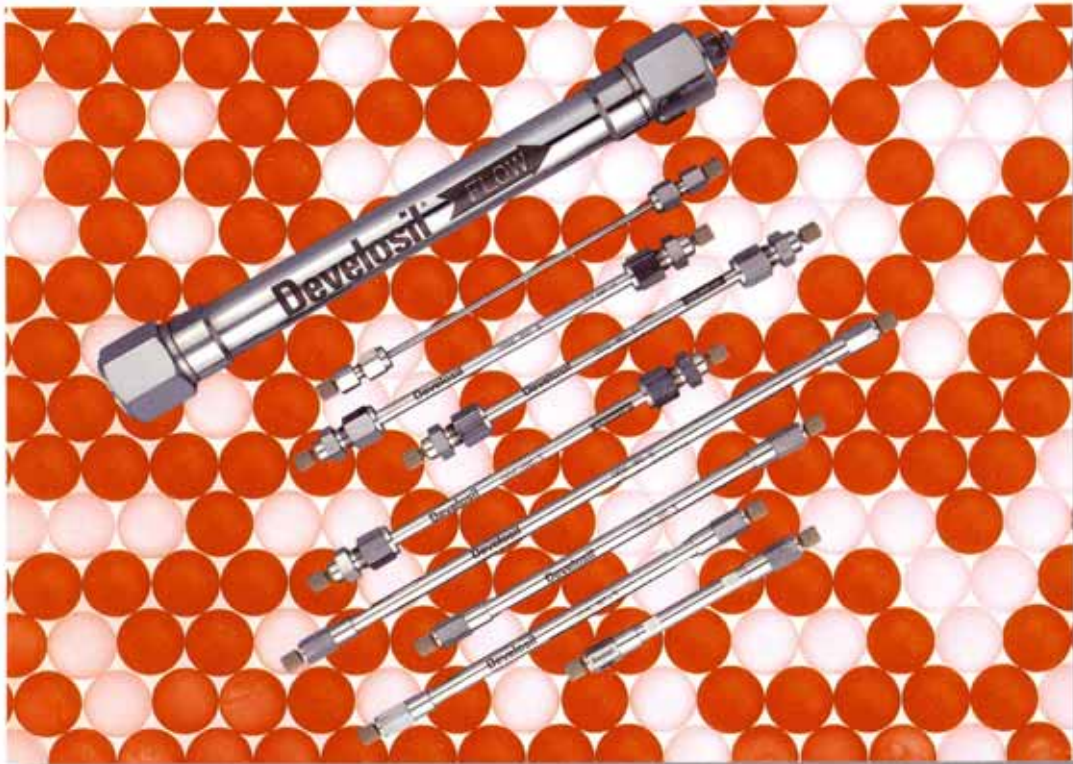


Columns for HPLC

Develosil™



Nomura Chemical Co., Ltd.

北京慧德易科技有限责任公司
<http://www.herbs-extract.com>

Nomura Chemical

Nomura Chemical Co. started and has continued till present as a maker of Develosil HPLC column since 1979. We manufacture from silica gel to a final column, and also provide Develosil silica gel or Develosil ODS phases to the other HPLC makers. We are one of leading companies for HPLC column in the world. Especially our patented C30 phase has a unique characteristics and has been used by many pharmaceuticals. Develosil columns are available in the world through our distributors in North America, Europe and etc.

Develosil HPLC column

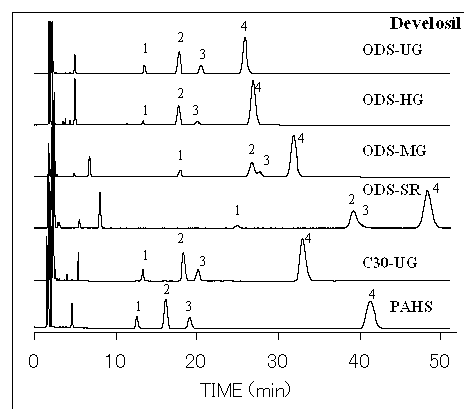
Columns in 3 kinds of mode such as Reversed, Gel filtration and Normal phase are commercially available. We have 5 kinds of C30 phase, 6 kinds of C18 (ODS) phase and 2 kinds of C8 phase. 300ODS-HG, 300C8-HG and 300C4-HG phases have pores with 25 nm diameter and are for separation of proteins. We have 4 kinds of silica gel. Especially Develosil 30 (silica gel) has 3nm pores. Its pore size is the smallest, and it has very large surface area and shows large retention.

