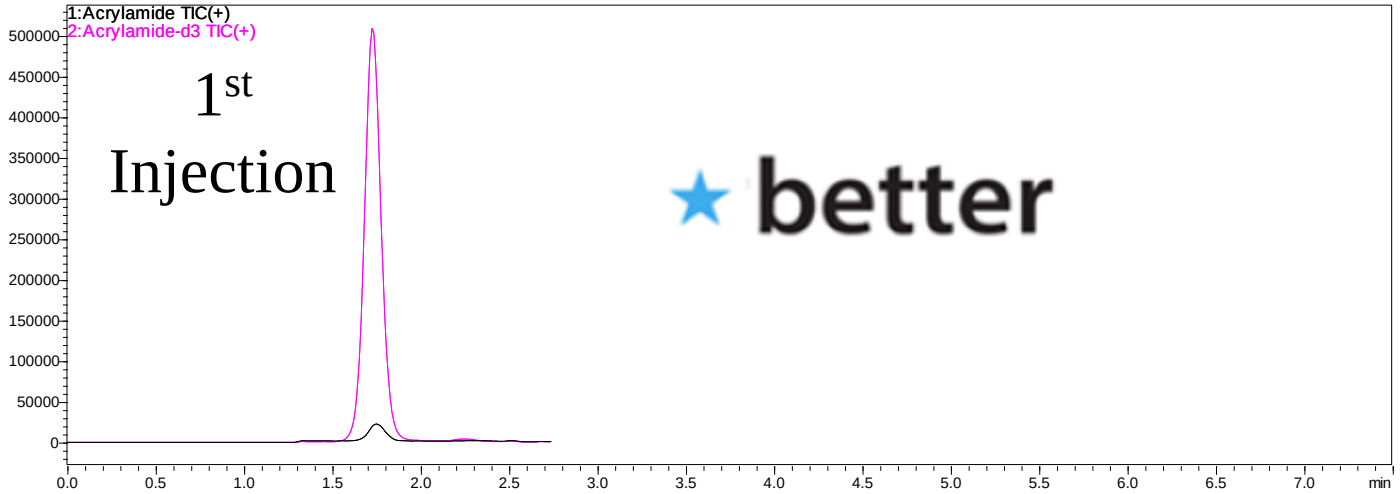
Allure Acrylamide LC Column

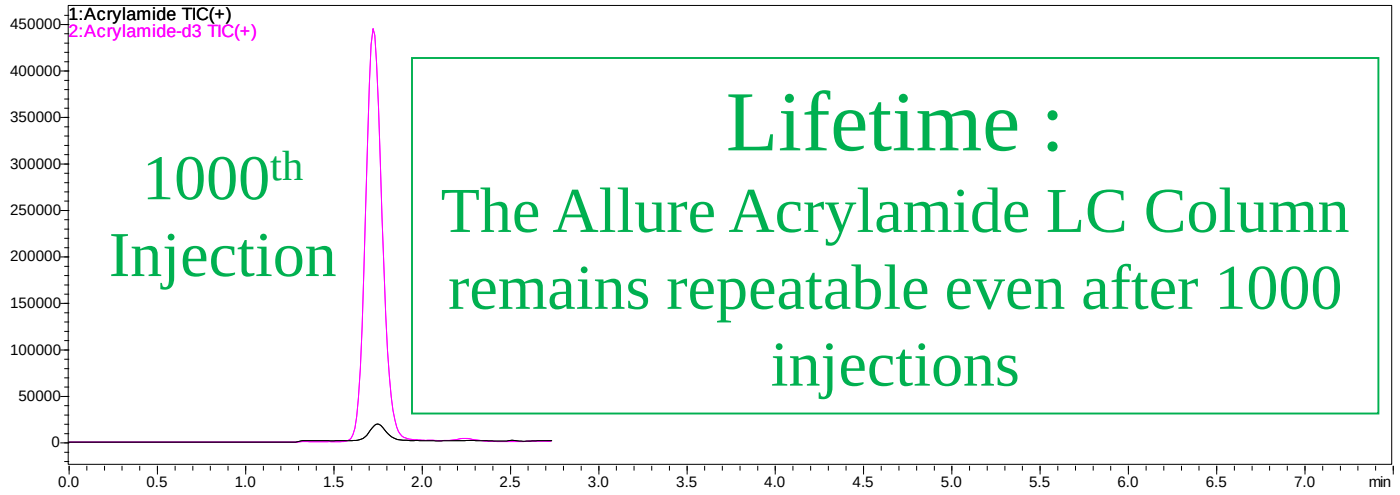
Particle: 5 µm, spherical
Pore size : 60 Å
Carbon load : proprietary
End-capping: no
Surface area: 450 m^2/g
pH range: 2.5 to 8
Max. Temperature: 80 °C
Equivalent Phases: Unique
Length: **50 mm**
Internal diameter: **2.1 mm**

NB : are also included in the Restek solution : deuterated internal standard (Ref. 30153), acrylamide standard (ref. 30494) & guard column 10mm (Trident)

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column



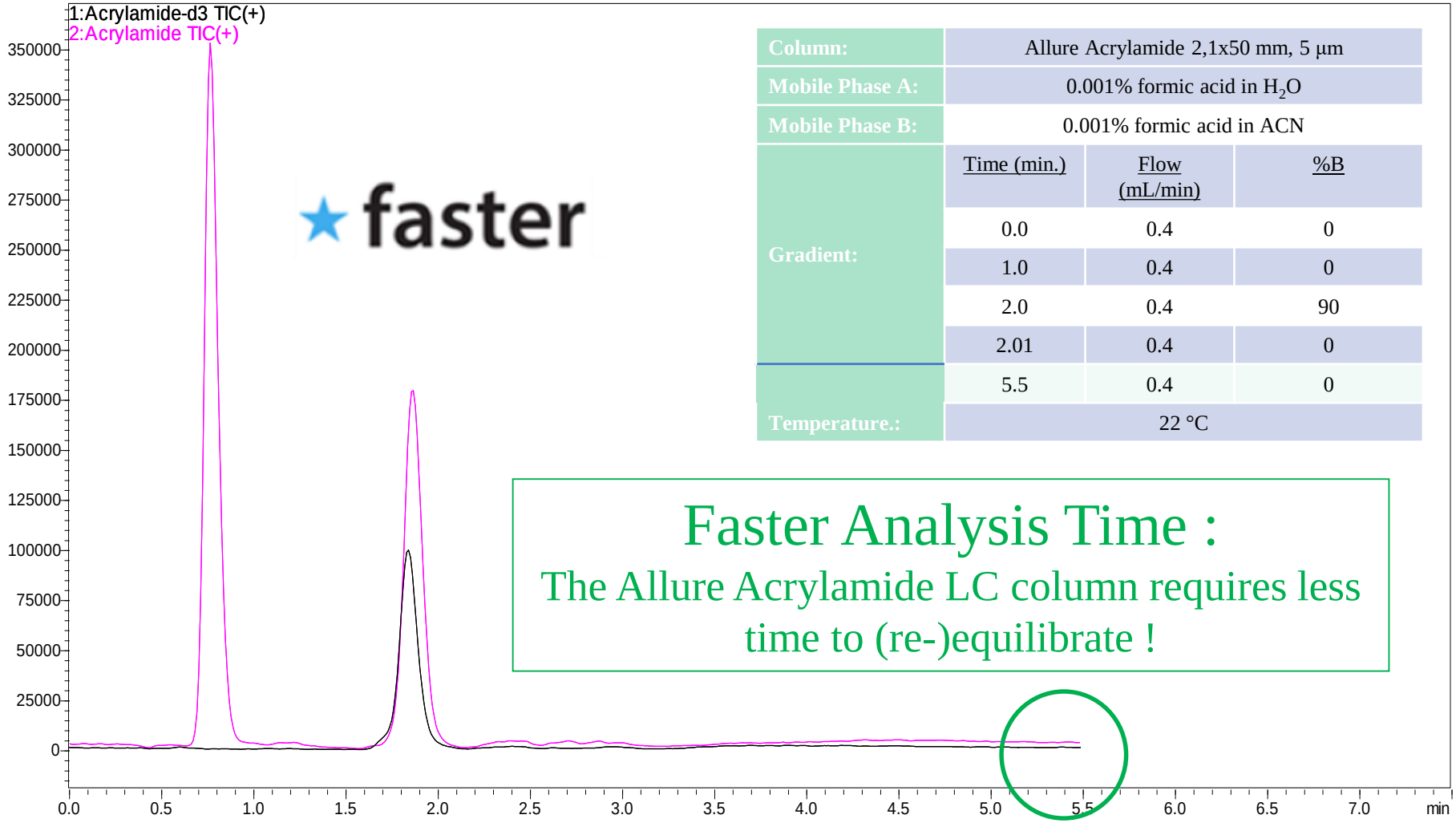


Lifetime :

The Allure Acrylamide LC Column remains repeatable even after 1000 injections

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column



★ faster

Column:	Allure Acrylamide 2,1x50 mm, 5 µm		
Mobile Phase A:	0.001% formic acid in H_2O		
Mobile Phase B:	0.001% formic acid in ACN		
Gradient:	Time (min.)	Flow (mL/min)	%B
	0.0	0.4	0
	1.0	0.4	0
	2.0	0.4	90
	2.01	0.4	0
	5.5	0.4	0
Temperature.:	22 °C		

Faster Analysis Time :

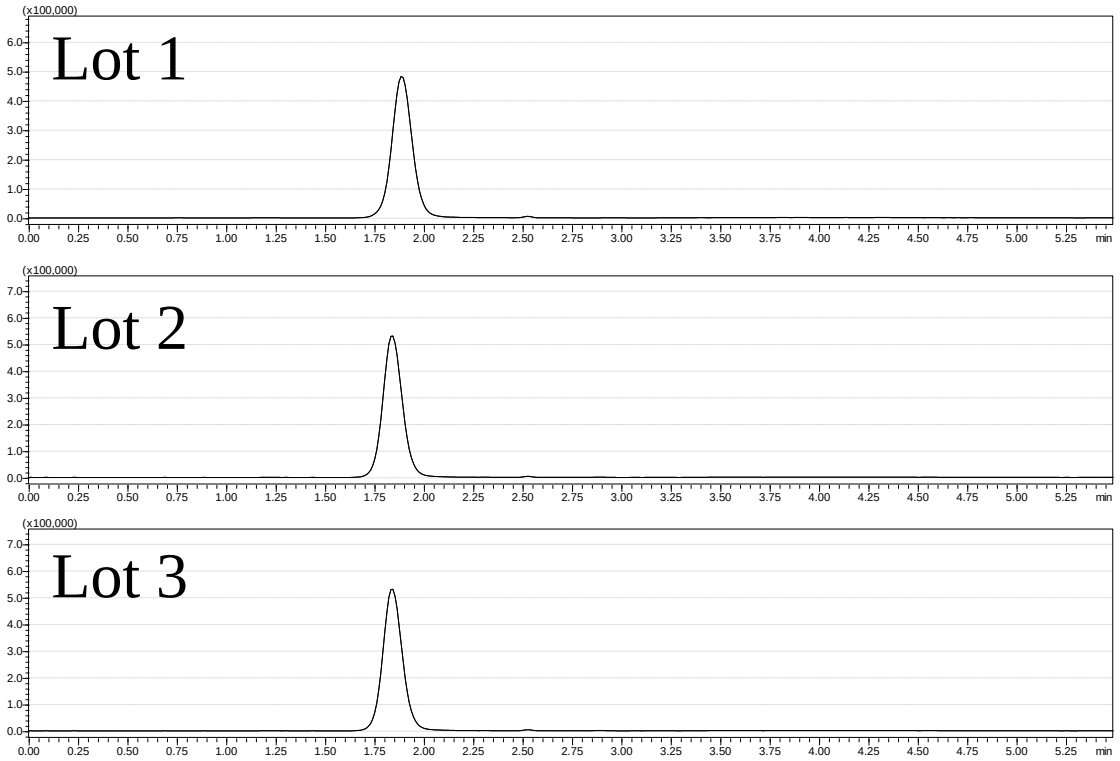
The Allure Acrylamide LC column requires less time to (re-)equilibrate !

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

Reliability & Robustness

Lot-to-lot variability is excellent (~3% RSD) and the retention time stability makes data processing easier and data quality more reliable



Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

Cleaning Procedure

- PGC columns
- The recommended cleaning procedure to regenerate a column is long and complicated and it does not generally completely « restore » the column.

- Allure Acrylamide
- Cleaning included in each analysis increases column robustness – less instrument downtime to regenerate or change the column

★ simpler

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

Lead Times

- PGC columns
- 3 to 4 weeks lead time are not unusual for this type of column. This means you have to maintain a “buffer” stock or suspend the analysis until column reception.

- Allure Acrylamide
- Restek always has the needed material on hand (stock & manufacturing) and is ready to ship the products as soon as you need them.

