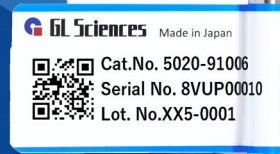
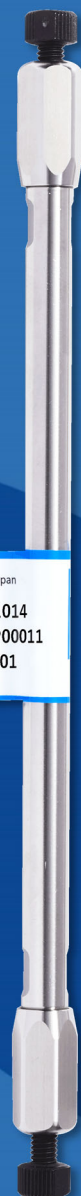
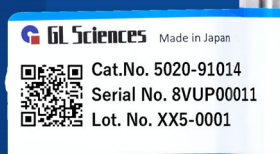


HPLC and LC/MS Columns

InertSustain AX-C18



Recommended for Separation and Analysis of Acidic Compounds!

InertSustain AX-C18

Features

- Retention behavior that combines both reversed phase and anion exchange
- Excellent retention for acidic compounds
- Excellent reproducibility and stable quality

Recommended when:

- You want to slightly change the separation pattern from C18 columns
- You want to analyze acidic compounds and anionic compounds
- You are having trouble with reproducibility and lot-to-lot variation in anion exchange columns

Specifications

• Base Material	: High purity spherical silica gel (ES silica)
• Particle Size	: 3 μm , 5 μm
• Surface Area	: 200 m^2/g
• Pore Size	: 200 \AA (20 nm)
• Pore Volume	: 1.00 mL/g
• Functional Group	: Octadecyl group + tertiary amino group
• End-capping	: Yes
• Carbon Loading	: 8 %
• USP Code	: L1, L78
• Recommended pH Range for Use	: 1 to 9



A mixed-mode column where C18 groups and tertiary amino groups are chemically bonded to silica gel

Feature
1

Retention behavior that combines
both reversed phase and anion exchange

Compared to typical C18 columns

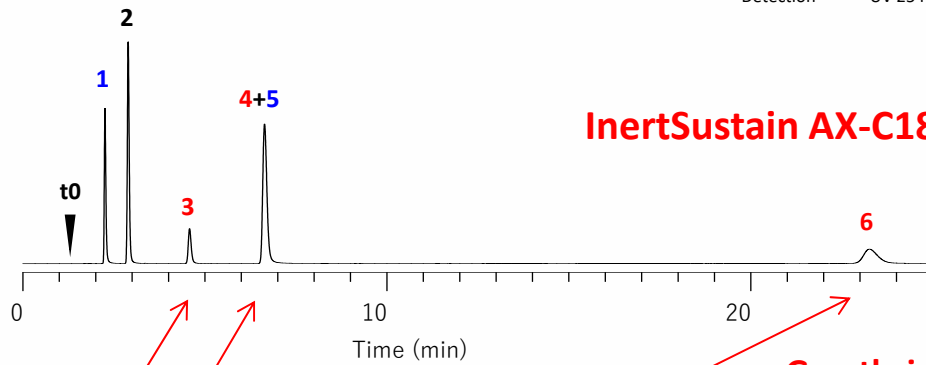


⊙ Increased retention of
acidic compounds

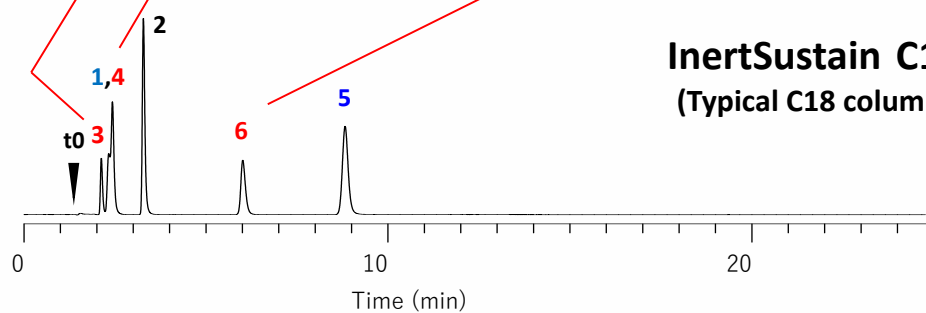
Analysis of nucleobases
and monophosphates

Conditions

Column : 3 μ m, 150 \times 2.1 mm I.D.
Eluent : 25 mM NaH₂PO₄ in H₂O
Flow Rate : 0.2 mL/min
Col. Temp. : 40 $^{\circ}$ C
Detection : UV 254 nm



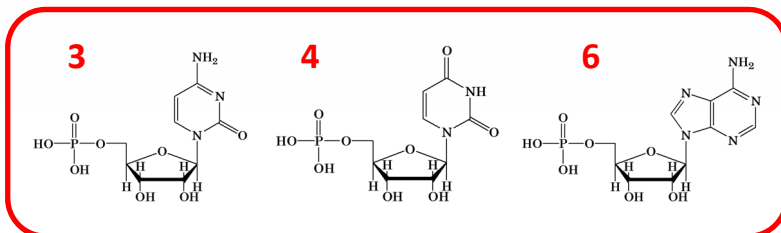
Greatly improved retention
of acidic compounds!



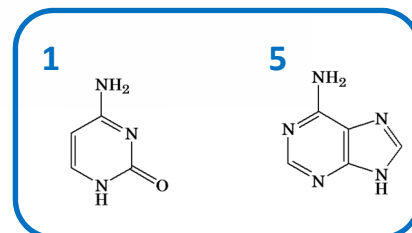
* Decreased retention of
basic compounds

Sample :

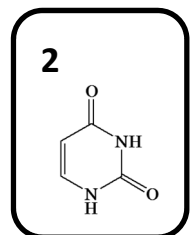
1. Cytosine (basic) 2. Uracil (neutral) 3. CMP (acidic) 4. UMP (acidic) 5. Adenine (basic) 6. AMP (acidic)



