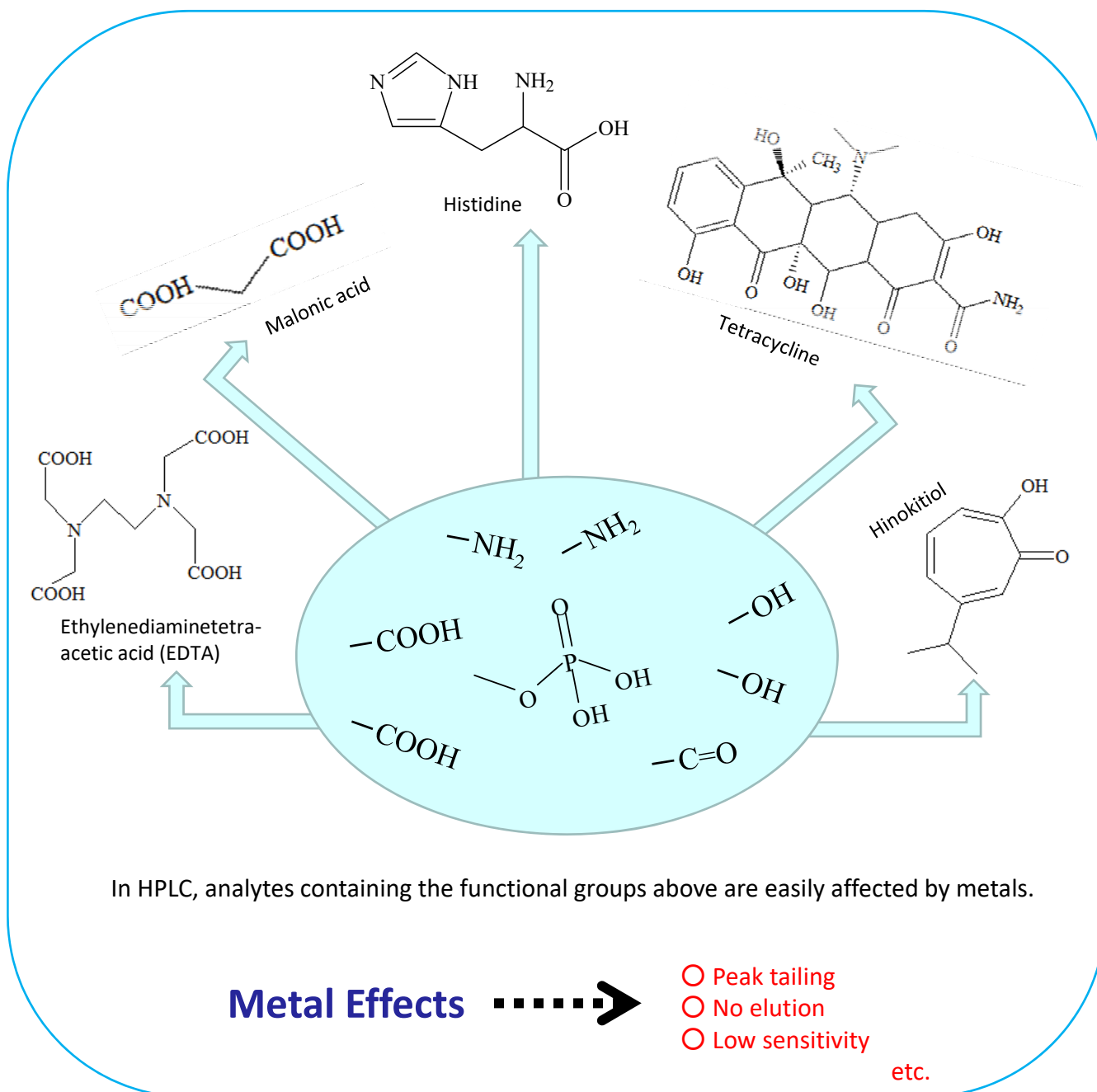


Some amino acids, organic acids, tetracyclines and organophosphates are metal chelators. Chelation during analysis of these compounds can distort the peak shapes and worsen the quantification accuracy. Residual metals on the packing surface were assumed to cause these problems. However, in the case of 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)