| | | | |
|----------------------|--------------------|---------------------------|-------------------------|
| Reversed phase | C30 phase | Develosil C30-UG | 3 um, 5 um |
| | | Develosil PRPAQUEOUS | 3 um, 5 um |
| | | Develosil Combi-RP | 3 um, 5 um |
| | | Develosil RPFULLERENE | 3 um, 5 um |
| | | Develosil RPAQUEOUS-AR | 3 um, 5 um |
| | Develosil ERP20 | 15/30 um | |
| | C18 phase | Develosil ODS-UG | 3 um, 5 um and 15/30 um |
| | | Develosil ODS-HG | 3 um, 5 um and 15/30 um |
| | | Develosil ODS-MG | 3 um, 5 um and 15/30 um |
| | | Develosil ODS-SR | 3 um, 5 um and 15/30 um |
| | | Develosil PAHS | 3 um, 5 um |
| | | Develosil 300ODS-HG | 5 um |
| | C8 phase | Develosil C8-UG | 3 um, 5 um |
| Develosil 300C8-HG | | 5 um | |
| C4 phase | Develosil 300C4-HG | 5 um | |
| C1 phase | Develosil TMS-UG | 3 um, 5 um | |
| Phenyl phase | Develosil Ph-UG | 3 um, 5 um | |
| Cyano phase | Develosil CN-UG | 5 um | |
| Gel filtration phase | Diol phase | Develosil 300Diol | 5 um |
| | | Develosil 100Diol | 5 um |
| Normal phase | Cyano phase | Develosil CN-UG | 5 um |
| | Amino phase | Develosil NH ₂ | 5 um |
| | Silica | Develosil 30 | 3 um, 5 um and 15/30 um |
| | | Develosil 60 | 3 um, 5 um and 15/30 um |
| Develosil 100 | | 3 um, 5 um and 15/30 um | |
| | | Develosil SILICA-HILIC() | 3 um, 5 um |

C18 phase

| Develosil | ODS-UG | ODS-HG | ODS-MG | ODS-SR | PAHS |
|--|--------------------|-------------------|------------------|---------------------|-----------------------------|
| Functionality of C18 | Monofunctional | Trifunctional | Difunctional | Difunctional | Trifunctional and polymeric |
| Ligand density (umol/g) | 3.2 | 3.4 | 1.6 | --- | 4.5 |
| Carbon content (%) | 18 | 18 | 15 | 18 | 23 |
| Endcapping (TMS) | Yes | Yes | Yes | Yes | No |
| Pore diameter of silica (nm) | 14 | 14 | 10 | 8 | 12 |
| Surface area of silica (m ² /g) | 300 | 300 | 450 | --- | 350 |
| Hydrogen bonding capacity k(caffeine)/k(phenol) | 0.38 | 0.38 | 0.48 | 0.48 | 0.40 |
| Hydrophobic consistency k'amylbenzene)/k'(butyl benzene) | 1.59 | 1.58 | 1.60 | 1.66 | 1.58 |
| Steric selectivity k(triphenylene)/k'(o-terphenyl) | 1.50 | 1.58 | 1.20 | 1.21 | 2.72 |
| Stability | Very good (pH1-10) | Very good (pH1-9) | Good (pH2-7.5) | Good (pH2-7.5) | Good (pH2-7.5) |
| Retention | Average | Average | Long (1.3 times) | Very long (2 times) | Average |

Characteristics of ODS phases are showed in the above table. Develosil ODS-UG is the most stable under alkaline conditions, and can be used under pH1 to pH10. Develosil ODS-HG is the most stable under acidic conditions, and can be used even under 0.5% TFA. Develosil ODS-MG shows medium performance and suitable for all samples. Develosil ODS-SR shows long retention, and suitable for LC/MS because organic in the mobile phase increase and sensitivity increases. Develosil PAHS is a real polymeric ODS, and has the highest steric selectivity.



Conditions

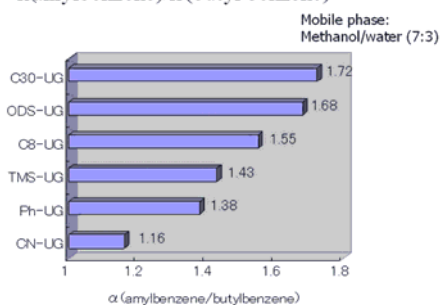
Column size: 150 x 4.6 mm i.d.
 Mobile phase: Methanol/water (75:25)
 Temperature: 30C
 Detection: UV@254nm
 Sample:
 1=Butylbenzene
 2=o-Terphenyl
 3=Amylbenzene
 4=Triphenylene