Acidic compounds



Basic compounds



Neutral
compounds

Excellent retention for acidic compounds

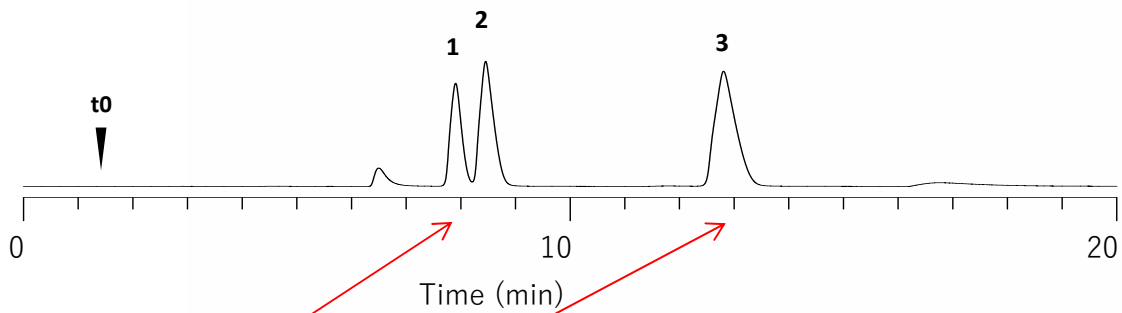
No ion pair reagent in eluent
↓
⊙ Highly polar acidic compounds retained

Analysis of sugar-nucleotides

Eluent : 20 mM HCOONH₄ in H₂O
(Ion pair not used)

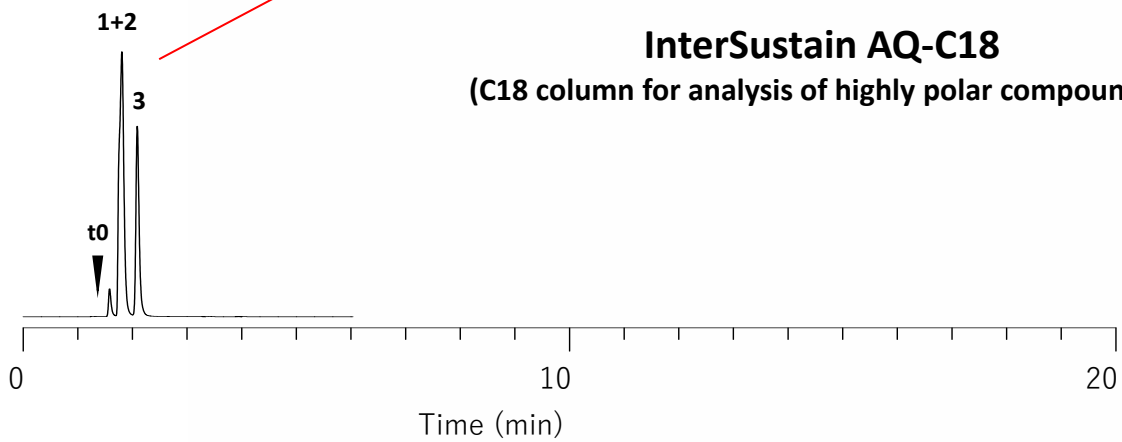
Conditions
Column : 3 μm, 150 × 2.1 mm I.D.
Flow Rate : 0.2 mL/min
Col. Temp. : 40 °C
Detection : UV 254 nm
Sample : 1. UDP-Glu 2. UDP-Gal 3. GDP-Man

InertSustain AX-C18



InterSustain AQ-C18

(C18 column for analysis of highly polar compounds)



Strongly retains highly polar acidic compounds which cannot normally be retained by C18 column for analysis of highly polar compounds!

Even if the salt concentration of the eluent is low



- ⊙ Acidic compounds strongly retained
- ⊙ Compatible with MS analysis

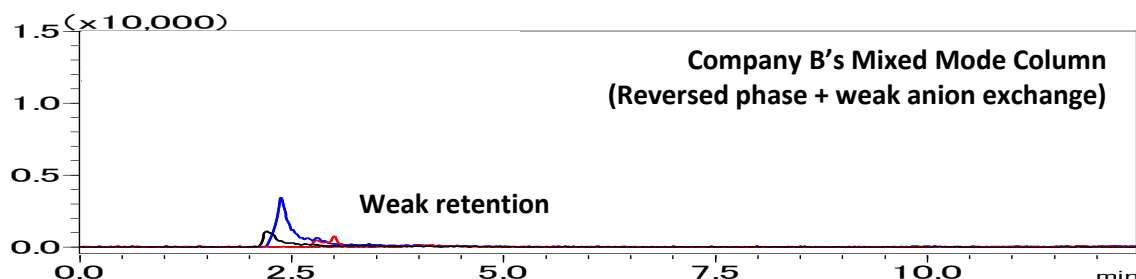
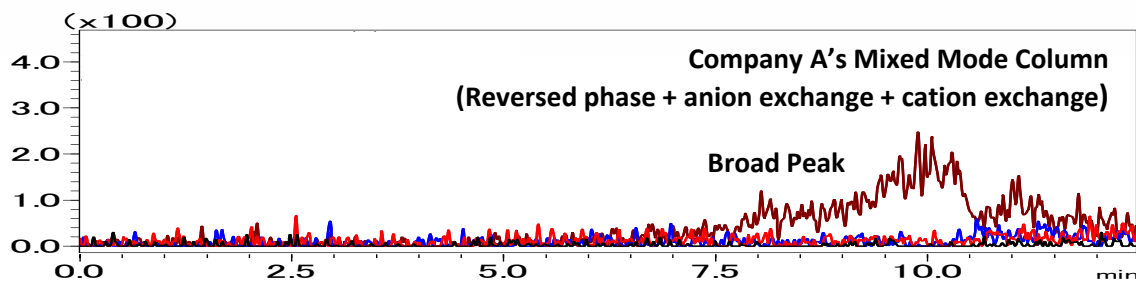
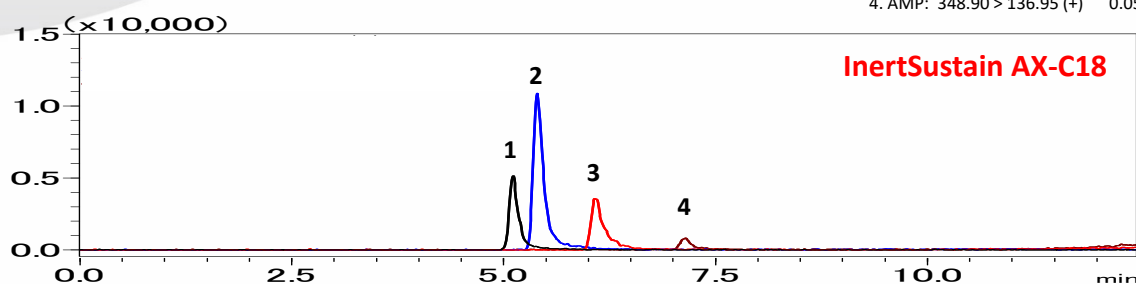
Analysis of monophosphoric acid

