

Comparison of Inertsil CN-3 and ODS-3 in separation of VMA and HVA in human urine (Inertsil CN-3)

Conditions

Column: Inertsil CN-3 4.6 mm I.D. x 150 mm length

Column temperature: 30°C

Injected sample volume: 10µl

Mobile phase: Acetonitrile / 50 mM KH_2PO_4 (pH3.0) = 1 / 99

Flow rate: 1 ml / min

Detection : ECD 700 mV vs. Ag / AgCl

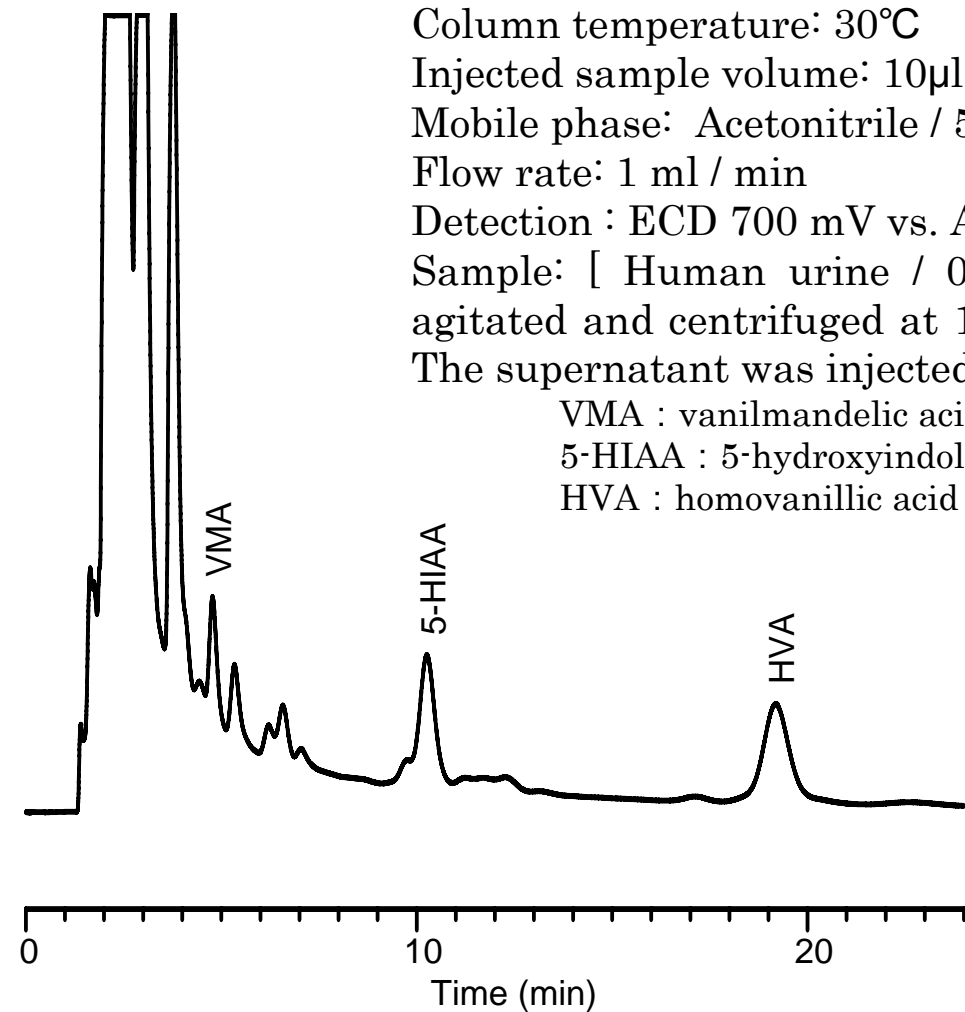
Sample: [Human urine / 0.1 M HClO_4 = 1 / 1] mixture was agitated and centrifuged at 1200 rpm for 5 min prior to injection.

The supernatant was injected.

VMA : vanilmandelic acid

5-HIAA : 5-hydroxyindoleacetic acid

HVA : homovanillic acid



These catecholamine metabolites are analyzed for the diagnosis of tumors such as neuroblastoma. Concentrations of these compounds reach a high level in patient urine. It is important to separate these target compounds from the matrix compounds in urine.

With ODS columns, it is difficult to separate the target compounds from urine background compounds by isocratic elution. With the Inertsil CN-3 column, however, the target compounds can be separated from the background compounds by isocratic elution due to the specific selectivity and the running time is shorter than the separation with the ODS column (see next page). Inertsil CN-3 is suitable for mass screening assaying of VMA, 5-HIAA and HVA in human urine.

In this application, an electrochemical detector is employed.

Comparison of Inertsil CN-3 and ODS-3 in Separation of VMA and HVA in human urine (Inertsil ODS-3)

- Column : Inertsil ODS-3 (4.6mmI.D. X 150mm)
- Sample : [Human urine / 0.1 M HClO₄ = 1 / 1] mixture was agitated and centrifuged at 1200 rpm for 5 min prior to injection. The supernatant was injected.

Conditions

Column: Inertsil ODS-3, 4.6 mm I.D. x 150 mm length

Column temperature: 30°C

Injected sample volume: 10µl

Mobile phase:

Acetonitrile / 50 mM KH₂PO₄ (pH3.0) = 8 / 92

Flow rate: 1 ml / min

Detection : ECD 700 mV vs. Ag / AgCl

VMA : vanilmandelic acid

5-HIAA : 5-hydroxyindoleacetic acid

HVA : homovanillic acid

Conditions

Column: Inertsil ODS-3 4.6 mm I.D. x 150 mm length

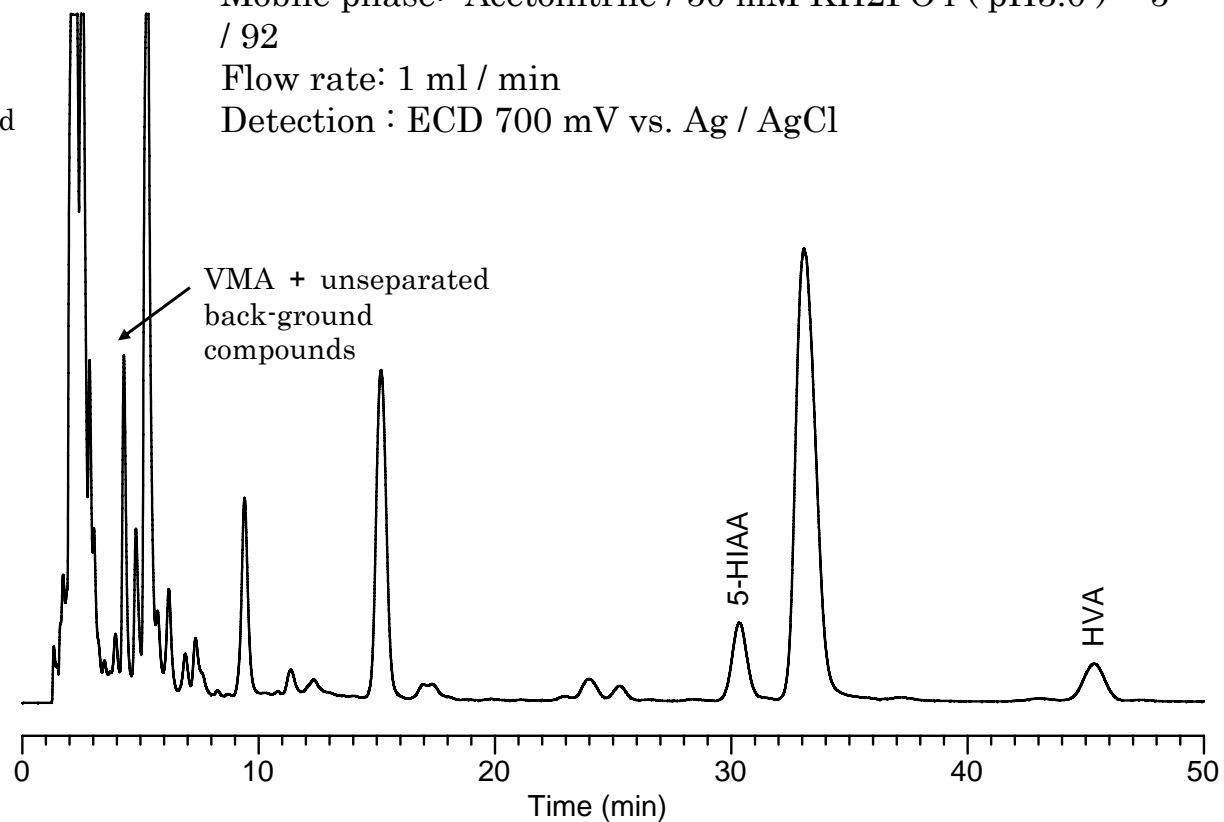
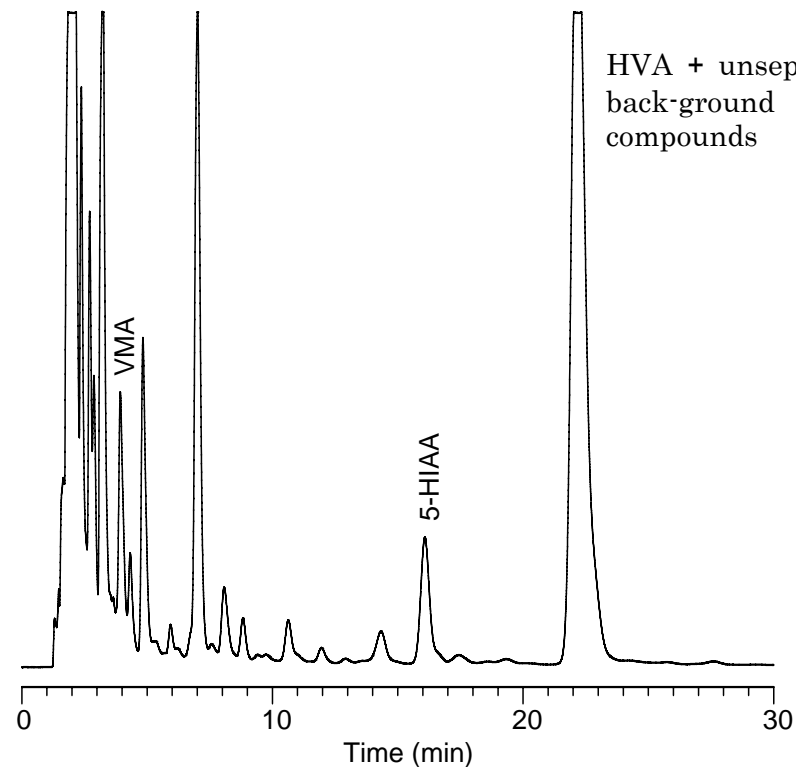
Column temperature: 30°C

Injected sample volume: 10µl

Mobile phase: Acetonitrile / 50 mM KH₂PO₄ (pH3.0) = 5 / 92

Flow rate: 1 ml / min

Detection : ECD 700 mV vs. Ag / AgCl



Comparison of CN columns in separating steroids

Conditions

Column dimensions:

4.6 mm I.D. x 150 mm length

Mobile phase:

n-Hexane : Ethanol = 90 : 10

Flow rate: 1 ml / min

Column temperature: 40°C

Detection: UV 254 nm

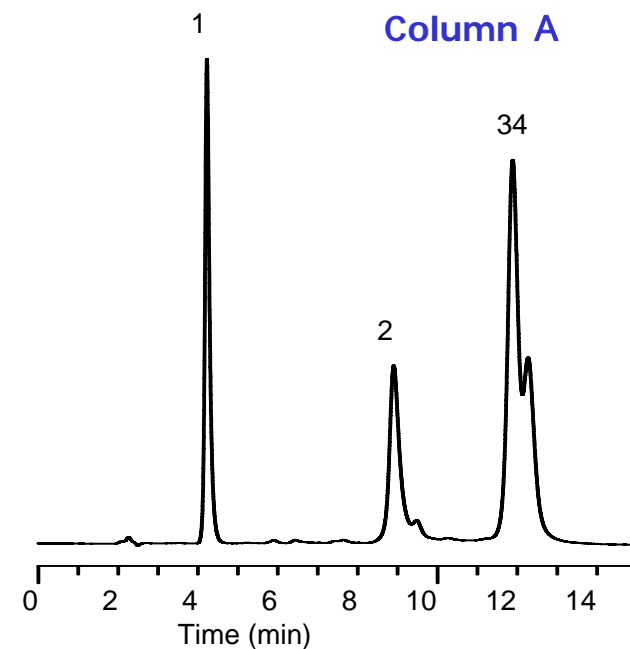
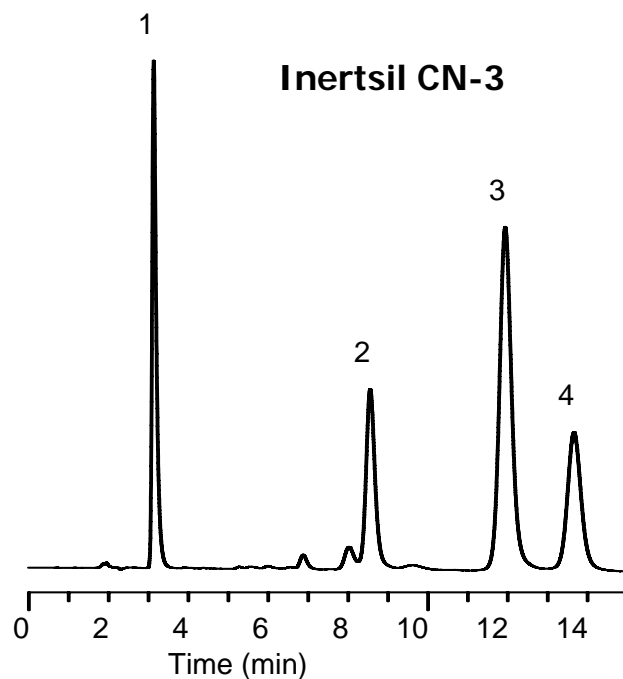
Peak identification

1 Progesterone

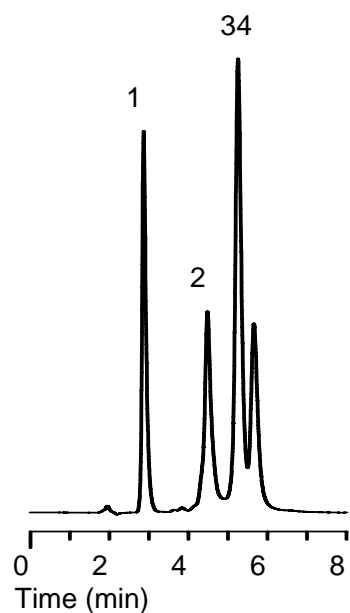
2 Corticosterone

3 Prednisone

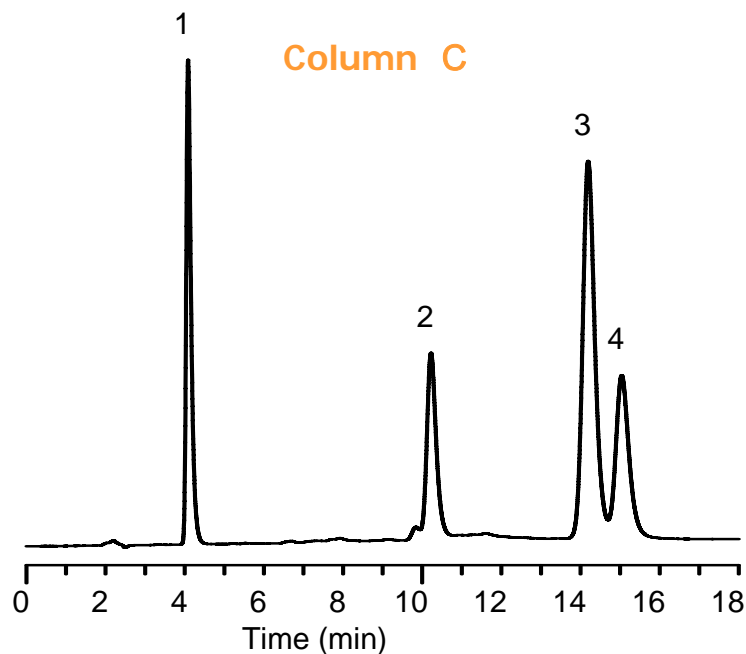
4 Prednisolone



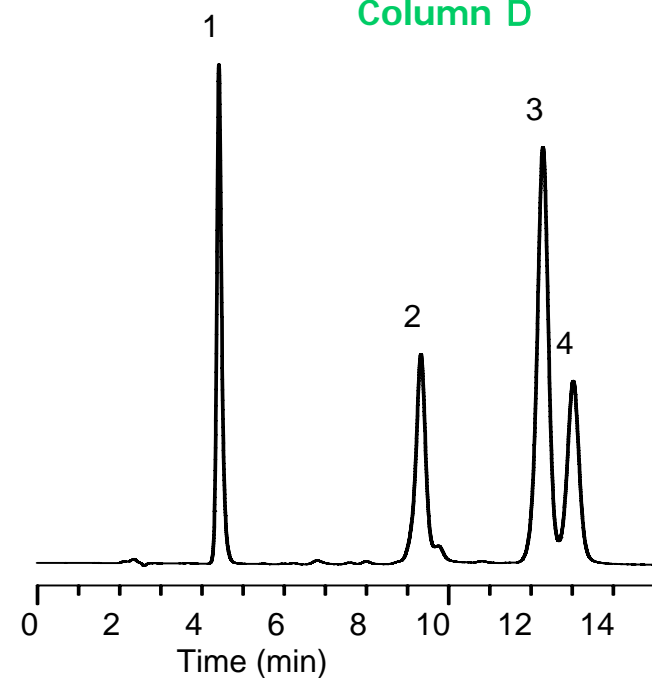
Column B



Column C



Column D



Separation of estrogens using the Inertsil CN-3 column in both normal and reversed-phase partition modes

Column : **Inertsil CN-3** (5 μ m,250 \times 4.6mmI.D.)

