

Columns for HPLC

# Develosil™



## 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.

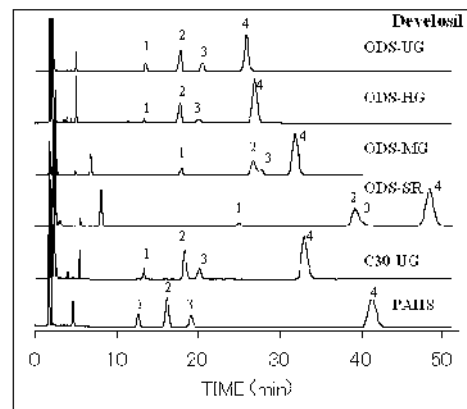
## Develosil HPLC column

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 NH2	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( I )		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 <sup>2</sup> /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



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

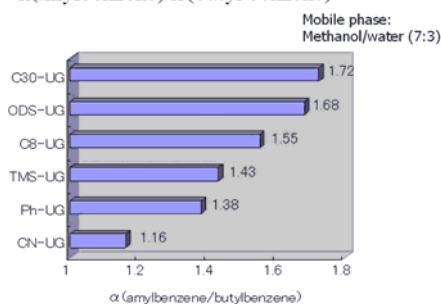
Characteristics of ODS phases are showed in the above table. Develosil ODS-UG is the most stable under alkaline conditions, and can be used under pH1 to pH10. Develosil ODS-HG is the most stable under acidic conditions, and can be used even under 0.5% TFA. Develosil ODS-MG shows medium performance and 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.

## UG series phases

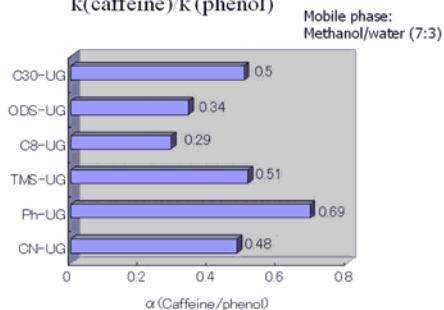
All phase are monomerically bonded and endcapped on the same silica base. We can use and compare with phases which has only different ligand each other.

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

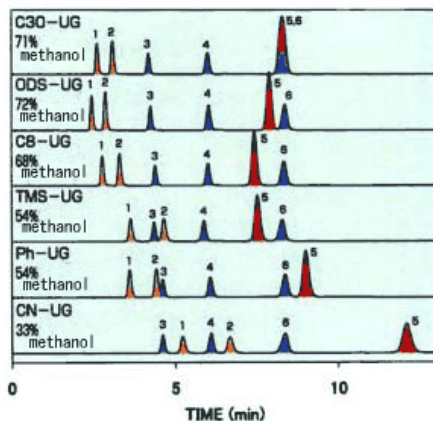
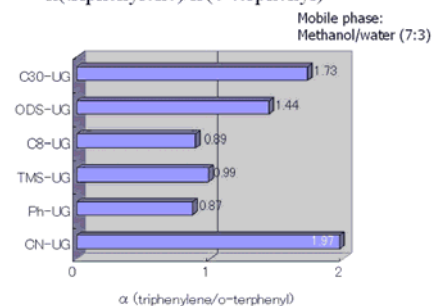
Hydrophobic consistency  
k(amybenzene)/k(butyl benzene)



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=Ethylbezene

## Develosil silica gel

	Particle size	Surface area (m <sup>2</sup> /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( I )	3 um, 5 um	300	1.15	14

Develosil 30, 60 and 100 silica gels are type A. But Develosil SILICA-HILIC( I ) 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

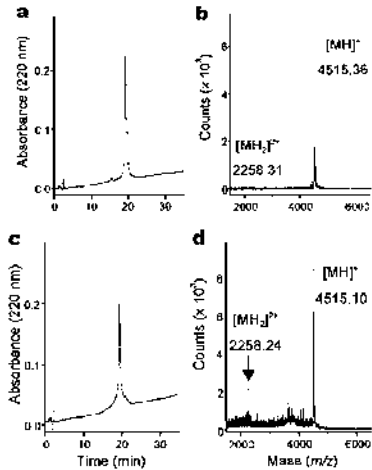
Capillary column (0.075 mm i.d.) to preparative column (28 mm i.d. for 5 um particle, 50 mm i.d. for 15/30 um particle) are available.