UG series phases

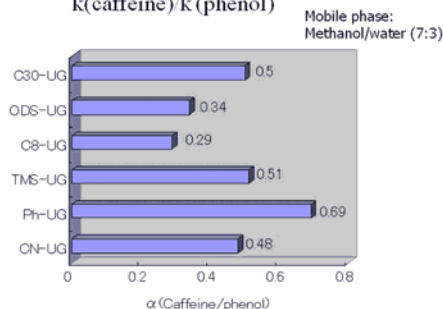
All phase are monomerically bonded and endcapped on the same silica base. We can use and compare with phases which has only different ligand each other.

| | Particle size | Ligand | End-capping (TMS) | Carbon content (%) | Silica | | |
|------------------|---------------|--|-------------------|--------------------|----------------------------------|--------------------|--------------------|
| | | | | | Surface area (m ² /g) | Pore volume (mL/g) | Pore diameter (nm) |
| Develosil C30-UG | 3 um, 5 um | -Si (CH ₃) ₂ C ₃₀ H ₆₁ | Yes | 18 | 300 | 1.15 | 14 |
| Develosil ODS-UG | 3 um, 5 um | -Si (CH ₃) ₂ C ₁₈ H ₃₇ | Yes | 18 | 300 | 1.15 | 14 |
| Develosil C8-UG | 3 um, 5 um | -Si (CH ₃) ₂ C ₈ H ₁₇ | Yes | 11 | 300 | 1.15 | 14 |
| Develosil TMS-UG | 3 um, 5 um | -Si (CH ₃) ₃ | Yes | 4.5 | 300 | 1.15 | 14 |
| Develosil Ph-UG | 3 um, 5 um | -Si (CH ₃) ₂ C ₆ H ₅ | Yes | 8 | 300 | 1.15 | 14 |
| Develosil CN-UG | 5 um | -Si (CH ₃) ₂ C ₃ H ₆ CN | Yes | 7 | 300 | 1.15 | 14 |

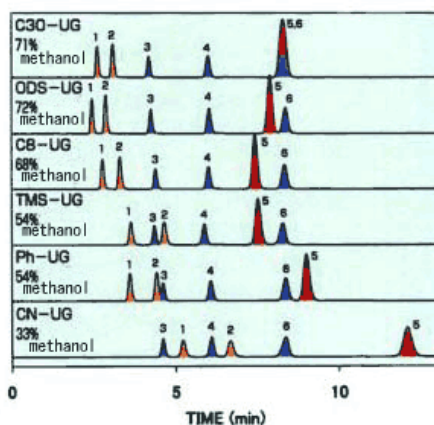
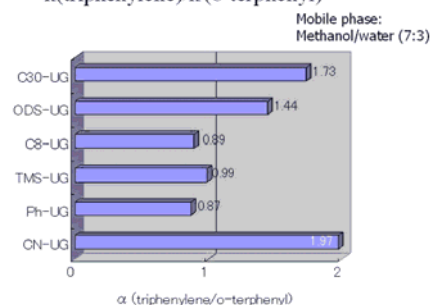
Hydrophobic consistency
k(amylybenzene)/k(butyl benzene)



Hydrogen bonding capacity
k(caffeine)/k(phenol)



Steric selectivity
k(triphenylene)/k(o-terphenyl)



Comparison of chromatograms

Conditions
 Column size: 150 x 4,6 mm i.d.
 Mobile phase: Methanol/water (methanol percent described in figure) (Retention time of peak 6 was adjusted at 8.5 min.)
 Flow rate: 1.0 mL/min
 Temperature: 30C
 Detection: UV@254nm
 Sample
 1=Methyl parabene
 2=Ethyl parabene
 3=Benzene
 4=Toluene
 5=Naphthalene
 6=Ethylbebenze

Develosil silica gel

| | Particle size | Surface area (m ² /g) | Pore volume (mL/g) | Pore diameter (nm) |
|---------------------------|---------------------|----------------------------------|--------------------|--------------------|
| Develosil 30 | 3 um, 5 um, 15/30um | 700 | 0.5 | 3 |
| Develosil 60 | 3 um, 5 um, 15/30um | 500 | 0.75 | 6 |
| Develosil 100 | 3 um, 5 um, 15/30um | 350 | 1.0 | 12 |
| Develosil SILICA-HILIC() | 3 um, 5 um | 300 | 1.15 | 14 |

Develosil 30, 60 and 100 silica gels are type A. But Develosil SILICA-HILIC() is type B and also for HILIC mode.

