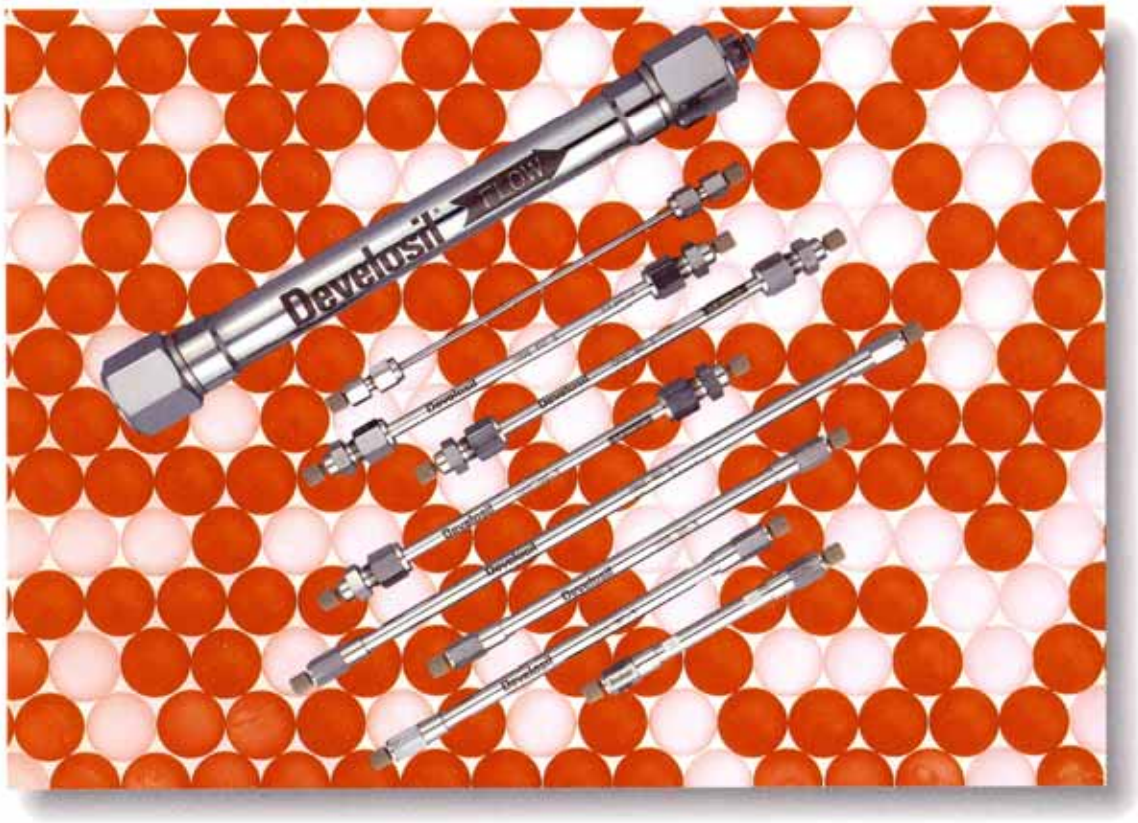
Columns for HPLC



Develosil™

Nomura Chemical

Nomura Chemical Co. was founded as a manufacturer of HPLC columns in1979. 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 the leading companies of HPLC columns in the world. Especially our patented C30 phase has a quite unique characteristic and has been used in many pharmaceutical corporations, laboratories and universities. Develosil columns are available in the world through our distributors in North America, Europe and Asia.

Develosil HPLC Columns

Columns in 3 kinds of mode such as Reversed, Gel filtration and Normal phase are commercially available.

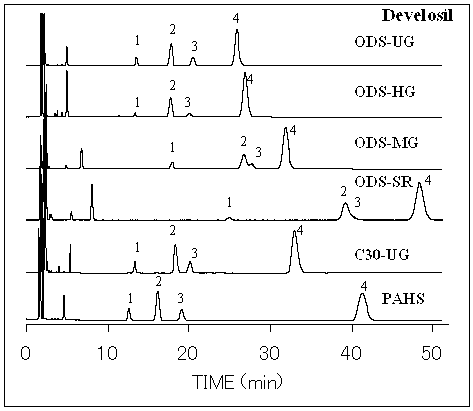
We have 5 kinds of C30 phase, 6 kinds of C18 (ODS) phase and 2 kinds of C8 phase. 300ODS-HG, 300C8-HG and 300C4-HG phases have pores with 25 nm diameter and are for separation of proteins or polypeptides.