Flow rate : 1.0mL/min

Detection : UV 220nm

Samples : Estrone , β -Estradiol, Ethynylestradiol , Diethylstilbestrol , Estriol

in Ethanol

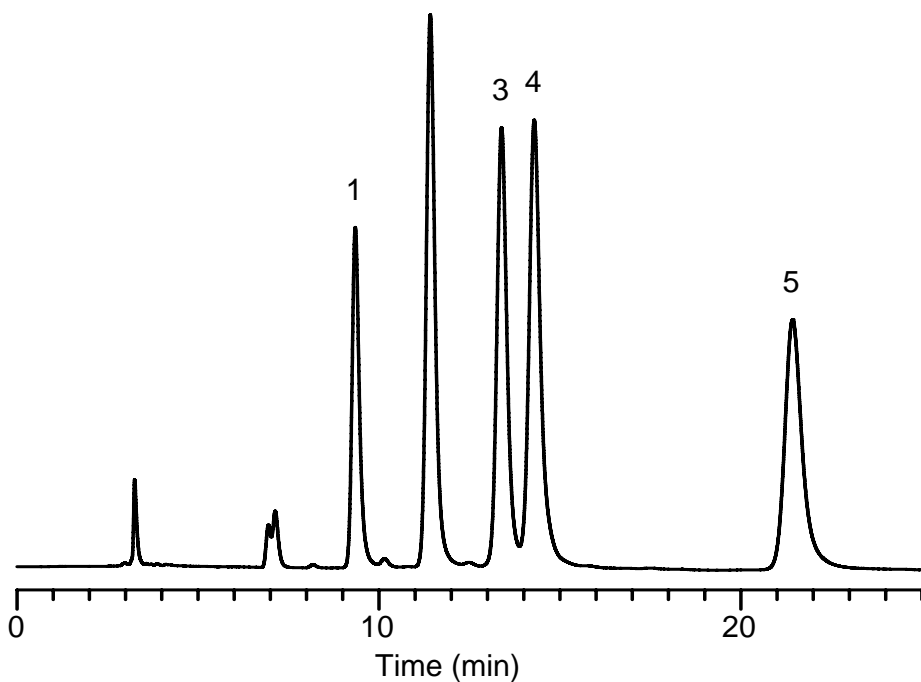
Sample

volume : 1 μ L

Normal phase partition mode

Mobile phase : Hexane / Ethanol = 90 / 10

Column temp.: 40 $^{\circ}$ C

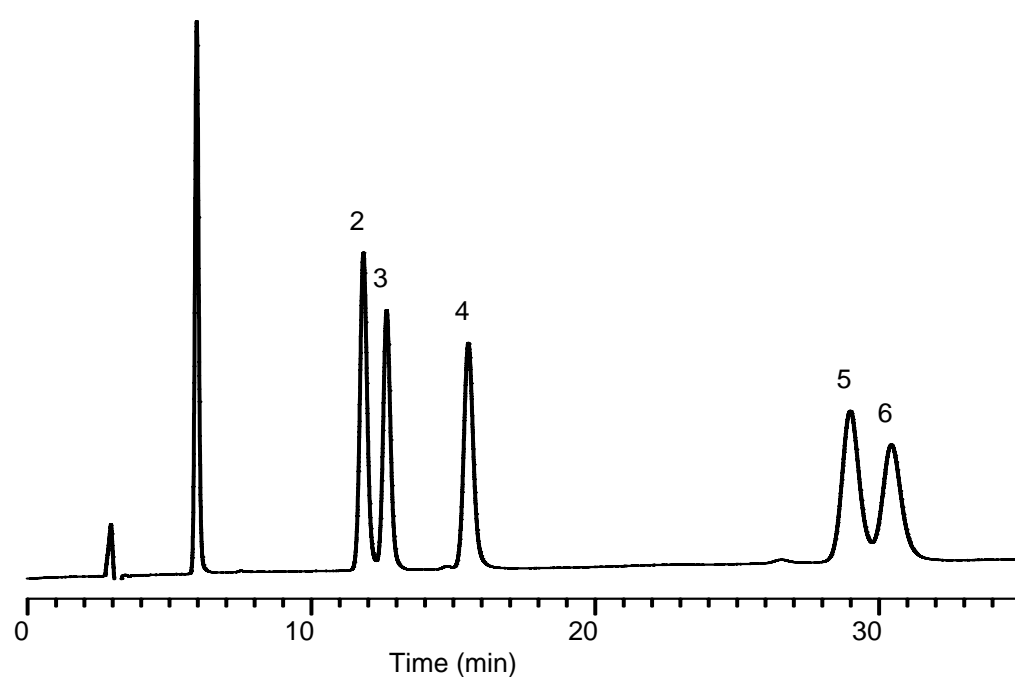


No.	Peak Name	R.Time	Area	Efficiency
1	Estrone	9.343	528435	10716.7
2	Estradiol	11.417	1.01414e+06	11140.7
3	Ethynylestradiol	13.387	930519	11463.8
4	Diethylstilbestrol	14.290	1.09679e+06	10597.4
5	Estriol	21.427	903730	10446.8

