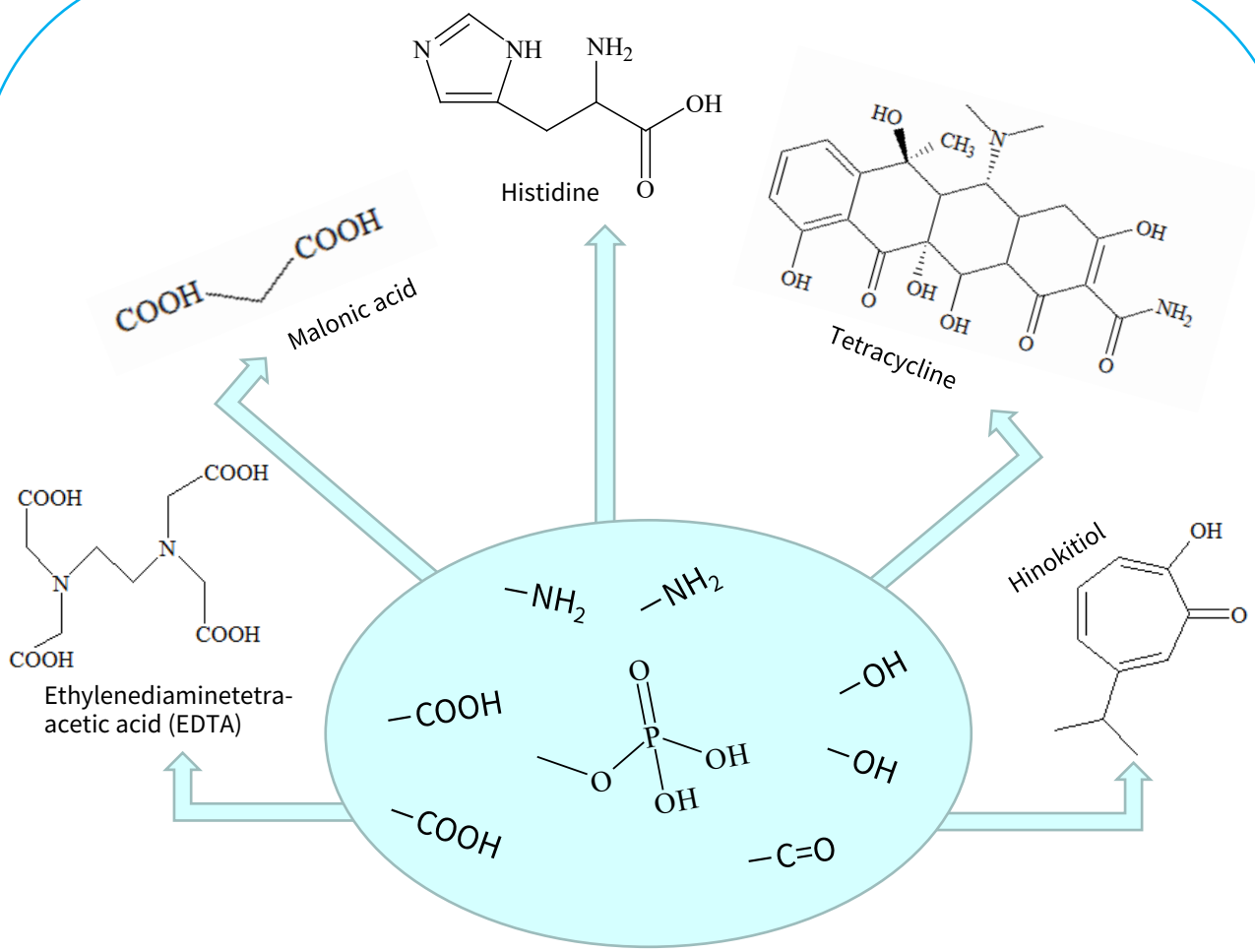
Metal-free Columns for HPLC Part 1

Some amino acids, organic acids, tetracyclines and organophosphates are metal chelators. Chelation during analysis of these compounds can distort the peak shapes and worsen the quantification accuracy. Residual metals on the packing surface were assumed to cause these problems. However, in the case of microanalysis, it has turned out that metals on the wetted parts such as tubing or frits also has a strong influence. Phosphate buffers are widely used in metal chelator analysis to improve the peak shapes, however, phosphate buffers cannot be used in LC/MS(/MS) because non-volatile salts precipitate from these buffers.
Metal-free columns have wetted parts made of PEEK instead of metal. In this technical note, some applications using metal-free columns are shown and the advantages of metal-free columns are described.