★faster

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

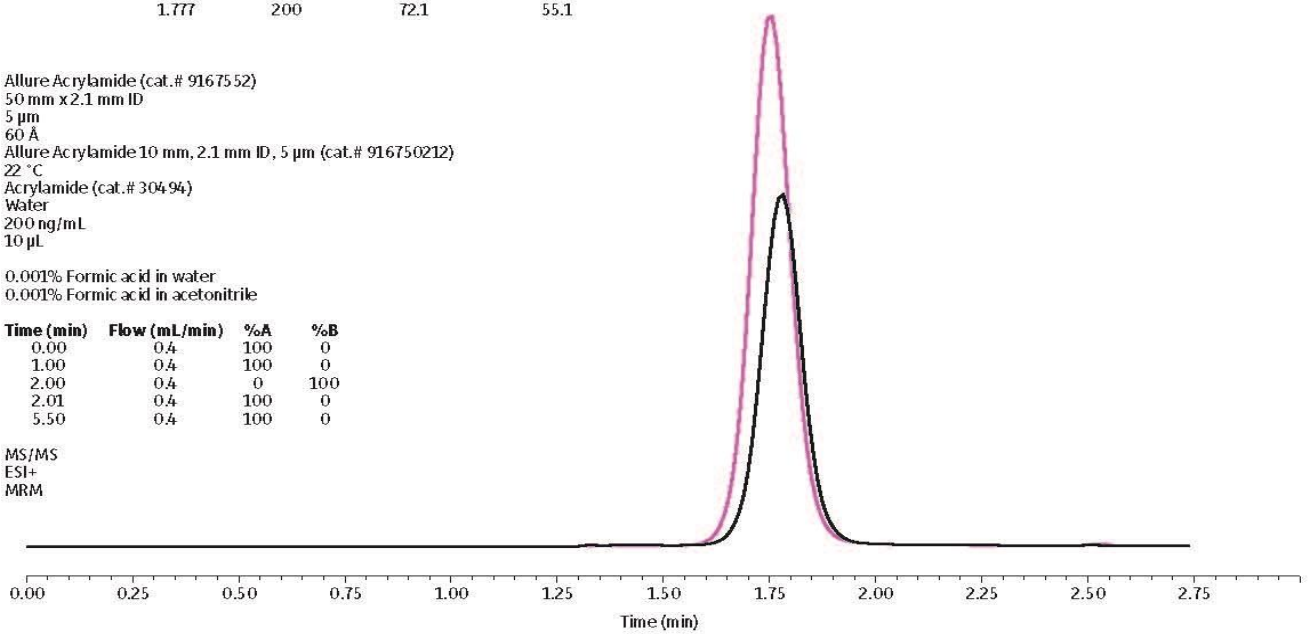
Acrylamide Reference Standard (200 ppb) and IS on Allure Acrylamide Column

Peaks	t_R (min)	Conc. (ng/mL)	Precursor	Product
1. Acrylamide-2,3,3-d3	1.739	200	75.1	58.1
2. Acrylamide	1.777	200	72.1	55.1

Column	Allure Acrylamide (cat.# 9167552)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	5 µm
Pore Size:	60 Å
Guard Column:	Allure Acrylamide 10 mm, 2.1 mm ID, 5 µm (cat.# 916750212)
Temp.:	22 °C
Sample	Acrylamide (cat.# 30494)
Diluent:	Water
Conc.:	200 ng/mL
Inj. Vol.:	10 µL
Mobile Phase	
A:	0.001% Formic acid in water
B:	0.001% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	100	0
1.00	0.4	100	0
2.00	0.4	0	100
2.01	0.4	100	0
5.50	0.4	100	0

Detector	MS/MS
Ion Mode:	ESI+
Mode:	MRM



LC_FS0529

Acrylamide in Food Matrices

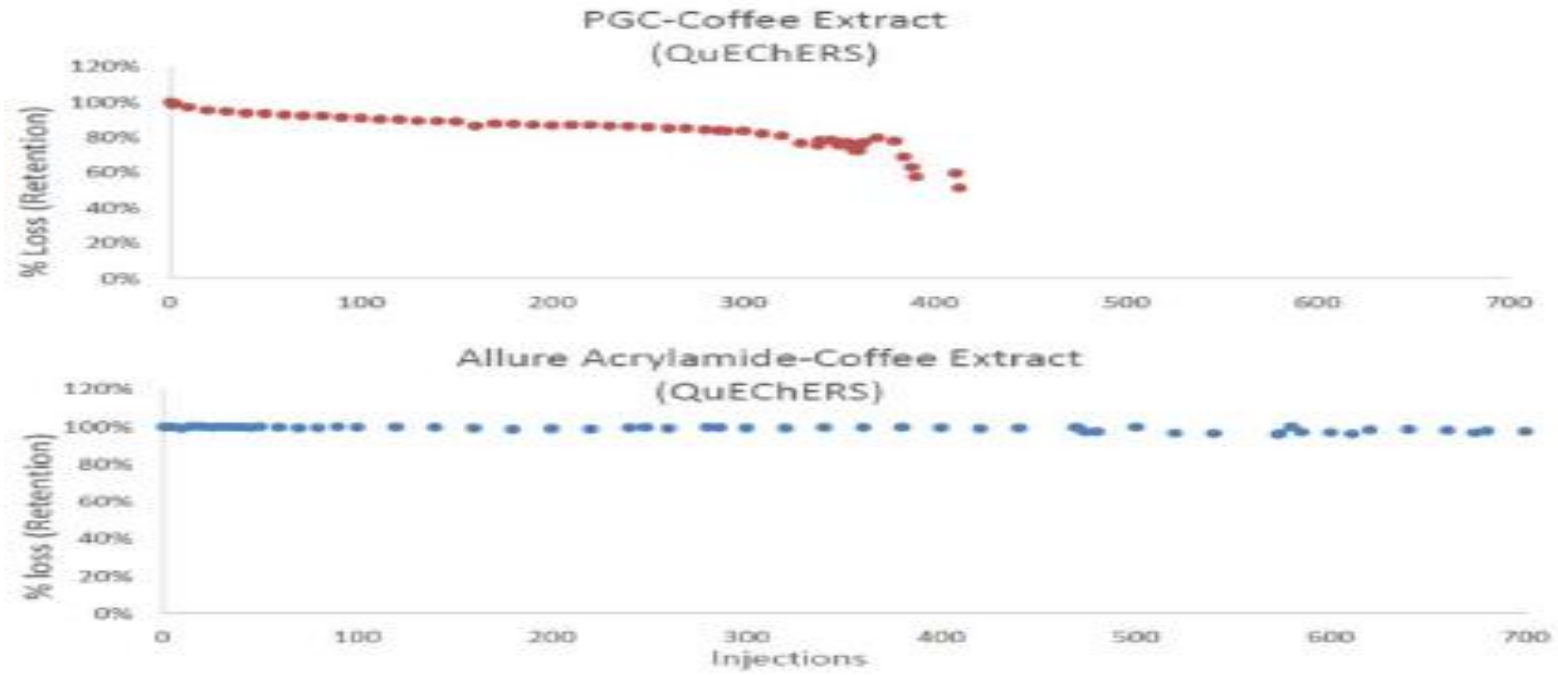
RESTEK Solution : Allure Acrylamide LC Column

Product characteristics	Benefits	**Restek Allure Acrylamide	PGC 1 Column	PGC 2 Column	C18 SB Column	« Polar » C18 column
***Fast analysis times**	High Throughput, faster results	Yes	No	No	No	Yes
***Retention time stability after repeated matrix injections**	Longer Lifetime	Yes	No	No	Uncertain	Uncertain
Retention and separation of interfering matrix compounds	Peak Quantification Simplification	Yes	Yes	Yes	Yes	No
Limited peak distortion after repeated matrix injections	Peak Quantification Simplification	Yes	No	No	Uncertain	Uncertain

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

- « Robustness » :

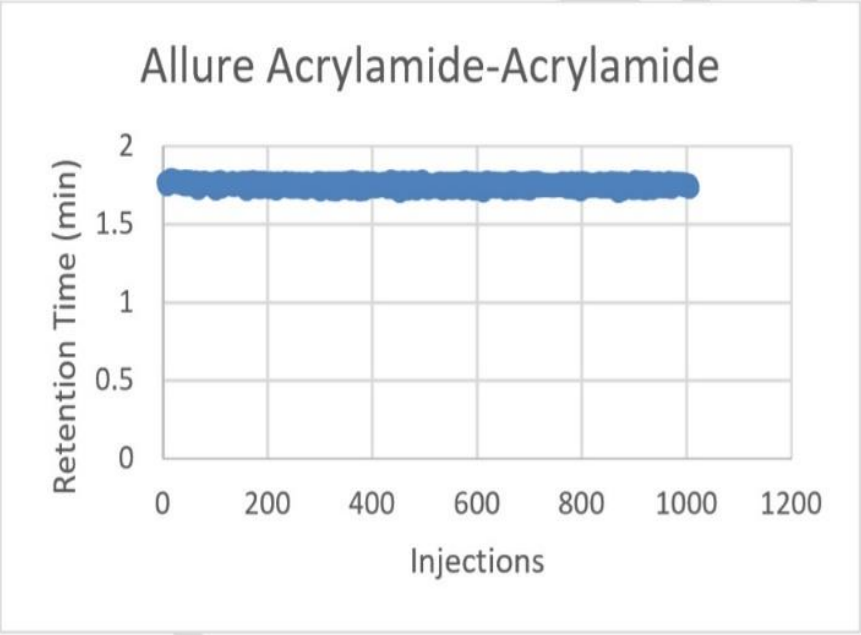


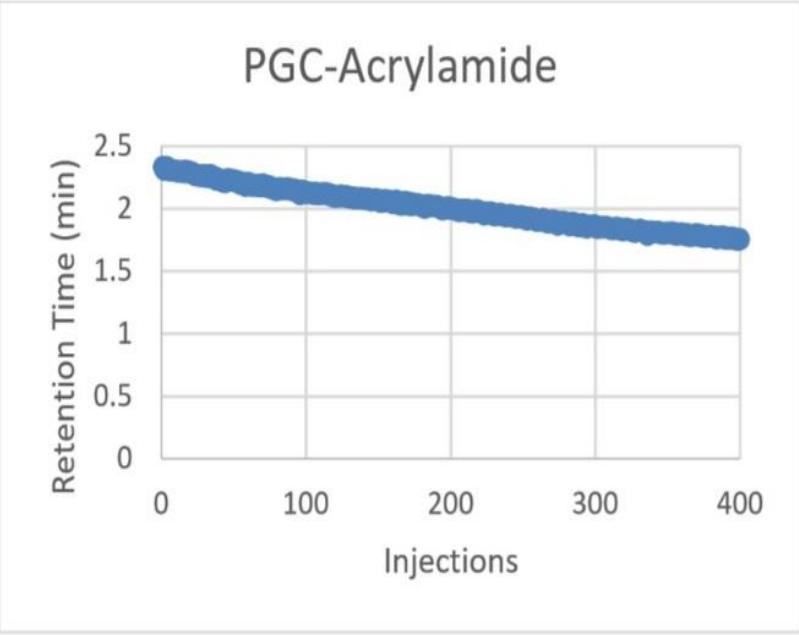
Lifetime test for PGC & Allure Acrylamide columns, after QuEChERS extraction of a coffee matrix

Acrylamide in Food Matrices

RESTEK Solution : Allure Acrylamide LC Column

- « Robustness » :

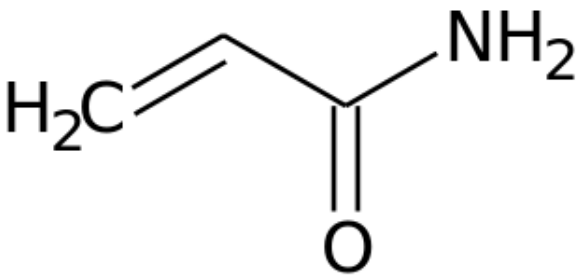




Lifetime test for PGC & Allure Acrylamide columns, after SPE extraction (2 steps – EN 16618 method) of a coffee matrix

Acrylamide in Water

New Restek Allure Acrylamide LC column, standards & LC-MS/MS method



Acrylamide in Water Matrices

Regulations and Limits examples

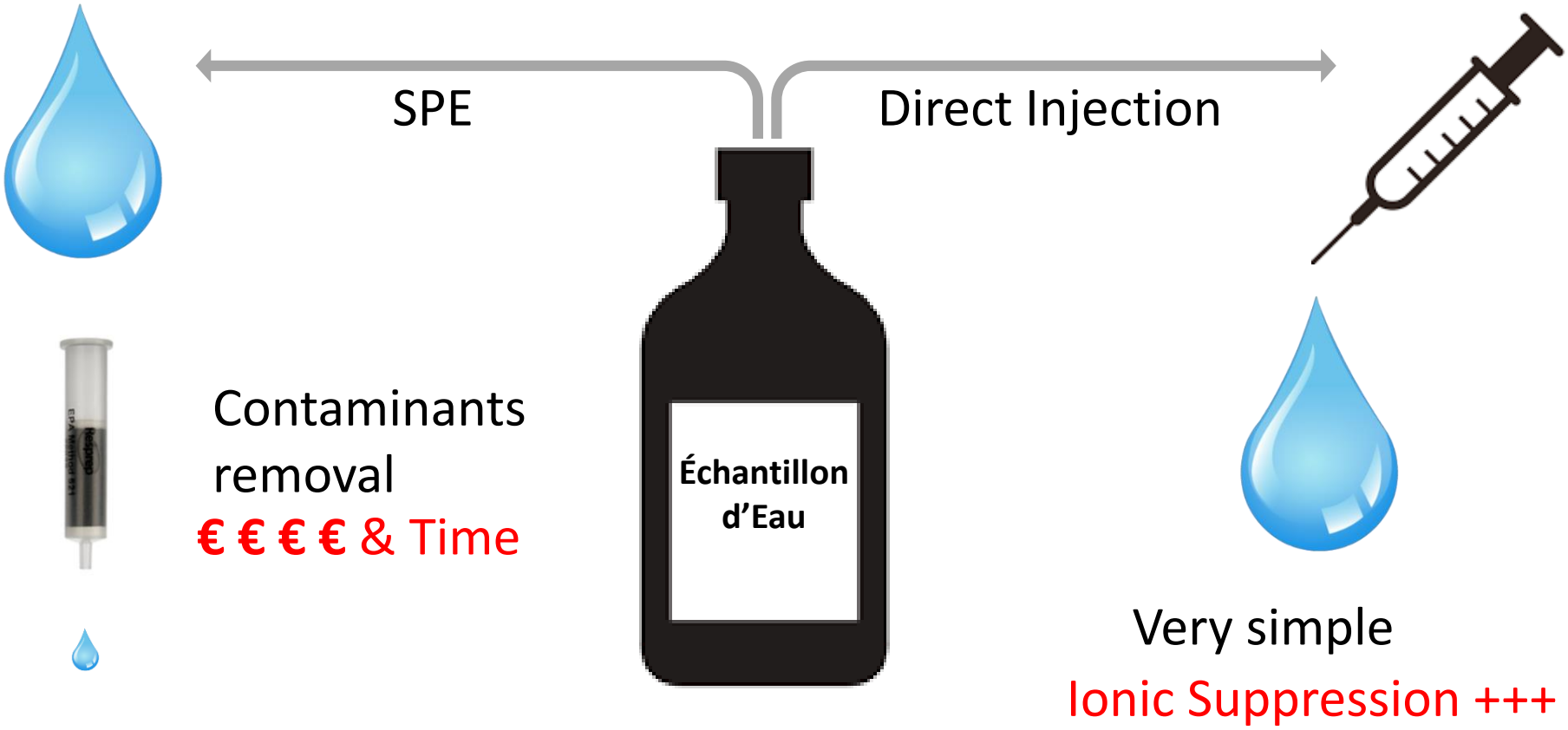
- EU 98/93/EC Directive : 100 ppt (0,1µg/L)