Eluent : A) 10 mM HCOONH₄ in CH₃OH
B) 10 mM HCOONH₄ in H₂O
A/B = 5/95 - 10 min - 100/0 - 5 min - 100/0, v/v

Conditions

Column : 3 μm, 150 × 2.1 mm I.D.
Flow Rate : 0.2 mL/min
Col. Temp. : 40 °C
Inj. Volume : 5 μL
Detection : MS/MS (ESI, Positive, MRM)

Sample: Q1 > Q3
1. CMP: 324.90 > 111.95 (+) 0.5 mg/L
2. GMP: 364.90 > 153.00 (+) 0.5 mg/L
3. UMP: 325.90 > 96.90 (+) 1.0 mg/L
4. AMP: 348.90 > 136.95 (+) 0.05 mg/L



**Strongly retains highly polar acidic compounds
even when salt concentration is as low as 10 mM!
Sharp elution peaks!**

**Can be analyzed at low salt concentration.
Compatibility with MS!**

Excellent reproducibility and quality

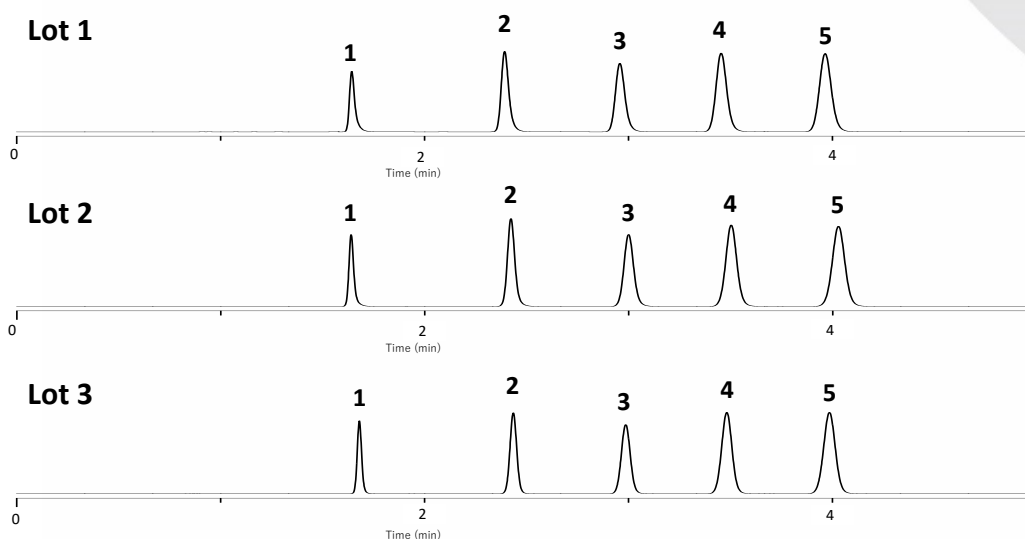
Reproducibility issues with mixed mode



are solved by GL Sciences' original packing and manufacturing technology

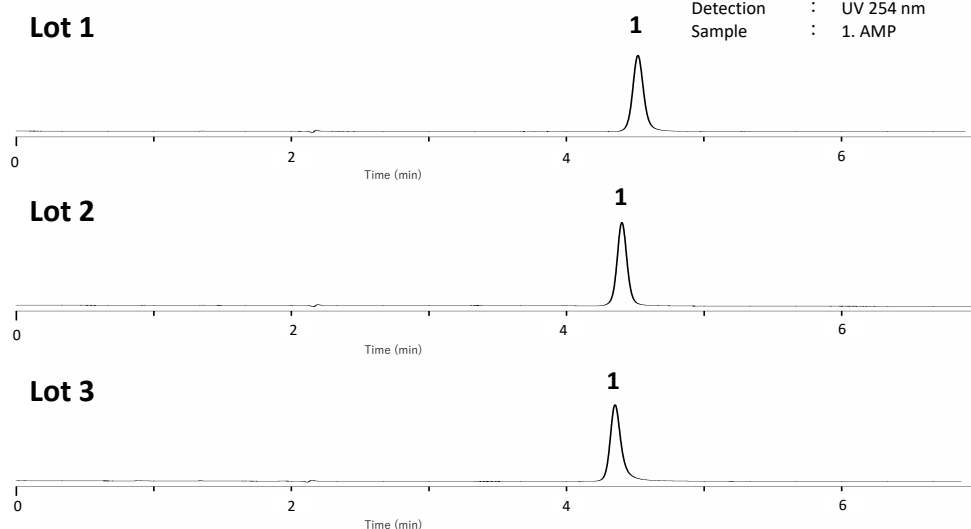
Shipping inspection for evaluation of lot-to-lot variation

Column : 3 μ m, 150 \times 4.6 mm I.D.
 Eluent : CH₃CN/H₂O = 65/35, v/v
 Flow Rate : 1.0 mL/min Col. Temp. : 40 $^{\circ}$ C
 Detection : UV 254 nm
 Sample : 1. Uracil 2. Acetophenone 3. Benzene
 4. Toluene 5. Naphthalene



Evaluation of lot-to-lot variation in acidic compounds

Column : 3 μ m, 150 \times 4.6 mm I.D.
 Eluent : CH₃CN/0.1% HCOOH = 10/90, v/v
 Flow Rate : 1.0 mL/min
 Col. Temp. : 40 $^{\circ}$ C
 Detection : UV 254 nm
 Sample : 1. AMP



Excellent reproducibility of retention time in mixed-mode columns where reproducibility is difficult to control!