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))



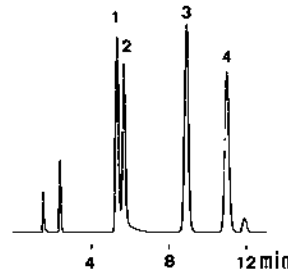
Data: Hiroyuki Fukuda, PE Biosystems Japan

Column: Develosil ODS-UG-3 50 x 2.0 mm  
 Mobile phase:  
 A) 0.1% NH4OH pH10  
 B) Acetonitrile

Time	0 min	35 min
%B	15%	36%

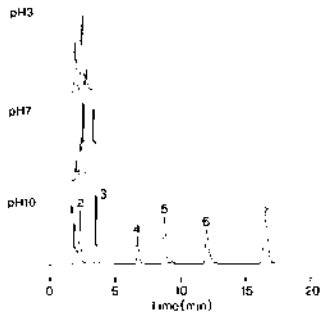
Flow rate: 0.2 mL/min  
 Detection: UV@220 nm and MALDI-TOF-MS  
 Sample: Amyloid peptide  
 DAEFRHDSGYEVHHQKLVFFAEDVGSNKG  
 AIIGLMVGGVVIA (a,b:synthetic)

## Separation of tricyclic antidepressants



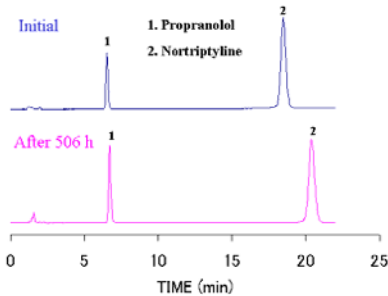
Column: Develosil ODS-UG-5 150 x 4.6 mm  
 Mobile phase:  
 Methanol/0.025MKH2PO4,pH7.5(80:20)  
 Flow rate: 1.0 mL/min  
 Temperature: 30 C  
 Detection: UV@254nm  
 Sample:  
 1. Doxepin  
 2. Nortriptyline  
 3. Amitriptyline  
 4. Trimipramine

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



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

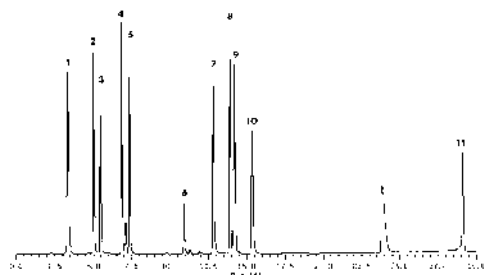
## Separation of tricyclic antidepressant (Stability test)



Column: Develosil ODS-UG-5 4.6x150 mm + 4.0x20mm guard column  
 Mobile phase: 0.05M Pyrrolidine-Hydrochloric acid pH11.5/acetoneitrile, 50/50  
 Flow rate: 1.0 mL/min  
 Temperature: 30°C  
 Detection: UV @215 nm  
 Sample volume: 2 uL

	Peak Number	USP Tailing Factor
Initial	1.Propranolol	1.2
	2.Nortriptyline	1.1
After 506 h	1.Propranolol	1.2
	2.Nortriptyline	1.2

## Separation of color additives

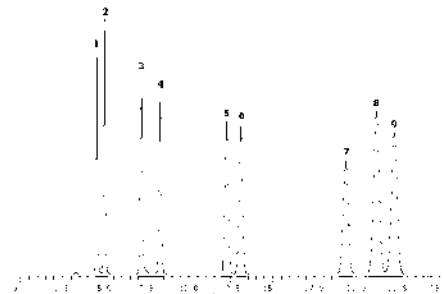


Column: Develosil ODS-UG-5 150 x 4.6 mm  
 Mobile phase:  
 A) 10mM ammonium acetate pH6.0  
 B) Acetonitrile

Time	0 min	30 min
%B	5%	100%

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

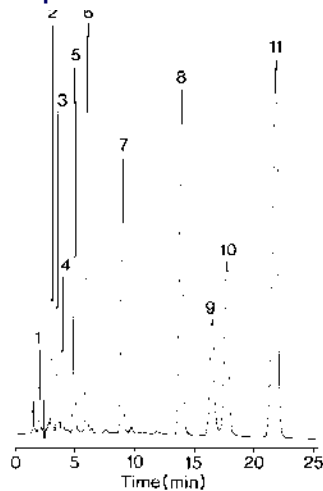
## Separation of food preservatives



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°C  
 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