(K.Kanno)



In HPLC, analytes containing the functional groups above are easily affected by metals.

Metal Effects ⋯⋯▶

- ○ Peak tailing
- ○ No elution
- ○ Low sensitivity

etc.

*Special inertization was carried out for the metal wetted parts of the HPLC systems used for this technical note. Please contact for details.

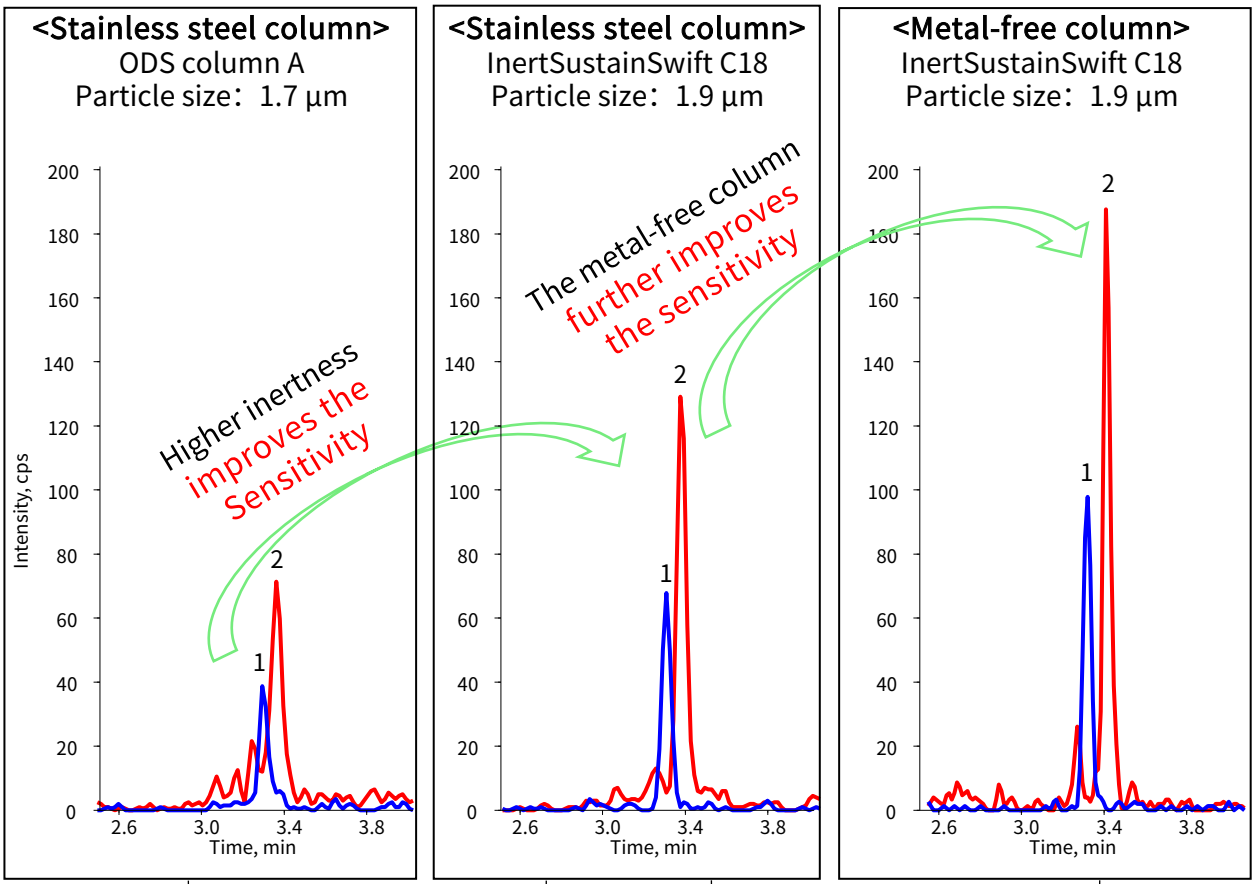
GL Sciences

RP Example 1. Tetracyclines

Tetracyclines are metal chelators, and their adsorption onto residual metals on the packing surface and metal parts of the column results in poor performance.
By using a highly inert column with minimized residual metals, high sensitivity becomes possible with formate buffers, which are often used in LC/MS(/MS). Further sensitivity improvement is possible with a metal-free column.

1. Oxytetracycline
2. Tetracycline

2 mg/L each



Conditions

Column : InertSustainSwift C18 (1.9 µm, 50 x 2.1 mm I.D.)
