High purity silica gel base HPLC column

Mightysil

Kanto Reagents

Kanto Kagaku

Mightysil Series

In the Mightysil series lineup, a C8 and C4 with lower hydrophobicity is also offered mainly with silica gel based reversed-phase fillers, particularly for ODS fillers that are mostly used for general purposes. Including the test items concerning the elution behavior of each filler batch which were implemented in the GP series, additional items by analysis subject according to its application were also implemented for the columns by application.

GP Series

Mightysil RP-18GP Ⅱ	This is a difunctional ODS column which consists of an octadecylsilyl (ODS) agent bonded with 2 adjacent silanol groups.
Mightysil RP-18GP	This is high performance filler which is chemically bonded with monofunctional ODS group.
Mightysil RP-18GP Aqua	This is an ODS filler which demonstrates a stable retention time when using up to 100% water-based solutions as eluents. This filler is effective particularly for analysis using a high polarity eluent, such as for the separation of samples with high hydrophilicity.
Mightysil RP-18(H)GP Mightysil RP-18(L)GP	Compared with the conventional Mightysil RP-18 GP, this is an ODS filler introduced with octadecyl ligands in a high density (H) or low density (L).
Mightysil RP-8GP Mightysil RP-4GP	This filler consists of a highly purified spherical silica gel chemically bonded with the octyl group and butyl group. Since the hydrophobicity ligands are shorter than the RP-18 (ODS), the retentivity becomes weaker for substances with a high level of hydrophobicity.

Filler Name	Particle Size (μm)	Pore Size (nm)	Specific Surface Area (m^2/g)	Modification Group	Carbon Content	Modification Type
Mightysil RP-18GP Ⅱ	5	14	270	C18	15%	Difunctional
Mightysil RP-18GP	3, 5	12.5	330	C18	19%	Monofunctional
Mightysil RP-18GP Aqua	3, 5	13.5	270	C18	15%	Monofunctional
Mightysil RP-18(H)GP	5	12.5	390	C18	23%	Monofunctional
Mightysil RP-18(L)GP	5	13.5	270	C18	15%	Monofunctional
Mightysil RP-8GP	5	12.5	330	C8	12%	Monofunctional
Mightysil RP-4GP	5	12.5	330	C4	4%	Monofunctional

Column by Use, Normal Phase Column

Mightysil RP-18MS (Support LC/MS)	This is an improved column of the conventional "Mightysil GP" series, and can be used reliably for LC/MS. This column achieved lower back pressure, while maintaining high theoretical plates. Moreover, the effluent which can be observed at the initial usage of the column has been reduced to the utmost, by revising the cleaning process and filling method of the filler.
Mightysil RP-18PA (For analysis of agricultural chemicals)	This ODS filler is for analysis of agricultural chemicals where the nonspecific adsorption of oxine copper has been suppressed to an extremely low level, by revising the deactivation method of the residual silanol groups. This filler can be used as a common ODS column, and can also be reliably used for other analyses of agricultural chemicals.
Mightysil DNPH	This column is for DNPH aldehydes measurement.
Mightysil Si60BDF	This column is for petroleum products test.
Mightysil NH_2	This filler which monomeric modified aminopropylsilyl based on a high purity silica-gel. Aminopropyl type filler is often used in the analysis of the saccharide in particular, but is also applied as the weak anion exchange filler and the reverse phase separation which used specific selectivity.
Mightysil Si60	This column is normal phase column using purity over 99.99% of high purity silica-gel.

Filler Name	Particle Size (μm)	Pore Size (nm)	Specific Surface Area (m^2/g)	Modification Group	Carbon Content	Modification Type
Mightysil RP-18MS	5	12.5	330	C18	18%	Monofunctional
Mightysil RP-18PA	3、5	13.5	270	C18	15%	Monofunctional
Mightysil RP-18DNPH	5	12.5	330	C18	19%	Monofunctional
Mightysil Si60BDH	5	6	525	-	-	-
Mightysil NH_2	5	7	525	NH_2	-	Monofunctional
Mightysil Si60	5	6	525	-	-	-

Mightysil GP

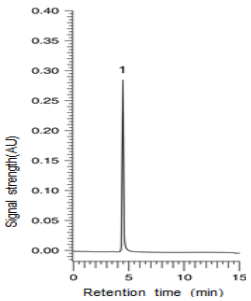
Supporting analysis validation Reversed-phase HPLC column

High purity silica gel･Full end capping Reversed-phase fillers

The "Mightysil GP" series uses a highly purified silica gel with an extremely low amount of metal impurities, and the polar group on the surface of the filler has been deactivated to the utmost by the unique synthetic reaction of our company. This product can be used reliably for any analysis, without concerns about the physical properties of a sample.