And, we have 4 kinds of silica gel. Especially, Develosil 30 (silica gel) has 3 nm pores. Its pore size is the smallest, and it has very large surface area and shows large retention.

MODE	Ligand	PACKING MATERIAL	PARTICLE SIZE
Reversed phase	C30	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	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	Develosil C8-UG	3 um, 5 um
		Develosil 300C8-HG	5 um
	C4	Develosil 300C4-HG	5 um
	C1	Develosil TMS-UG	3 um, 5 um
	Phenyl	Develosil Ph-UG	3 um, 5 um
	Cyano	Develosil CN-UG	5 um
Gel filteration phase	Diol	Develosil 300Diol	5 um
		Develosil 100Diol	5 um
Normal phase	Cyano	Develosil CN-UG	5 um
	Amino	Develosil NH2	5 um
	None (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(I) and (II)	3 um, 5 um

Comparison of Develosil ODS (C18) Columns

	ODS-UG	ODS-HG	ODS-MG	ODS-SR	PAHS
Functionatrity of C18	Monofunctional	Trifunctional	Difunctional	Difunctional	Trifunctional and polymeric
Ligand density (umol/g)	3.2	3.4	1.6	---	4.5
Carboncontent (%)	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 (pH2-10)	Very good (pH1-9)	Good (pH2-7.5)	Good (pH2-7.5)	Good (pH2-7.5)
Retention	Moderate	Moderate	Long (1.3 folds)	Very long (2 folds)	Moderate



Analytical Conditions
Column dimension: 150 x 4.6 mm i.d.
Mobile phase: Methanol / water = 75 / 25
Temperature: 30 °C
Detection: UV at 254 nm
Sample: 1 = Butylbenzene
2 = *o* - Terphenyl
3 = Amylbenzene
4 = Triphenylene

The characteristic of each ODS phase is showed in the above table.

Develosil ODS-UG is the most stable under alkaline conditions, and can be used under pH2 - 10.

Develosil ODS-HG is the most stable under acidic conditions (pH1 - 9), and can be used even under 0.5% TFA.

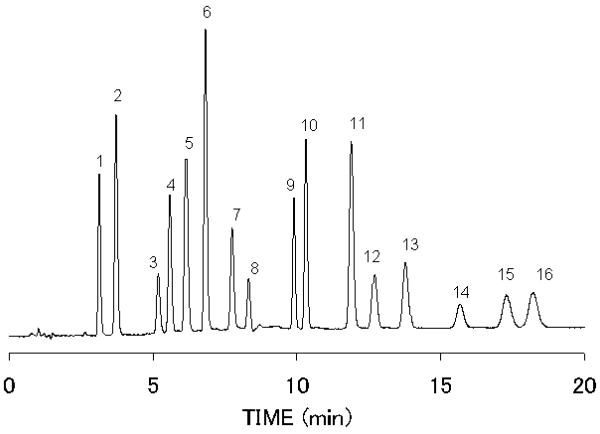
Develosil ODS-MG shows medium performance and suitable for all samples and time to equilibrate is very quick.

Develosil ODS-SR shows the longest retention in our ODS phases, and suitable for LC/MS because much organic solvent obtain high sensitivity.

Develosil PAHS is a real polymeric ODS, and has the highest steric selectivity.

Applications

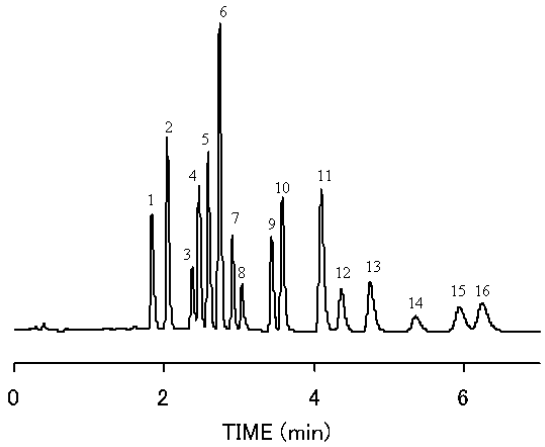
Separation of Polyaromatichydrocarbons (PAHs)