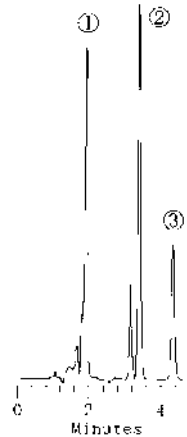
# Applications

## Separation of water-soluble vitamins



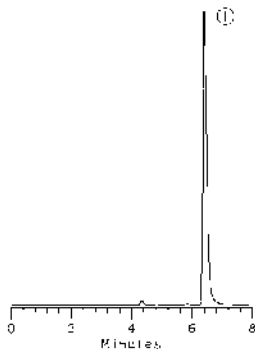
Column: Develosil **ODS-UG-5** 150 x 4.6 mm  
 Mobile phase: Acetonitrile/5mM 1-hexansulfonic acid sodium salt + 20mM phosphate acid(8:92)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV @210 nm  
 sample:  
 1. Ascorbic acid  
 2. Nicotinic acid  
 3. Nicotinamide  
 4. Calcium (+) -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

## Separation of steroids



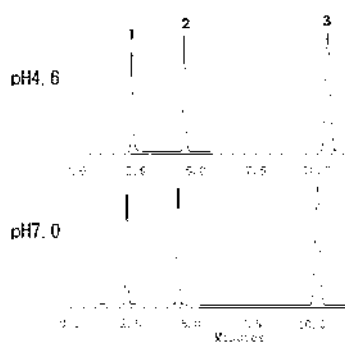
Column: Develosil **ODS-UG-5** 150 x 4.6 mm  
 Mobile phase: Acetonitrile/water (55:45)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV @254 nm  
 sample:  
 1. Estriol  
 2.  $\beta$ -Estradiol  
 3. Estron

## Separation of berberine chloride



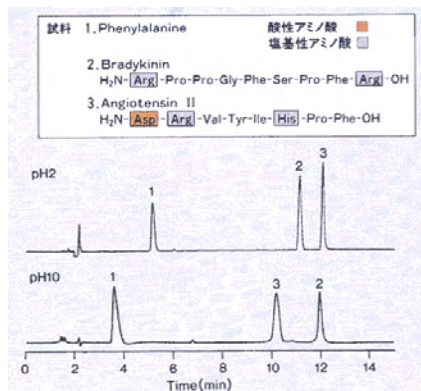
Column: Develosil **ODS-UG-5** 150 x 4.6 mm  
 Mobile phase: Acetonitrile/10mM NaH<sub>2</sub>PO<sub>4</sub> + 100mM NaClO<sub>4</sub> (40:60)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV @340 nm  
 Sample:  
 1. Berberine chloride

## 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°C  
 Detection: UV @254 nm  
 Sample:  
 1. Thiabendazole (TBZ)  
 2. o-Phenylphenol (OPP)  
 3. Diphenyl (DP)

## Separation of peptides (effect of pH of mobile phase)



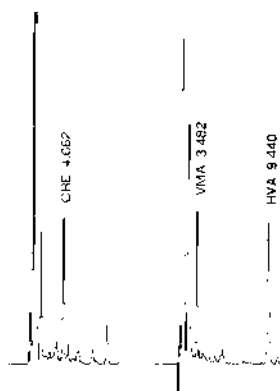
Column: Develosil **ODS-UG-5** 150 x 4.6 mm  
 Mobile phase:  
 A) 0.1% Trifluoroacetic acid pH2.0, or 30mM Ammonium acetate pH10  
 B) Acetonitrile

Time	0 min	20 min
%B	10%	50%

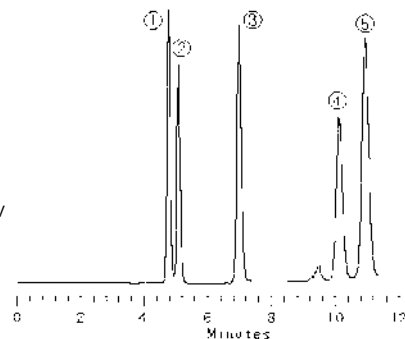
Flow rate: 1.0 mL/min  
 Temperature: 30°C  
 Detection: UV @215 nm  
 sample:  
 1. Phenylalanine  
 2. Bradykinin  
 3. Angiotensin II