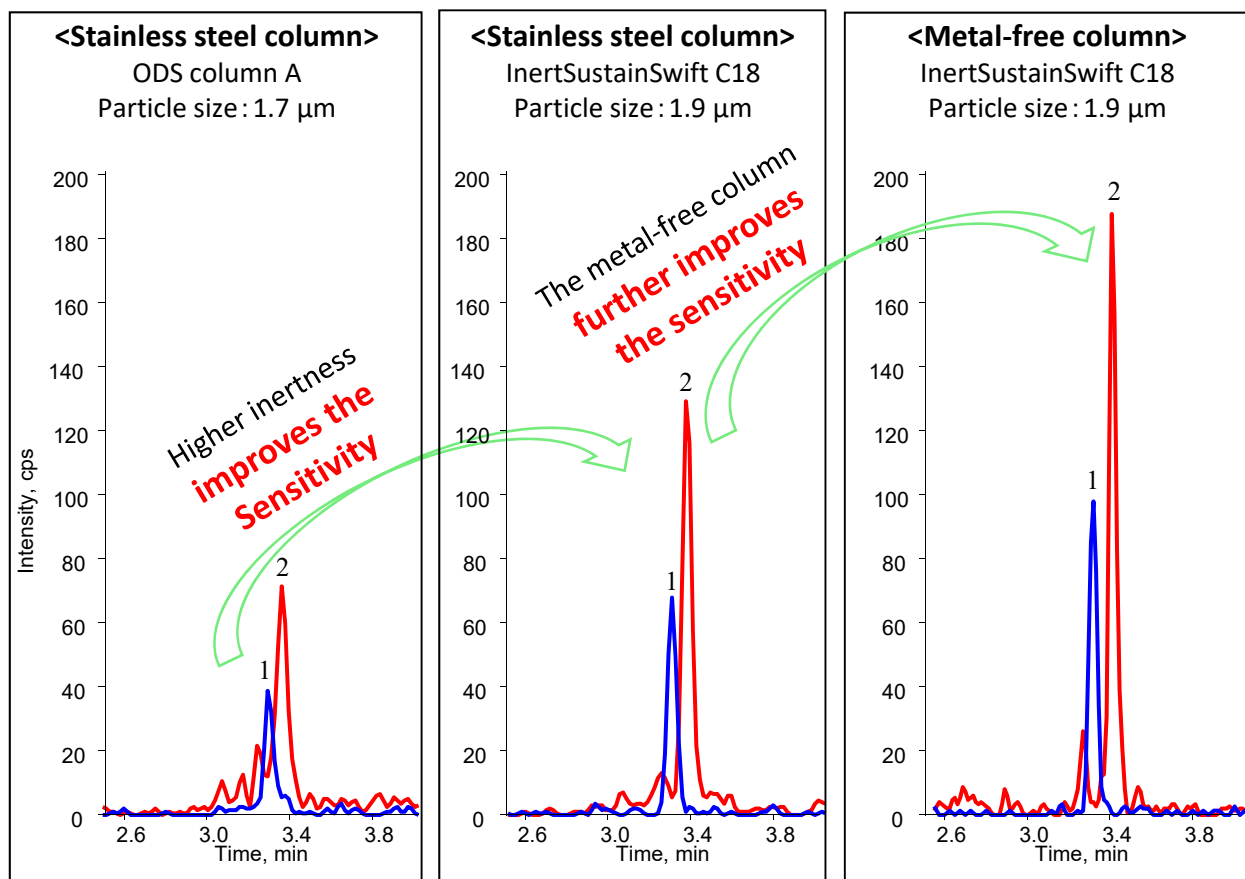
*Special inertization was carried out for the metal wetted parts of the HPLC systems used for this technical note. Please contact for details.

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



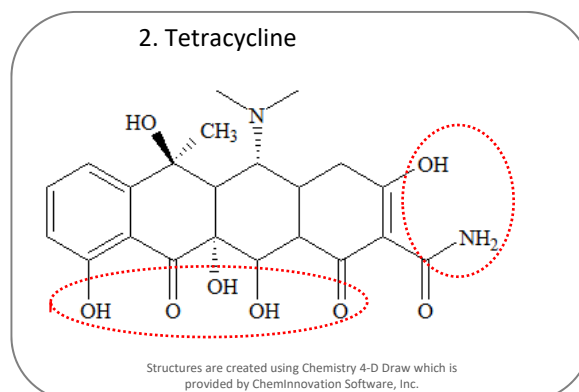
Different packing inertness

Different column material
Stainless steel or PEEK

Conditions

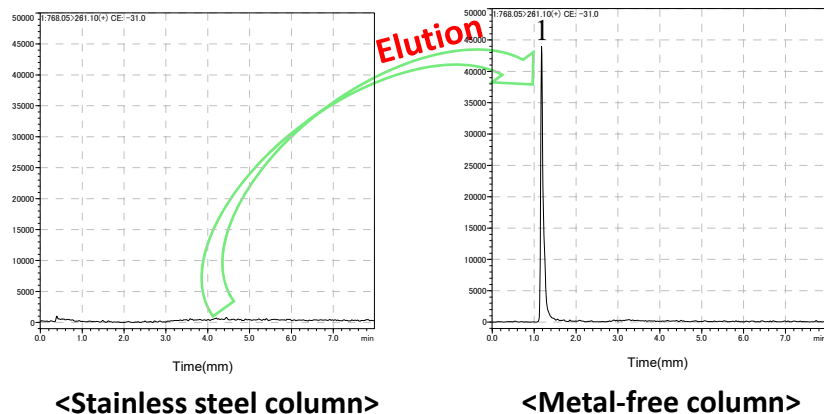
- Column** : InertSustainSwift C18 (1.9 μm, 50 x 2.1 mm I.D.)
 ODS column A (1.7 μm, 50 x 2.1 mm I.D.)
- Mobile phase** : A) 0.1 % HCOOH in CH₃CN
 B) 0.1 % HCOOH in H₂O
 A/B = 10/90 – 1.5 min – 10/90 – 2.5 min – 90/10 , v/v
- Flow rate** : 0.4 mL/min
- Temperature** : 40 °C
- Detection** : LC/MS/MS (ESI, 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

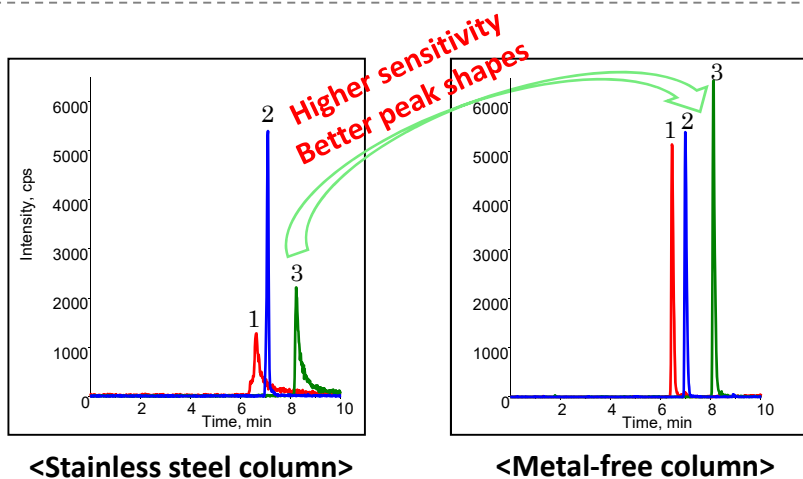
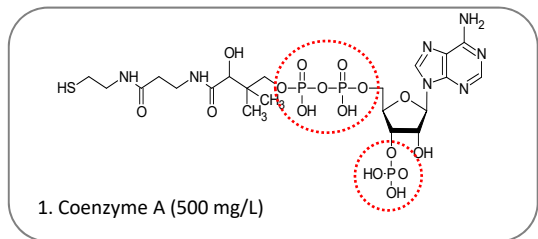