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 ºC
Detection:UV at 254 nm
Sample: 1 = Naphthalene
2 = Acenaphthaylene
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

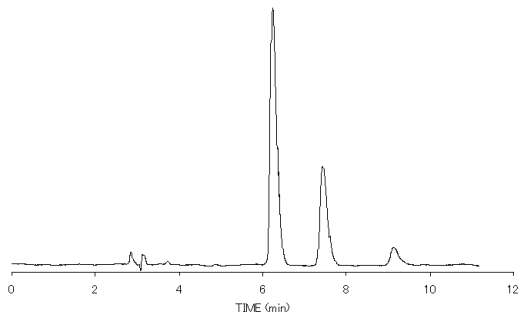


Column: Develosil **PAHS-3** 75 x 4.6 mm (3 um particle)
Mobile phase:
A) Water
B) Methanol

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

Flow rate: 1.5 mL/min
Temperature: 30 ºC
Detection: UV at 254 nm
Sample: 1 = Naphthalene
2 = Acenaphthaylene
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

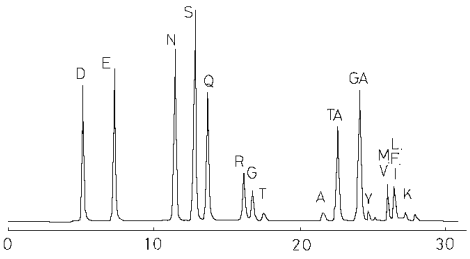
Separation of Benzalkonium chloride



Column: Develosil **CN-UG-5** 250 x 4.6 mm
Mobile phase: Methanol / 100 mM CH_3COONa, pH5.5 = 70 / 30
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 265 nm
Sample: 1 = benzalkonium chloride

Applications

Separation of Amino acids (OPA)

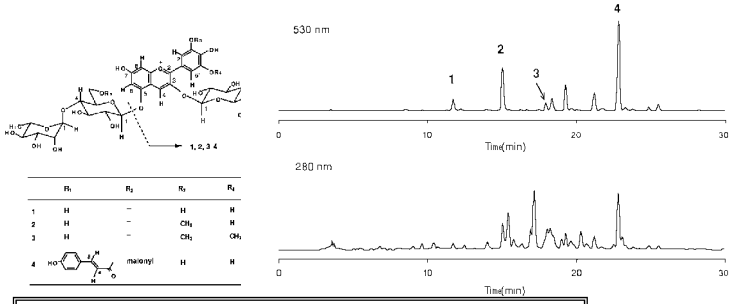


Data:Tetsuhisa Goto, National Food Research Institute
Column: Develosil **ODS-HG-5** 150 x 4.6 mm + 10 x 4.0 mm (guard)
Mobile phase:
A) 5 mM Citrate buffer (pH6.0) / acetonitrile = 19 / 1
B) 5 mM Citrate buffer (pH6.0) / acetonitrile = 3 / 7

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

Flow rate: 1.0 mL/min
Temperature:40 ºC
Detection: Florescence Ex. at 340nm、Em. at 450nm

Separation of Anthocyanins

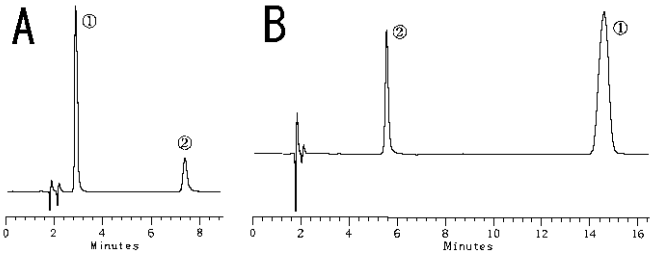


Data: Dr. Kumi Yoshida, Nagoya University
Column: Develosil **ODS-HG-5** 250 x 4.6 mm
Mobile phase:
A) 0.5% TFA
B) TFA / acetonitrile = 0.5 / 99.5

Time	0 min	30 min
%B	10%	30%

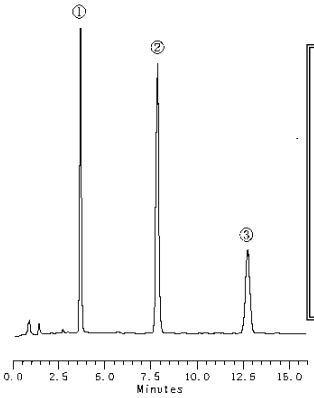
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV at 530 nm and 280 nm
Sample: Extract of purplish blue spicate flower petal of *Muscari armeniacum*