If you are having trouble analyzing highly polar compounds using C18 columns...

You can choose the right column according to your target:

Want to strengthen overall retention



InertSustain AQ-C18

Want to specifically retain acidic compounds



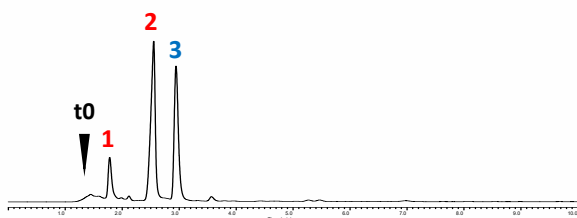
InertSustain AX-C18

Want to specifically retain basic compounds



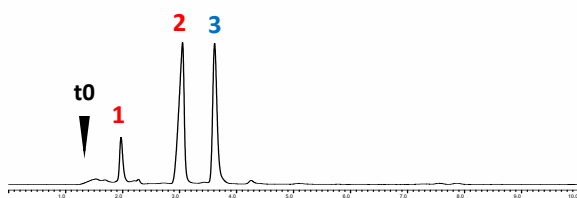
InertSustain PFP

InertSustain C18 (Typical C18 column)

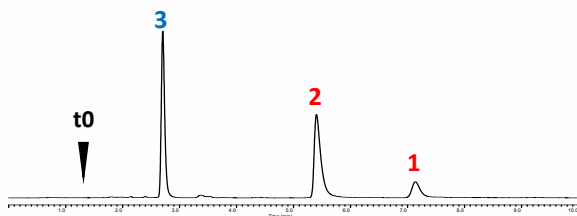


Column : 3 μ m, 150 \times 2.1 mm I.D.
 Eluent : A) CH₃CN
 B) 25 mM NaH₂PO₄ in H₂O
 A/B = 20/80, v/v
 Flow Rate : 0.2 mL/min
 Col. Temp. : 40 $^{\circ}$ C
 Detection : UV 270 nm

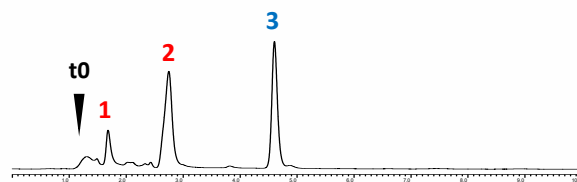
InertSustain AQ-C18



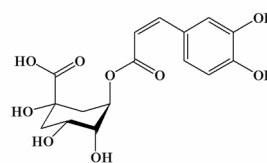
InertSustain AX-C18



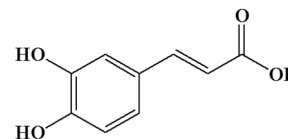
InertSustain PFP



1. Chlorogenic acid

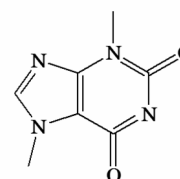


2. Caffeic acid



Acidic compounds

3. Caffeine



Basic compounds

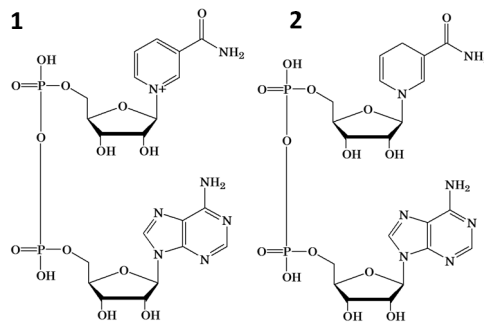
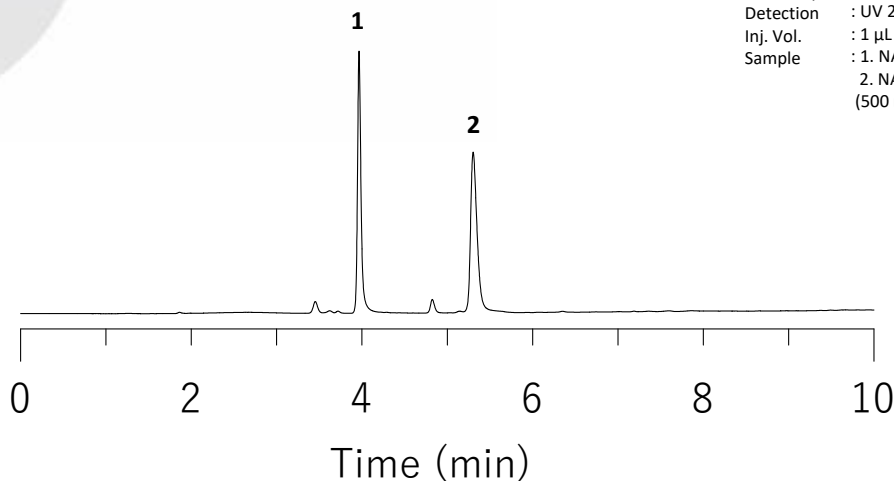
InertSustain AX-C18 Applications