Expression of stationary phase

Develosil + stationary phase name (ODS-UG or C8-UG) + particle size (μm) e.g. Develosil ODS-HG-5

Size of Develosil column

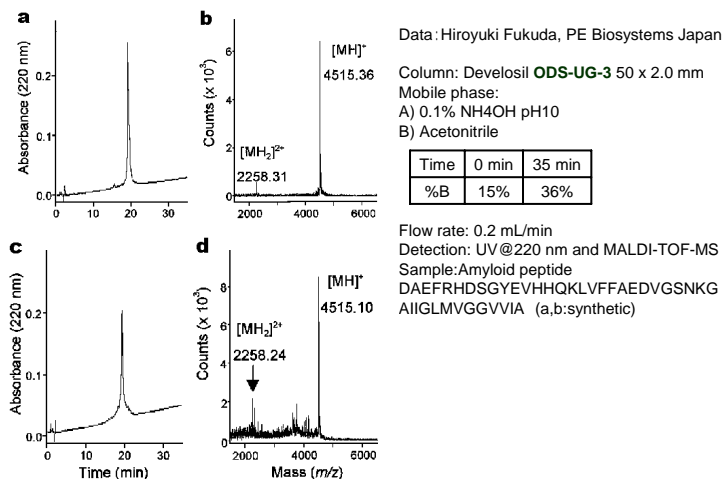
Capillary column (0.075 mm i.d.) to preparative column (28 mm I.d. for 5 um particle, 50 mm i.d. for 15/30 um particle) are available.

Available Inner diameters are shown as follows:

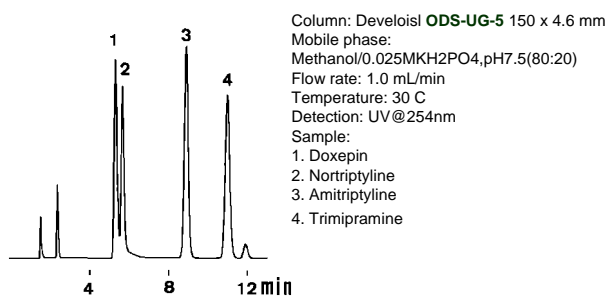
0.075 mm, 0.15 mm, 0.3 mm, 0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, 3.0 mm, 4.0 mm, 4.6 mm, 6.0 mm, 8.0 mm, 10 mm, 20 mm, 28 mm, 50mm

Applications

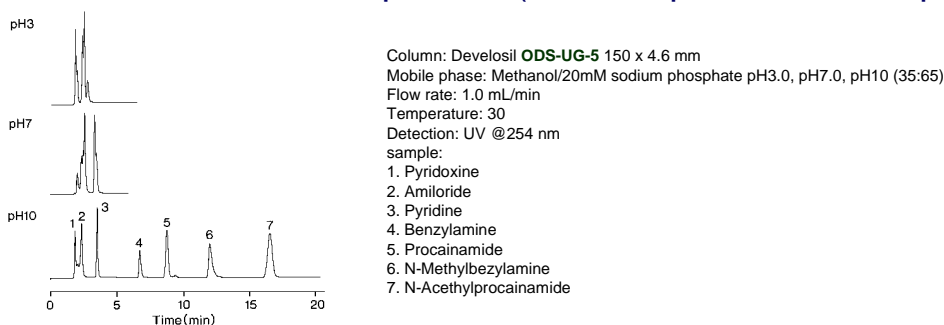
Separation of amyloid peptide (LC/MS(3))



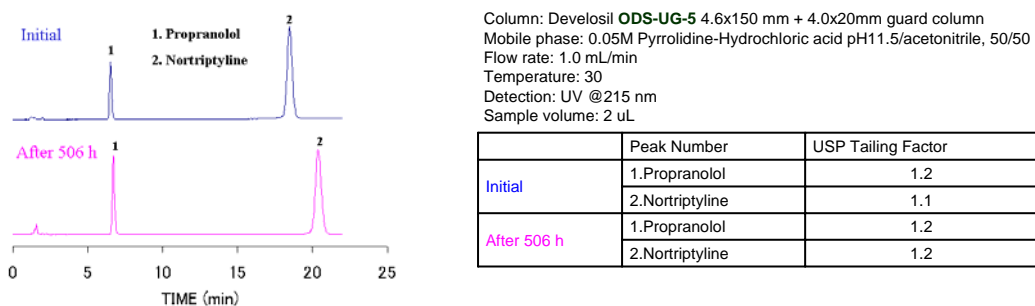
Separation of tricyclic antidepressants



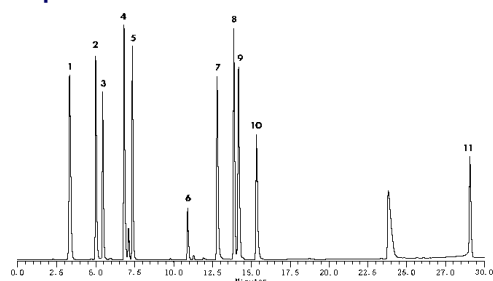
Separation of basic compounds (effect of pH of a mobile phase)