Separation of Agricultural chemicals 1



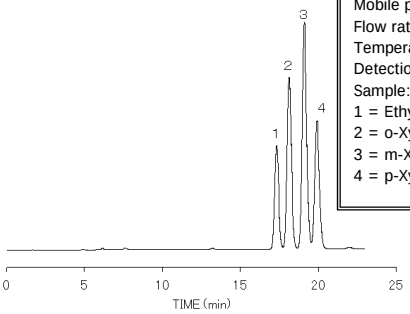
Column: Develosil **ODS-HG-5** 150 x 4.6 mm
Mobile phase:
A) Acetonitrile / 20 mM phosphoric acid = 10 / 90
B) Acetonitrile / 5 mM octansulfonic acid sodium salt + 20 mM phosphoric acid = 10 /90
Flow rate: 1.0 mL/min
Temperature: 40**℃**
Detection: UV at 250 nm
Sample:
1= Copper 8-quinolinolate
2 = Methyl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

Separation of Agricultural chemicals 2



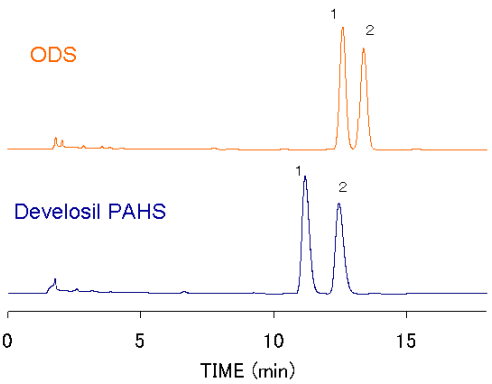
Column: Develosil **ODS-HG-5** 150 x 4.6 mm
Mobile phase: Acetonitrile / water = 55 /45
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV at 230 nm
Sample:
1 = Bis(dimethylthiocarbamoyl)disulfide (Thiram)
2 = Iprodione
3 = 0,0-Di-isopropyl S-2-benzensulphonamidoethylphosphorodithioate (Bensulide)

Separation of Xylenes



Column: Develoisl **PAHS-5** 250 x 4.6 mm
Mobile phase: Methanol / water = 75 / 25
Flow rate: 1.0 mL/min
Temperature: 20 ºC
Detection: UV at 254 nm
Sample:
1 = Ethylbenzene
2 = o-Xylene
3 = m-Xylene
4 = p-Xylene

Separation of Vitamin D2 and D3



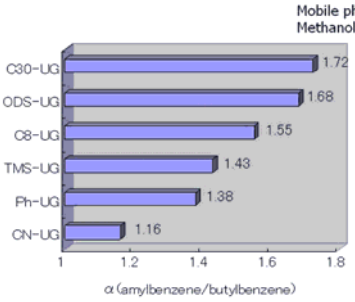
Analytical Consitions
Column: Develoisl **PAHS-5** 250 x 4.6 mm
ODS 250 x 4.6 mm
Mobile phase: Acetonitrile
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 254 nm
Sample:
1 = Vitamin D2
2 = Vitamin D3

UG Series Phases

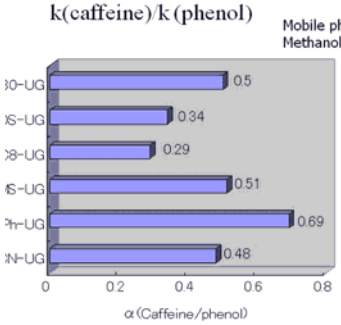
All phase are monomerically bonded and fully endcapped on the same silica base material.
Therefore, UG phases can be compared concerning the difference of separation between each ligand.