NAD⁺, NADH

Nicotinamide Adenine Dinucleotide

Conditions

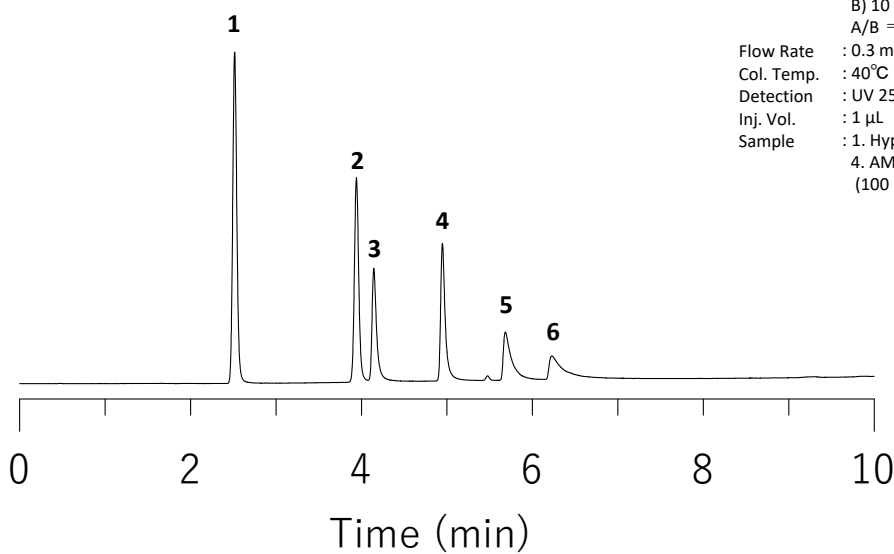
Column : 3 μm, 150 × 2.1 mm.I.D. (High pressure resistant UHPLC PEEK column)
 Eluent : A) 10 mM HCOONH₄ in (CH₃CN/H₂O = 90/10, v/v)
 B) 10 mM HCOONH₄ in H₂O
 A/B = 2/98 – 10 min – 80/20, v/v
 Flow Rate : 0.3 mL/min
 Col. Temp. : 40°C
 Detection : UV 254 nm
 Inj. Vol. : 1 μL
 Sample : 1. NAD⁺
 2. NADH
 (500 mg/L each)



ATP Decomposer

Conditions

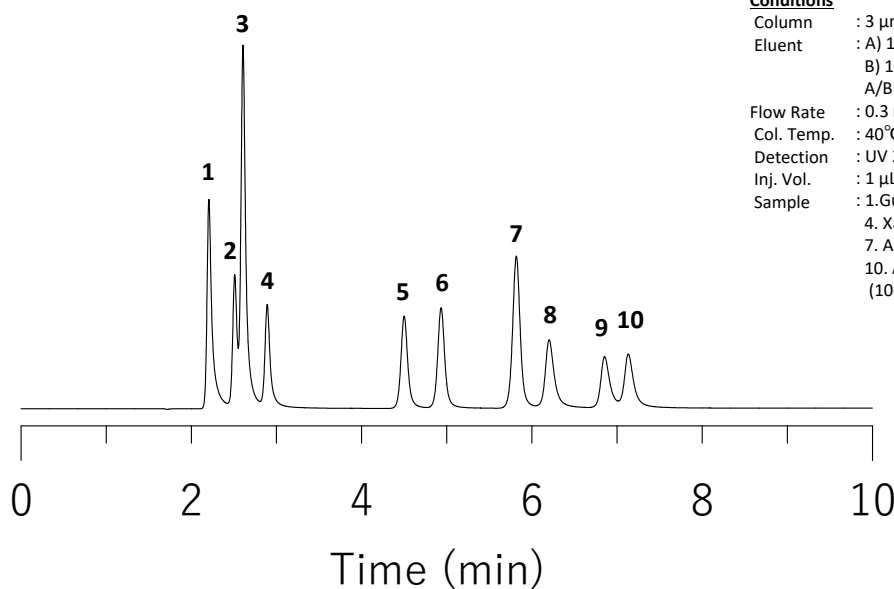
Column : 3 μm, 150 × 2.1 mm.I.D. (High pressure resistant UHPLC PEEK column)
 Eluent : A) 200 mM HCOONH₄ in (CH₃CN/H₂O = 50/50, v/v)
 B) 10 mM HCOONH₄ in H₂O
 A/B = 2/98 - 10 min – 50/50, v/v
 Flow Rate : 0.3 mL/min
 Col. Temp. : 40°C
 Detection : UV 254 nm
 Inj. Vol. : 1 μL
 Sample : 1. Hypoxanthine 2. Inosine 3. IMP
 4. AMP 5. ADP 6. ATP
 (100 mg/L each)



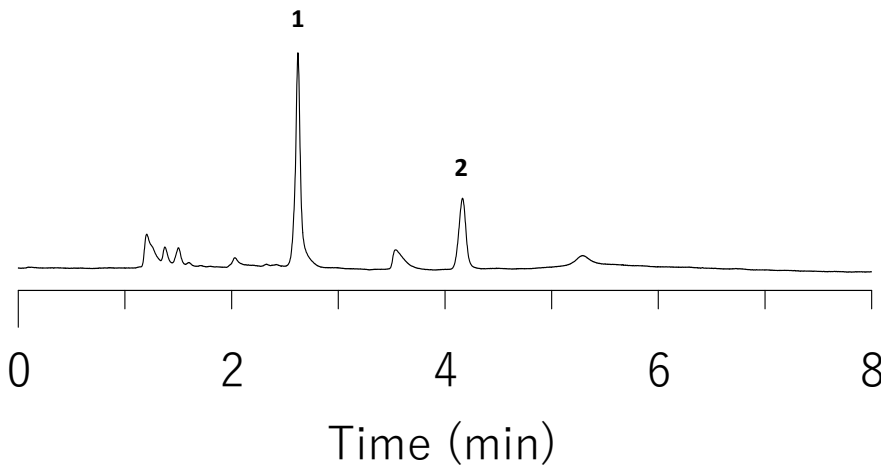
Uric Acid Metabolism

Conditions

Column : 3 μm, 150 × 2.1 mm.I.D. (High pressure resistant UHPLC PEEK column)
 Eluent : A) 100 mM HCOONH₄ + 0.1% HCOOH in (CH₃CN/H₂O = 50/50, v/v)
 B) 10 mM HCOONH₄ + 0.1% HCOOH in H₂O
 A/B = 2/98 - 10 min – 15/85, v/v
 Flow Rate : 0.3 mL/min
 Col. Temp. : 40°C
 Detection : UV 254 nm
 Inj. Vol. : 1 μL
 Sample : 1. Guanine 2. Uric acid 3. Hypoxanthine
 4. Xanthine 5. Inosine 6. Guanosine
 7. Adenosine 8. IMP 9. GMP
 10. AMP
 (100 mg/L each)