Separation of tricyclic antidepressant (Stability test)



Separation of color additives

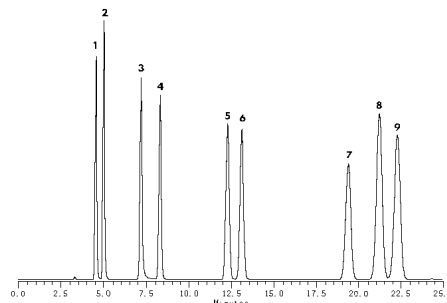


Column: Develosil ODS-UG-5 150 x 4.6 mm
 Mobile phase:
 A) 10mM ammonium acetate pH6.0
 B) Acetonitrile

| Time | 0 min | 30 min |
|------|-------|--------|
| %B | 5% | 100% |

Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @254 nm
 sample:
 1.Tetrazine)
 2.Amaranth
 3.Indigocarmine
 4.Nwe coccine
 5.Sunset yellow FCF
 6.Fast green
 7.Erythrosine B
 8.Acid red
 9.Phloxine B
 10.Rose bengal
 11.Brilliant green

Separation of food preservatives

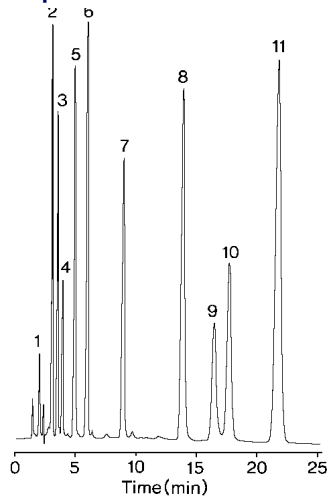


Column: Develosil ODS-UG-5 250 x 4.6 mm
 Mobile phase: Acetonitrile/20 mM sodium acetate (pH4.2)
 Flow rate: 1.0 mL/min
 Temperature: 30
 Detection: UV @254 nm
 Sample:

1.Benzoic acid (BA)
 2.Sorbic acid (SOA)
 3.Dehydroacetic acid (DHA)
 4.p-Hydroxybenzoic acid ethyl ester
 5.p-Hydroxybenzoic acid iso-propyl ester
 6.p-Hydroxybenzoic acid n-propyl ester
 7.p-Hydroxybenzoic acid sec-butyl ester
 8.p-Hydroxybenzoic acid iso-butyl ester
 9.p-Hydroxybenzoic acid n-butyl ester

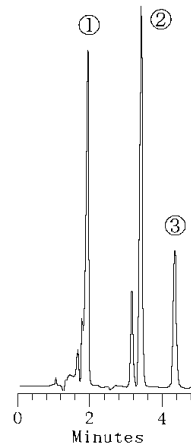
Applications

Separation of water-soluble vitamins



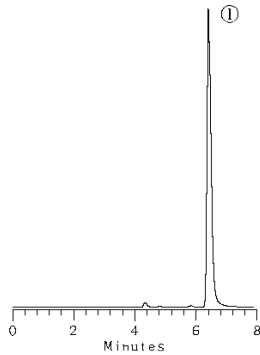
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
 Mobile phase: Acetonitrile/5mM 1-hexansulfonic acid sodium salt + 20mM phosphate acid(8:92)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @210 nm
 sample:
 1. Ascorbic acid
 2. Nicotinic acid
 3. Nicotinamide
 4. Calcium (+) - pantothenate
 5. Pyridoxal hydrochloride
 6. Pyridoxine hydrochloride
 7. Pyridoxamine hydrochloride
 8. Thiamin
 9. Folic acid
 10. (+)-Biotin
 11. Riboflavin

Separation of steroids



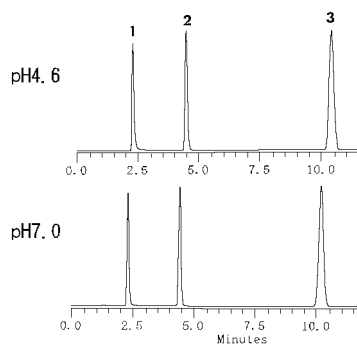
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
 Mobile phase: Acetonitrile/water (55:45)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @254 nm
 sample:
 1. Estriol
 2. -Estradiol
 3. Estron