RP Example 2. Phosphate group-containing compounds

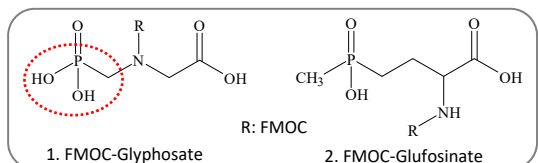
Phosphate group-containing compounds such as organophosphate pesticides and nucleotides easily form metal chelates. With a stainless steel column, analytes may not be able to elute from the column or distorted peaks may be obtained. In this case, a metal-free column can be an effective tool.



Conditions
 Column : InertSustainSwift C18 (1.9 μm, 50 × 2.1 mm I.D.)
 Mobile phase : A) 5 mM HCOONH₄ in H₂O
 B) 5 mM HCOONH₄ in CH₃CN
 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)

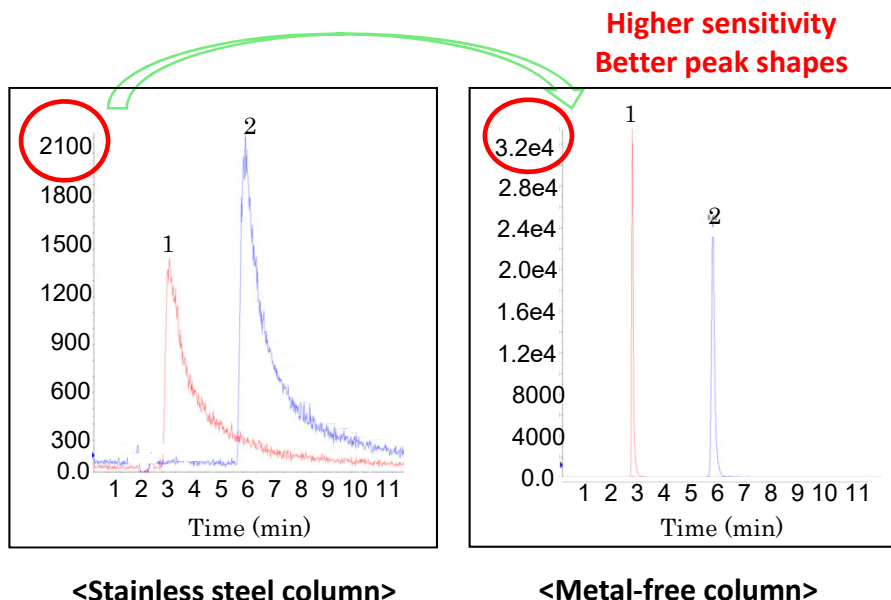


Conditions
 Column : InertSustain C18 (3 μm, 150 × 2.1 mm I.D.)
 Mobile phase : A) 5 mM HCOONH₄ in H₂O
 B) CH₃CN
 A/B=95/5-9.3 min-37/63
 Flow rate : 0.25 mL/min
 Temperature : 40 °C
 Detection : LC/MS/MS (ESI, Negative, MRM)
 Injection volume : 10 μL
 Analyte : 1. FMOC-Glyphosate (Q1/Q3 = 390/168)
 2. FMOC-Glufosinate (Q1/Q3 = 402/190)
 3. FMOC-AMPA (Q1/Q3 = 332/110) 2 μg/L each

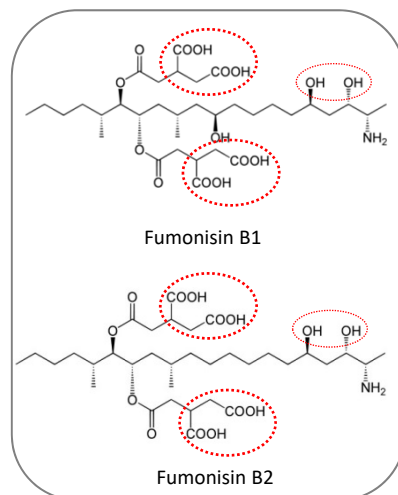


RP Example 3. Fumonisins

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.