	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 $(CH_3)_2C_{30}H_{61}$	Double	18	300	1.15	14
Develosil ODS-UG	3 um, 5 um	-Si $(CH_3)_2C_{18}H_{37}$	Double	18	300	1.15	14
Develosil C8-UG	3 um, 5 um	-Si $(CH_3)_2C_8H_{17}$	Double	11	300	1.15	14
Develosil TMS-UG	3 um, 5 um	-Si $(CH_3)_3$	Double	4.5	300	1.15	14
Develosil Ph-UG	3 um, 5 um	-Si $(CH_3)_2C_6H_5$	Double	8	300	1.15	14
Develosil CN-UG	5 um	-Si $(CH_3)_2$ C_3H_6CN	Double	7	300	1.15	14

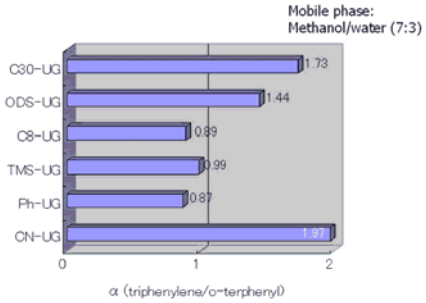
Hydrophobic consistency
k(amylbenzene)/k(butyl benzene)

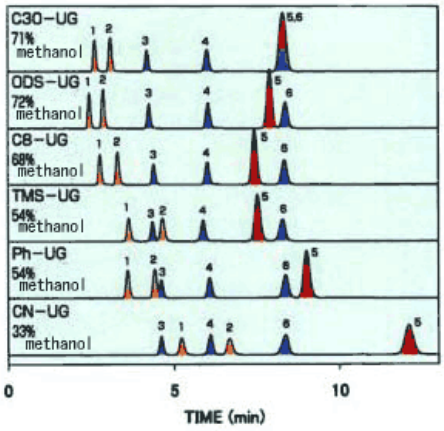


Hydrogen bonding capacity
k(caffeine)/k(phenol)



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





Comparison of chromatograms for standard samples

Conditions
Column size: 150 x 4.6 mm i.d.
Mobile phase: Methanol/ water
(methanol ratio is described in figure) (Retention time of peak No. 6 was adjusted at 8.5 min.)

Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 254 nm
Sample
1 = **Methyl parabene**
2 = **Ethyl parabene**
3 = **Benzene**
4 = **Toluene**
5 = **Naphthalene**
6 = **Ethylbebzene**

Develosil Silica Gel

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

Develosil 30, 60 and 100 silica gels are type A silica.

Develosil SILICA-HILIC(Ⅰ) is type B and also for HILIC mode.

Expression of Stationary Phases

"Develosil" + stationary phase name (ODS-UG or C8-UG) + particle size (μm)
e.g. Develosil C8-UG-5, Develosil ODS-HG-3, Develosil ODS-UG-5, Develosil C30-UG-5

Dimension of Develosil Columns

Available inner diameters are shown as follows:

Semi-micro column: 1.0 mm i.d., 1.5 mm i.d., 2.0 mm i.d.

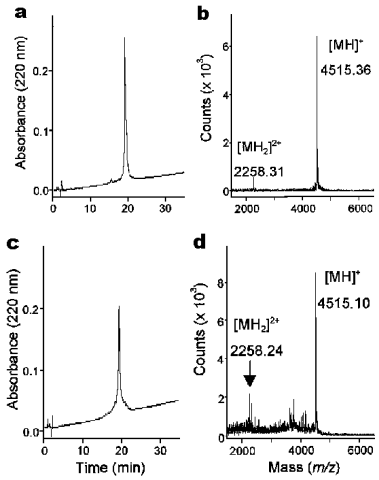
Conventional column: 3.0 mm i.d., 4.0 mm i.d., 4.6 mm i.d., 6.0 mm i.d.

Preparative column: 8.0 mm i.d., 10 mm i.d., 20 mm i.d., 28 mm i.d.

Large scale preparative column: 50 mm i.d. (for >10 or 15/30 um particle)

Applications

Separation of Amyloid peptide (LC/MS)



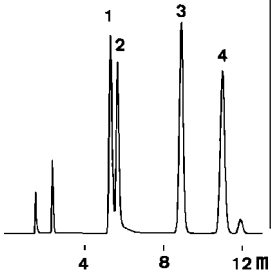
Data：Hiroyuki Fukuda, PE Biosystems Japan

Column: Develosil **ODS-UG-3** 50 x 2.0 mm
Mobile phase:
A) 0.1% NH_4OH (pH10)
B) Acetonitrile