DIN 38413-6 Method (2007)	US EPA 8316 Method (1994)
LC-MS/MS	LC-UV
SPE	**Direct Injection**
Internal Calibration	External Calibration (w/o Internal Standard)
Calibration Range: 0.03 – 0.3 ug/L	MDL: 10 ug/L
Guideline Limit Value (Drinking Water):	Guideline Limit Value (Drinking Water):
0.1 ug/L	**0.5 ug/L**

Acrylamide in Water Matrices

Challenges

- The main problem in water matrices is the presence of many potential contaminants → Ionic Suppression

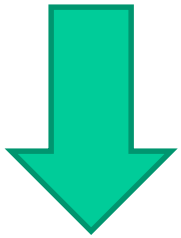


Acrylamide in Water Matrices

Challenges

- Low LOQ in Europe (100 ppt for drinking water)

- Will to simplify sample preparation



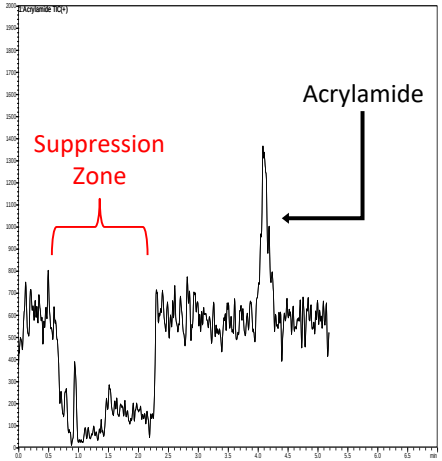
Large Volume Direct Injection

* Column Dimensions * Stationary Phase Chemistry * Particle Type

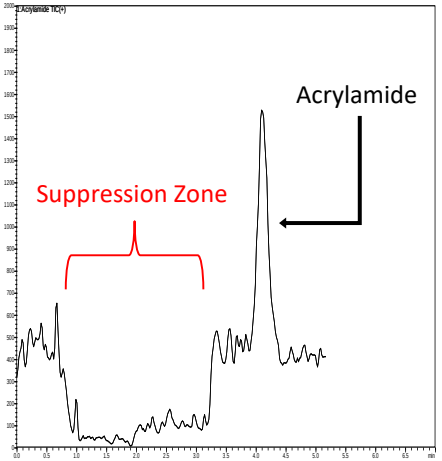
Acrylamide in Water Matrices

RESTEK Solution : Allure Acrylamide LC Column

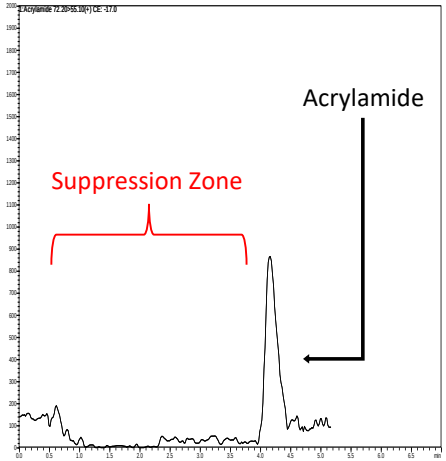
50 µL Inj Vol



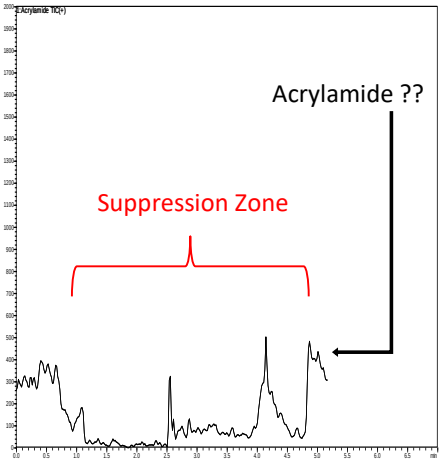
100 µL Inj Vol



150 µL Inj Vol



200 µL Inj Vol



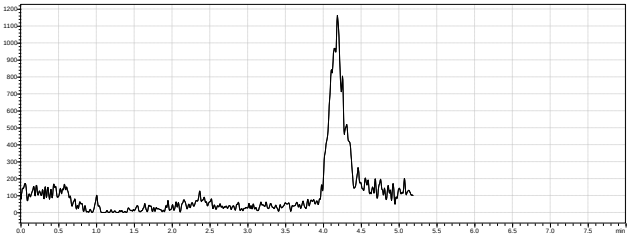
- 3,0x150mm 5µm Column
- Isocratic - 0,001% Formic Acid in Water
- Concentration : 100 ppt (spiked)

Acrylamide in Water Matrices

RESTEK Solution : Allure Acrylamide LC Column

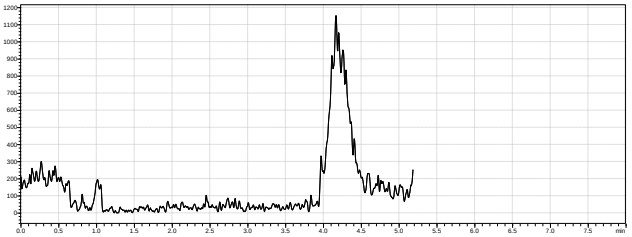
Tap water

Injected Volume : 150 µL



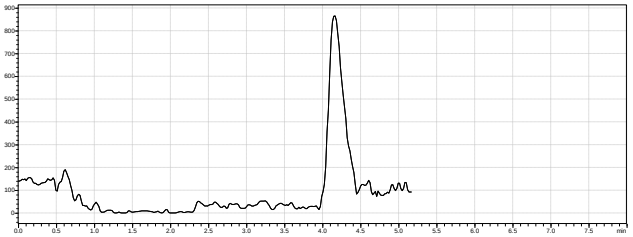
River water

Injected Volume : 200 µL



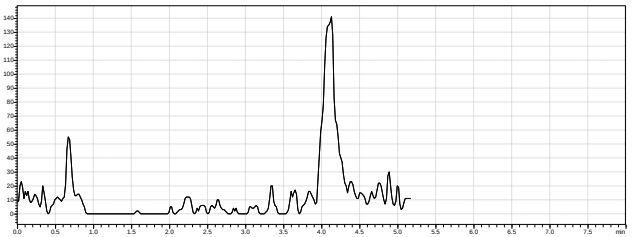
Wastewater

Injected Volume : 150 µL



Underground water

Injected Volume : 150 µL



Concentration 100 ppt

Acrylamide in Water Matrices

RESTEK Solution : Allure Acrylamide LC Column

- More retentive than usual reverse phase columns
- Longer lifetime than PGC columns
- Faster – no re-equilibration between runs
- Simpler – **direct injection**

Acrylamide in Water Matrices

RESTEK Solution : Allure Acrylamide LC Column

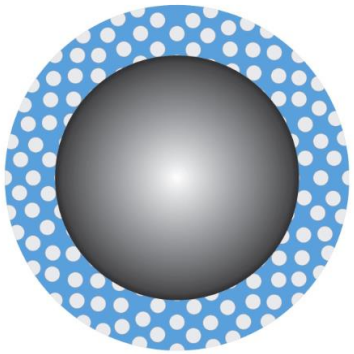
Product characteristics	Benefits	**Restek Allure Acrylamide	PGC 1 Column	PGC 2 Column	C18 SB Column	« Polar » C18 column
***Fast analysis times**	High Throughput, faster results	Yes	No	No	No	Yes
***Retention time stability after repeated matrix injections**	Longer Lifetime	Yes	No	No	Uncertain	Uncertain
Retention and separation of interfering matrix compounds	Peak Quantification Simplification	Yes	Yes	Yes	Yes	No
Limited peak distortion after repeated matrix injections	Peak Quantification Simplification	Yes	No	No	Uncertain	Uncertain

Raptor
Colonnes LC

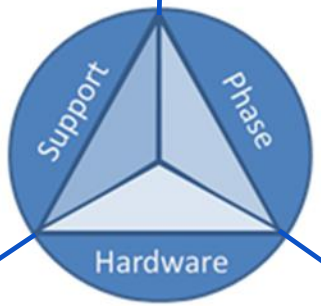
RESTEK Raptor Polar X LC Column

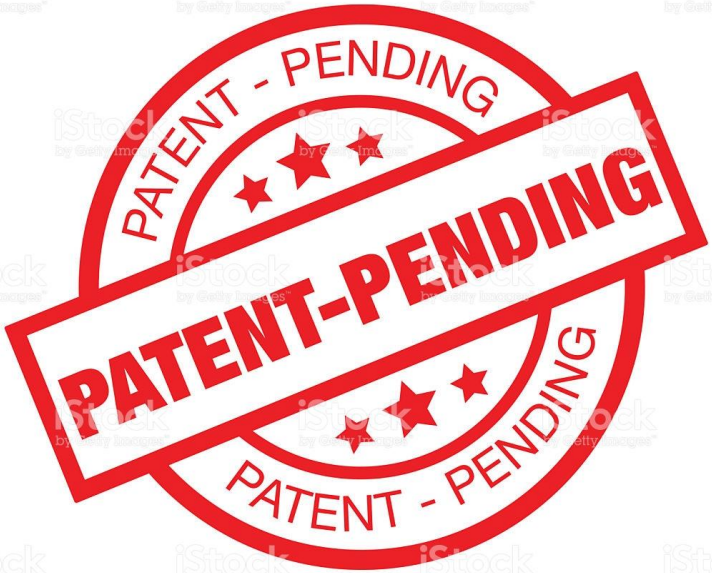
Dual selectivity for the analysis of polar compounds

Raptor Polar X LC Column



"Core-Shell" particle



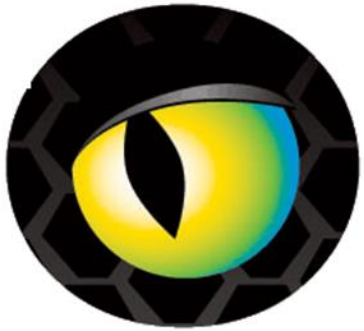


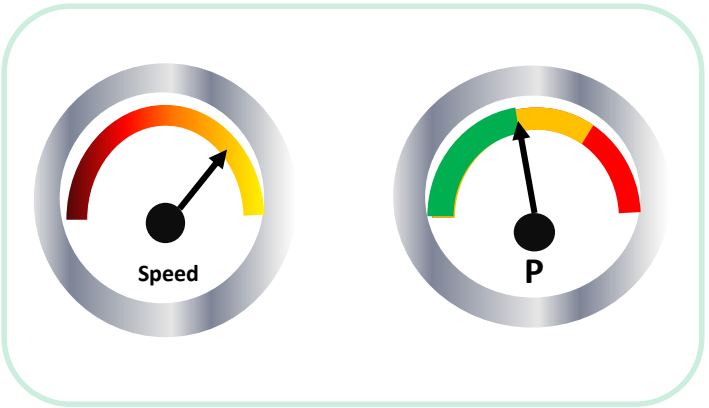
New manufacturing process

Raptor Polar X LC Column

Particle

- High Purity (Type B) Superficially Porous Particle (« Core-Shell »)
- Particle Size : 2.7 µm
- Pore Size : 90 Å
- Surface Area : 130 m^2/g



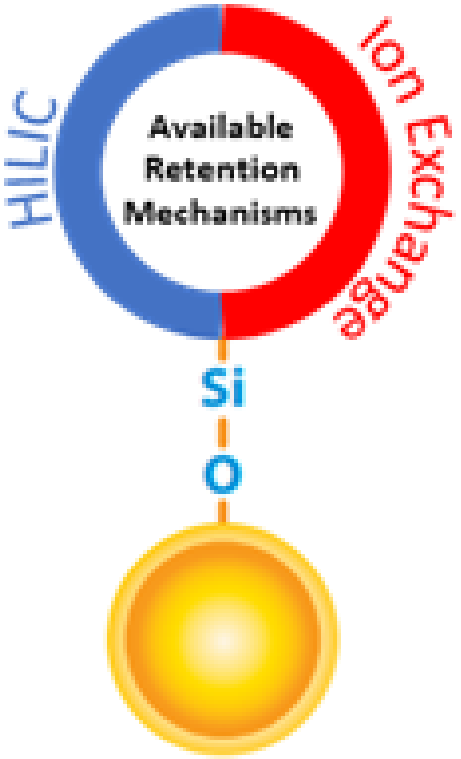


- High efficiency
- Low backpressure
- Faster separations
- HPLC <-> Fast LC <-> UHPLC
- **Robustness VS matrix**

Raptor Polar X LC Column
Chemistry

- « Dual » HILIC and IEX retention mechanisms offering unique selectivity and versatility for the retention and separation of polar compounds who are usually difficult to retain or separate in classical reverse phase, ion pairing, IC or HILIC.

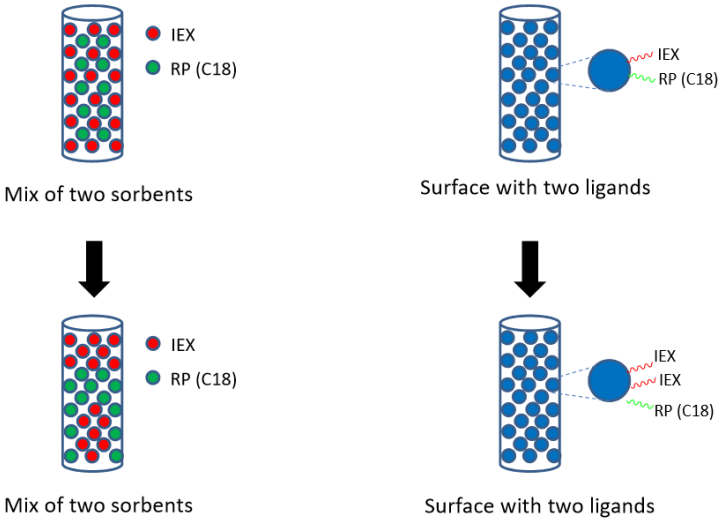
- Works on the “native” polarity of the compounds or on their charge to get retention and separation, with no need for ion pairing reagents, extreme pHs or “exotic” chromatographic conditions.