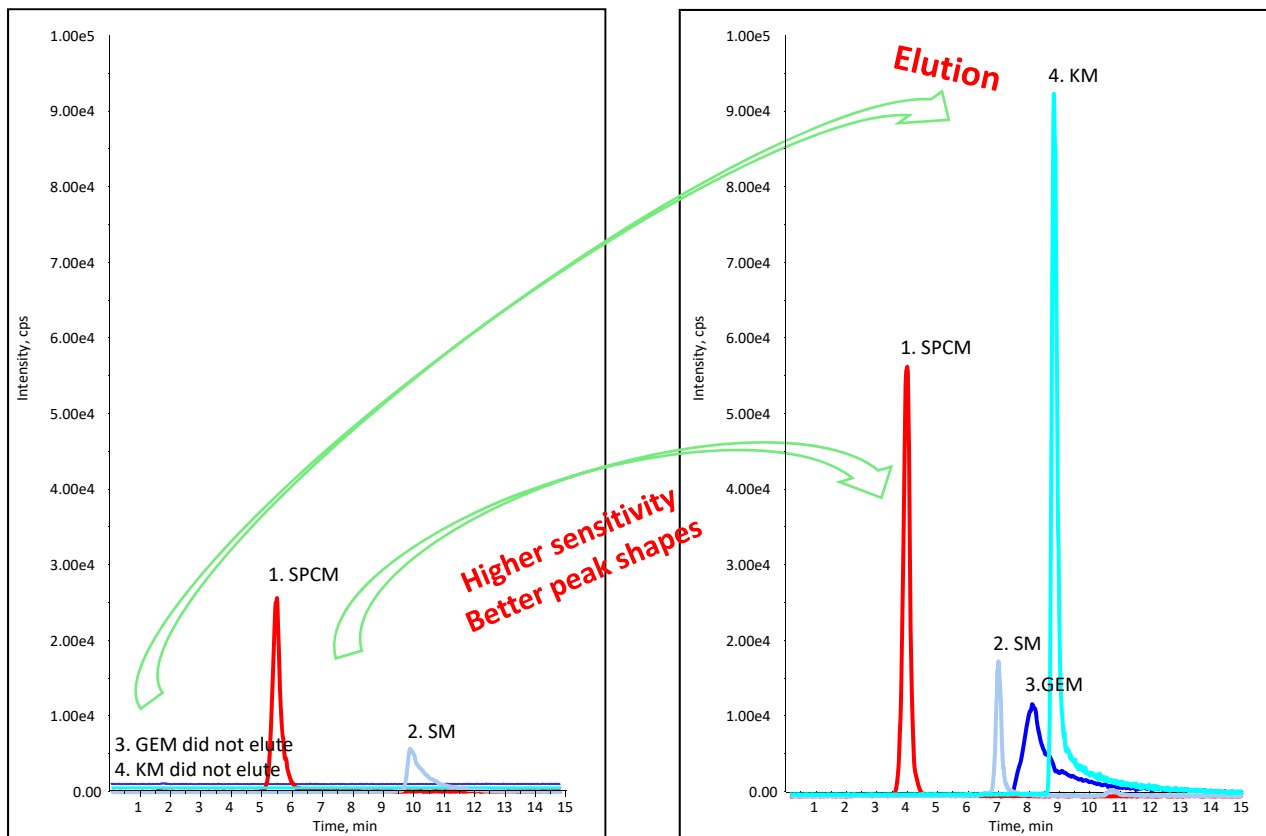


Conditions
 Column : InertSustain C18 (3 μm, 100 × 2.1 mm I.D.)
 Mobile phase : A) 0.1 % HCOOH, 10 mM HCOONH₄
 B) CH₃CN
 A/B = 60/40, v/v
 Flow rate : 0.2 mL/min
 Temperature : 40 °C
 Detection : LC/MS/MS (ESI, Positive, MRM)
 Analyte : 1. Fumonisin B1 (Q1/Q3 = 722/334)
 2. Fumonisin B2 (Q1/Q3 = 706/336)



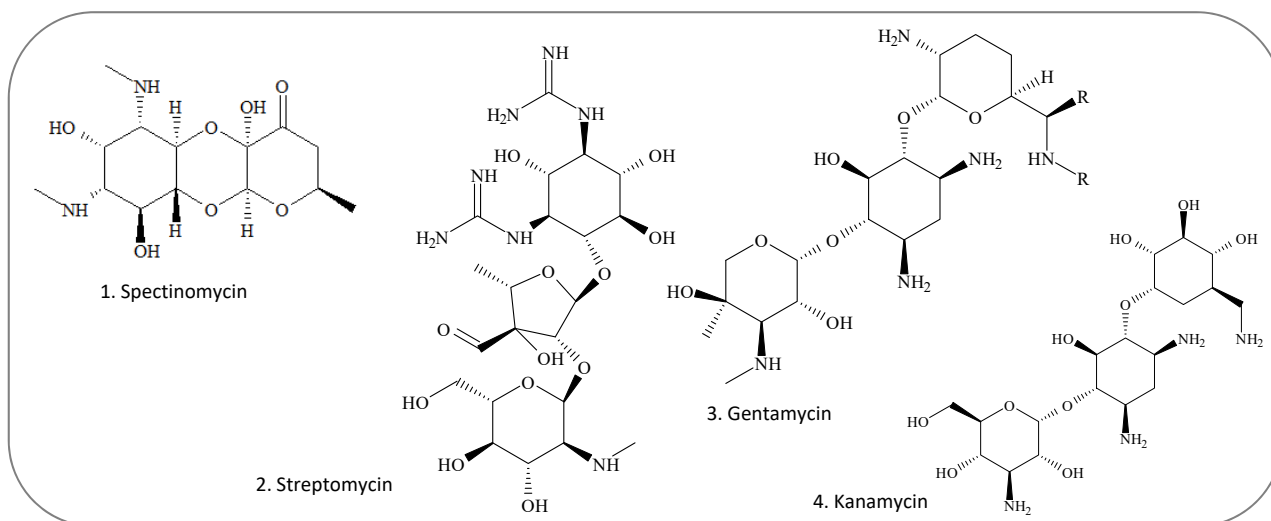
HILIC Example 1. Aminoglycosides

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 CH₃CN
B) 0.1% HCOOH in H₂O
A/B = 70/30 – 10 min – 50/50, v/v