High purity silica gel

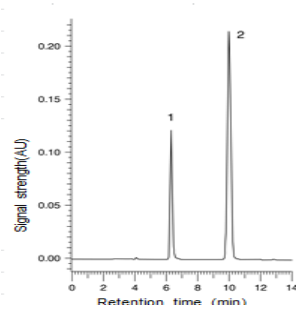
Since a silica gel with a purity of 99.99% (SiO_2) or more is used, it does not indicate nonspecific adsorption against metal coordination compounds.



Column; Mightysil RP-18 GP 150-4.6, 5μm
Eluent; Acetonitrile/ 20mM H_3PO_4=5/95
Flow rate; 1.0mL/min
Temp; 40℃
Detection; UV250nm
Inj. Vol; 5μL
Sample; 1. Oxine-copper 40μg/mL

Full end capping

The unique synthetic reaction of our company is used for the end capping of the residual silanol groups. Basic substances are not only detected as a sharp peak, but there is no change in the peak shape by the interaction with acidic substances which can be observed in some reversed-phase fillers.



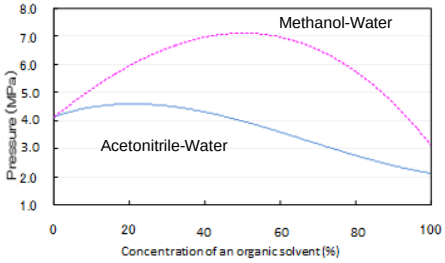
Column; Mightysil RP-18 GP 150-4.6, 5μm
Eluent; Methanol/ 20mM Buffer (pH7.6)=40/60
Flow rate; 0.5mL/min
Temp; 40℃
Detection; UV250nm
Inj. Vol; 5μL
□ample; 1. Procainamide 40μg/mL
2.Acetyl Procainamide 45μg/mL

*pH adjust; add 20mM $K_2HPO_4 \cdot 3H_2O$ to 20mM KH_2PO_4

High durability

The use of a true spherical silica gel selected by high precision classification provides sufficiently high theoretical plates under low pressure operation. Since the surface of the filler is covered to the utmost, outstanding stability is demonstrated from the acidic region to the basic region.

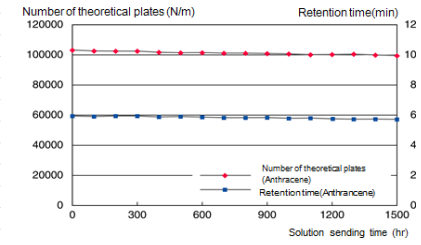
Variation of back pressure of column



Column; Mightysil RP-18 MS 150-2.0, 5μm
Flow rate; 1.0mL/min

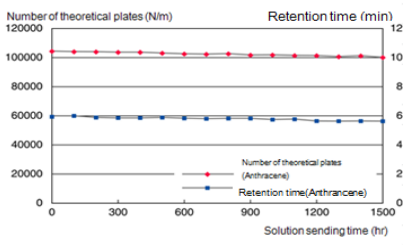
Durability test

Acidic eluent (pH1.5)



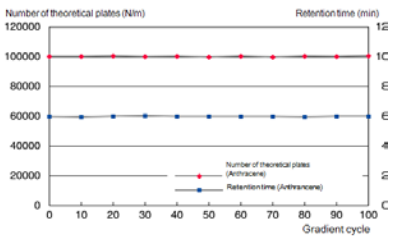
Column; Mightysil RP-18 GP 150-4.6, 5μm
Eluent; Methanol/ 0.3M H_3PO_4(pH1.5)=1/1
Flow rate; 1.0mL/min
Temp; Ambient
Sample; Anthracene

Basic eluent(pH10.0)



Column; Mightysil RP-18 GP 150-4.6, 5μm
Eluent; Methanol/ 20M K_2HPO_4(pH10.0)=1/1
Flow rate; 1.0mL/min
Temp; Ambient
Sample; Anthracene