Separation of berberine chloride



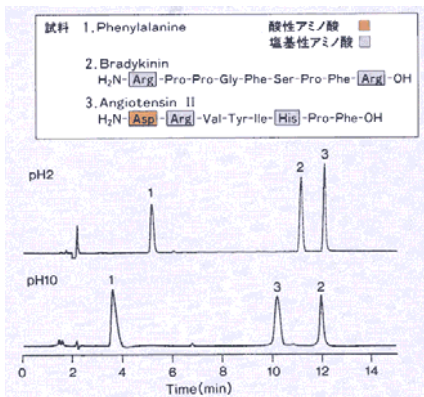
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
 Mobile phase: Acetonitrile/10mM NaH₂PO₄ + 100mM NaClO₄ (40:60)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @340 nm
 Sample:
 1. Berberine chloride

Separation of fungicides



Column: Develosil **ODS-UG-5** 4.6 x 150 mm
 Mobile phase: Acetonitrile/20mM sodium phosphate (pH4.6, pH7.0) (60:40)
 Flow rate: 1.0 mL/min
 Temperature: 30
 Detection: UV @254 nm
 Sample:
 1. Thiabendazole (TBZ)
 2. o-Phenylphenol (OPP)
 3. Diphenyl (DP)

Separation of peptides (effect of pH of mobile phase)



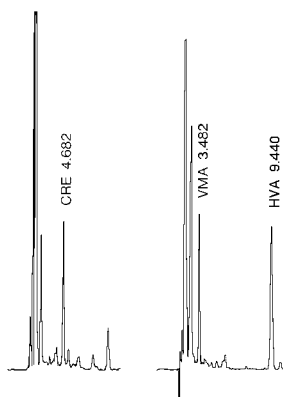
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
 Mobile phase:
 A) 0.1% Trifluoroacetic acid pH2.0, or 30mM Ammonium acetate pH10
 B) Acetonitrile

| Time | 0 min | 20 min |
|------|-------|--------|
| %B | 10% | 50% |

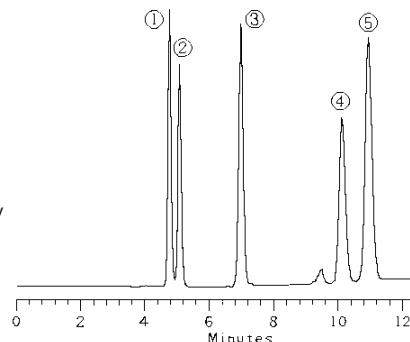
Flow rate: 1.0 mL/min
 Temperature: 30
 Detection: UV @215 nm
 sample:
 1. Phenylalanine
 2. Bradykinin
 3. Angiotensin

Separation of hippuric and methylhippuric acids

Separation of creatinine, VMA and HVA



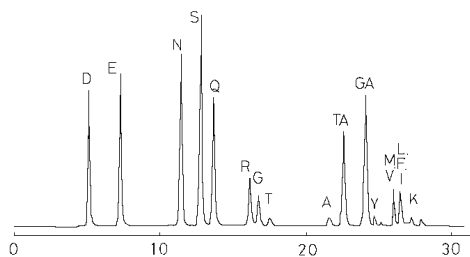
Column: Develosil **ODS-HG-5** 250 x 4.6 mm
 Mobile phase: Acetonitrile/3mM 1-hexansulfonic acid sodium salt + 50mM potassium phosphate + EDTA10mg/L pH2.0 (93:1000)
 Flow rate: 1.0 mL/min
 Temperature: 65
 Detection: Electrochemical 5100A, ESA (USA), Detector1 0.2V, Detector2 0.26V, Guard cell 0.31V, UV @235 nm
 sample:
 1. Creatinine
 2. VMA
 3. HVA



Column: Develosil **ODS-HG-5** 150 x 4.6 mm
 Mobile phase: Acetonitrile/20mM phosphate buffer(pH2.7) + 10mM cyclodextrin (20:80)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @210 nm
 sample:
 1. Mandelic acid
 2. Hippuric acid
 3. o-Methylhippuric acid
 4. p-Methylhippuric acid
 5. m-Methylhippuric acid

Applications

Separation of amino acids (OPA)

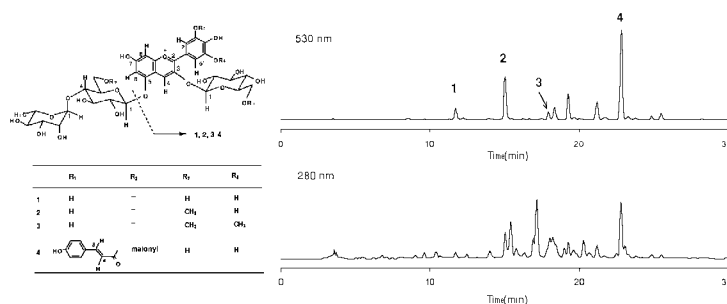


Data: Tetsuhisa Goto, National Food Research Institute
 Column: Develosil **ODS-HG-5** 150 x 4.6 mm + 10 x 4.0 mm (guard)
 Mobile phase:
 A) 5 mM Citrate buffer (pH6.0)/acetonitrile (19:1)
 B) 5 mM Citrate buffer (pH6.0)/acetonitrile (3:7)

| Time | 0 min | 5 min | 20 min | 25 min |
|------|-------|-------|--------|--------|
| %B | 5% | 12% | 22% | 95% |

Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: Ex@340nm, Em@450nm

Separation of anthocyanins



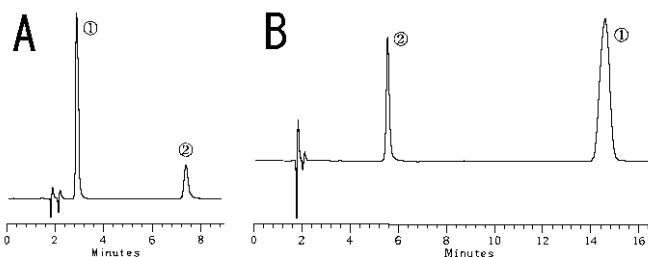
Data by Dr Kumi Yoshida, Nagoya University

Column: Develosil **ODS-HG-5** 250 x 4.6 mm
 Mobile phase:
 A) 0.5% TFA
 B) TFA/acetonitrile(0.5:99.5)

| Time | 0 min | 30 min |
|------|-------|--------|
| %B | 10% | 30% |

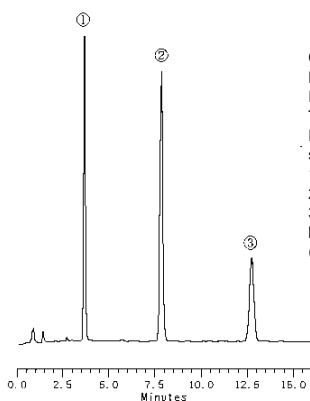
Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @530 nm and 280 nm
 Sample: Extract of purplish blue spicate flower petal of *Muscari armeniacum*

Separation of agricultural chemicals 1



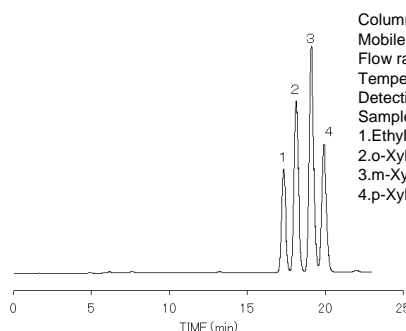
Column: Develosil **ODS-HG-5** 150 x 4.6 mm
 Mobile phase:
 A) Acetonitrile/20 mM phosphoric acid (10:90)
 B) Acetonitrile/5 mM octansulfonic acid sodium salt + 20 mM phosphoric acid (10:90)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @250 nm
 sample:
 1. Copper 8-quinolinolate
 2. Methyl sulfanilylcarbamate (Asulam)

Separation of agricultural chemicals 2



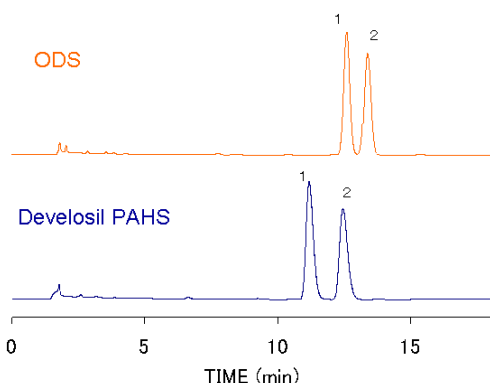
Column: Develosil **ODS-HG-5** 150 x 4.6 mm
 Mobile phase: Acetonitrile/water (55:45)
 Flow rate: 1.0 mL/min
 Temperature: 40
 Detection: UV @230 nm
 sample:
 1. Bis(dimethylthiocarbamoyl)disulfide (Thiram)
 2. Iprodione
 3. 0,0-Di-isopropyl S-2-benzensulphonamidoethylphosphorodithioate (Bensulide)

Separation of xylenes



Column: Develosil **PAHS-5** 250 x 4.6 mm
 Mobile phase: Methanol/water(75:25)
 Flow rate: 1.0 mL/min
 Temperature: 20 C
 Detection: UV @254nm
 Sample:
 1. Ethylbenzene
 2. o-Xylene
 3. m-Xylene
 4. p-Xylene