Time	0 min	35 min
%B	15%	36%

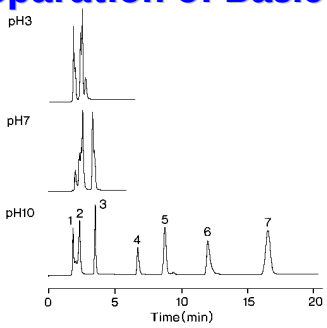
Flow rate: 0.2 mL/min
Detection: UV at 220 nm and MALDI-TOF-MS
Sample: Amyloid peptide
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVVIA (a,b:synthetic)

Separatin of Tricyclic antidepressants



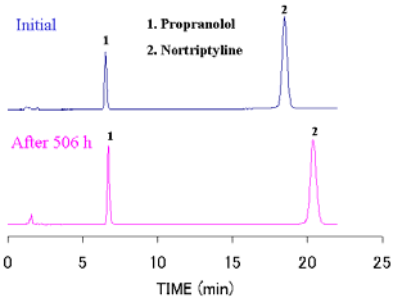
Column: Develoisl **ODS-UG-5** 150 x 4.6 mm
Mobile phase:
Methanol / 25 mM KH_2PO_4 (pH7.5) = 80 / 20
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 254 nm
Sample:
1 = Doxepin
2 = Nortriptyline
3 = Amitriptyline
4 = Trimipramine

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



Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase: Methanol / 20 mM sodium phosphate (pH3.0), (pH7.0), (pH10) = 35 /65
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 254 nm
sample:
1 = Pyridoxine
2 = Amiloride
3 = Pyridine
4 = Benzylamine
5 = Procainamide
6 = N-Methylbezylamine
7 = N-Acethylprocainamide

Separatin of Tricyclic antidepressant (Stability test)

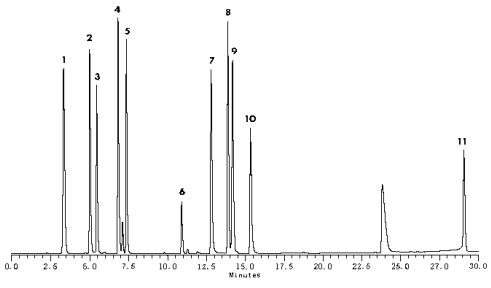


Column: Develosil **ODS-UG-5** 150 x 4.6 mm + 20 x 4.0 mm guard column
Mobile phase: 0.05M Pyrrolidine-Hydrochloric acid (pH11.5) / acetonitrile = 50 / 50
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 215 nm
Sample volume: 2 uL

	Peak Number	USP Tailing Factor
Initial	1 = Propranolol	1.2
	2 = Nortriptyline	1.1
After 506 hours	1 = Propranolol	1.2
	2 = Nortriptyline	1.2

Peak shape is no change after 506 hours.

Separation of Color additives



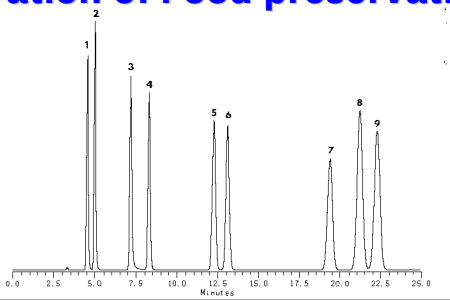
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase:
A) 10 mM Ammonium acetate (pH6.0)
B) Acetonitrile

Time	0 min	30 min
%B	5%	100%

Flow rate: 1.0 mL/min
Temperature: 40 oC
Detection: UV at 254 nm

sample:
1 = Tertrazine
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

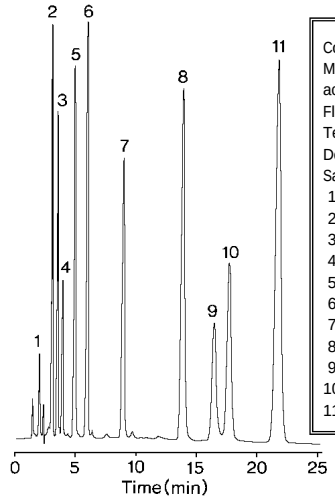
Separation of Food preservatives



Column: Develosil **ODS-UG-5** 250 x 4.6 mm
Mobile phase: Acetonitrile / 20 mM sodium acetate (pH4.2) = 40 / 60
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 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