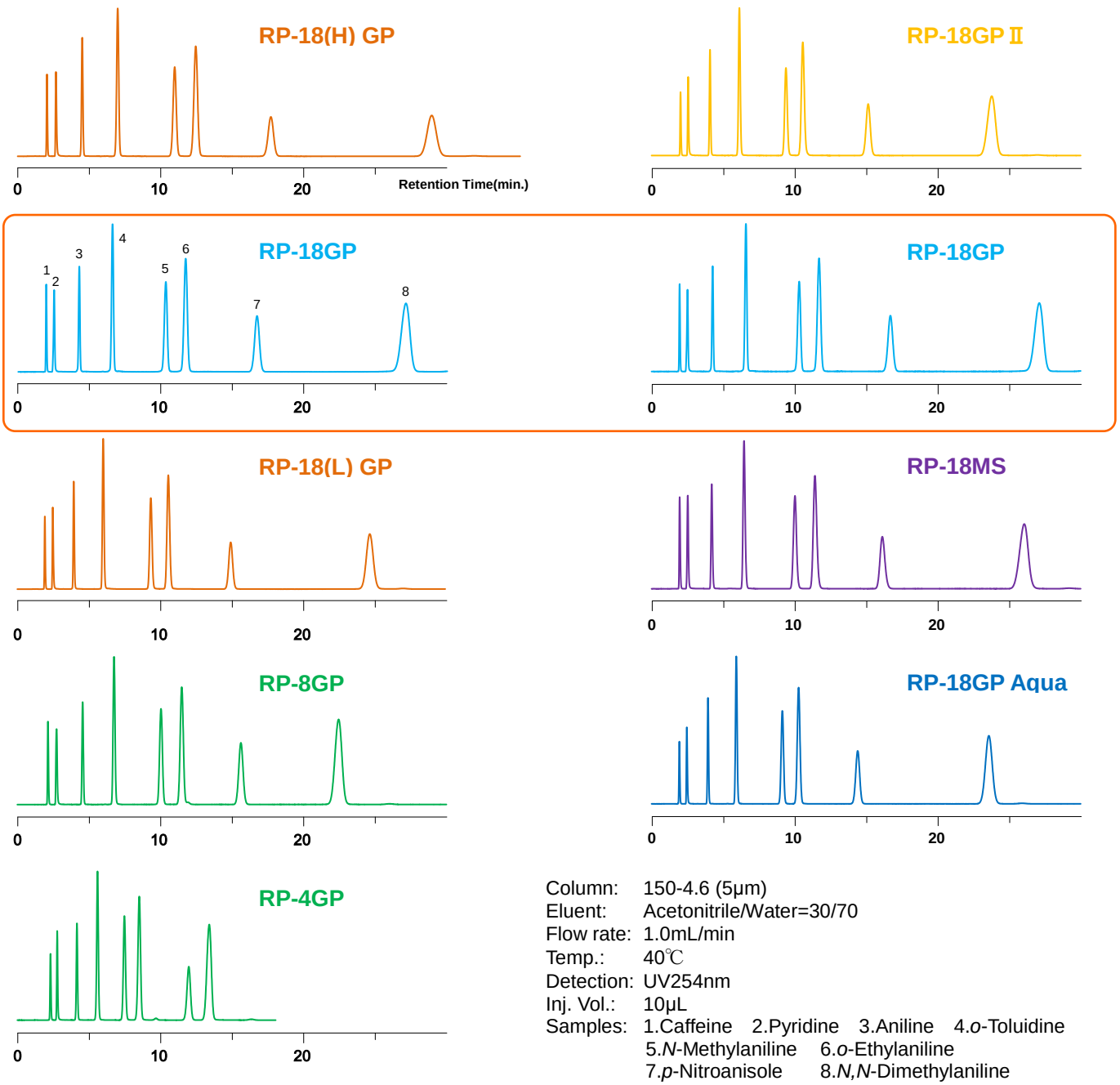
Gradient test



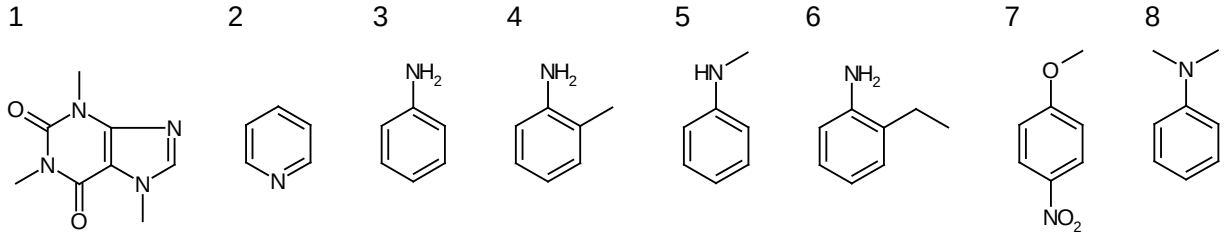
Column; Mightysil RP-18 GP 150-4.6, 5μm
Eluent; A; 10mM Phosphate buffer(pH4.7)
B; 20%Methanol
C; 80%Methanol
Gradient; A→B→C Linear; each 30min
Flow rate; 1.0mL/min
Temp; Ambient
Sample; Anthracene