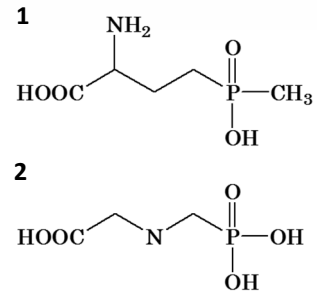


Glyphosate and Glufosinate

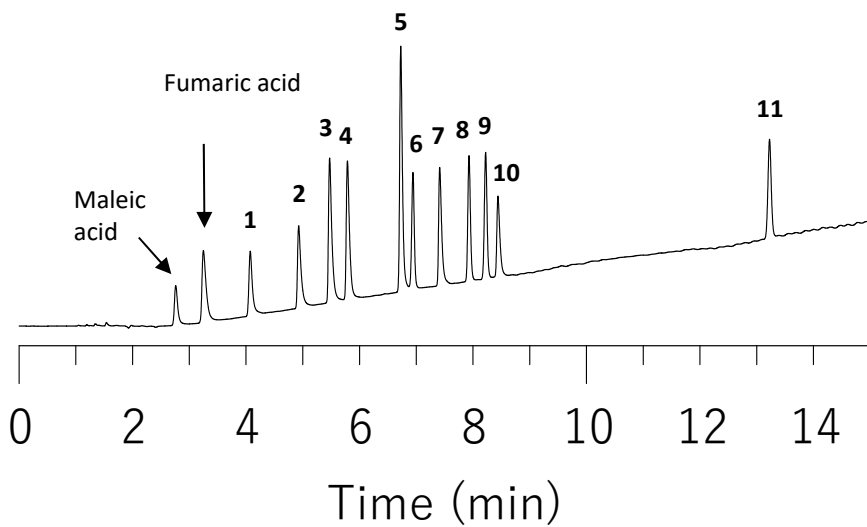


Conditions

Column : 3 μm , 150 \times 2.1 mm I.D.
 Eluent : 0.1 % H_3PO_4 in H_2O
 Flow Rate : 0.2 mL/min
 Col. Temp. : 40°C
 Detection : UV 210 nm
 Inj. Vol. : 1 μL
 Sample : 1. Glufosinate
 2. Glyphosate
 (1000 mg/L each)



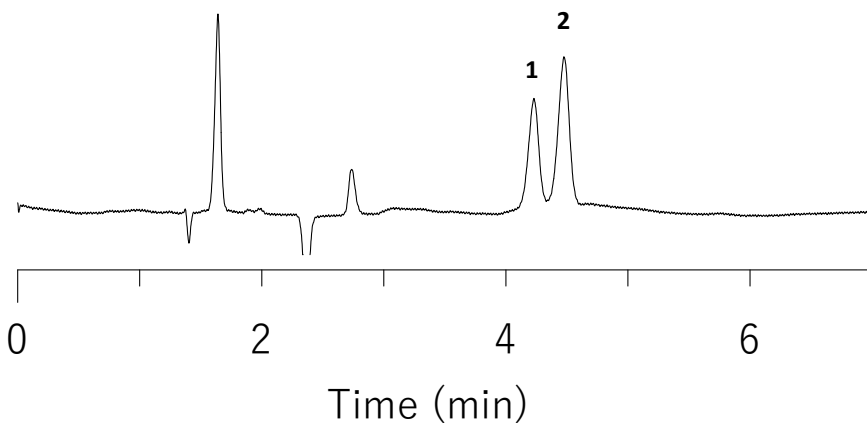
Antihistamines



Conditions

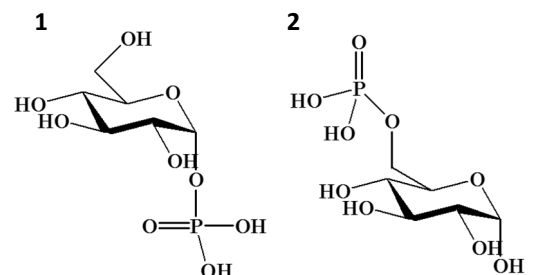
Column : 3 μm , 150 \times 2.1 mm I.D.
 Eluent : A) 10 mM HCOONH_4 (in $\text{CH}_3\text{CN}/\text{H}_2\text{O} = 90/10$, v/v)
 : B) 10 mM HCOONH_4 in H_2O
 A/B = 20/80 - 15 min - 90/10, v/v
 Flow Rate : 0.3 mL/min
 Col. Temp. : 40°C
 Detection : UV 230 nm
 Inj. Vol. : 2 μL
 Sample : 1. Epinastine
 2. Chlorpheniramine * 1
 3. Triprolidine
 4. Hydroxyzine
 5. Azelastine
 6. Ketotifen * 2
 7. Loratadine
 8. Homochlorcyclizine
 9. Clemastine * 2
 10. Cetirizine
 11. Fexofenadine
 (50 mg/L each)
 *1 Maleate *2 Fumarate

Glucose Phosphates



Conditions

Column : 3 μm , 150 \times 4.6 mm I.D.
 Eluent : 10 mM HCOONH_4 + 0.1 % HCOOH in H_2O
 Flow Rate : 1.0 mL/min
 Col. Temp. : 40°C
 Detection : RI
 Inj. Vol. : 5 μL
 Sample : 1. Glucose 1-phosphate
 2. Glucose 6-phosphate
 (10000 mg/L each)