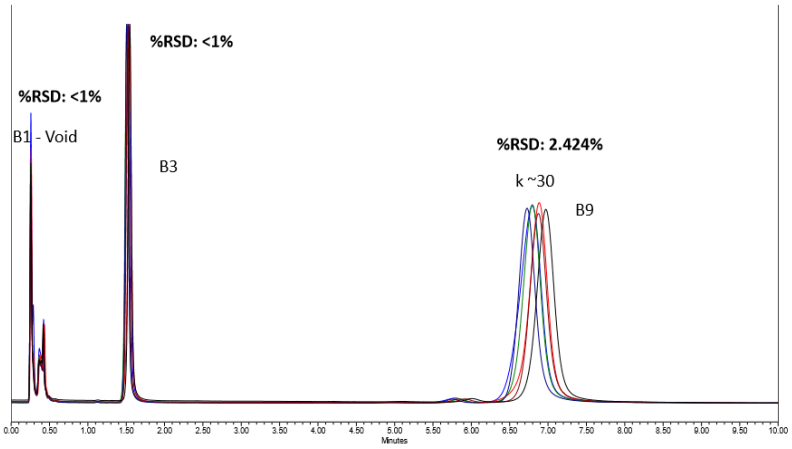
Raptor Polar X LC Column
Chemistry

- Advantages of a stationary phase with a bi-functional ligand / mixed « packing » :
  - Better control of the manufacturing process
  - Homogenous surface
  - Better lot-to-lot reproducibility
  - More stable surface : better analysis-to-analysis repeatability

Multimodal Chromatography



Comparison of Bonded Materials



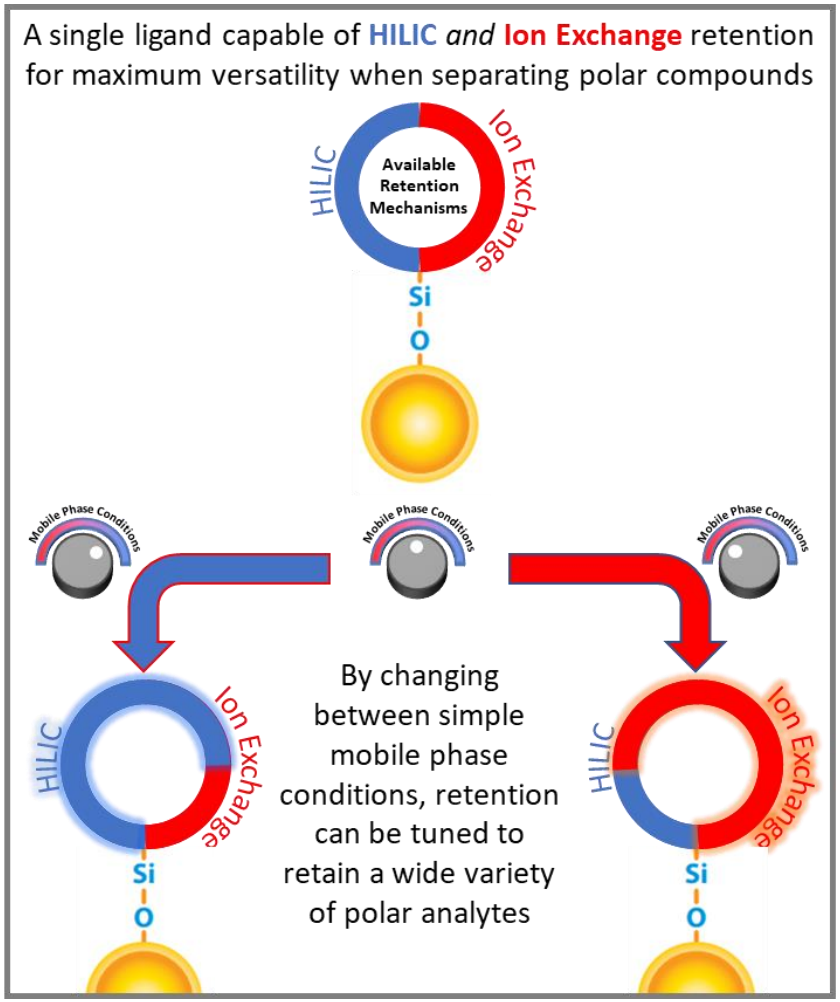
Two chemists, Multiple months, Multiple Silica Lots

Raptor Polar X LC Column

Chemistry

- High % ACN Mobile Phases → HILIC « partitioning » → charged polar compounds and neutral polar compounds

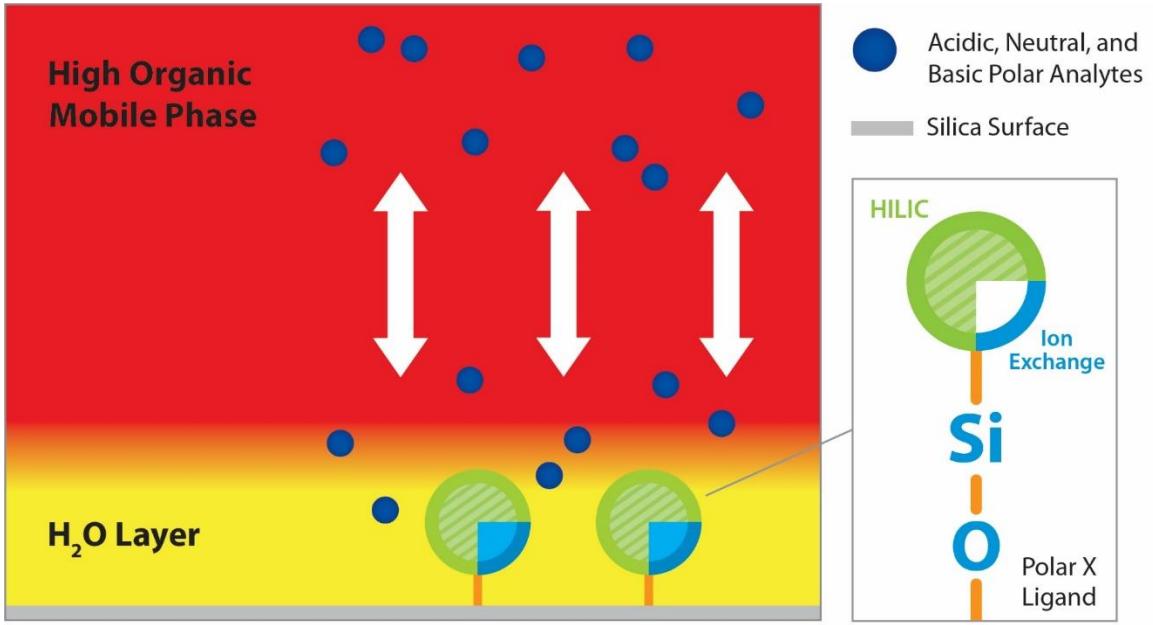
- If HILIC « partitioning » is not necessary, simple changes in the mobile phase composition will allow access to the ion exchange characteristics of the phase



Raptor Polar X LC Column

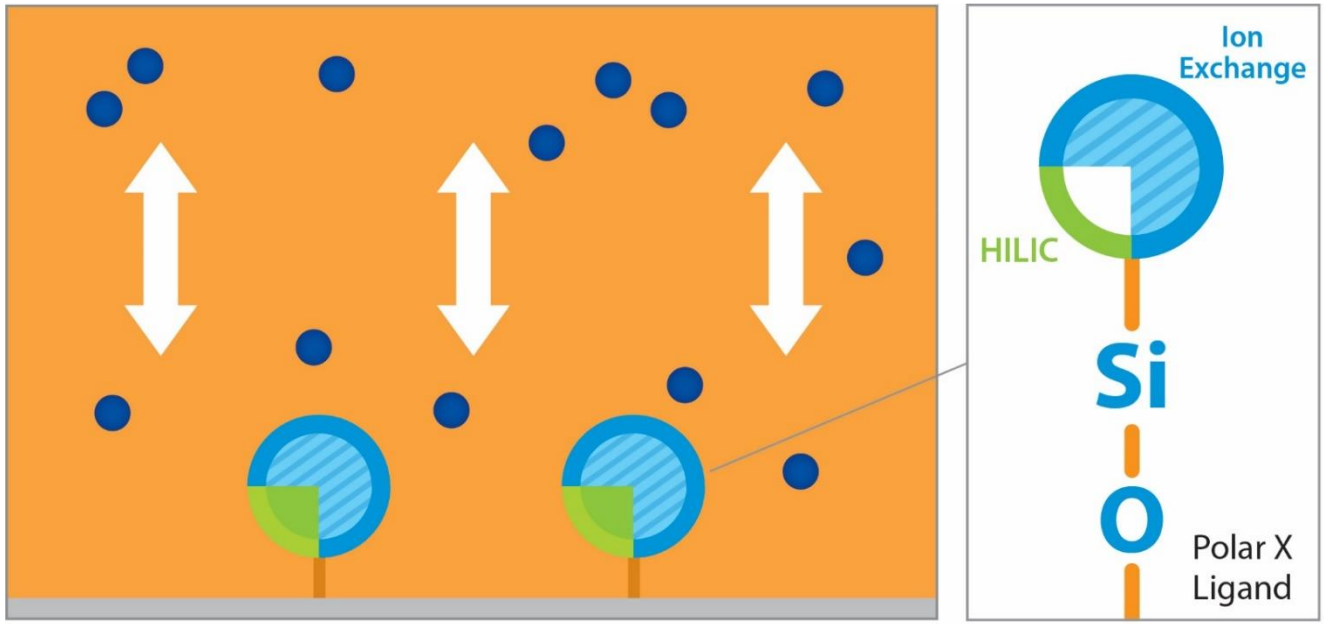
Chemistry – HILIC mode

- **Hydrophilic Interaction Liquid Chromatography (HILIC) is a technique used in LC for retention and analysis of polar compounds**
- **It combines a polar stationary phase with mobile phases with a high organic solvent content (typically ACN, above 60%), which creates a small aqueous layer on the surface of the particle**
- **Water soluble polar analytes are partitioning into the aqueous layer where they can interact with the ligand and the particle surface**
- **Partitioning is the main retention mechanism of the HILIC mode. It is a very efficient technique for the retention and separation of a wide variety of polar compounds (acidic, neutral and basic)**



Raptor Polar X LC Column

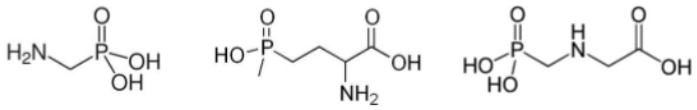
Chemistry – IEX mode



- Ion Exchange chromatography is based on the ionic interactions between the analyte and the stationary phase
- Mixtures are separated according to their affinity and their interaction with the functional groups present on the bonded phase
- In ion exchange, water will be the strong solvent, so to elute the compounds of interest, it will need higher aqueous mobile phase % than in HILIC mode

Raptor Polar X LC Column

Applications : Glyphosate & Co.

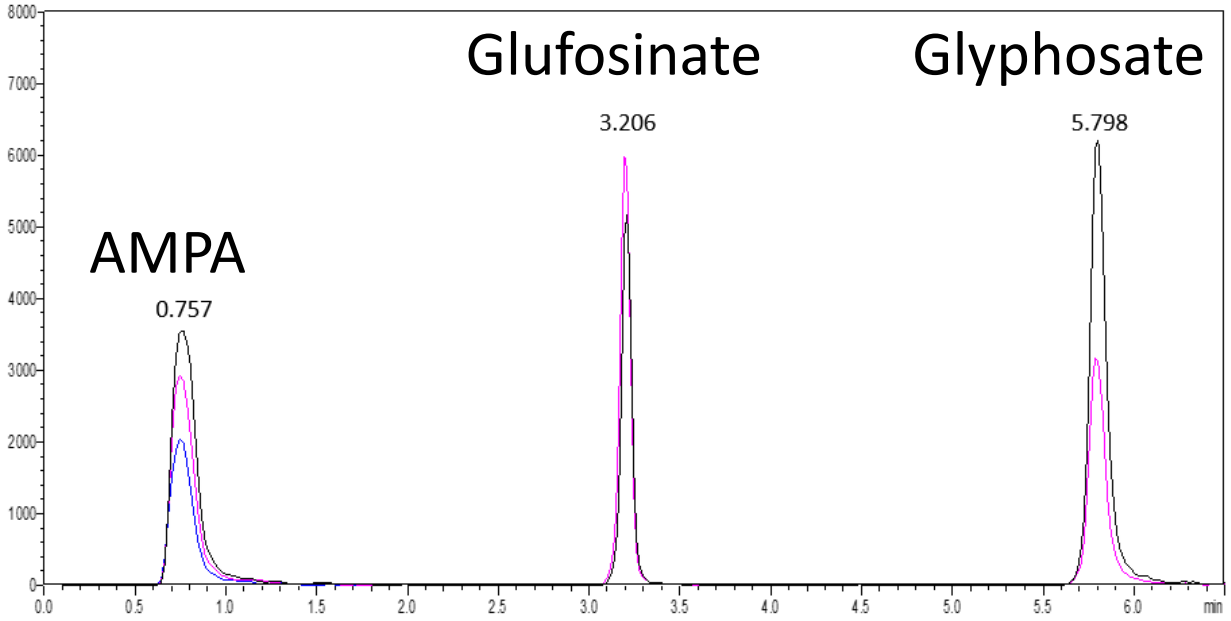


AMPA Glufosinate Glyphosate

- ***No derivatization***, filtration and ***direct injection*** for Water matrices !
- Mechanism(s) : IEX
- Main application and historical reason for the creation of the column
- Compounds : Glyphosate, Glufosinate & AMPA
- Important problem linked to glyphosate in the US and in Europe (carcinogenic potential)
- Our solution : 8 minutes cycle time, re-equilibration included, with H2O/CAN mobile phases (each with 0,5% formic acid), compatible with MS detection

Raptor Polar X LC Column

Applications : Glyphosate & Co.