Difference in separation characteristics

Retention Time



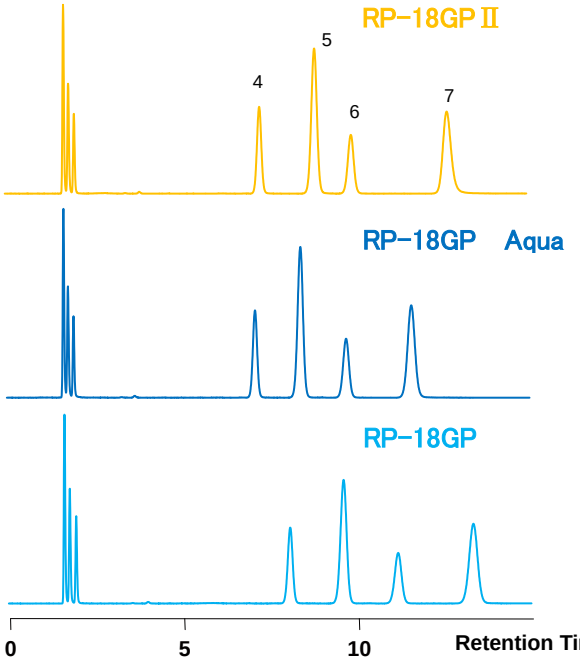
Column: 150-4.6 (5μm)
Eluent: Acetonitrile/Water=30/70
Flow rate: 1.0mL/min
Temp.: 40℃
Detection: UV254nm
Inj. Vol.: 10μL
Samples: 1.Caffeine 2.Pyridine 3.Aniline 4.*o*-Toluidine
5.*N*-Methylaniline 6.*o*-Ethylaniline
7.*p*-Nitroanisole 8.*N,N*-Dimethylaniline



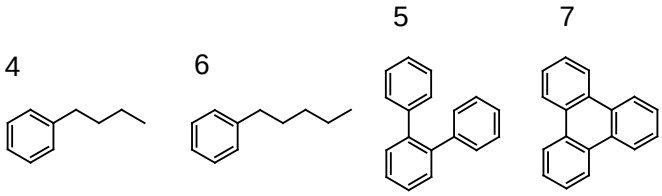
If the functionality of the modified group is the same, the retention time can be changed without impairing the stereoselectivity.
Since the hydrophobicity groups of the RP-8 and RP-4 fillers are shorter than the RP-18 (ODS), the retentivity becomes weaker against substances with a high level of hydrophobicity. In the case of a sample where the retentivity becomes excessive if an ODS column is used, the elution time can be reduced while maintaining a degree of separation by changing to the RP-8 or RP-4 column.

Difference in separation characteristics

Stereoselectivity



Column: 150-4.6 (5μm)
Eluent; Methanol/Water=80/20
Flow rate; 1.0mL/min
Temp.; 40℃
Detection; UV254nm
Inj. Vol.; 5μL
Samples: 1.Uracil, 2.Caffeine, 3.Phenol, 4.*n*-Butylbenzene, 5.*o*-Terphenyl, 6.*n*-Amylbenzene, 7.Tripheniylene



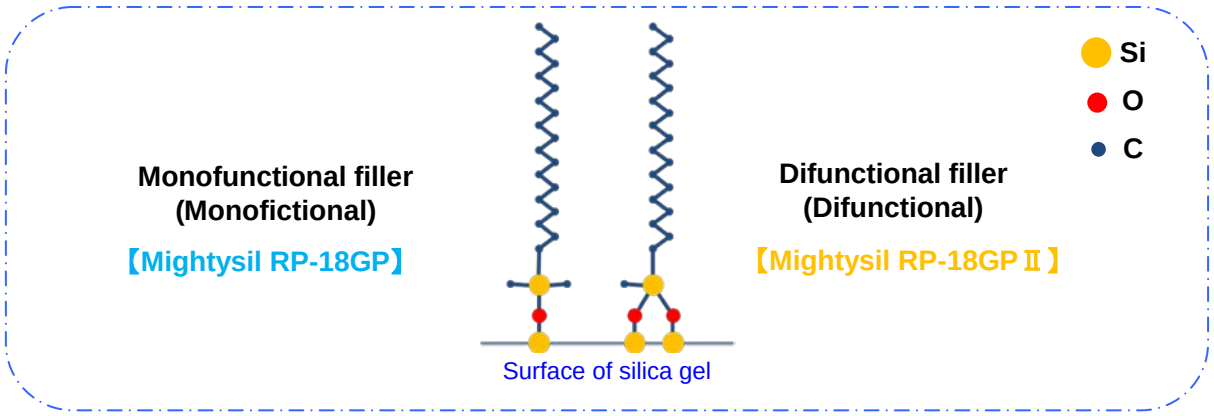
	RP-18GP Ⅱ	RP-18GP Aqua	RP-18GP
$k'_{Amylbenzene}$	4.92	4.99	5.98
$k'_{Amylbenzene}/k'_{Butylbenzene}$	1.47	1.47	1.48
$k'_{Triphenylene}/k'_{o\text{-}Terphenyl}$	1.53	1.47	1.47