Flow rate : 0.2 mL/min

Temperature : 40 °C

Injection volume : 10 mL

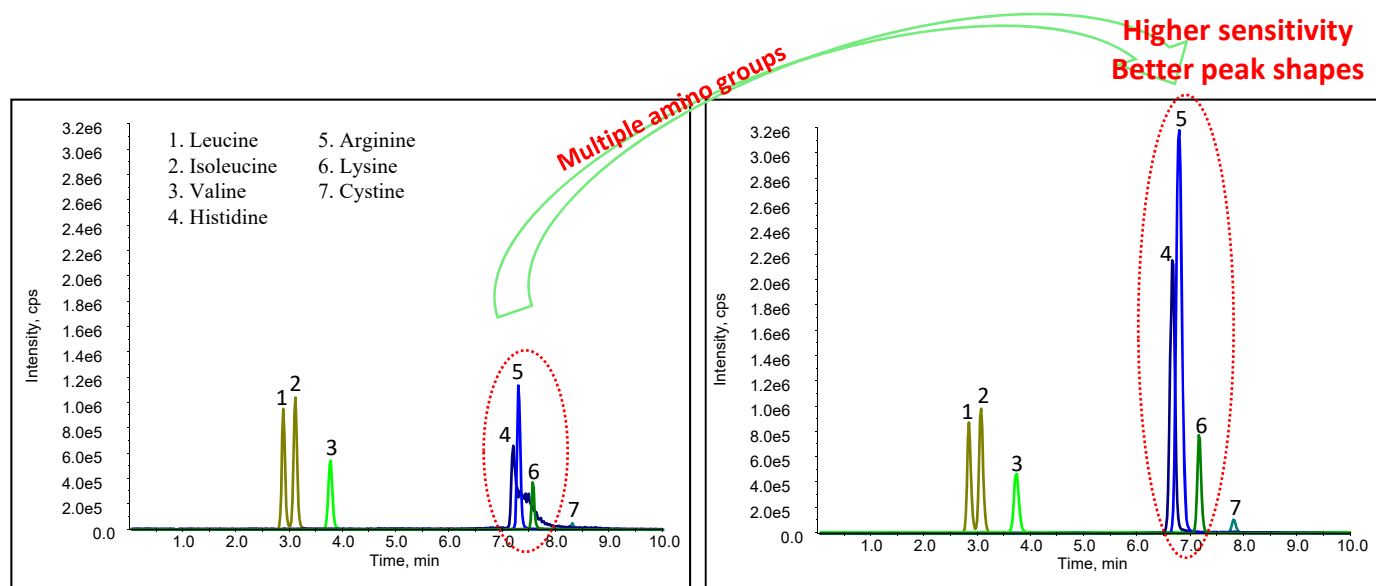
Detection : LC/MS/MS (ESI, Positive, MRM)

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

Concentration : 500 mg/L

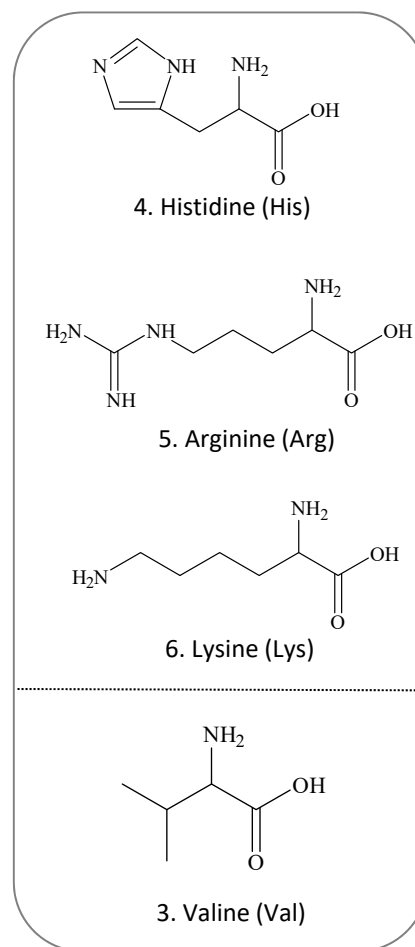
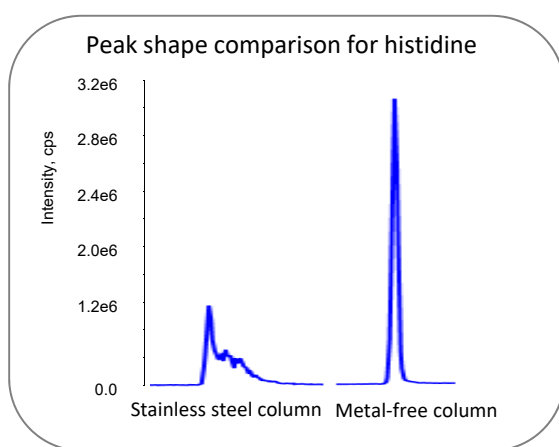
HILIC example 2. Amino acids

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 HCOONH₄, 0.1 % HCOOH in 75 % CH₃CN