ODS column A (1.7 µm, 50 x 2.1 mm I.D.)

Mobile phase : A) 0.1 % HCOOH in CH_3CN
B) 0.1 % HCOOH in H_2O
A/B = 10/90 – 1.5 min – 10/90 – 2.5 min – 90/10 , v/v

Flow rate : 0.4 mL/min

Temperature : 40 °C

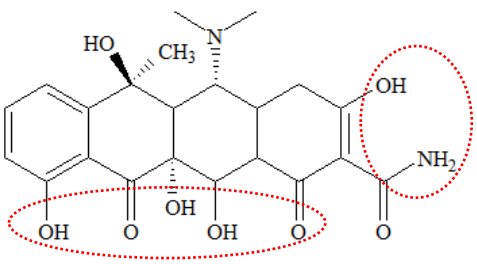
Detection : LC/MS/MS (ESI, Positive, MRM)

Injection volume : 10 mL

Concentration : 2.0 mg/L

	Q1	Q3
1. Oxytetracycline(OTC)	460.9	426.2
2. Tetracycline(TC)	445.0	154.0

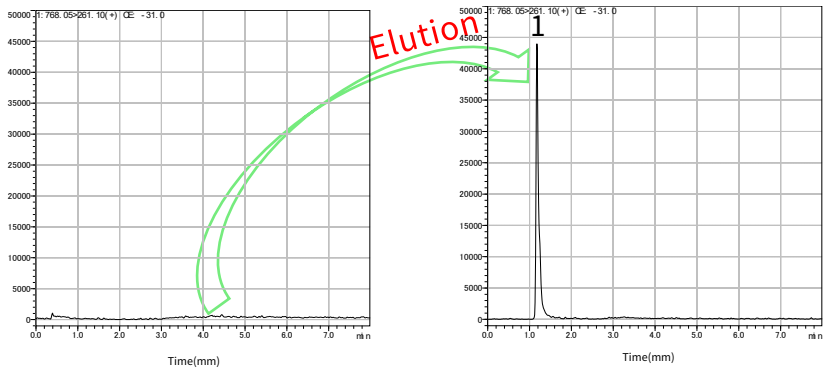
2. Tetracycline



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

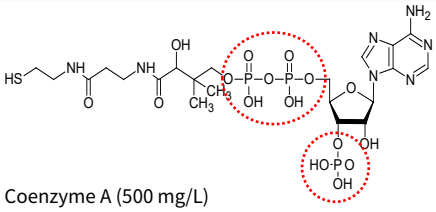
RP Example 2. Phosphate group-containing compounds

Phosphate group-containing compounds such as organophosphate pesticides and nucleotides easily form metal chelates. With a stainless steel column, analytes may not be able to elute from the column or distorted peaks may be obtained. In this case, a metal-free column can be an effective tool.