In the above data, it is clear that the stereoselectivity of the difunctional ODS is higher than the monofunctional ODS.

(The base material physical properties are almost the same in the RP-18 GP and RP-18 GP Aqua.)

Difference in Mightysil RP-18GP・GP Ⅱ

■Figure of fillers



【Mightysil RP-18GP Ⅱ】

The ODS base of the monofunctional silica gel bonded with the silanol group 1:1, may be desorbed due to the base being hydrolyzed under acidic conditions. Accordingly, a worsening of the peak shape and reduction of the retention time occur, and the function as a filler may not be achieved sufficiently.

The difunctional ODS silica gel consists of an ODS group bonded with a silanol group by about 1:2. Therefore, the hydrolysis tendency at the base can be suppressed even under acidic conditions, and chemical stability improves compared with a monofunctional ODS silica gel.

Since the Mightysil RP-18 GP Ⅱ is a difunctional ODS silica gel, stable analysis results can be acquired under acidic conditions compared with the Mightysil RP-18 GP.

Guarantee contents

Supporting in analysis validations in GLP･GMP

In analysis validations in GLP and GMP, it is necessary to prove the appropriateness of analytical methods. Since the filler physical properties and elution characteristics of the Mightysil GP series are uniform at a high level, it can be used reliably for any analysis that requires validation.

【Severe quality control】

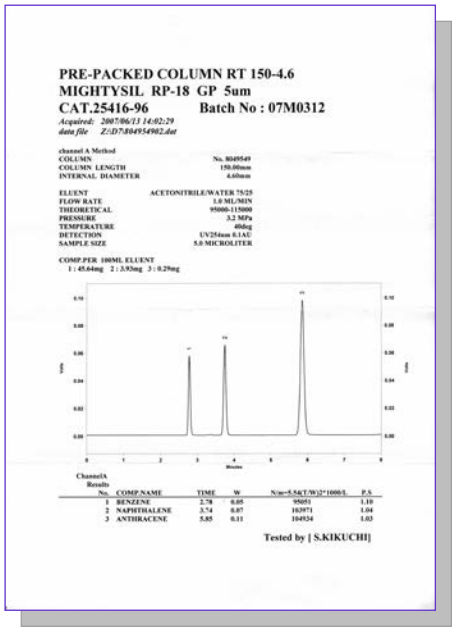
We set severe standards for the deviation between filler batches and between column lots regarding the factors which influence the analysis accuracy, and perform high precision filling. High repeatability of the characteristics is maintained for each filler batch and column lot, and columns with the same elution characteristics and separability can be used for long periods of time from the start of examinations of analytical methods to the end of a routine analysis.

【Three filler batches is prepared at all times】

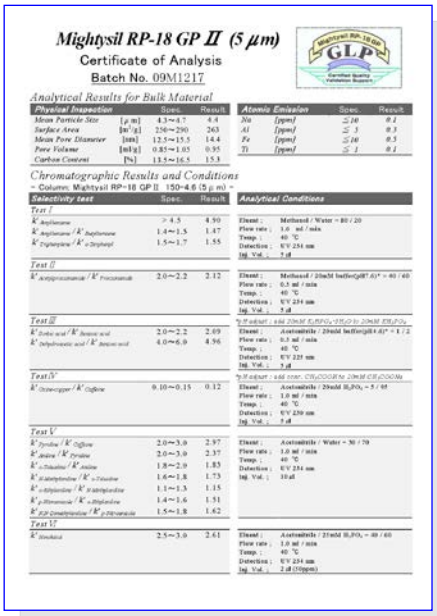
Since we have a structure to supply three columns with different filler batches at all times, the existence of differences between filler batches can be examined in advance, when examining the analytical methods of pharmaceuticals and etc.