B) 100 mM HCOONH₄, 0.1 % HCOOH in H₂O

A/B = 100/0 – 3 min – 100/0 – 5.5 min – 70/30 , v/v

Flow rate : 0.3 mL/min

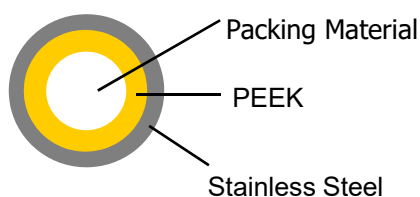
Temperature : 40 °C

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

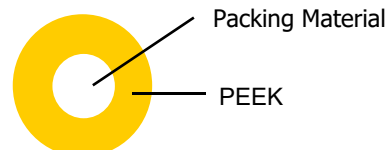
Injection Volume : 5.0 μL

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

Inertsil Series

- Inertsil ODS-4
- Inertsil C8-4
- Inertsil WP300 C4
- Inertsil NH2
- Inertsil ODS-3
- Inertsil C8-3
- Inertsil Peptides C18
- Inertsil WP300 Diol
- Inertsil ODS-SP
- Inertsil Ph-3
- Inertsil HILIC
- Inertsil SIL-100A
- Inertsil ODS-P
- Inertsil WP300 C18
- Inertsil Amide
- Inertsil WP300 SIL
- Inertsil ODS-EP
- Inertsil WP300 C8
- Inertsil Diol
- 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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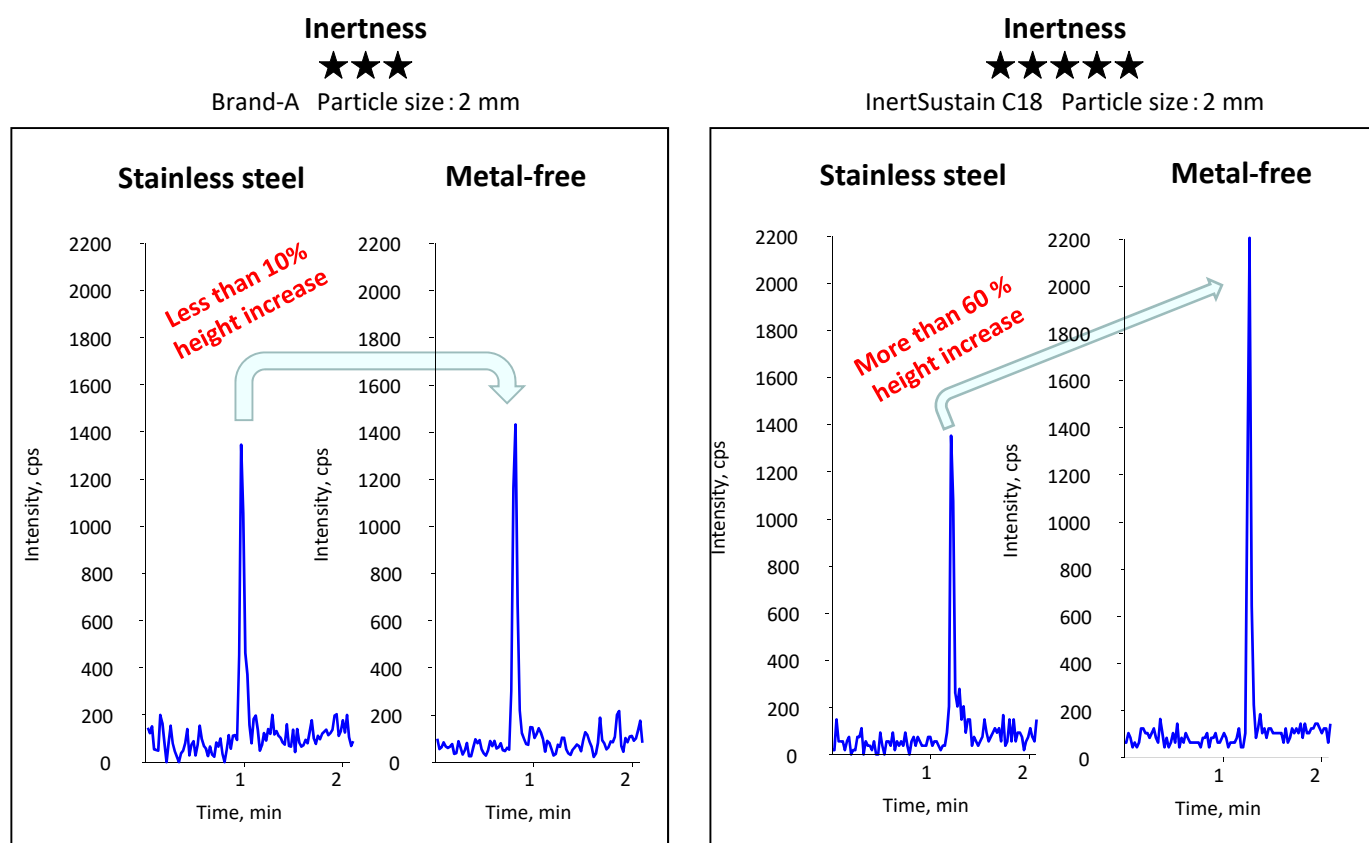
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The advantages of metal-free columns are described in LT152 "Metal-free columns for HPLC Part1". This technical note focuses on the packing inertness (e.g. residual metals) as a sequel to LT152.

(K.Kanno)

RP Example 1. Oxine copper

Oxine copper, a kind of pesticide, is a metal chelator and a better peak is expected to be obtained with a metal-free column. In the chromatograms below, two different packings are compared between stainless steel and metal-free columns. The highly inert packing increases the peak height more significantly, and the effects of metal-free columns are more pronounced.



Conditions

Column : ODS columns (2 μ m, 50 x 2.1 mm I.D.)

Mobile phase : A) 0.1 % HCOOH in CH₃CN

B) 0.1 % HCOOH in H₂O

A/B = 5/95 – 5 min – 100/0, v/v

Flow rate : 0.4 mL/min

Temperature : 40 °C

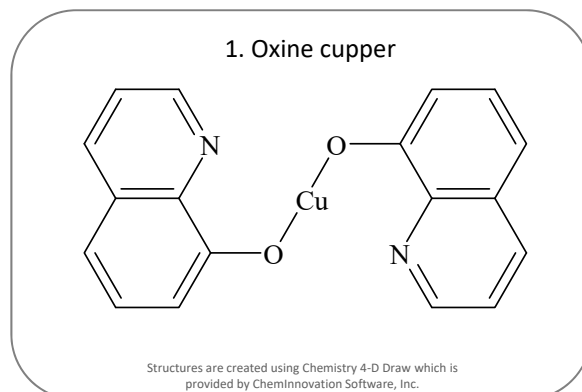
Detection : LC/MS/MS (ESI, Positive, MRM)

Injection volume : 5 μ L

Analyte : Q1 Q3

1. Oxine copper 146 128

Concentration : 2.0 mg/L



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

RP Example 2. Pesticides

Inertness of the packing and the column material is compared in analysis of 17 pesticides. The differences in the peak heights are smaller than the case of RP Example 1. Oxine copper, however, the more highly inert columns generally yield higher peak heights. Even for analysis of compounds not categorized as metal chelators, a more highly inert packing can highlight the effects of metal-free columns.