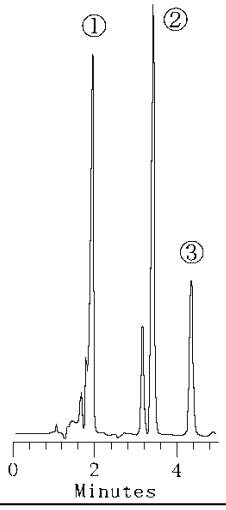
Applications

Separation of Water-soluble Vitamins



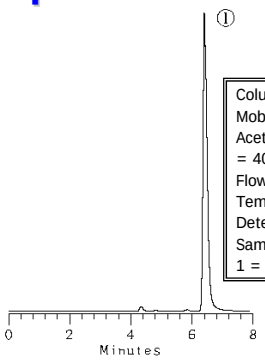
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase: Acetonitrile / 5 mM 1-hexansulfonic acid sodium salt + 20 mM phosphate acid = 8 / 92
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV at 210 nm
Sample:
1 = Ascorbic acid
2 = Nicotinic acid
3 = Nicotinamide
4 = Calcium (+) - pantothenate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

Separation of Steroids



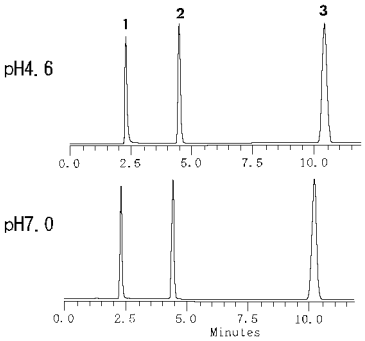
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase: Acetonitrile / water = 55 /45
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV at 254 nm
Sample:
1 = Estriol
2 = β-Estradiol
3 = Estron

Separation of Berberine chloride



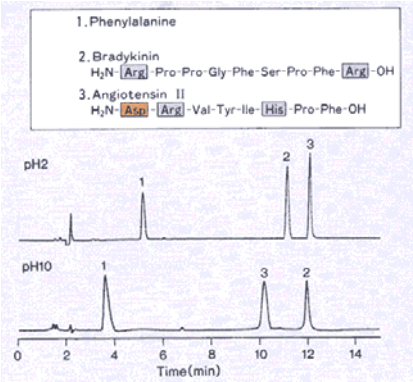
Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase:
Acetonitrile / 10 mM NaH_2PO_4 + 100 mM $NaClO_4$ = 40 /60
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV at 340 nm
Sample:
1 = Berberine chloride

Separation of Fungicides



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

Separation of Peptides (pH effect of mobile phase)

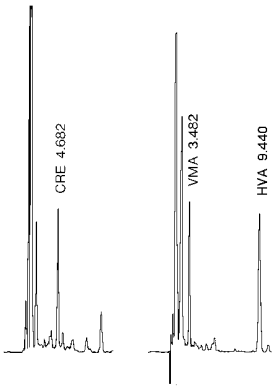


Column: Develosil **ODS-UG-5** 150 x 4.6 mm
Mobile phase:
A) 0.1% Trifluoracetic acid (pH2.0), or 30 mM Ammonium acetate (pH10)
B) Acetonitrile

Time	0 min	20 min
%B	10%	50%

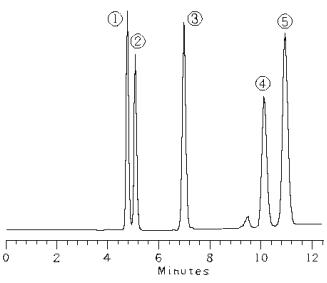
Flow rate: 1.0 mL/min
Temperature: 30 ºC
Detection: UV at 215 nm
Sample:
1 = Phenylalanine
2 = Bradykinin
3 = Angiotensin **II**

Separation of Creatinine, VMA and HVA



Column: Develosil **ODS-HG-5** 250 x 4.6 mm
Mobile phase:
Acetonitrile / 3m M 1-hexansulfonic acid sodiumsalt + 50 mM potassium phosphate + EDTA 10 mg/L (pH2.0) = 93 / 1000
Flow rate: 1.0 mL/min
Temperature: 65 ºC
Detection: Electoricchemical 5100 A, ESA (USA),
Detector1 0.2V,
Detector2 0.26V, Guard cell 0.31V,
UV at 235 nm
Sample:
1 = Creatinine
2 = VMA
3 = HVA

Separation of Hippuric and Methylhippuric acids



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