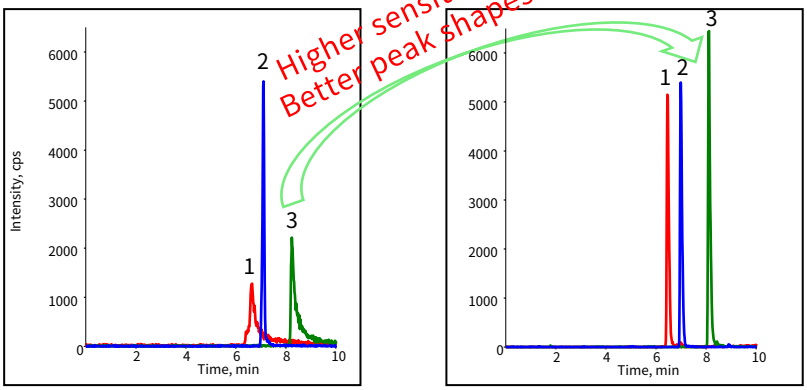


<Stainless steel column> <Metal-free column>

Conditions
Column : InertSustainSwift C18 (1.9 µm, 50 × 2.1 mm I.D)
Mobile phase : A) 5 mM $HCOONH_4$ in H_2O
B) 5 mM $HCOONH_4$ in CH_3CN
A/B=98/2-2 min-80/20
Flow rate : 0.4 mL/min
Temperature : 40 °C
Detection : LC/MS/MS (ESI, Positive, MRM)
Injection volume : 10 µL
Analyte : Coenzyme A (Q1/Q3 = 768/261)

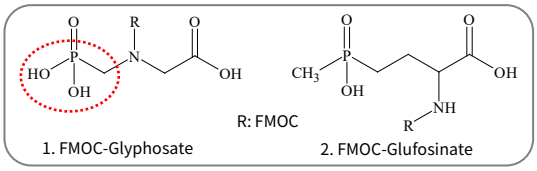


1. Coenzyme A (500 mg/L)



<Stainless steel column> <Metal-free column>

Conditions
Column : InertSustain C18 (3 µm, 150 × 2.1 mm I.D)
Mobile phase : A) 5 mM $HCOONH_4$ in H_2O
B) CH_3CN
A/B=95/5 -9.3 min - 37/63
Flow rate : 0.25 mL/min
Temperature : 40 °C
Detection : LC/MS/MS (ESI, Negative, MRM)
Injection volume : 10 µL
Analyte : 1. FMOC-Glyphosate (Q1/Q3 = 390/168)
2. FMOC-Glufosinate (Q1/Q3 = 402/190)
3. FMOC-AMPA (Q1/Q3 = 332/110) 2 mg/L each