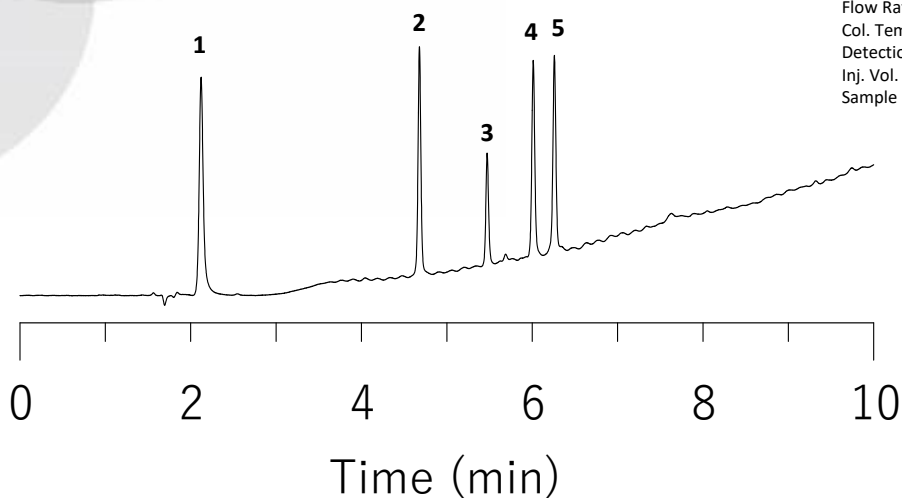


InertSustain AX-C18 Applications

Peptides

Conditions

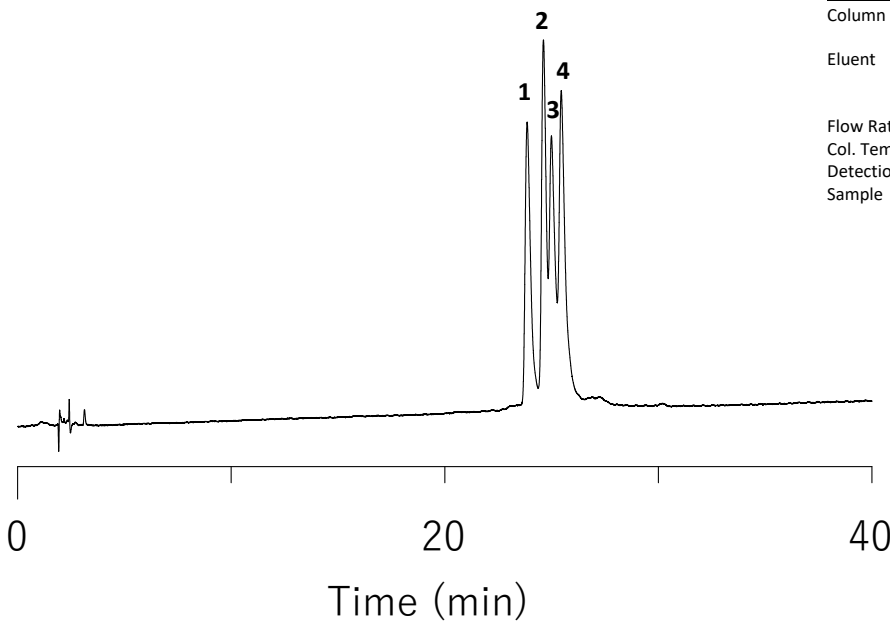
Column : 3 μ m, 150 \times 2.1 mm.I.D.
 Eluent : A) 10 mM HCOONH₄ (0.1% HCOOH) in CH₃CN/H₂O =90/10, v/v
 : B) 10 mM HCOONH₄ (0.1% HCOOH) in H₂O
 A/B = 2/98 - 10 min - 80/20, v/v
 Flow Rate : 0.3 mL/min
 Col. Temp. : 40°C
 Detection : UV 254 nm
 Inj. Vol. : 1 μ L
 Sample : 1. Gly-Tyr
 2. Val-Tyr-Val
 3. Angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe)
 4. Met-Enkephalin (Tyr-Gly-Gly-Phe-Met)
 5. Lue-Enkephalin (Tyr-Gly-Gly-Phe-Lue)
 (500 mg/L each)



Oligonucleic Acid

Conditions

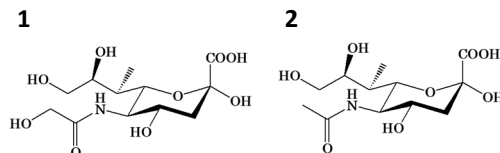
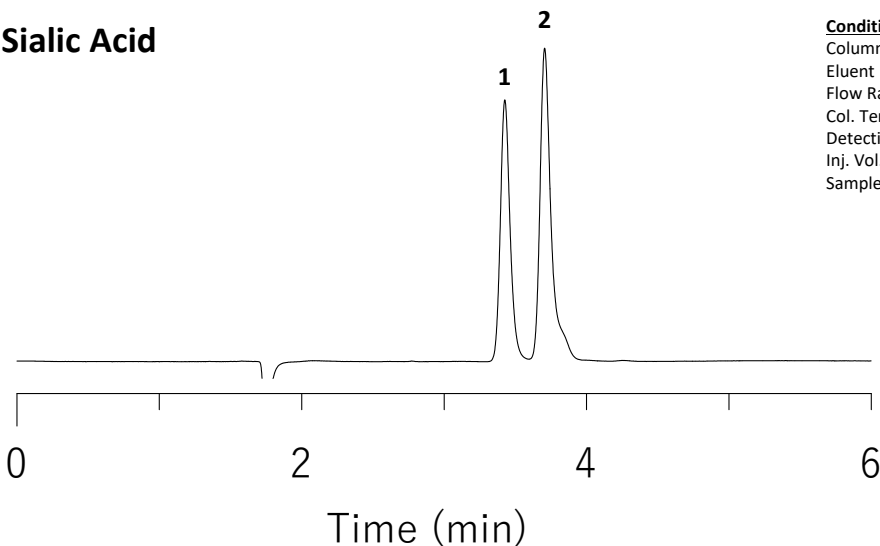
Column : 3 μ m, 150 \times 2.1 mm.I.D. (High pressure resistant UHPLC PEEK column)
 Eluent : A) 200 mM HCOONH₄ in (CH₃CN/H₂O = 50/50, v/v)
 : B) 200 mM HCOONH₄ in H₂O
 A/B = 10/90 - 40 min - 40/60, v/v
 Flow Rate : 0.2 mL/min
 Col. Temp. : 40°C
 Detection : UV 260 nm
 Sample : 1. 5'-CATGACGTTCCCTGATGCT-3' (18 mer)
 2. 5'-GCTACGACGTTCCCTGATGCT-3' (20 mer)
 3. 5'-TATGACGTTCCCTGATGCT-3' (18 mer)
 4. 5'-GCTATGACGTTCCCTGATGCT-3' (20 mer)



Sialic Acid

Conditions

Column : 3 μ m, 150 \times 2.1 mm.I.D.
 Eluent : 10 mM HCOONH₄ + 0.1 % HCOOH in H₂O
 Flow Rate : 1.0 mL/min
 Col. Temp. : 40°C
 Detection : RI
 Inj. Vol. : 2 μ L
 Sample : 1. Neu5Gc (N-Glycolylneuraminic acid)
 2. Neu5Ac (N-Acetylneuraminic acid)
 (500 mg/L each)



Columns lineup

InertSustain AX-C18 Analytical Columns

HP series Particle size: 3 µm	Length/internal diameter (mm)	2.1	3.0	4.6
	150	5020-91053	5020-91055	5020-91057
	250	5020-91054	5020-91056	5020-91058

Note: The recommended maximum pressure of the column is 50 MPa.