Reversed-phase partition mode

Mobile phase : Acetonitrile / Water = 35 / 65

Temp₁ : 25 $^{\circ}$ C



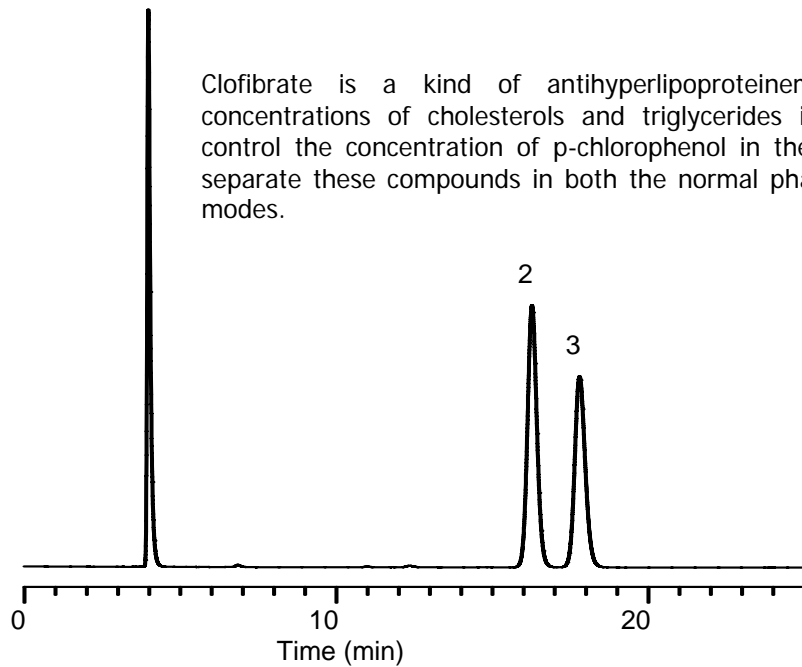
No.	Peak Name	R.Time	Area	Efficiency
1	Estriol	5.957	799655	9485.92
2	Estradiol	11.827	876105	10322.6
3	Estrone	12.647	736611	11417.6
4	Ethynylestradiol	15.527	807169	10597.2
5	Hexestrol	28.983	979656	10641.8
6	Diethylstilbestrol	30.433	829107	10072.5

Separation of clofibrate using the Inertsil CN-3 column in both normal and reversed-phase partition modes

Column : Inertsil CN-3 (5um,250×4.6mmI.D.) Flow rate : 1.0mL/min Detection : UV 275nm
 Samples : Clofibrate 、 p-Chlorophenol 、 p-Ethoxyphenol in Ethanol Sample volume : 1μL

Normal phase mode

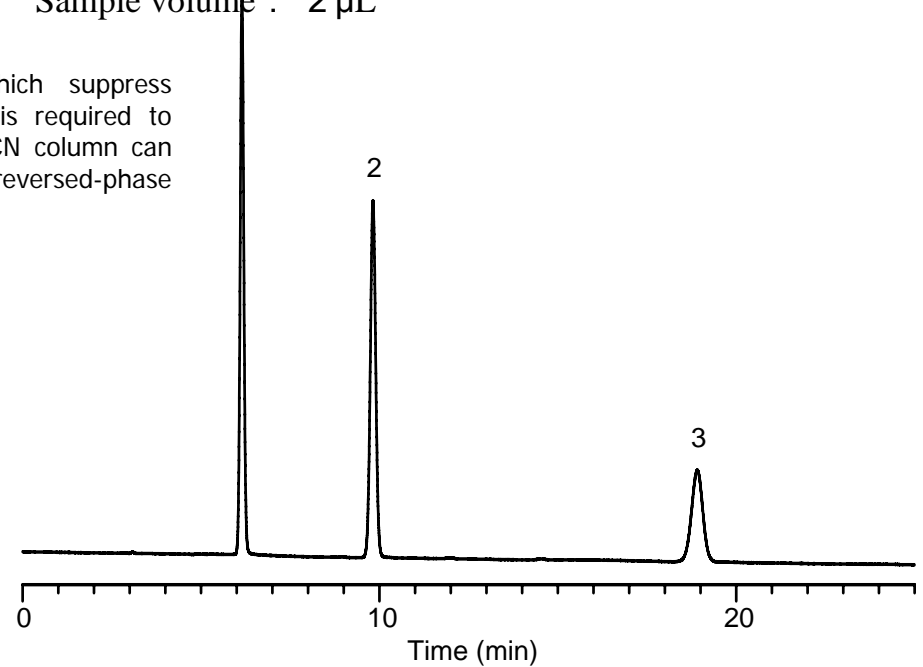
Mobile phase : hexane / THF / acetic acid = 1800 / 200 / 1
 Column temp. : 25°C
 Sample volume : 1μL



No.	Peak Name	R.Time	Area	Efficiency
1	Clofibrate	3.970	1.38749e+06	7719.79
2	p-Chlorophenol	16.263	1.93655e+06	13881.3
3	p-Ethoxyphenol	17.790	1.5836e+06	13270.6

Reversed-phase partition mode

Mobile phase : acetonitrile / 20mM potassium phosphate buffer (pH3.0) = 30 / 70
 Column temp.1: 40°C
 Sample volume : 2 μL