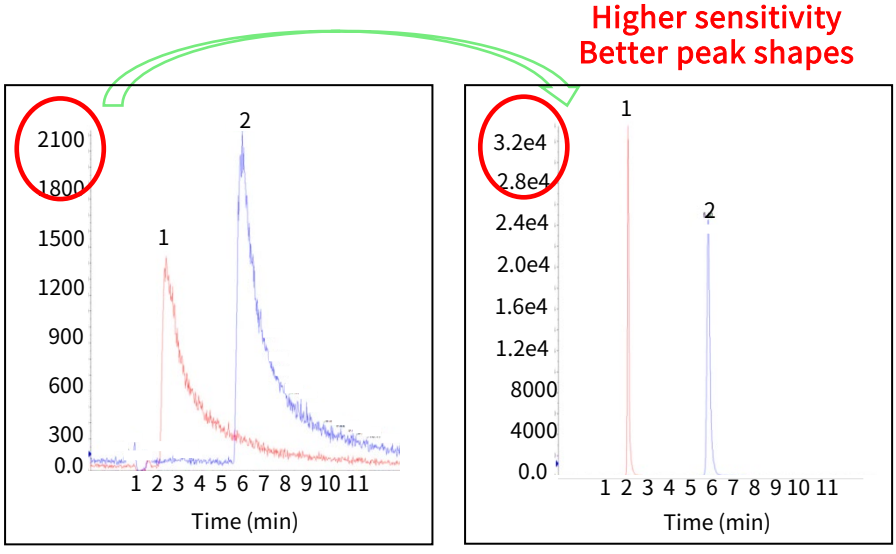


1. FMOC-Glyphosate 2. FMOC-Glufosinate

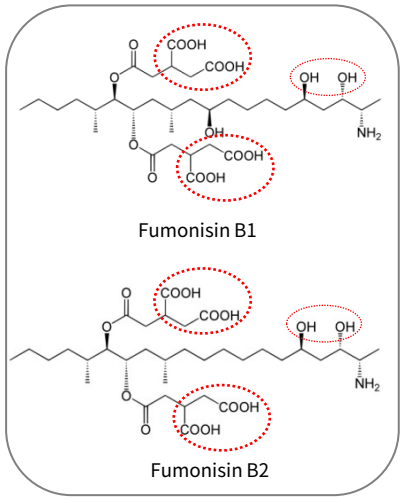
RP Example 3. Fumonisins

Fumonisins, kinds of mycotoxins, are strong metal chelators because of their adjacent carboxyl and hydroxyl groups. Adsorption of fumonisins onto the column inner wall and the filters of stainless steel columns distorts the peak shapes.

Conditions
Column : InertSustain C18 (3 µm , 100 ×2.1 mm I.D.)
Mobile phase : A) 0.1 % HCOOH ,10 mM $HCOONH_4$
B) CH_3CN
A/B = 60/40,v/v
Flow rate : 0.2 mL/min
Temperature : 40 °C
Detection : LC/MS/MS (ESI , Positive. , MRM)
Analyte : 1.Fumonisin B1 (Q1/Q3 = 722/334)
2.Fumonisin B2 (Q1/Q3 = 706/336)