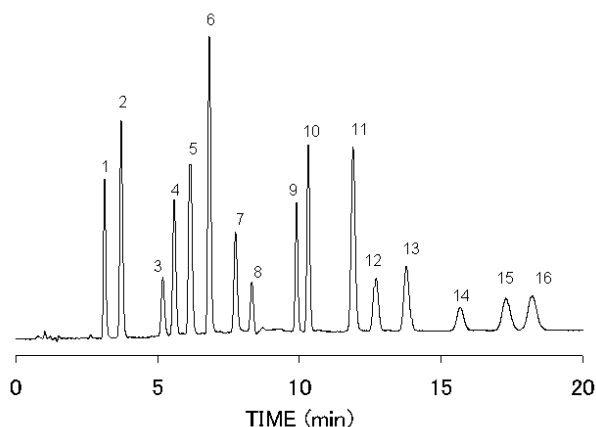
Separation of vitamin D2 and D3



Column: Develosil **PAHS-5** 250 x 4.6 mm
 ODS 250 x 4.6 mm
 Mobile phase: Acetonitrile
 Flow rate: 1.0 mL/min
 Temperature: 30 C
 Detection: UV @254nm
 Sample:
 1. Vitamin D2
 2. Vitamin D3

Applications

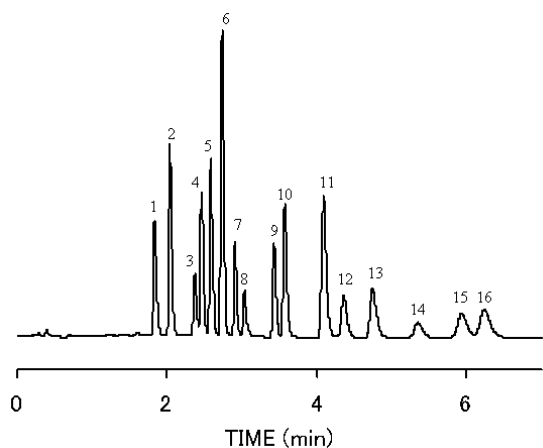
Separation of polyaromatic hydrocarbons (PAHs)



Column: Develosil **PAHS-5** 150 x 4.6 mm
 Mobile phase:
 A)Water
 B)Methanol

| Time | 0 min | 3.4 min | 8.1 min | 20min |
|------|-------|---------|---------|-------|
| %B | 80% | 80% | 100% | 100% |

Flow rate: 1.5 mL/min
 Temperature: 30 C
 Detection:UV@254nm
 Sample:1)Naphthalene 2)Acenaphthylene 3)Acenaphthene 4)Fluorene 5)Phenanthrene 6)Anthracene 7)Fluoranthene 8)Pyrene 9)Benzo (a) anthracene 10)Chrysene 11)Benzo (b) fluoranthene 12)Benzo (k) fluoranthene 13)Benzo (a) pyrene 14)Dibenzo (a,h) anthracene 15)Benzo (g,h,i) perylene 16)Indeno (1,2,3-cd) pyrene

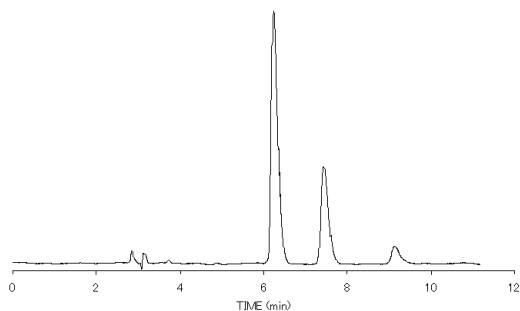


Column: Develosil **PAHS-3** 75 x 4.6 mm (3 um particle)
 Mobile phase:
 A)Water
 B)Methanol

| Time | 0 min | 0.5 min | 2.5 min | 7min |
|------|-------|---------|---------|------|
| %B | 70% | 70% | 100% | 100% |

Flow rate: 1.5 mL/min
 Temperature: 30 C
 Detection:UV@254nm
 Sample:1)Naphthalene 2)Acenaphthylene 3)Acenaphthene 4)Fluorene 5)Phenanthrene 6)Anthracene 7)Fluoranthene 8)Pyrene 9)Benzo (a) anthracene 10)Chrysene 11)Benzo (b) fluoranthene 12)Benzo (k) fluoranthene 13)Benzo (a) pyrene 14)Dibenzo (a,h) anthracene 15)Benzo (g,h,i) perylene 16)Indeno (1,2,3-cd) pyrene

Separation of benzaikonium chloride



Column: Develosil **CN-UG-5** 250 x 4.6 mm
 Mobile phase: Methanl/100mM CH3COONa, pH5.5=70:30
 Flow rate: 1.0 mL/min
 Temperature: 30 C
 Detection: UV@265nm
 Sample:
 1=benzaikonium chloride