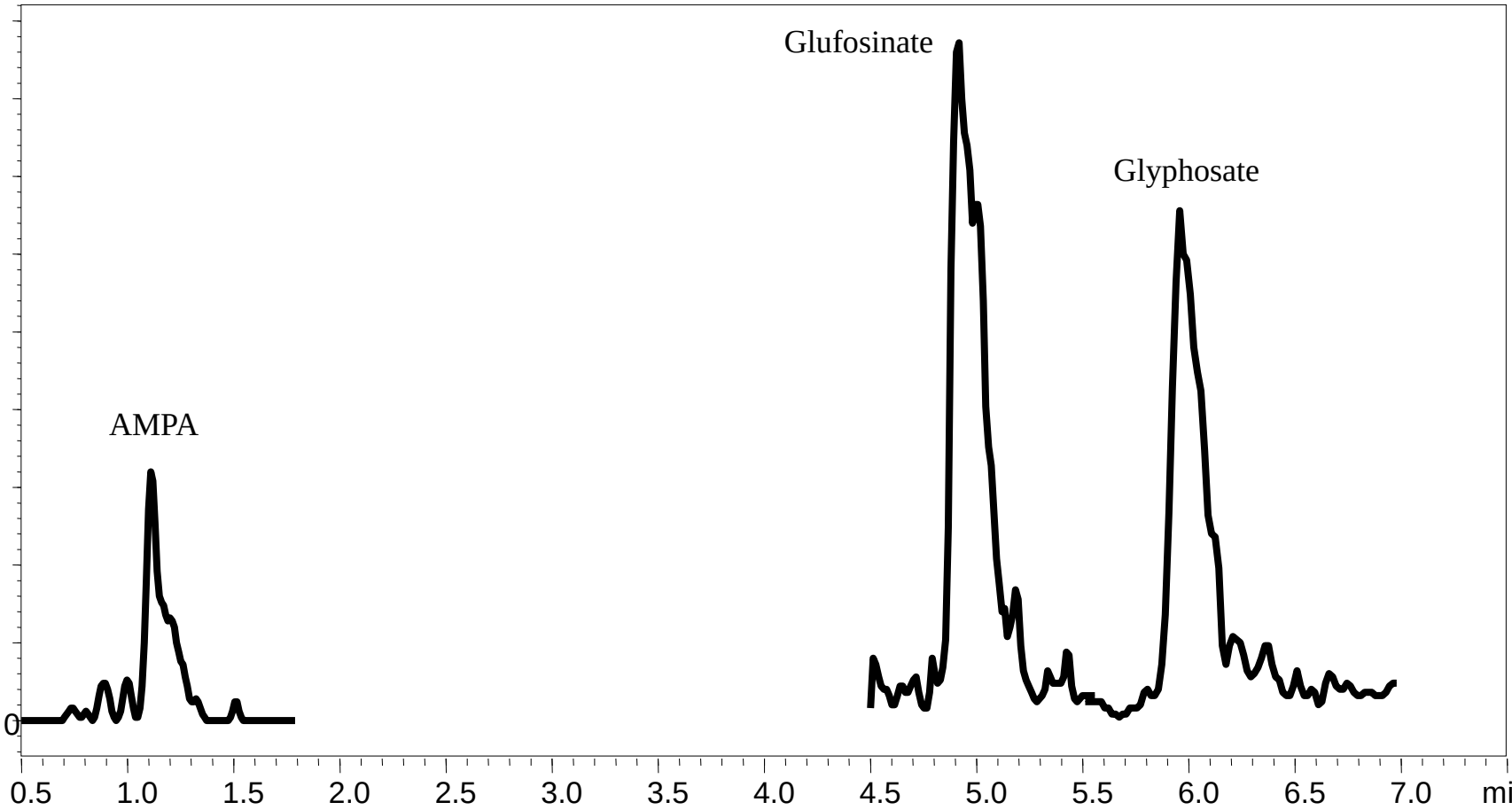


Conditions:		
Mobile Phase A:	0.5% formic acid in H2O	
Mobile Phase B:	0.5% formic acid in ACN	
Flow:	0.5 mL/min	
Gradient:	Time (min)	%B
	0.0	65
	5.0	10
	6.5	10
	6.51	65
	8.0	65
Temp.:	35 °C	
Injection Volume:	5 µL	

Raptor Polar X LC Column

Applications : Glyphosate & Co.

20 ppt in water – Large Volume Injection (500µL)



Raptor Polar X LC Column

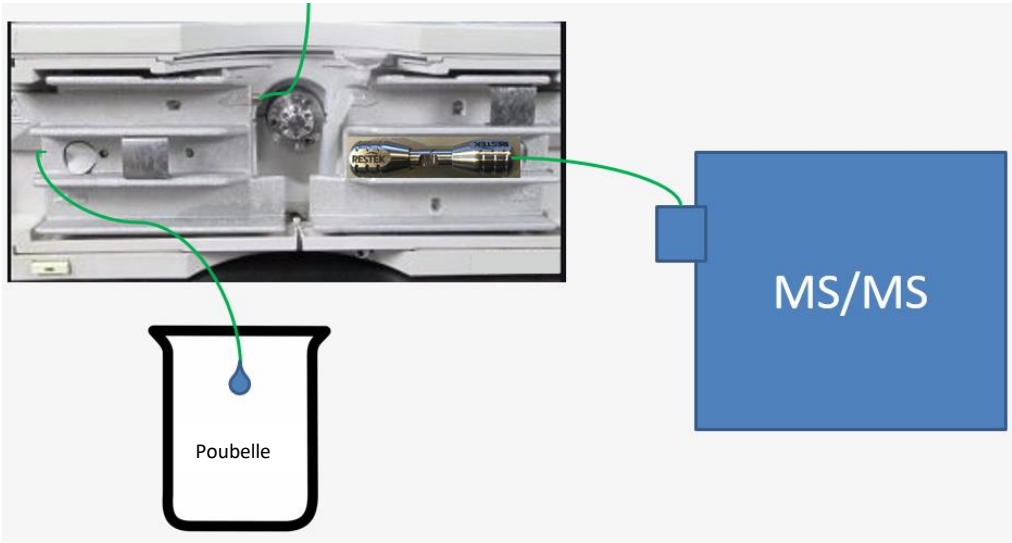
Applications : Glyphosate & Co.

- **NOTE :** phosphorylated compounds can chelate with metal ions present in the LC system → need for system passivation

Transfer into a vial

RESTEK LC Passivation Solution (#32475)

Inject several times through the injector and tubing to (but without) the column head and then to waste.

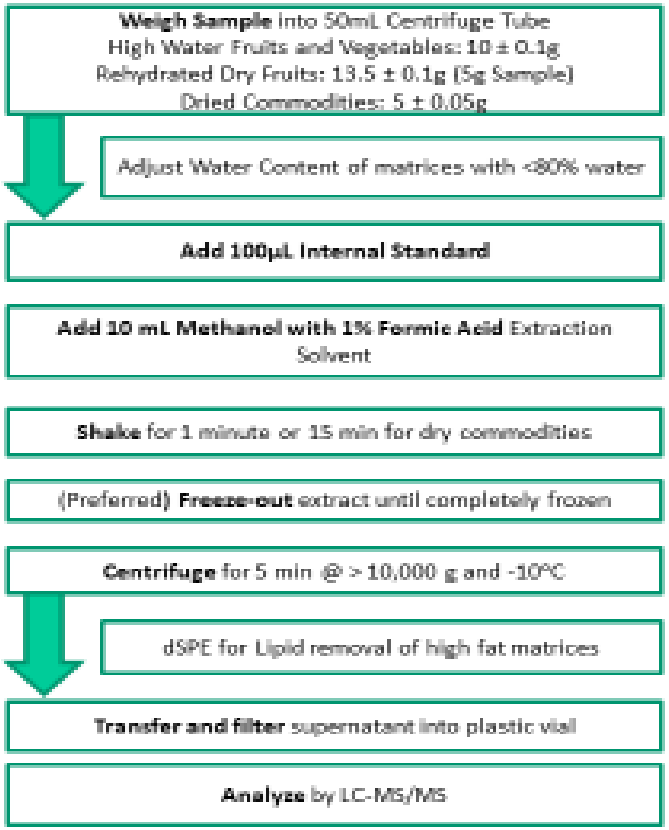


Raptor Polar X LC Column

Applications : QuPPe

QuPPe Sample Preparation Overview

Basically an Acidified Methanol Extraction

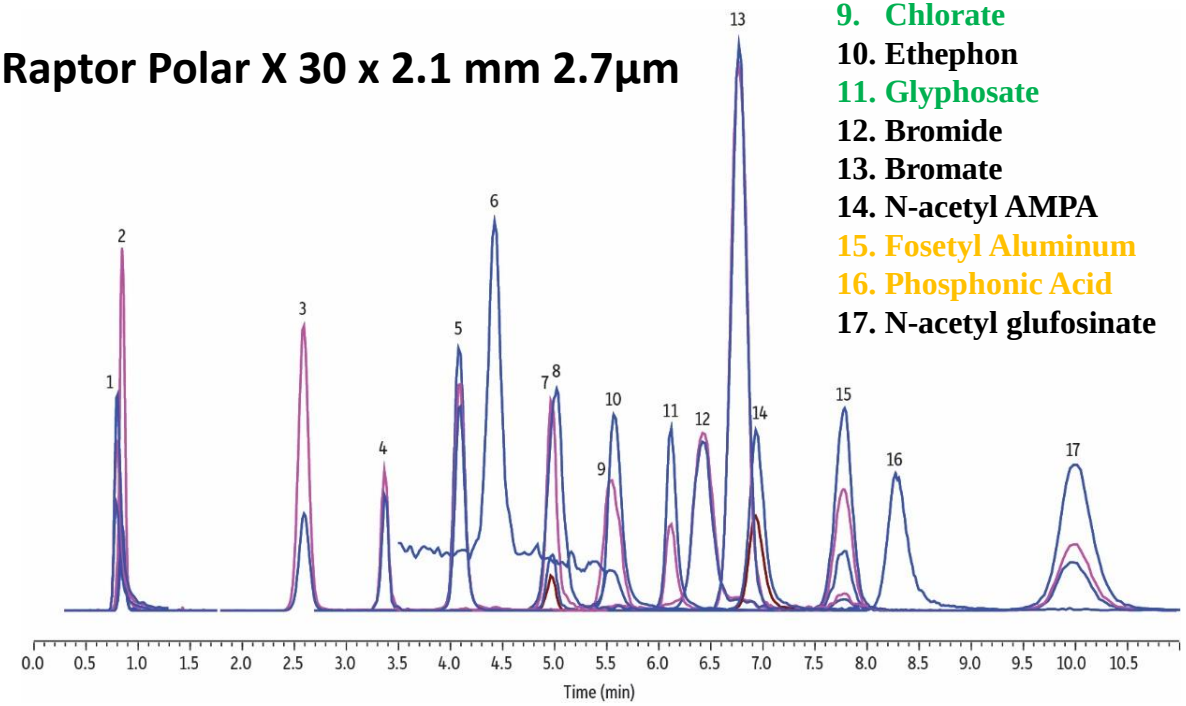


https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe_PO_V11(1).pdf

Raptor Polar X LC Column

Applications : QuPPe

Raptor Polar X 30 x 2.1 mm 2.7µm



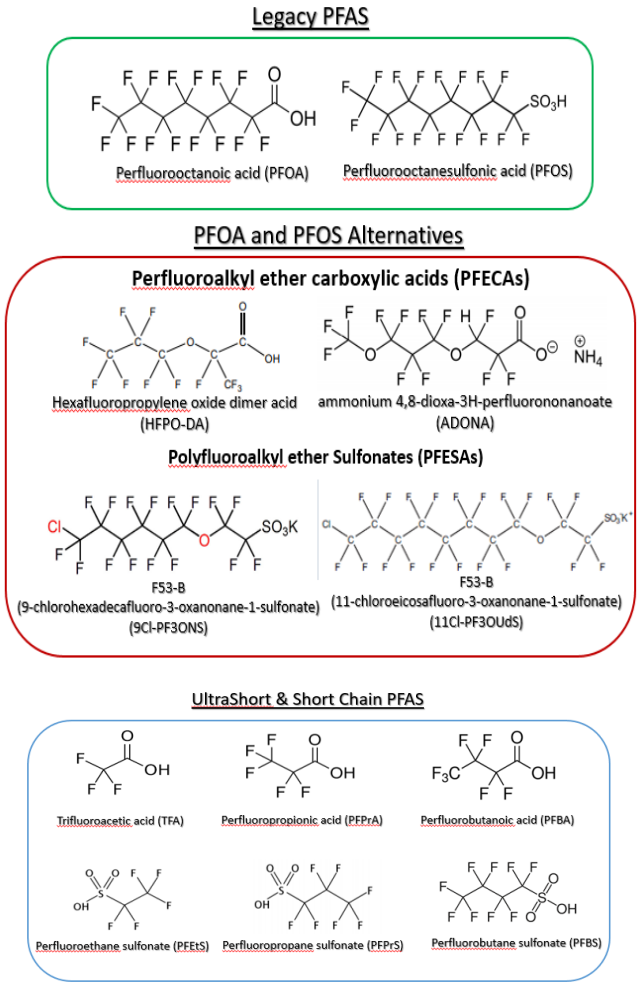
1. AMPA
2. Bialophos
3. Perchlorate
4. Glufosinate
5. MPPA
6. TFA
7. HEPA
8. Difluoroacetic acid
9. Chlorate
10. Ethephon
11. Glyphosate
12. Bromide
13. Bromate
14. N-acetyl AMPA
15. Fosetyl Aluminum
16. Phosphonic Acid
17. N-acetyl glufosinate

Conditions:		
Mobile Phase A:	0.5% formic acid in H2O	
Mobile Phase B:	0.5% formic acid in ACN	
Flow:	0.5 mL/min	
Gradient:	Time (min)	%B
	0.0	65
	5.0	10
	11.5	10
	11.51	65
	13	65
Temp.:	35 °C	
Injection Volume:	1 µL	

Raptor Polar X LC Column

Applications : PFAS

- PFAS are synthetic chemicals used as surfactants or raw materials in industrial applications and consumer products
- PFAS are now classified as persistent environmental pollutants with harmful effects on animal and human health.
- The US and the EU have banned the use and production of some PFAS (PFOA & PFOS), hence the emergence of alternative PFAS
- Recent interest on ultra-short chains PFAS (C2 –C3) in environmental samples
- Ultra-short chains PFAS are very polar and are therefore very difficult to retain with the usual reverse phase approaches