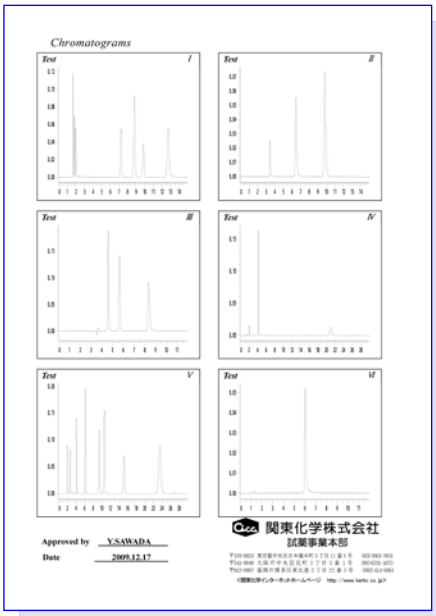
【Provide Certificate of Analysis】

The product is provided with an analysis table to prove the column filling condition, and a Certificate of Analysis (COA) to prove the physical properties of the filler and the elution characteristics, so that it can be used for various certifications of validation.


PRE-PACKED COLUMN RT 150-4.6
MIGHTYSIL RP-18 GP 5um
CAT.25416-96 Batch No : 07M0312

Tested by [S.KIKUCHI]


Mightysil RP-18 GP II (5 μm)
Certificate of Analysis
Batch No. 09M1217


Chromatograms

Approved by Y.SAWADA
Date 2009.12.17

関東化学株式会社
試薬事業本部

Filling condition【Theoretical plates】

Regarding the theoretical plates to be used as the index of the filling condition, the upper limit and lower limit are set to a standard value to control the difference between columns, and are provided with each column as an analysis table.

Test of physicochemical characteristics and the elution characteristics

Regarding the physicochemical characteristics and the elution characteristics of the filler itself, we conduct tests for each production of a filler, and the results are provided as a COA. In addition, the data is listed so that the elution characteristics can be determined from the chromatogram as well, not only by the characteristic values.

◆Test contents and standard value of each filler batches

Test of the physicochemical characteristics		RP-18	RP-8	RP-4	Measurement method
Mean Particle Size	[μm]	4.3~4.7			Coulter counter method
Surface Area	[m²/g]	300~360			BET method
Mean Pore Diameter	[nm]	11~14			
Pore Volume	[mL/g]	0.9~1.15			
Carbon Content	[%]	18.0~20.0	11.0~13.0	3.5~5.5	Element analyzing method
Na	[ppm]	≦20			ICP method
Al	[ppm]	≦5			
Fe	[ppm]	≦10			
Ti	[ppm]	≦1			