<Stainless steel column> <Metal-free column>

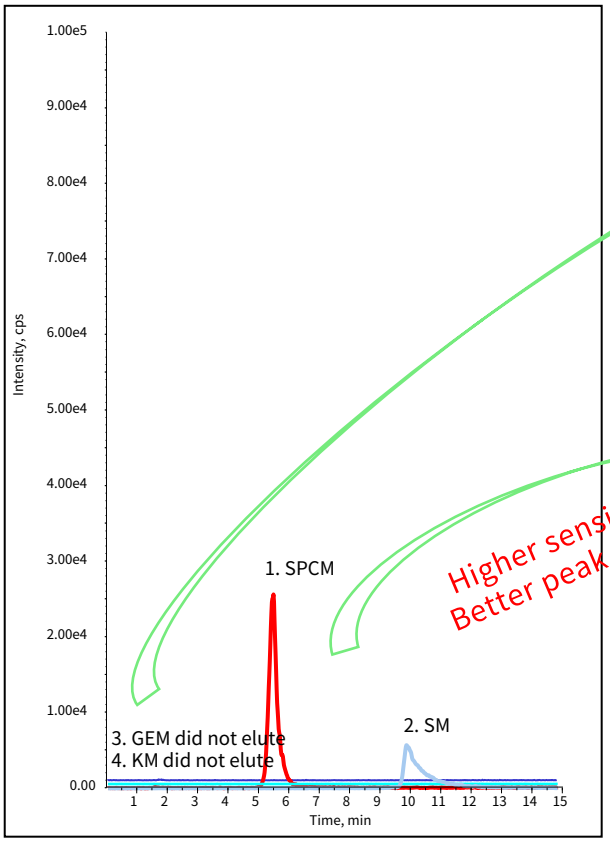


Fumonisin B1

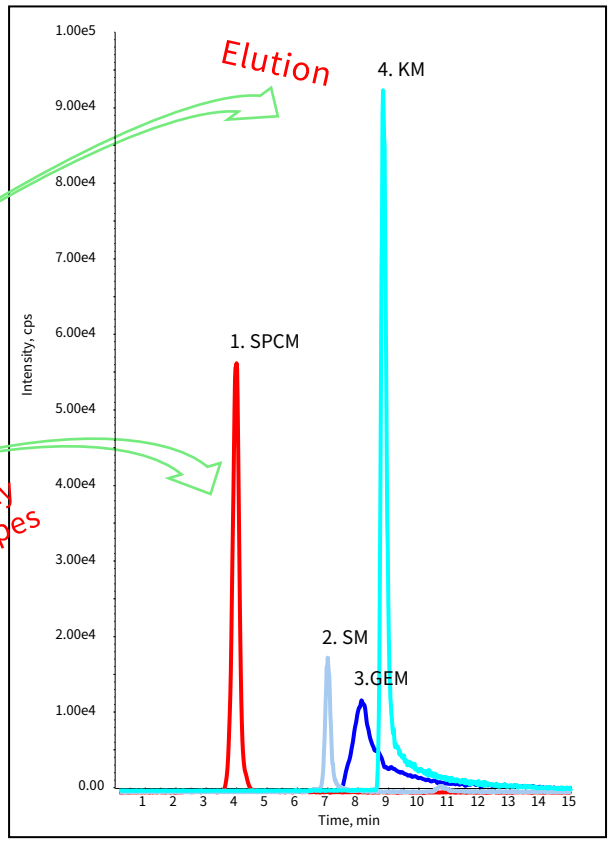
Fumonisin B2

HILIC Example 1. Aminoglycosides

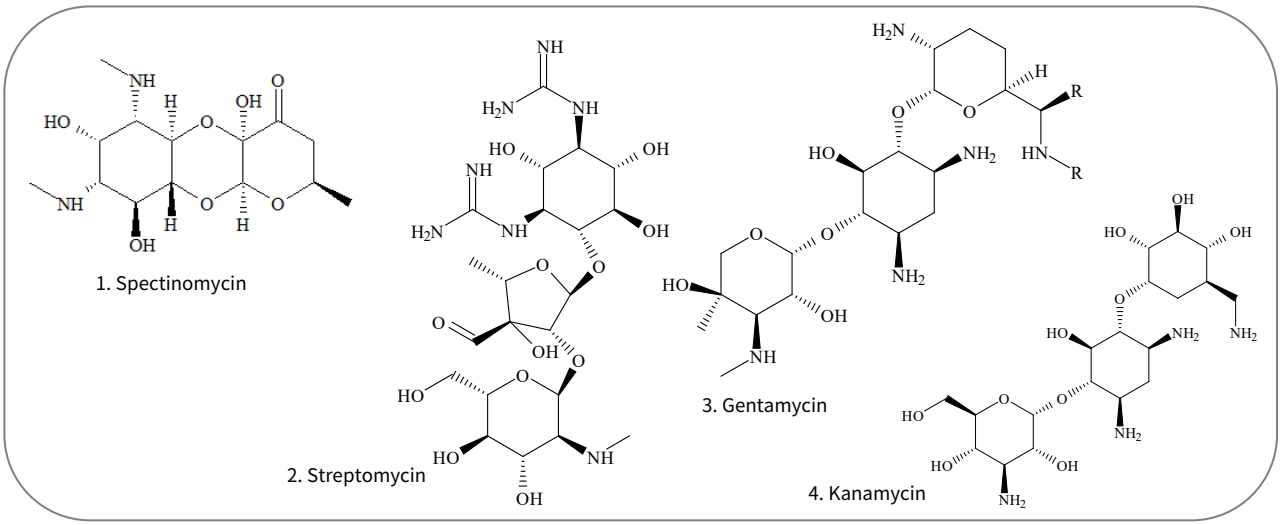
Aminoglycoside antibiotics such as streptomycin possess amino sugar substructures and their adjacent hydroxyl and amino groups contribute to metal chelation. The effects of stainless steel depends on the analyte; kanamycin does not elute from the column, whereas spectinomycin elutes as a broad peak.



<Stainless steel column>



<Metal-free column>



Conditions

Column : Inertsil Amide (5 µm, 150 x 2.1 mm I.D.)