Inertness

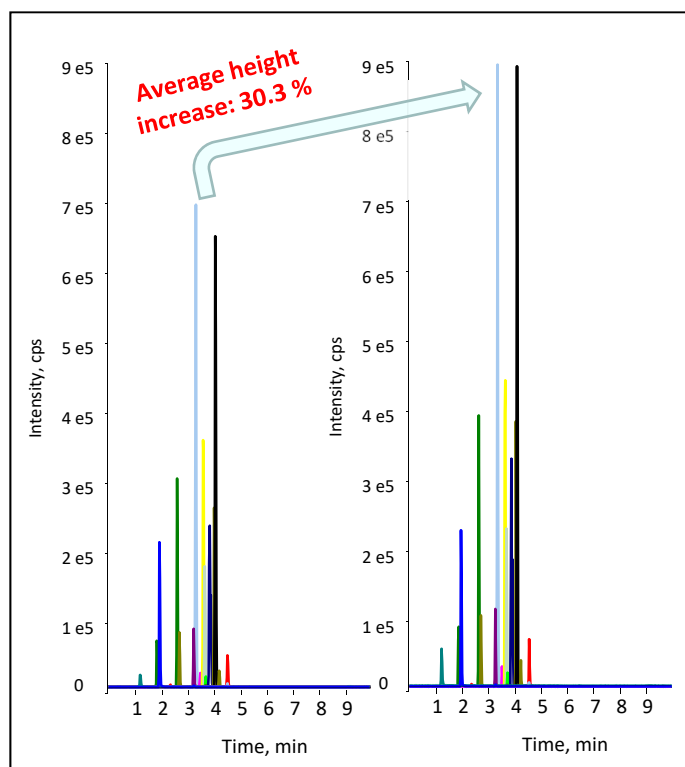
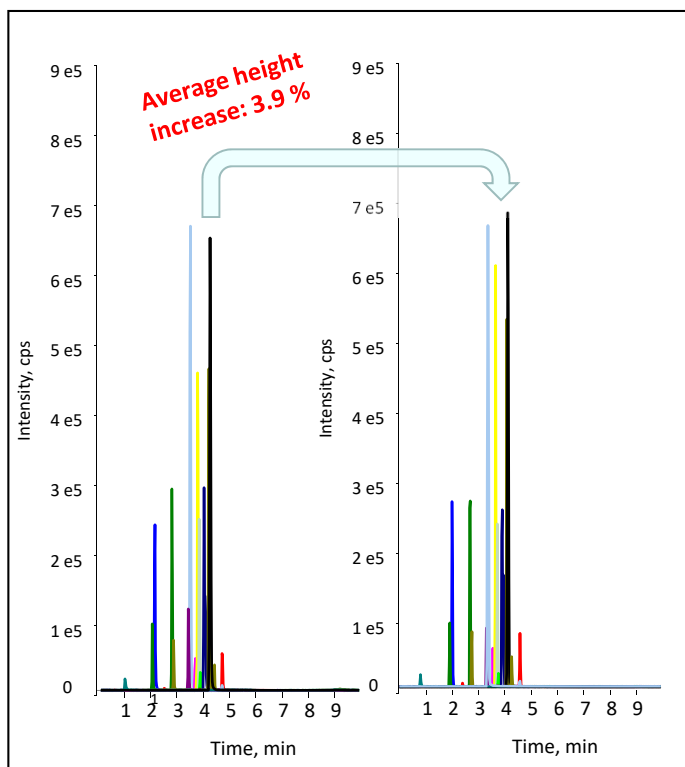


Brand-A Particle size : 2 mm

Inertness



InertSustain C18 Particle size : 2 mm



Conditions

Column : ODS columns (2 μm, 50 x 2.1 mm I.D.)

Mobile phase : A) 0.1 % HCOOH in CH₃CN

B) 0.1 % HCOOH in H₂O

A/B = 5/95 – 5 min – 100/0 – 3 min , v/v

Flow rate : 0.4 mL/min

Temperature : 40 °C

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

CUR	CAD	IS	TEM	GS1	GS2
10	7	5500	500	80	80

Injection volume : 5.0 mL

Concentration : 20 mg/L each

	Q1	Q3	DP	CE
1. Oxine Copper	146	128	80	34
2. Asulam	231	156	61	17
3. Methomyl	163	88	41	13
4. Tricyclazole	190	163	76	33
5. MPP oxon sulfone	295	217	60	20
6. Thiodicarb	355	88	56	33
7. Carbofuran	222	165	50	19
8. Thiuram	241	88	60	20
9. MPP oxon	263	231	60	20
10. Bensulfuron methyl	411	149	71	29
11. Flazasulfuron	408	182	60	20
12. Siduron	233	137	81	23
13. Azoxystrobin	404	372	65	19
14. Dymron	269	151	64	20
15. Iprodione	330	245	61	21
16. Bensulide	398	314	71	17
17. Carpropamid	334	139	66	27

As shown in LT152, tetracyclines easily adsorb onto residual metals on the packing surface or metal parts of the column by chelation, and this adsorption has a bad influence on the analysis. Like RP Example 1. Oxine copper, the less residual metals are on the packing, the more significantly the peak heights increase. In the analysis of metal chelators, not only the column material but also the inertness of the packing are important.