Test of the elution characteristics	RP-18	RP-8	RP-4	General description
***Test I* Hydrophobicity and stereoselectivity of the filler**				
$k'_{Amylbenzene}$	>5.5	>2.5	>1.0	The characteristics concerning the hydrophobicity and stereoselectivity of the filler are determined from the results of the elution of the aromatic hydrocarbons with different chain lengths of the side chain and spatial structure.
$k'_{Amylbenzene}/k'_{Butylbenzene}$	1.4~1.5	1.2~1.5	1.1~1.4	
$k'_{Triphenylene}/k'_{o\text{-}terphenyl}$	1.4~1.5	-	-	
***Test II* The degree of achievement of the end cap**				
$k'_{Acetylprocainamide}/k'_{Procainamide}$	1.9~2.2	1.8~2.2	1.9~2.3	The degree of achievement of the end cap for the residual silanol groups is determined from the elution behavior of strong base substances, such as procaineamide and acetylprocaineamide.
***Test III* Elution behavior of acidic substances**				
$k'_{Sorbic\ acid}/k'_{Benzoic\ acid}$	2.0~2.5	1.8~2.3	1.8~2.3	The peak tailing of acidic substances is rarely observed in a reversed-phase column. We have confirmed that there is no nonspecific interaction between three types of typical acidic substances and the filler.
$k'_{Dehydroacetic\ acid}/k'_{Benzoic\ acid}$	5.0~6.0	4.0~5.0	4.0~5.0	
***Test IV* Elution behavior of Complex compounds(1)**				
$k'_{Oxine\text{-}Copper}/k'_{Caffeine}$	0.10~0.15	0.1~0.2	0.04~0.07	Complex compounds, such as oxine copper and etc. are classified as metal impurities in the base silica gel, and may generate tailing. The Mightysil GP can be used reliably even for the separation of complex compounds.
***Test V* Elution behavior of basic substance**				
$k'_{Pyridine}/k'_{Caffeine}$	2.3~2.6	2.3~2.6	1.7~2.0	The strong base dimethylaniline is one of the substances which tend to change the peak shape by the effects of the residual silanol group. Since the elution behavior of eight typical substances has already been confirmed, it can be used reliably for analysis of basic samples including pharmaceuticals.
$k'_{Aniline}/k'_{Pyridine}$	2.6~2.9	2.5~2.8	2.2~2.5	
$k'_{o\text{-}Toluidine}/k'_{Aniline}$	1.8~2.0	1.6~1.9	1.5~1.7	
$k'_{N\text{-}Methylaniline}/k'_{o\text{-}Toluidine}$	1.6~1.8	1.5~1.8	1.3~1.6	
$k'_{2\text{-}Ethylaniline}/k'_{N\text{-}Methylaniline}$	1.1~1.3	1.0~1.3	1.0~1.3	
$k'_{3\text{-}Nitroanisole}/k'_{2\text{-}Ethylaniline}$	1.3~1.5	1.3~1.6	1.4~1.7	
$k'_{N,N\text{-}Dimethylaniline}/k'_{3\text{-}Nitroanisole}$	1.7~1.9	1.4~1.7	1.0~1.3	
***Test VI* Elution behavior of Complex compounds(2)**				
$k'_{Hinokitiol}$	3.0~3.5	2.8~3.1	1.6~1.9	We conduct elution behavior tests of complex compounds, such as hinokitiol for the same purpose as Test IV.

◆Test contents and standard value of filling condition

Guaranteed item		RP-18	RP-8	RP-4	General description
Guaranteed theoretical plates ($N_{Anthracene}/m$)	150-4.6 (5μm)	95,000~ 115,000	95,000~ 115,000	90,000~ 110,000	The upper and lower limits are set for the theoretical plates.
	100-1 (3μm)	70,000~ 90,000	-	-	
Symmetry (*Anthracene*)		0.9~1.4			

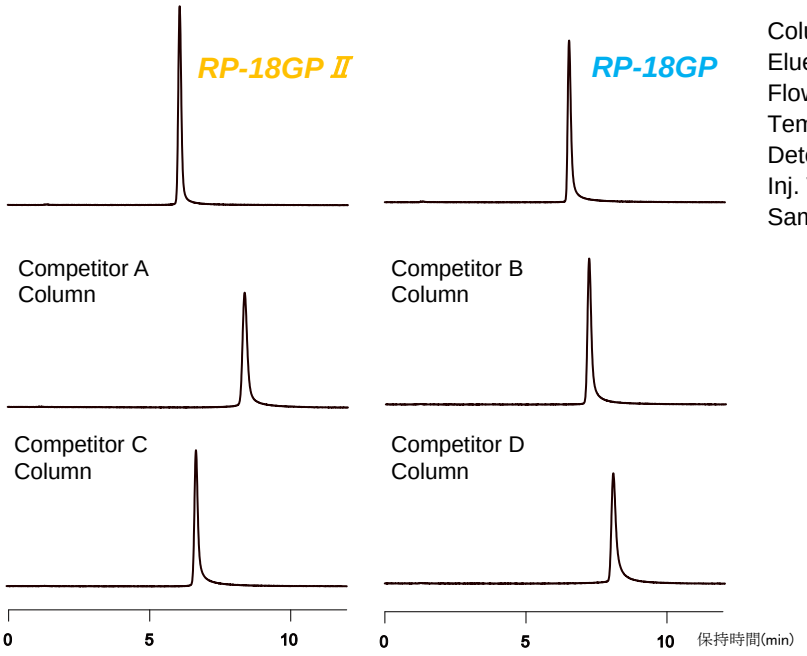
Mightysil RP-18 GPⅡ

Difunctional ODS silica gel

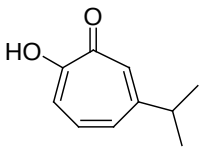
The "Mightysil RP-18 GPII" is a difunctional ODS column which consists of an octadecylsilyl (ODS) agent bonded with 2 adjacent silanol groups. Since the end capping is optimized to deactivate the residual silanol groups to the utmost, the peak tailing is suppressed to a minimum. Moreover, the high sensitivity of the hinokitiol which is known as a coordination compound was realized.
This product is excellent in durability and can be used for high precision analysis therefore it can be used reliably for analysis of extensive samples, including samples separated by the conventional "Mightysil RP-18 GP."