Mobile phase : A) 0.1 % HCOOH in CH_3CN
B) 0.1 % HCOOH in H_2O
A/B = 70/30 – 10 min – 50/50 , v/v

Flow rate : 0.2 mL/min

Temperature : 40 °C

Injection volume : 10 mL

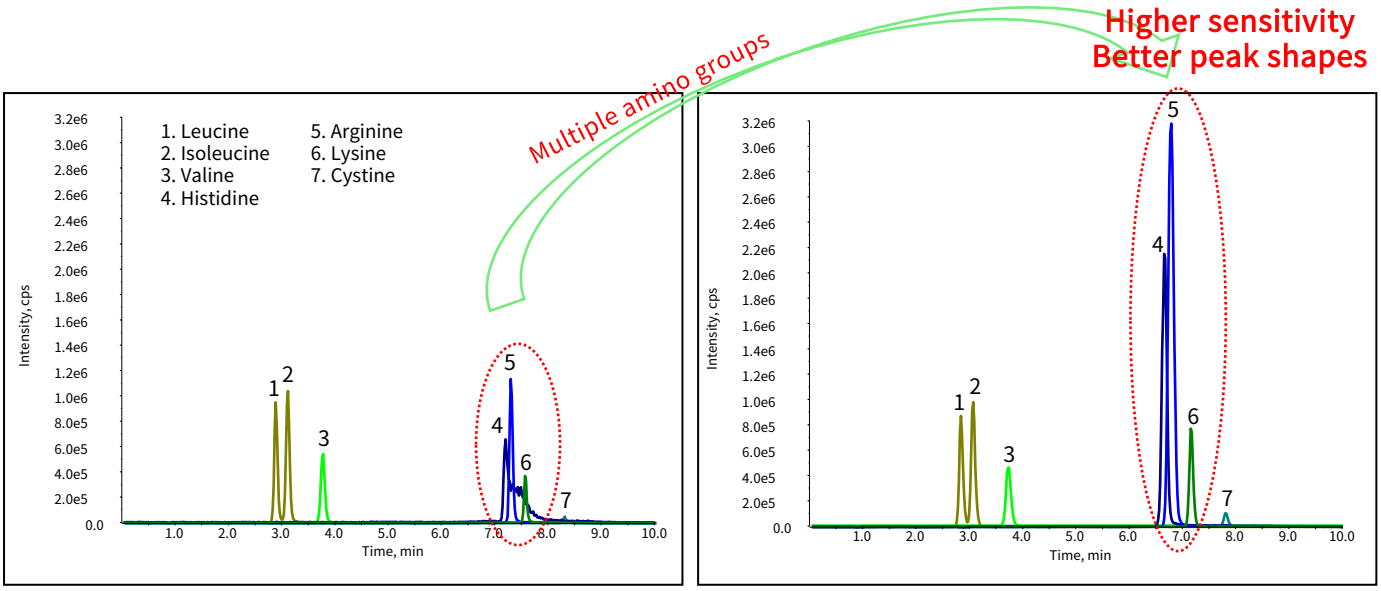
Detection : LC/MS/MS (ESI, Positive, MRM)

	Q1	Q3	
1. Spectinomycin(SPCM)	351	333	
2. Streptomycin(SM)		292	176
3. Gentamycin(GEM)		322	160
4. Kanamycin(KM)	243	162	