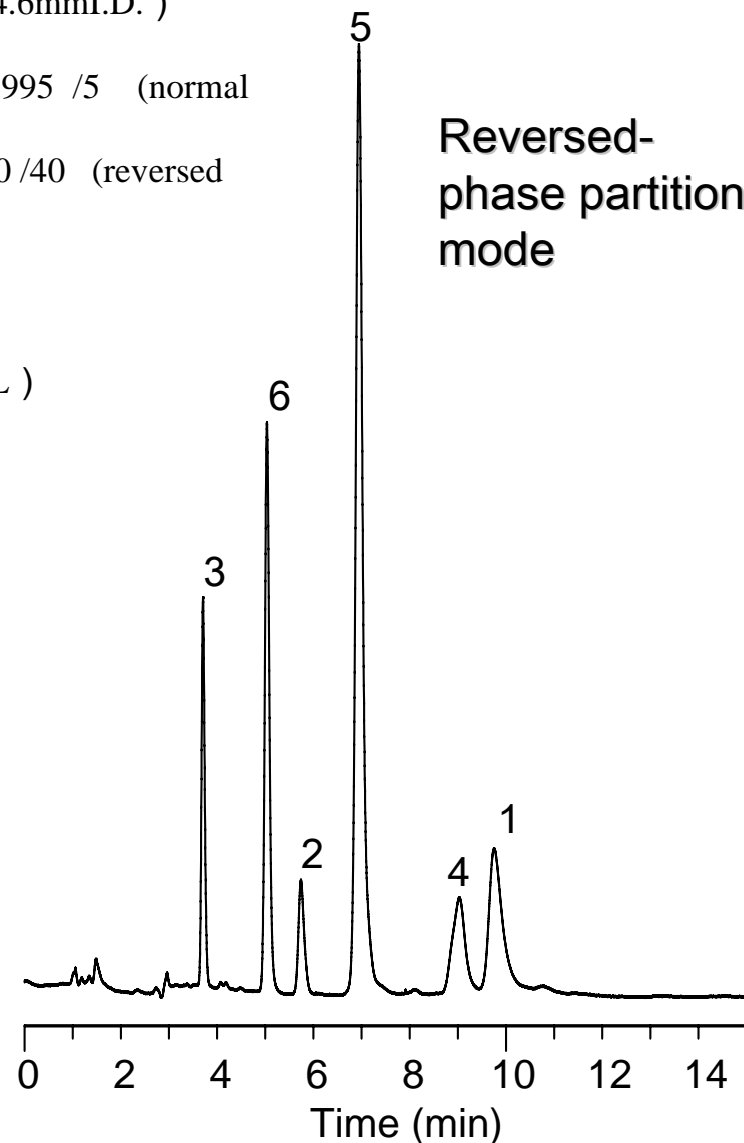
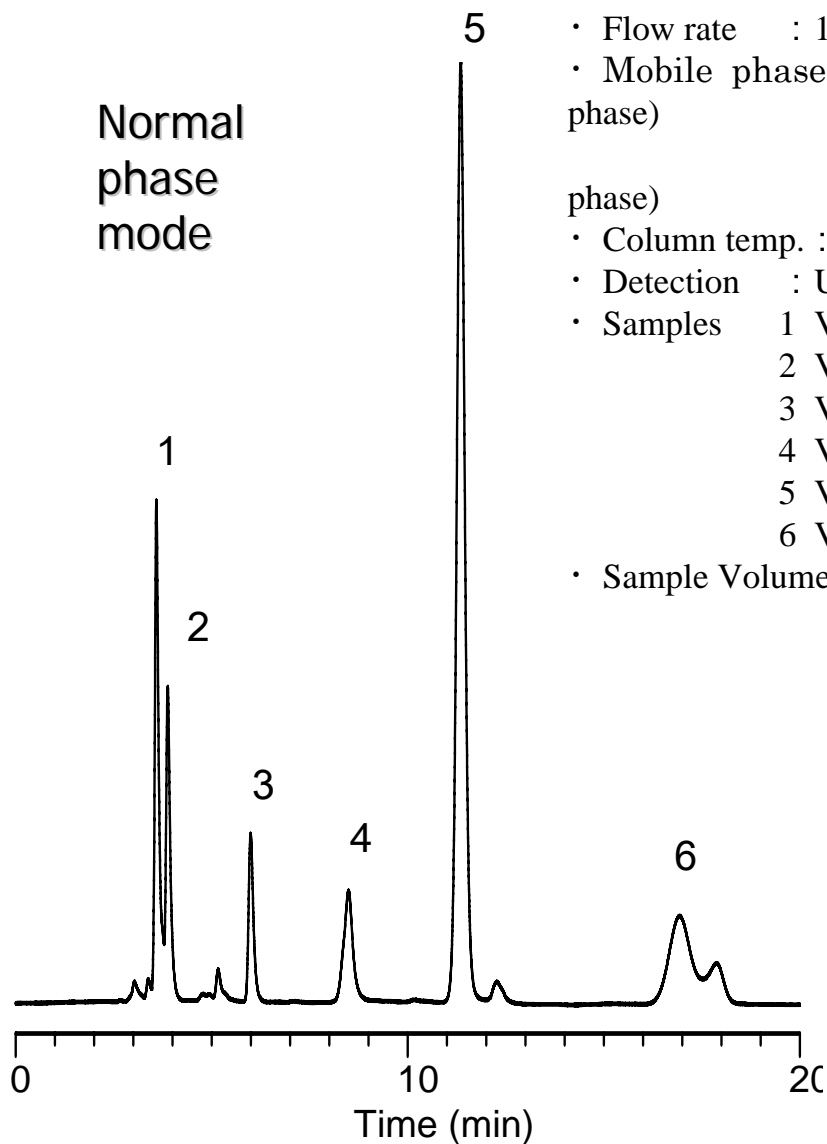


No.	Peak Name	R.Time	Area	Efficiency
1	p-Ethoxyphenol	6.147	569126	16544.8
2	p-Chlorophenol	9.820	539612	19473.4
3	Clofibrate	18.910	285969	16839.7

Separation of fat-soluble vitamins using the Inertsil CN-3 column in both normal and reversed-phase partition modes

Conditions

- Column : **Inertsil CN-3** (5 μ m,250 \times 4.6mmI.D.)
- Flow rate : 1.0mL/min
- Mobile phase : Hexane / Ethanol = 995 /5 (normal phase)
: Acetonitrile / Water = 60 /40 (reversed phase)
- Column temp. : 40°C
- Detection : UV 280nm
- Samples 1 Vitamin K1 (0.14mg/mL)
2 Vitamin A-Ac (0.14mg/mL)
3 Vitamin K3 (0.14mg/mL)
4 Vitamin E (0.14mg/mL)
5 Vitamin D3 (0.14mg/mL)
6 Vitamin A (0.14mg/mL)
- Sample Volume : 1 μ L