## Separation of hippuric and methylhippuric acids

## Separation of creatinine, VMA and HVA



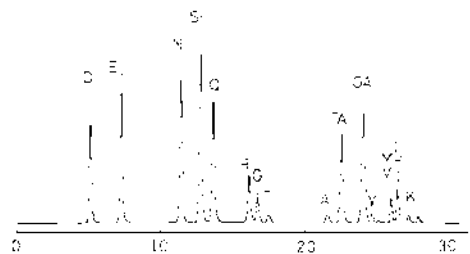
Column: Develosil **ODS-HG-5** 250 x 4.6 mm  
 Mobile phase: Acetonitrile/3mM 1-hexansulfonic acid sodium salt + 50mM potassium phosphate + EDTA 10mg/L, pH2.0 (93:1000)  
 Flow rate: 1.0 mL/min  
 Temperature: 65°C  
 Detection: Electrochemical 5100A, ESA (USA), Detector1 0.2V, Detector2 0.26V, Guard 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  $\beta$ -cyclodextrin (20:80)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 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)

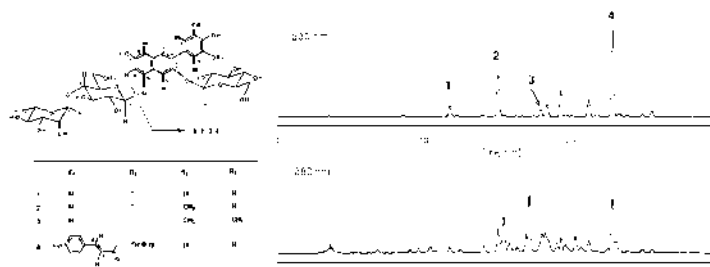


Data: Tetsuhisa Goto, National Food Research Institute  
 Column: Develosil **ODS-HG-5** 150 x 4.6 mm + 10 x 4.0 mm (guard)  
 Mobile phase:  
 A) 5 mM Citrate buffer (pH6.0)/acetonitrile (19:1)  
 B) 5 mM Citrate buffer (pH6.0)/acetonitrile (3:7)

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

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

## Separation of anthocyanins



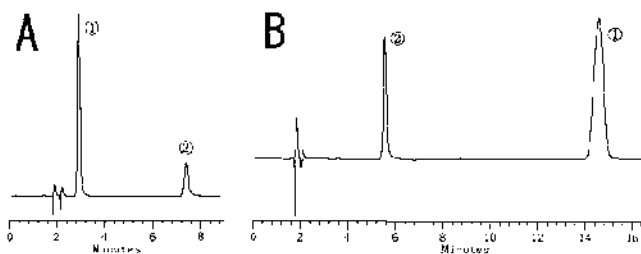
Data by Dr Kumi Yoshida, Nagoya University

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%

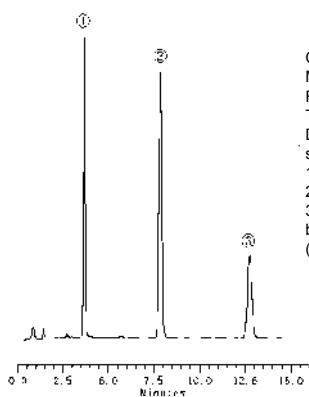
Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV@530 nm and 280 nm  
 Sample: Extract of purplish blue spicate flower petal of *Muscari armeniacum*

## Separation of agricultural chemicals 1



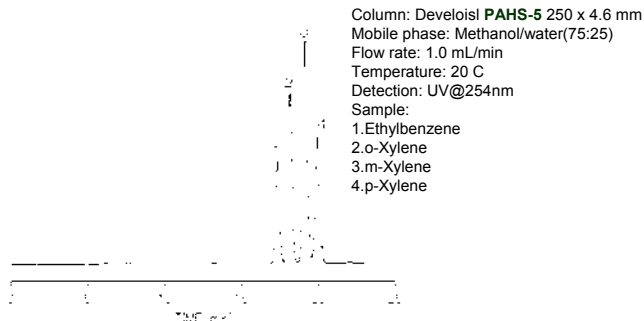
Column: Develosil **ODS-HG-5** 150 x 4.6 mm  
 Mobile phase:  
 A) Acetonitrile/20 mM phosphoric acid (10:90)  
 B) Acetonitrile/5 mM octansulfonic acid sodium salt + 20 mM phosphoric acid (10:90)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV @250 nm  
 sample:  
 1. Copper 8-quinolinolate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