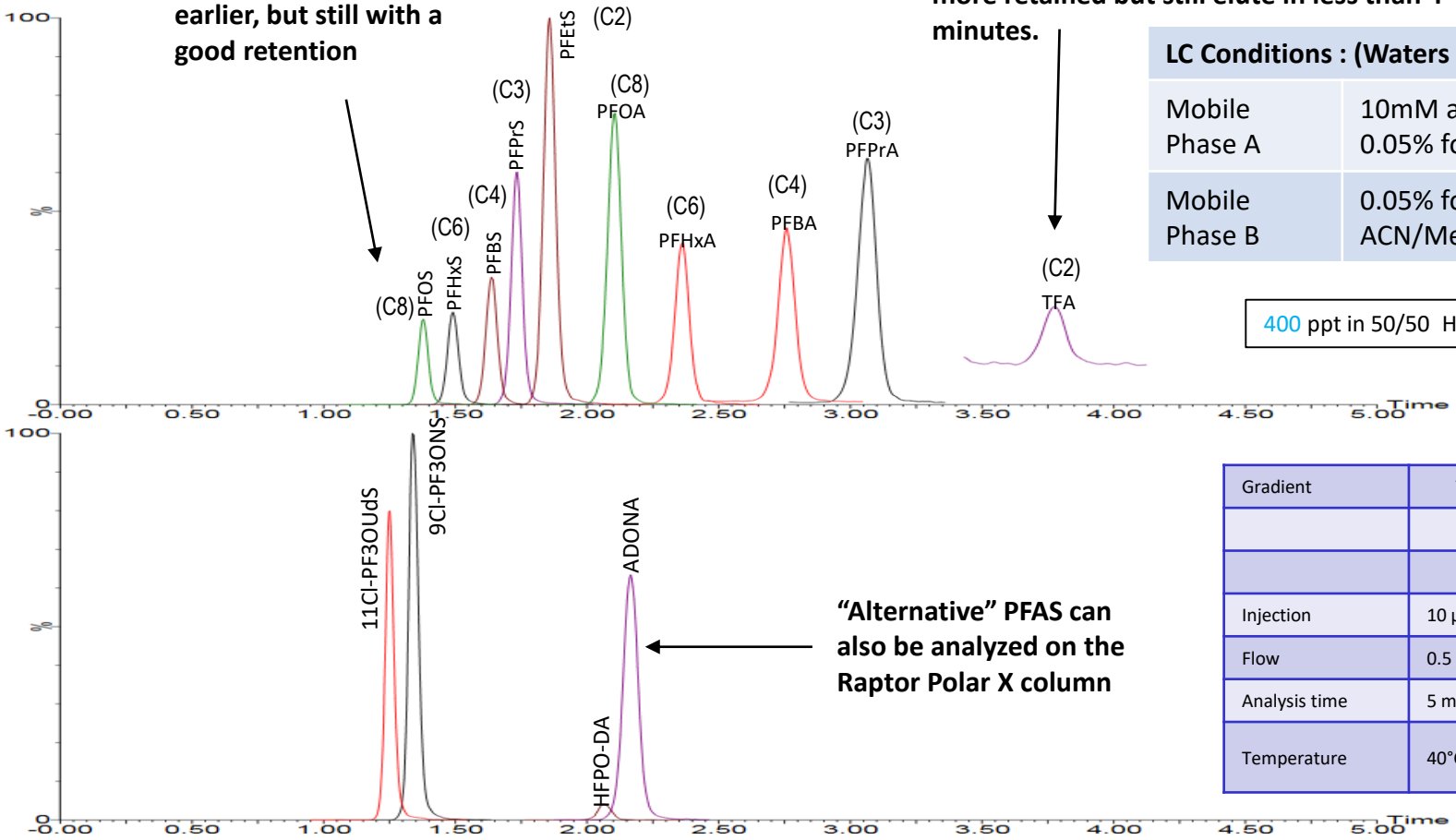


Raptor Polar X LC Column

Applications : PFAS

Long chains PFAS elute earlier, but still with a good retention

Short chains and ultra-short chains PFAS are more retained but still elute in less than 4 minutes.



"Alternative" PFAS can also be analyzed on the Raptor Polar X column

LC Conditions : (Waters Acquity UPLC)	
Mobile Phase A	10mM ammonium formate, 0.05% formic acid in H2O
Mobile Phase B	0.05% formic acid in 60/40 ACN/MeOH

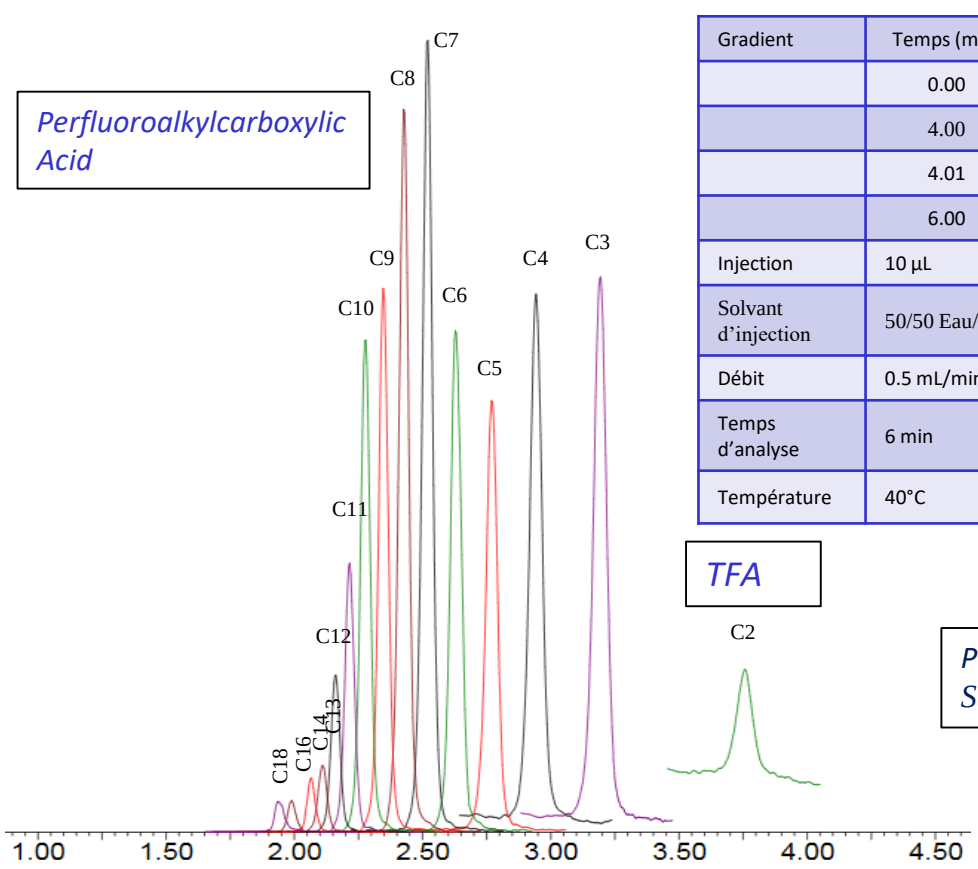
400 ppt in 50/50 H2O/MeOH

Gradient	Time (min)	%B
	0.00	85
	5.00	85
Injection	10 µL	
Flow	0.5 mL/min	
Analysis time	5 min	
Temperature	40°C	

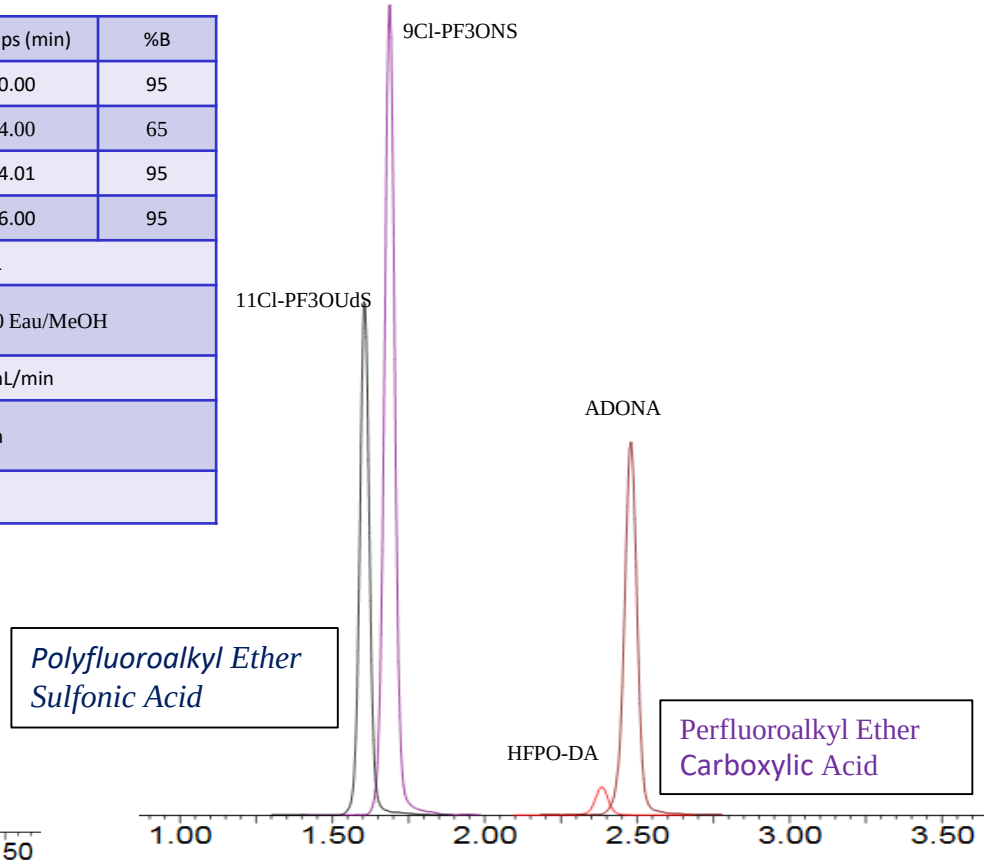
Raptor Polar X LC Column

Applications : PFAS

- Good retention and excellent peak shape for TFA (careful though with possible background noise linked to TFA usage - solvents and mobiles phases)



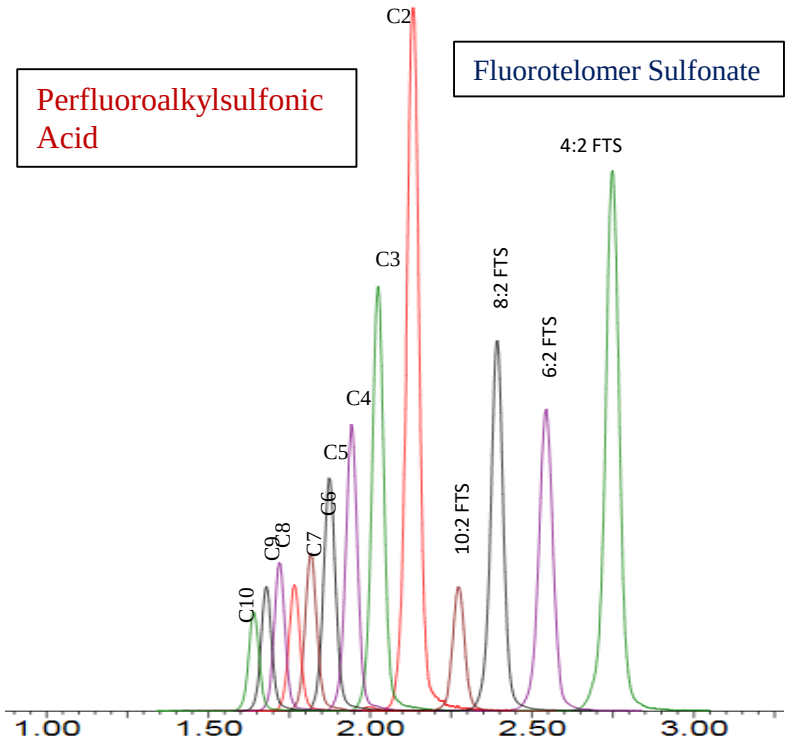
Gradient	Temps (min)	%B
	0.00	95
	4.00	65
	4.01	95
	6.00	95
Injection	10 µL	
Solvant d'injection	50/50 Eau/MeOH	
Débit	0.5 mL/min	
Temps d'analyse	6 min	
Température	40°C	

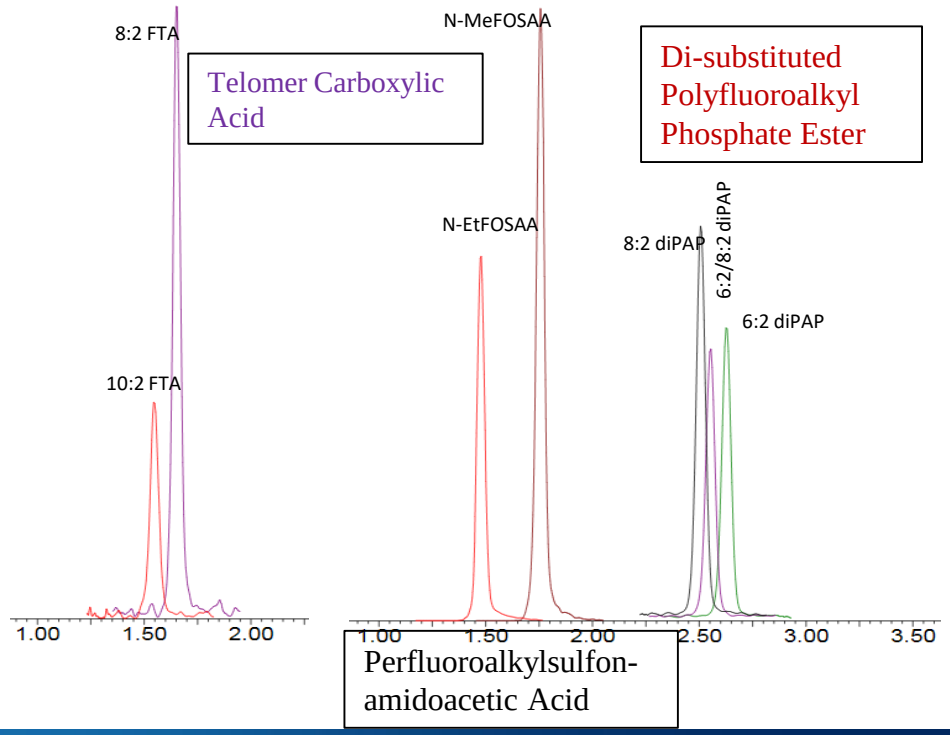


Raptor Polar X LC Column

Applications : PFAS

Polar X: 2.7µm - 50 x 2.1 mm





Raptor Polar X LC Column

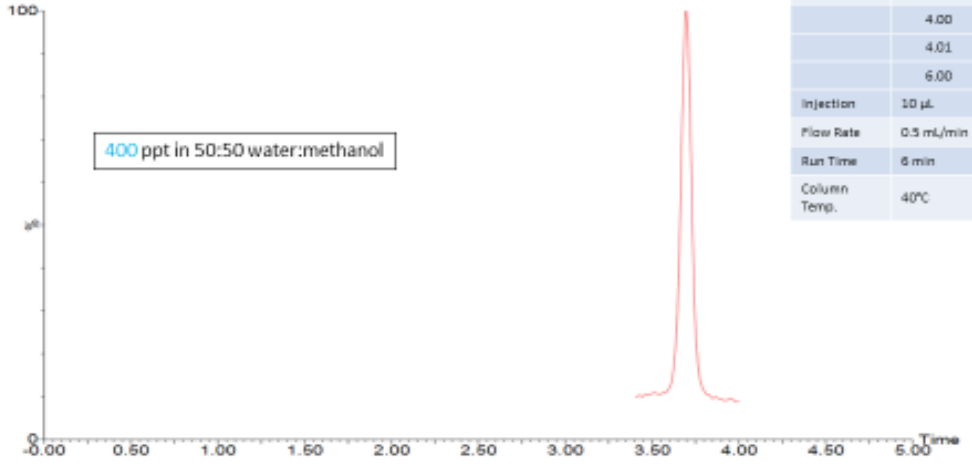
Applications : TFA

- Development of a direct injection method or D&S for water samples
- 2x dilution with MeOH
- Injection solvent : 50/50 H2O/MeOH