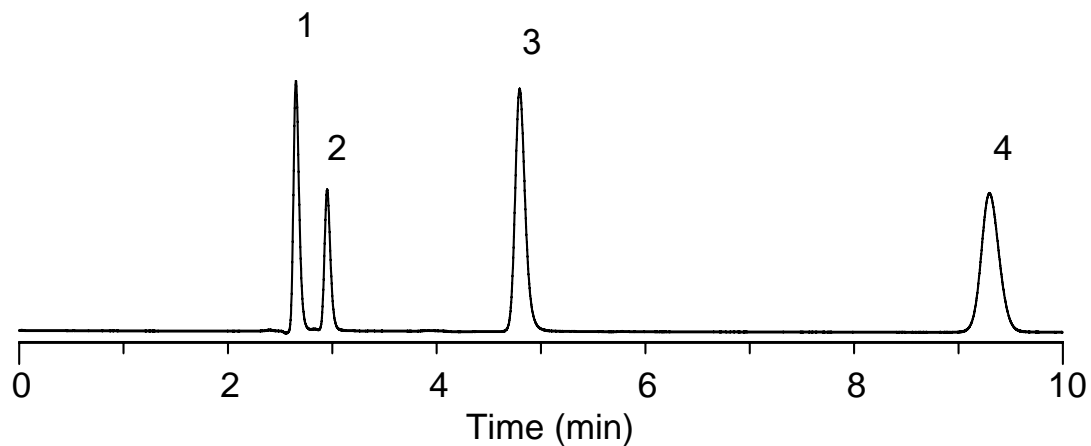


Separation of asparagine, aspartic acid and organic acids on the Inertsil CN-3 column

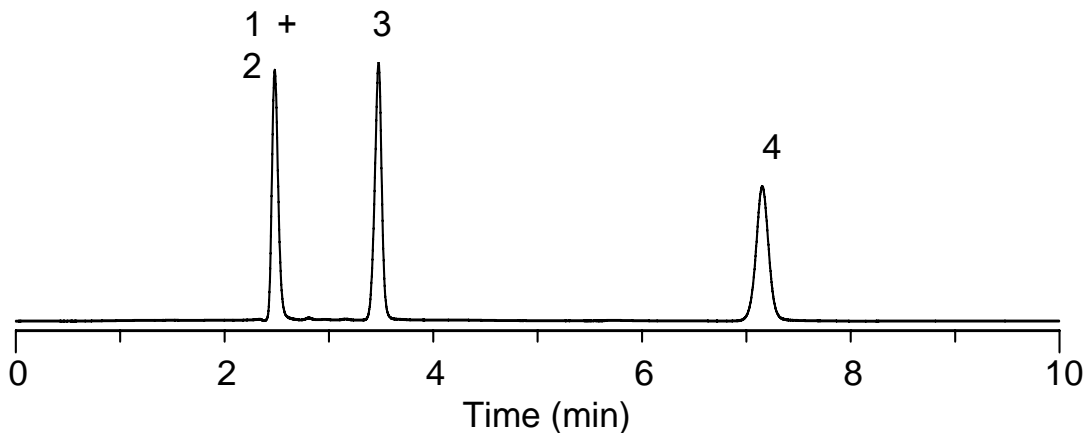
Asparagine and aspartic acid are contained in vegetables and beans. They are synthesized from their precursors fumaric acid and maleic acid, respectively.

Asparagine and aspartic acid cannot be separated in ODS columns without an ion-pair method. Using the Inertsil CN-3 column, they can be separated without the ion-pair method.

Inertsil CN-3



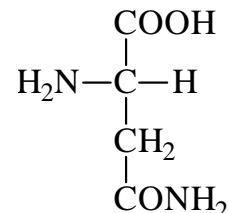
Inertsil ODS-3



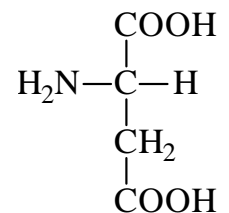
CONDITIONS

- Column : Inertsil CN-3 (5um,250×4.6mmI.D.)
Inertsil ODS-3 (5um,250×4.6mmI.D.)
- Flow rate : 1.0mL/min
- Eluent : 20mM Potassium phosphate buffer (pH4.0)
- Column temp. : 40°C
- Detection : UV 210 nm
- Samples : Asparagine:1H₂O (0.75mg/mL)
: Aspartic acid (0.75mg/mL)
: Fumaric acid (0.01mg/mL)
: Maleic acid (0.01mg/mL)
- Sample volume : 5uL

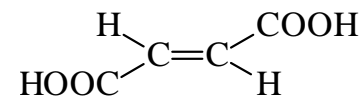
1 asparagine



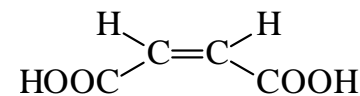
2 aspartic acid



3 fumaric acid



4 maleic acid



Separation of environmental endocrine disrupting compounds using the Inertsil CN-3 column in both normal and reversed-phase partition modes

Column : Inertsil CN-3 (5 μ m, 250 \times 4.6mm I.D.)

Flow rate : 1.0mL/min

Detection : UV 280nm

Column temp. : 40°C

Samples : Diethyl phthalate 、 4-Nonylphenol、 2,4-Dichlorophenol 、 Phenol 、 Bisphenol-A in Acetonitrile