## Separation of agricultural chemicals 2



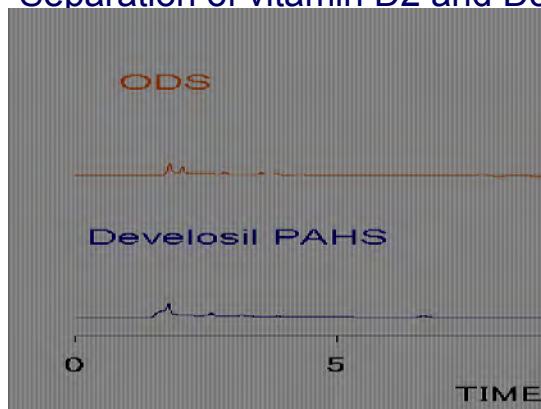
Column: Develosil **ODS-HG-5** 150 x 4.6 mm  
 Mobile phase: Acetonitrile/water (55:45)  
 Flow rate: 1.0 mL/min  
 Temperature: 40°C  
 Detection: UV @230 nm  
 sample:  
 1. Bis(dimethylthiocarbamoyl)disulfide (Thiram)  
 2. Iprodione  
 3. 0,0-Di-isopropyl S-2-benzensulphonamidoethylphosphorodithioate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

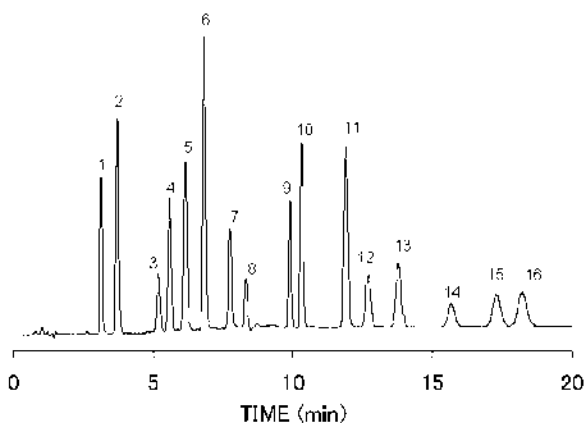
## Separation of vitamin D2 and D3



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

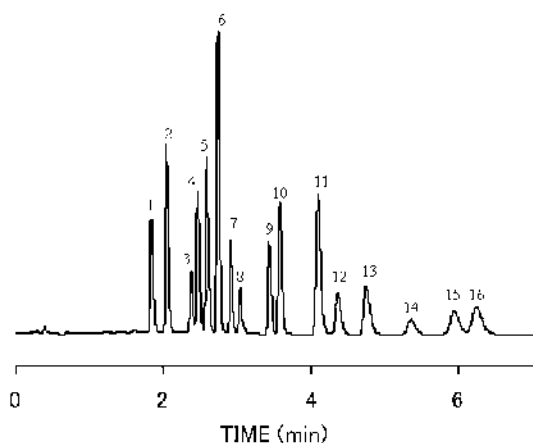
Time	0 min	3.4 min	8.1 min	20min
%B	80%	80%	100%	100%

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%

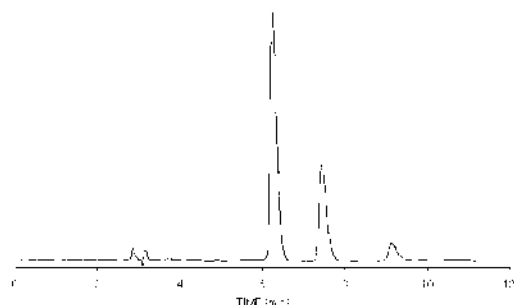
Flow rate: 1.5 mL/min

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 benzalkonium chloride



Column: Develosil **CN-UG-5** 250 x 4.6 mm

Mobile phase: Methanol/100mM CH<sub>3</sub>COONa, pH5.5=70:30

Flow rate: 1.0 mL/min

Temperature: 30 C

Detection: UV@265nm

Sample:

1=benzalkonium chloride



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