Particle size: 3 µm	Length/internal diameter (mm)	2.1	3.0	4.0	4.6
	50	5020-91037	5020-91041	5020-91045	5020-91049
	100	5020-91038	5020-91042	5020-91046	5020-91050
	150	5020-91039	5020-91043	5020-91047	5020-91051
	250	5020-91040	5020-91044	5020-91048	5020-91052
Particle size: 5 µm	Length/internal diameter (mm)	2.1	3.0	4.0	4.6
	50	5020-91000	5020-91004	5020-91008	5020-91012
	100	5020-91001	5020-91005	5020-91009	5020-91013
	150	5020-91002	5020-91006	5020-91010	5020-91014
	250	5020-91003	5020-91007	5020-91011	5020-91015

Note: Joint type is Parker type (UP type). The pressure resistance of the cartridge column is 20 MPa.

InertSustain AX-C18 Cartridge Guard Column E (Pressure Resistance: 20 MPa)

Internal diameter of relevant analytical column (mm)	Length (mm)	Internal diameter (mm)	Replacement Cartridge Guard Column E (2 cartridges)		Replacement Cartridge E Set (2 Cartridge E + 1 holder)	
			Particle size		Particle size	
			3 µm	5 µm	3 µm	5 µm
1.5、2.1	10	1.5	5020-91076	5020-91029	5020-91077	5020-91030
2.1、3.0		3.0	5020-91074	5020-91027	5020-91075	5020-91028
4.0、4.6		4.0	5020-91072	5020-91025	5020-91073	5020-91026
2.1、3.0	20	3.0	5020-91080	5020-91033	5020-91081	5020-91034
4.0、4.6		4.0	5020-91078	5020-91031	5020-91079	5020-91032
Cartridge Guard Column E Holder				For 10 mm length		5020-08500
				For 20 mm length		5020-08550

Note: Joint type is Parker type (UP type).

Guard Column for UHPLC (Max. Operating Pressure: 80 MPa)

Internal diameter or relevant analytical column (mm)	Length (mm)	Internal diameter (mm)	Replacement cartridge only (2 cartridges)	Set of 2 cartridges + 1 holder
			Particle size 3 µm	Particle size 3 µm
1.0	10	1.5	5020-91084	5020-91087
1.5、2.1		2.1	5020-91085	5020-91088
2.1、3.0		3.0	5020-91086	5020-91089
Guard Column for UHPLC Holder				5020-08630

Note: The end-fittings for the UHPLC holder are as follows.

Column connection side: Tube with 1/16 inch external diameter, 0.18 mm internal diameter, and 30 mm length is fixed in place.

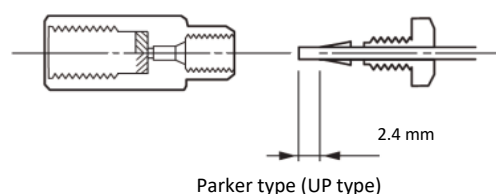
Instrument connection side: Tube tip is 2.4 mm long with 1/16 inch threads

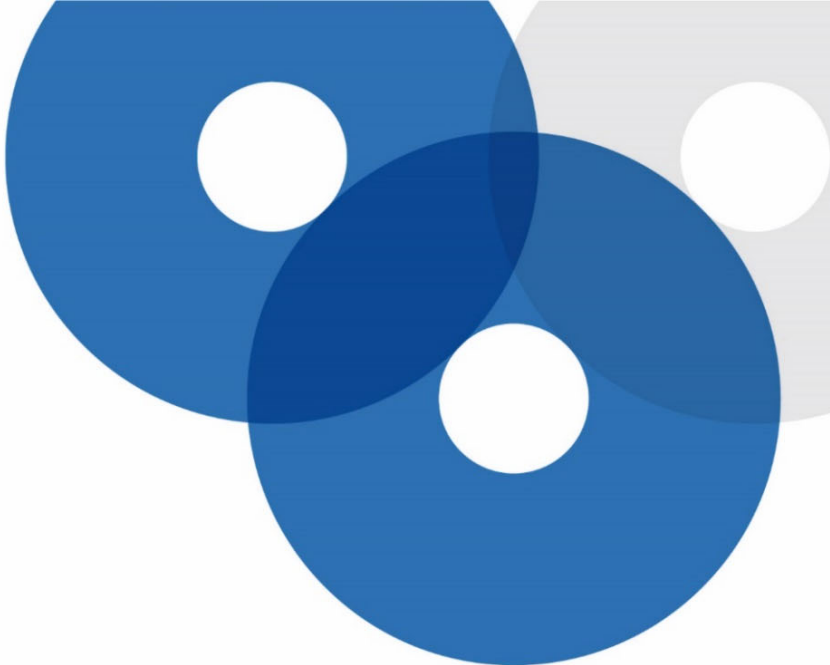
High Pressure Resistant UHPLC PEEK Column (Max. Operating Pressure: 80 MPa)

Particle size	Length (mm)	Internal diameter (mm)	
		2.1	4.6
3 µm	150	5020-91059	5020-91061
	250	5020-91060	5020-91062

Note: Joint type is Parker type (UP type).

The pressure resistance of 3 µm column is 50 MPa.





Usage Tips for Mixed Mode Columns

- Because of the ionic interactions, it is recommended to add 5-10 mM of salt to the eluent.
- When analyzing compounds that contain multiple anionic functional groups, a salt concentration gradient is recommended.
- After analysis of anionic compounds, the column should be washed thoroughly with 50-100 mM ammonium acetate in methanol solution or similar.
- For storage, replace with an organic solvent (methanol or acetonitrile) that does not contain salt.

HPLC Technical Information Website

<https://www.glsciences.com/technique/index.html>



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