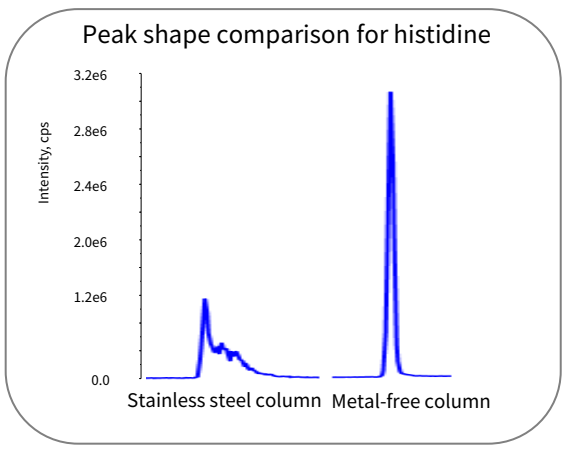
Concentration : 500 mg/L

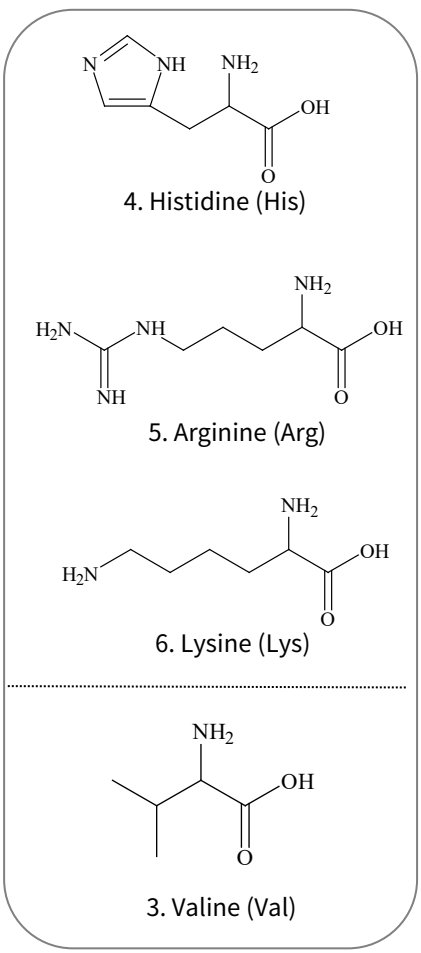
HILIC example 2. Amino acids

Amino acids containing multiple amino groups are easily affected by metals. There is no difference between stainless steel columns and metal-free columns in the sensitivity of amino acids possessing only one amino group, such as leucine and valine. On the other hand, the effects of column material are considerable on the sensitivity and the peak shapes of amino acids include several amino groups, such as histidine and arginine.



<Stainless steel column> <Metal-free column>





Conditions

Column : Inertsil Amide (3 µm, 150 x 2.1 mm I.D.)

Mobile phase : A) 100 mM $HCOONH_4$, 0.1 % HCOOH in 75 % CH_3CN
B) 100 mM $HCOONH_4$, 0.1 % HCOOH in H_2O
A/B = 100/0 – 3 min – 100/0 – 5.5 min – 70/30 , v/v

Flow rate : 0.3 mL/min

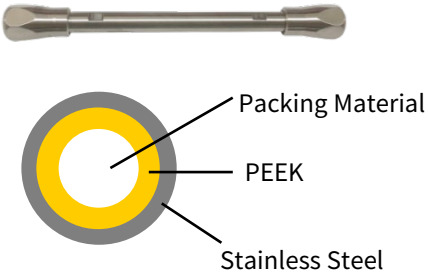
Temperature : 40 °C

Detection : LC/MS/MS (ESI, Positive, MRM)

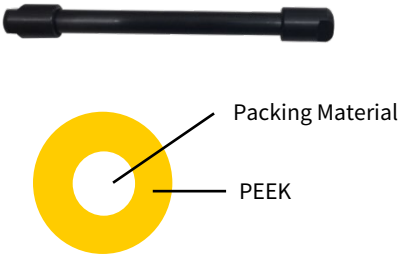
Injection Volume : 5.0 mL

Concentration : 5 mM

	Q1	Q3
1. Leucine	132	86
2. Isoleucine	132	86
3. Valine	118	72
4. Histidine	155	110
5. Arginine	175	70
6. Lysine	147	84
7. Cystine	241	152



UHPLC-PEEK Columns



PEEK Columns

Analytical Columns List

InertSustain Series

- InertSustain C18
- InertSustainSwift C18
- InertSustain AQ-C18
- InertSustain C8
- InertSustain NH2
- InertSustain Phenylhexyl
- InertSustain Phenyl

Inertsil Series

- Inertsil ODS-4
- Inertsil ODS-3
- Inertsil ODS-SP
- Inertsil ODS-P
- Inertsil ODS-EP
- Inertsil C8-4
- Inertsil C8-3
- Inertsil Ph-3
- Inertsil WP300 C18
- Inertsil WP300 C8
- Inertsil WP300 C4
- Inertsil Peptides C18
- Inertsil HILIC
- Inertsil Amide
- Inertsil Diol
- Inertsil NH2
- Inertsil WP300 Diol
- Inertsil SIL-100A
- Inertsil WP300 SIL
- Inertsil CN-3

*Other packing materials are on request.
*Check https://www.glsciences.com/product/lc_columns/01853.html for details.

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