1. Oxytetracycline
2. Tetracycline
3. Chlortetracycline

Inertness

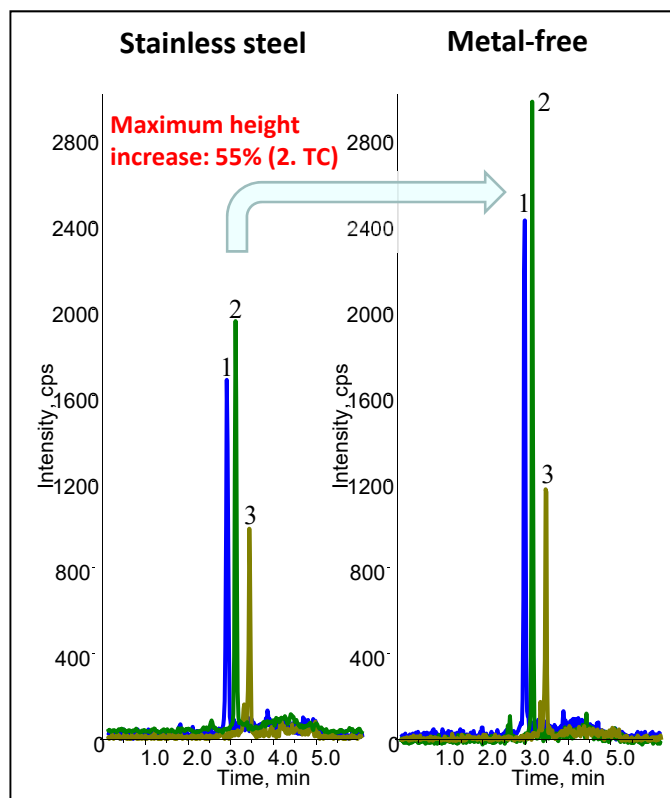
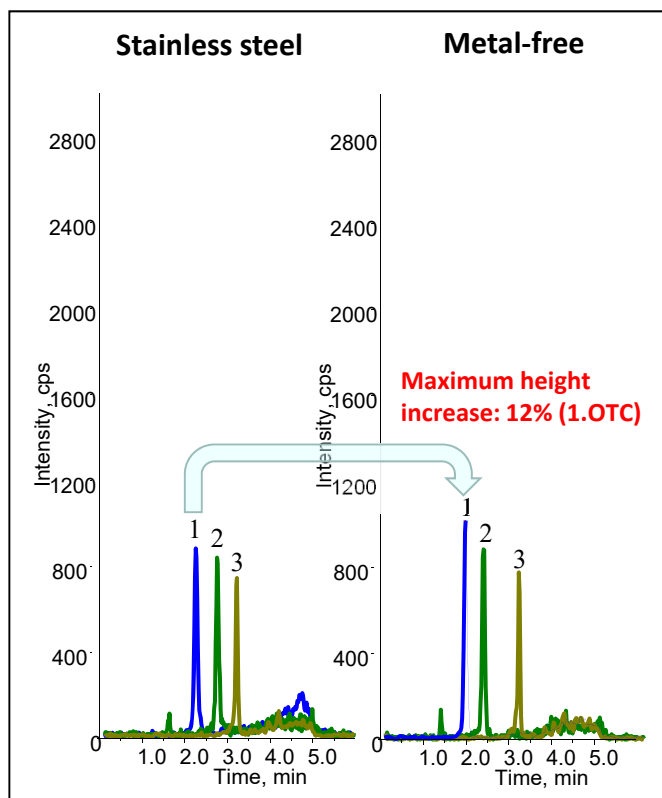


Brand-A Particle size: 2 mm

Inertness



InertSustain C18 Particle size: 2 mm



Conditions

Column : ODS columns (2 μm, 50 x 2.1 mm I.D.)

Mobile phase : A) 0.1 % HCOOH in CH₃CN

B) 0.1 % HCOOH in H₂O

A/B = 10/90 – 1 min – 10/90 – 3 min – 90/10 , v/v

Flow rate : 0.4 mL/min

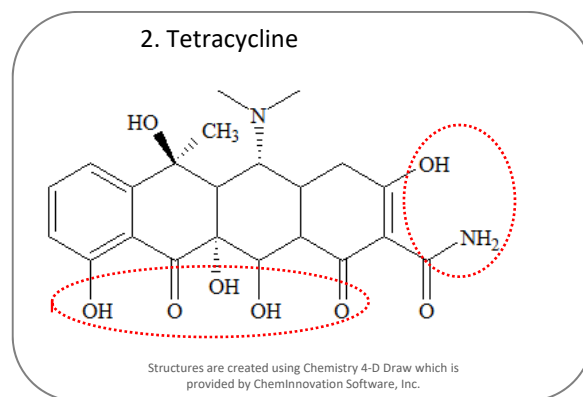
Temperature : 40 °C

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

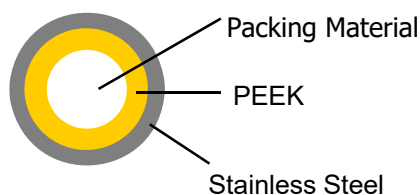
CUR	CAD	IS	TEM	GS1	GS2
10	7	5500	700	80	80

Injection volume : 10 mL

Analyte		Q1	Q3
1. Oxytetracycline(OTC)	460	426	
2. Tetracycline(TC)		445	410
3. Chlortetracycline(CTC)	479	444	



*Conditions are partly different from those of tetracycline analysis in LT152.



UHPLC-PEEK Columns



PEEK Columns

Analytical Columns List

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

Inertsil Series

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