TFA Analysis on Restek Polar column, 2.7um, 50x2.1mm

LC Conditions : (Waters Acquity UPLC)	
Mobile Phase A	10mM ammonium formate, 0.05% formic acid in water
Mobile Phase B	0.05% formic acid in 60:40 acetonitrile:methanol

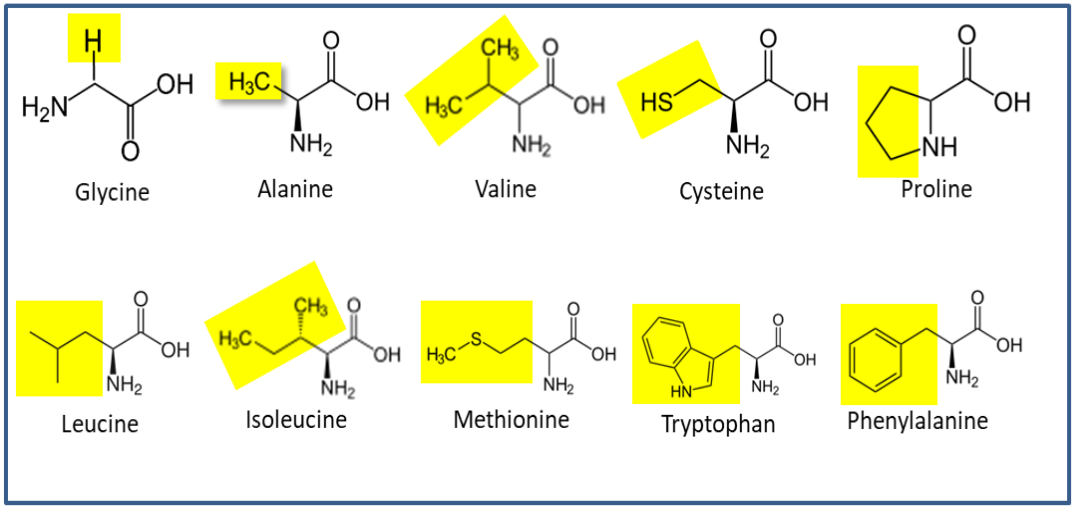
Gradient	Time (min)	%B
	0.00	95
	4.00	65
	4.01	95
	6.00	95
Injection	10 µL	
Flow Rate	0.5 mL/min	
Run Time	6 min	
Column Temp.	40°C	



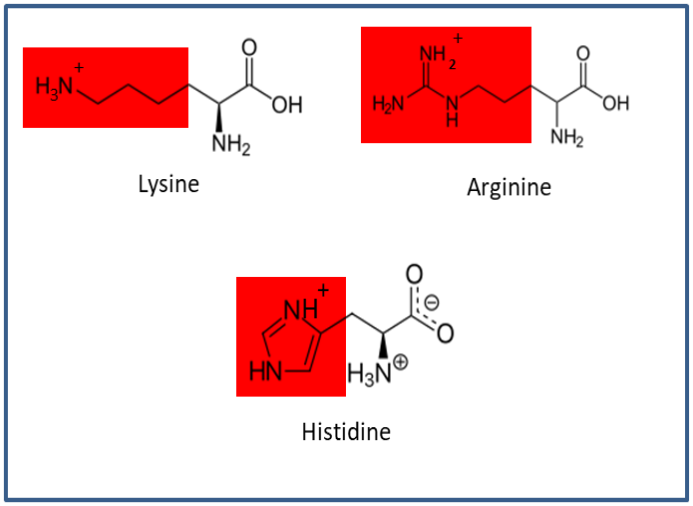
Raptor Polar X LC Column

Applications : Underivatized Amino Acids

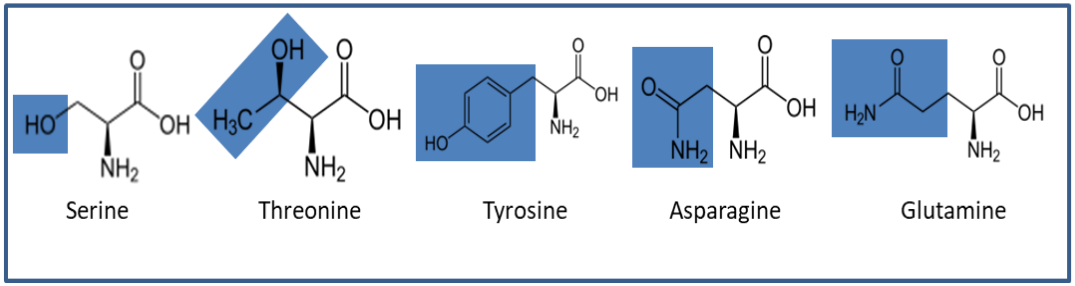
Non-Polar Amino Acids



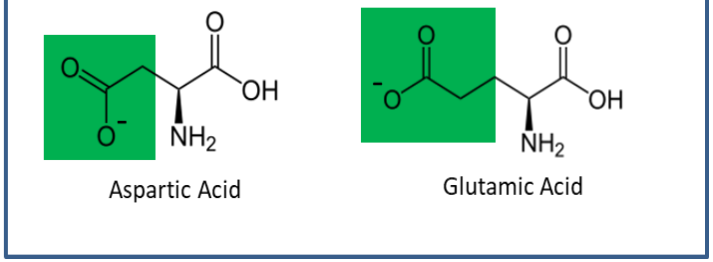
Positively Charged Amino Acids



Polar Amino Acids



Negatively Charged Amino Acids



Raptor Polar X LC Column

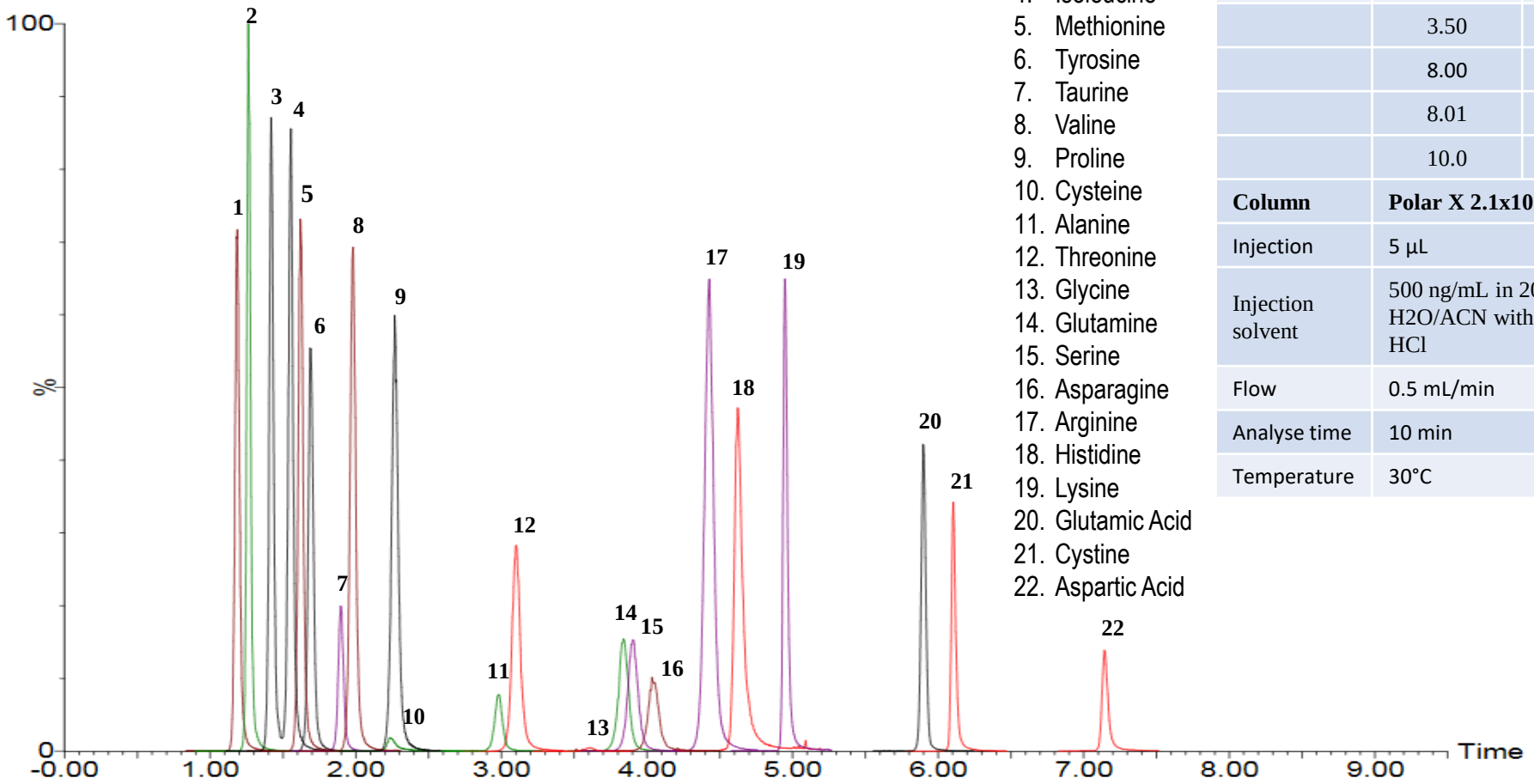
Applications : Underivatized Amino Acids

- LC analysis of free amino acids is particularly difficult because these compounds have many different physicochemical properties
- Amino acids can be classified into 4 groups based on the chemical properties of their side chains : non-polar, polar, positively charged and negatively charged
- In their native forms (underivatized), the retention and separation of some critical isobars won’t be good on a “ standard ” reverse phase column
- The Raptor Polar-X column was designed for a balanced retention of these compounds, while eliminating pre-column derivatization steps and using MS friendly mobile phases (improvement of ionization efficiency)

Raptor Polar X LC Column

Applications : Underivatized Amino Acids

- Mechanism(s) : IEX + HILIC



1. Tryptophan
2. Phenylalanine
3. Leucine
4. Isoleucine
5. Methionine
6. Tyrosine
7. Taurine
8. Valine
9. Proline
10. Cysteine
11. Alanine
12. Threonine
13. Glycine
14. Glutamine
15. Serine
16. Asparagine
17. Arginine
18. Histidine
19. Lysine
20. Glutamic Acid
21. Cystine
22. Aspartic Acid

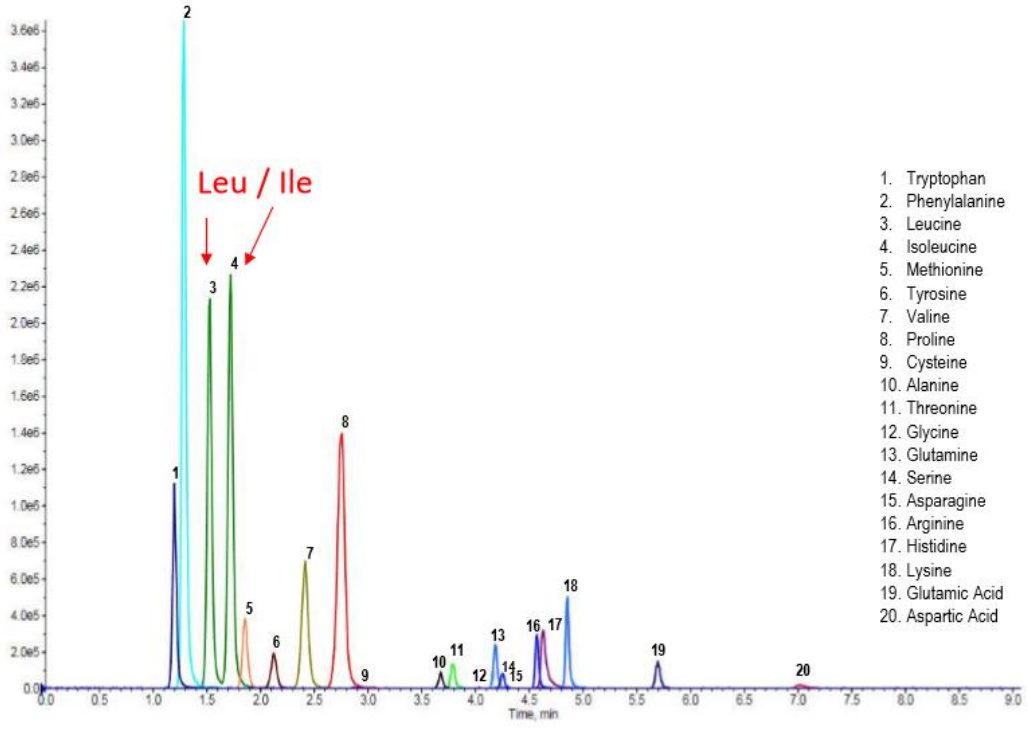
Gradient	Time (min)	%B
	0.00	88
	3.50	88
	8.00	30
	8.01	88
	10.0	88
Column	**Polar X 2.1x100 mm**	
Injection	5 µL	
Injection solvent	500 ng/mL in 20/80 H2O/ACN with 0.01 N HCl	
Flow	0.5 mL/min	
Analyse time	10 min	
Temperature	30°C	