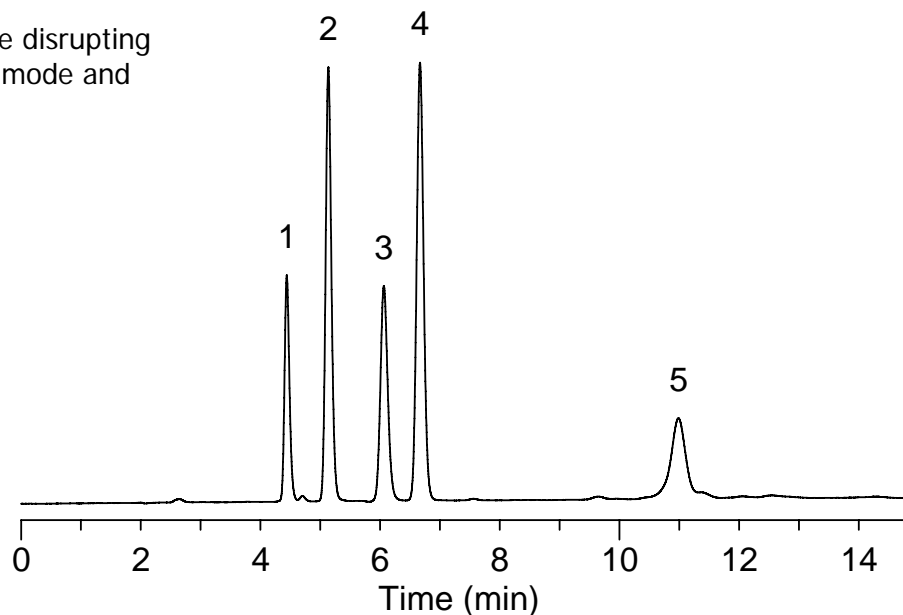
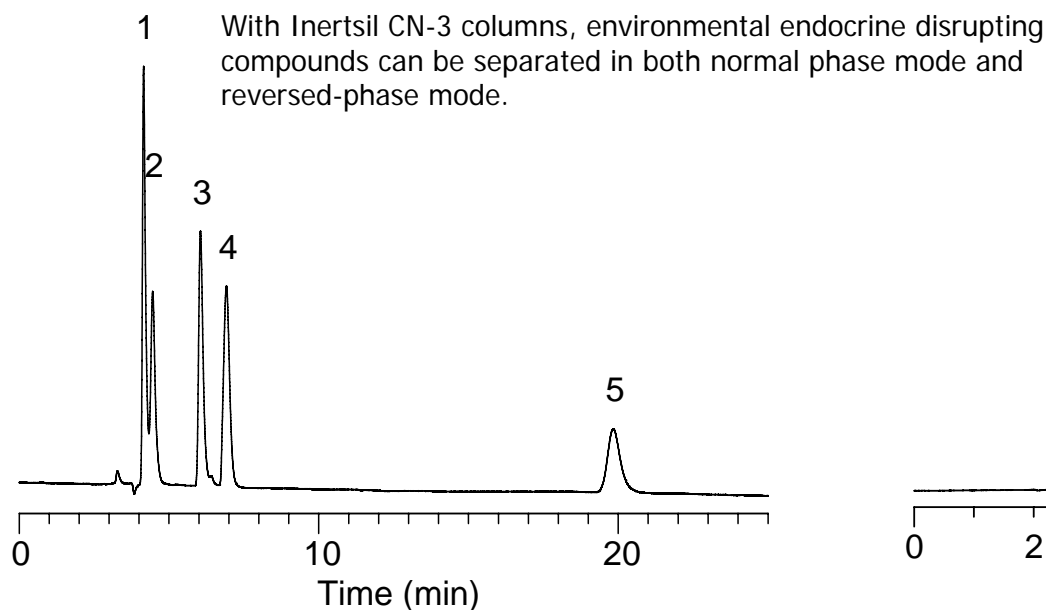
Sample volume : 1 μ L

Normal phase mode

Mobile phase : Hexane / Ethanol = 90 / 10

Reverse Phase Mode

Mobile phase : Acetonitrile / 20mM phosphate buffer(pH3.0) = 45 / 55



No.	Peak Name	R.Time
1	Diethyl phthalate	4.153
2	4-Nonylphenol	4.453
3	2,4-Dichlorophenol	6.053
4	Phenol	6.917
5	Bisphenol-A	19.840

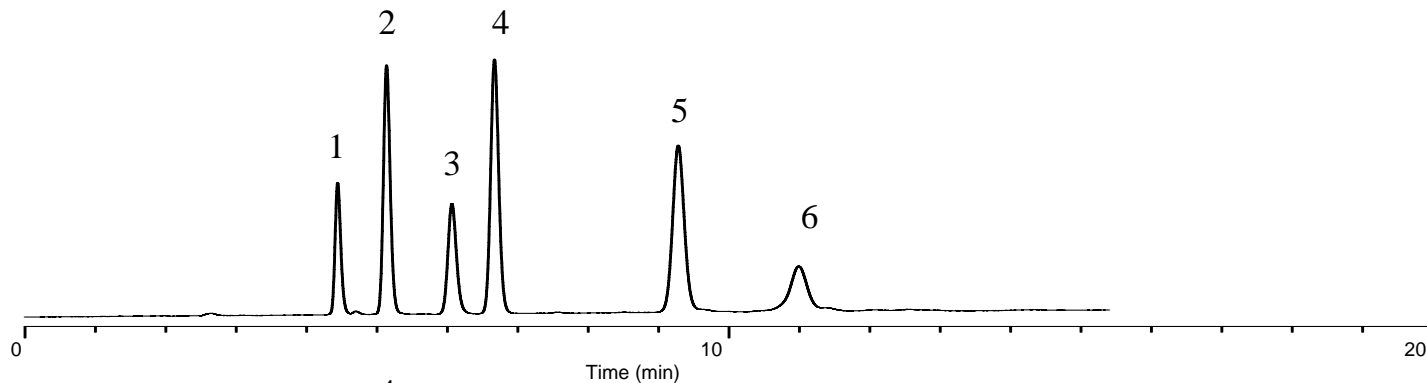
No.	Peak Name	R.Time
1	Phenol	4.440
2	Diethyl phthalate	5.133
3	Bisphenol-A	6.060
4	2,4-Dichlorophenol	6.667
5	4-Nonylphenol	10.987

Comparison of selectivity for environmental endocrine disrupting compounds between ODS and CN-3 columns

Conditions

- Column : **Inertsil CN-3** (5 μ m, 250 \times 4.6mm I.D.)
Inertsil ODS-3 (5 μ m, 250 \times 4.6mm I.D.)
 - Flow rate : 1.0mL/min
 - Mobile phase : **Inertsil CN-3**
Acetonitrile / 20mM Phosphate buffer(pH3.0) = 45 /55
Inertsil ODS-3
Acetonitrile / 20mM Phosphate buffer(pH3.0) = 70 /30
 - Oven Temp. : 40°C
 - Detection : UV 280nm
 - Sample Volume : 3 μ L
- Samples
 - 1 Phenol (0.06 mg/mL)
 - 2 Diethyl phthalate (0.12mg/mL)
 - 3 Bisphenol-A (0.02 mg/mL)
 - 4 2,4-Dichlorophenol (0.06 mg/mL)
 - 5 4-Octylphenol (0.12 mg/mL)
 - 6 4-Nonylphenol (0.12 mg/mL)

Inertsil CN-3



Inertsil ODS-3