Raptor Polar X LC Column

Applications : Underivatized Amino Acids

- Mechanism(s) : IEX + HILIC

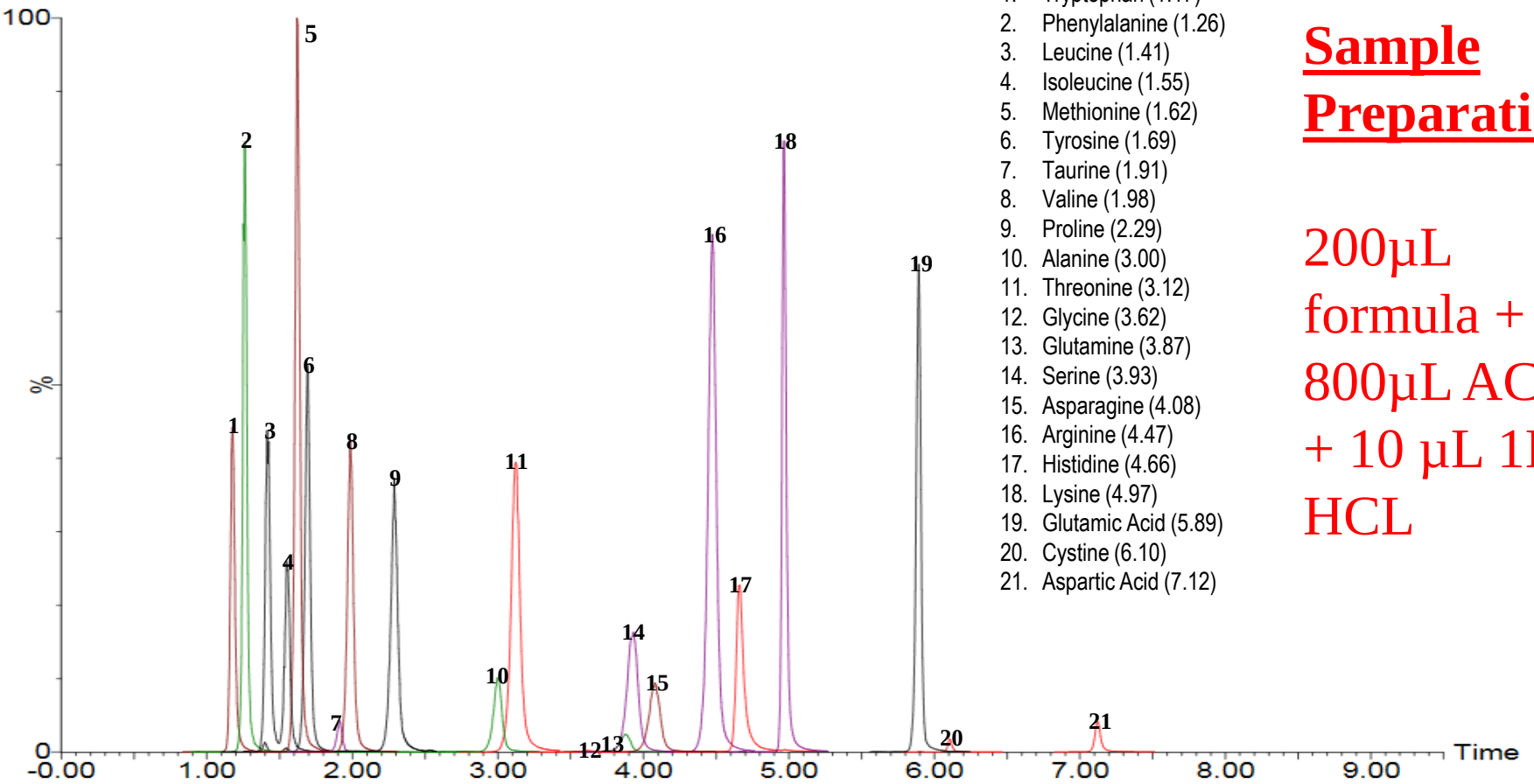
Critical separation Leu/Ile (isobaric compounds)



Raptor Polar X LC Column

Applications : Underivatized Amino Acids in infant formula

- Mechanism(s) : IEX + HILIC



1. Tryptophan (1.17)
2. Phenylalanine (1.26)
3. Leucine (1.41)
4. Isoleucine (1.55)
5. Methionine (1.62)
6. Tyrosine (1.69)
7. Taurine (1.91)
8. Valine (1.98)
9. Proline (2.29)
10. Alanine (3.00)
11. Threonine (3.12)
12. Glycine (3.62)
13. Glutamine (3.87)
14. Serine (3.93)
15. Asparagine (4.08)
16. Arginine (4.47)
17. Histidine (4.66)
18. Lysine (4.97)
19. Glutamic Acid (5.89)
20. Cystine (6.10)
21. Aspartic Acid (7.12)

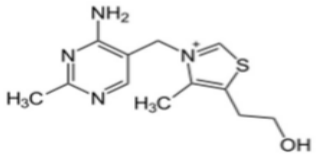
Sample Preparation:

200µL formula + 800µL ACN + 10 µL 1N HCL

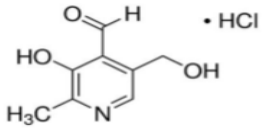
Raptor Polar X LC Column

Applications : Water Soluble Vitamins

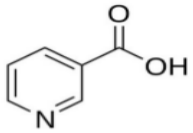
- Mechanism(s) : IEX → HILIC



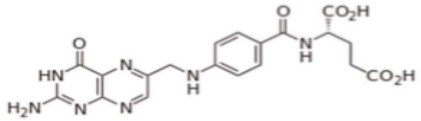
B1: Thiamine



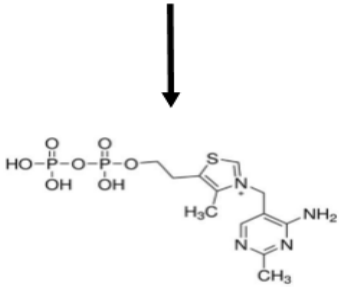
B6: Pyridoxal



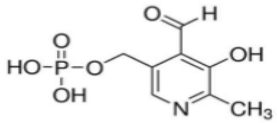
B3: Nicotinic Acid



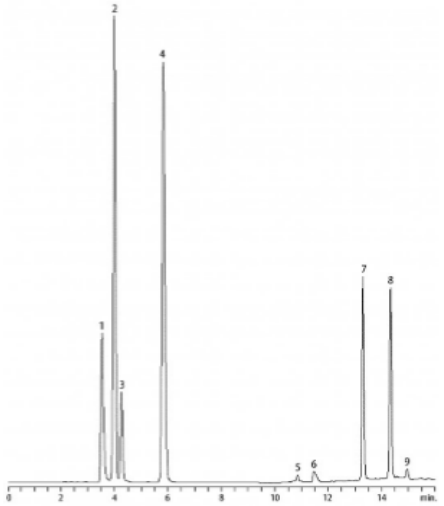
B9: Folic Acid



B1: Thiamine Pyrophosphate



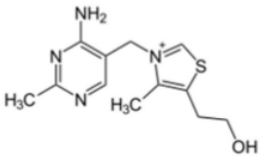
B6: Pyridoxal 5′-phosphate



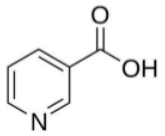
Raptor Polar X LC Column

Applications : Water Soluble Vitamins

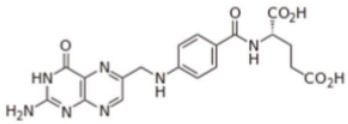
- Mechanism(s) : **IEX** → HILIC



B1: Thiamine

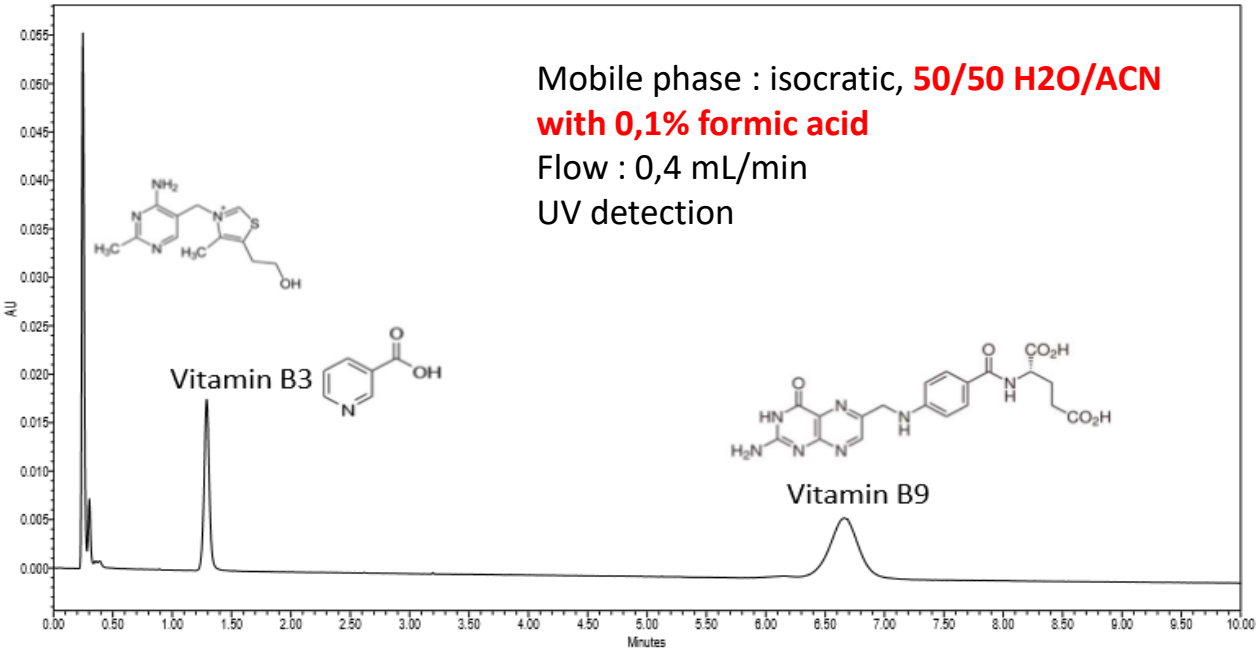


B3: Nicotinic Acid



B9: Folic Acid

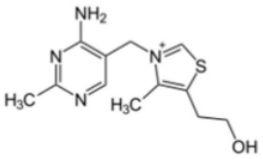
- No retention for vitamin B1
- IEX → electrostatic repulsion



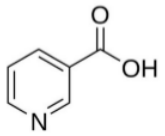
Raptor Polar X LC Column

Applications : Water Soluble Vitamins

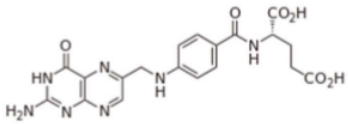
- Mechanism(s) : IEX → **HILIC**



B1: Thiamine

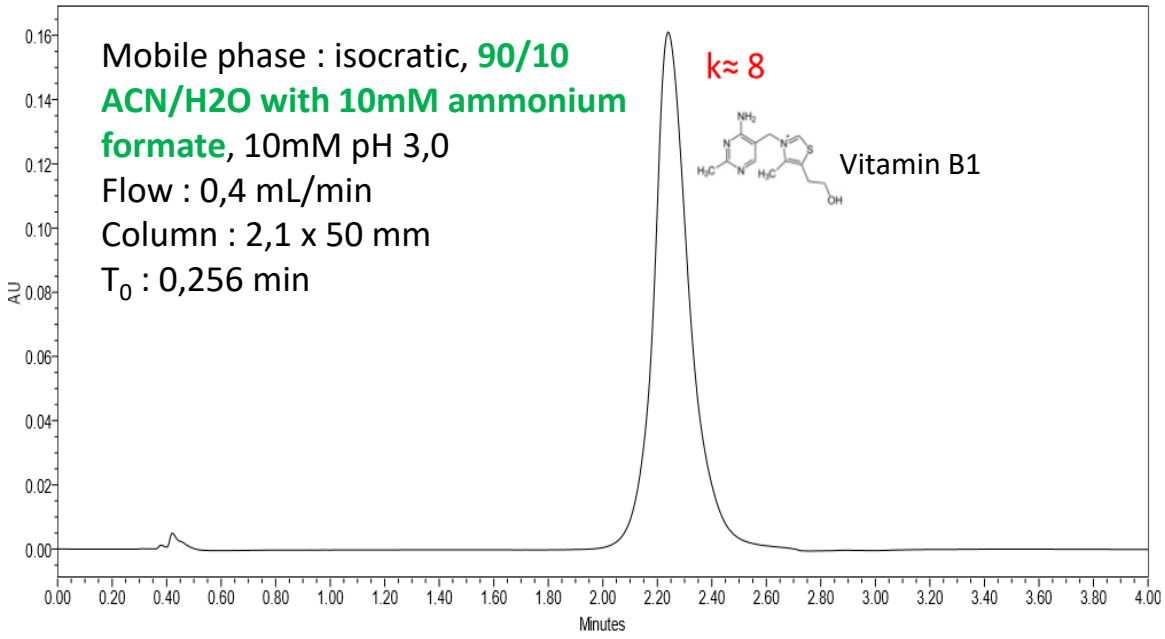


B3: Nicotinic Acid



B9: Folic Acid

- Vitamin B1 is retained
- Salts reduce electrostatic repulsion
- High organic % helps retention through « partitioning » (HILIC mode)
- HILIC mode becomes predominant

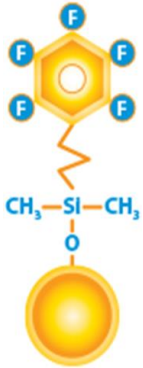


Raptor Polar X LC Column

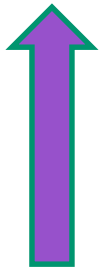
Key Advantages & Benefits

- Excellent retention for water soluble compounds (polar, anionic, hydrophilic)
- Reproducible retention times & better peak shapes = better data quality
- Excellent lot-to-lot and column-to-column reproducibility
- Used with LC-MS friendly mobile phases → less instrument downtime and maintenance
- Guard columns available
- No need for a complex and time consuming conditioning, no need to passivate the column
- LC Passivation Solution available when needed
- Large aqueous volume injection → Better sensitivity
- Many (other) possible applications

SPP Raptor LC Column Range

C18	ARC-18	Biphenyl	FluoroPhenyl	EtG/EtS	HILIC-Si	Polar X
				PROPRIETARY 		

REVERSED PHASE — MIXED MODE / HILIC / IEX



New Phase

Raptor
Colonnes LC

Thank you for your attention !

cyrille.lamboley@restek.com