High sensitivity

■Comparison with other products　< coordination compound (Hinokitiol)>



Column：　150-4.6 (5μm)
Eluent：　25mM Phosphoric acid/Acetonitrile=60/40
Flow rate：　1.0mL/min
Temp.：　40℃
Detection：　UV254nm
Inj. Vol.：　2μL
Sample：　Hinokitiol 50μg/mL

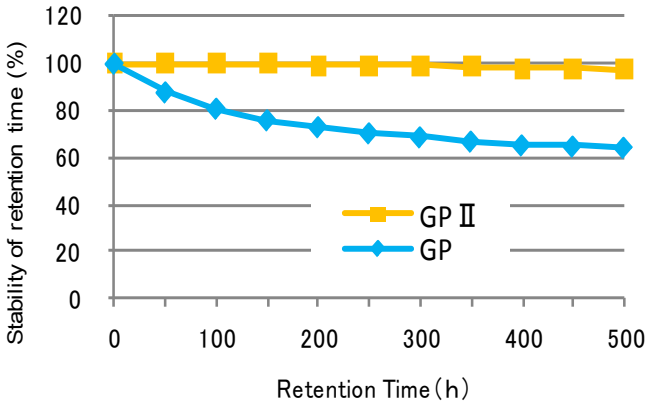


Hinokitiol

Hinokitiol	RP-18GP Ⅱ	RP-18GP	A	B	C	D
Theoretical plate number	104160	102257	87140	97560	114957	90473
Peak symmetry	1.49	3.23	1.61	3.49	3.40	1.92

Excellent durability

Generally, the ODS silica gel filler is known to be hydrolyzed by a trifluoroacetic acid (TFA) solution, and deteriorates the column performance irreversibly. Compared with the monofunctional ODS silica gel "Mightysil RP-18 GP," since the "Mightysil RP-18 GPII" consists of a structure where the ODS group is not separated easily, it demonstrates excellent durability even under severe acidic conditions, and stable retention times can be obtained.



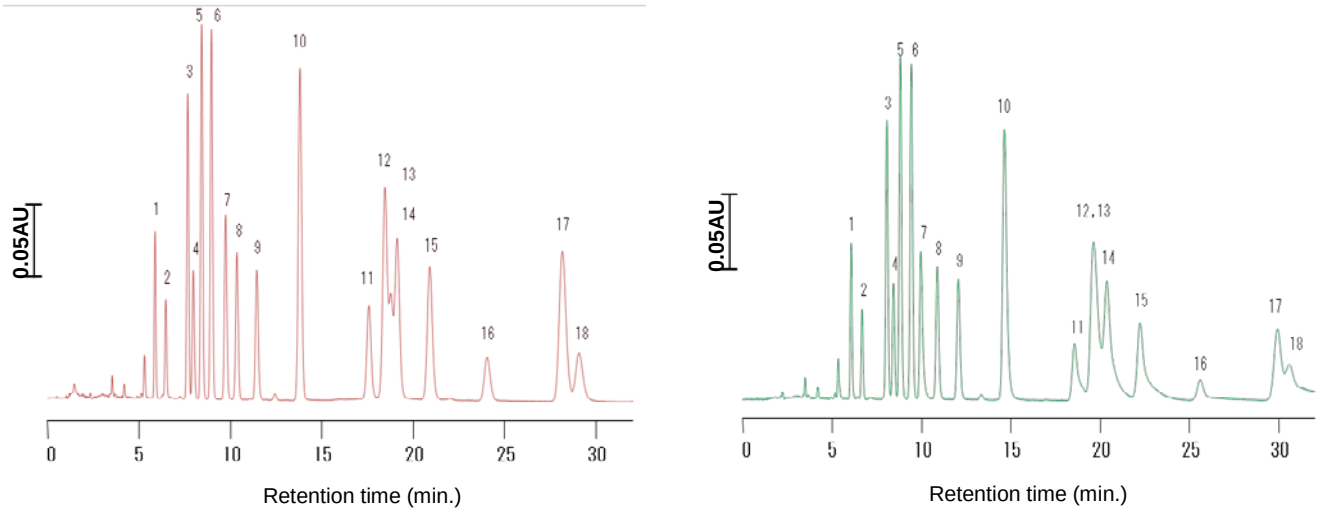
After a **0.5%** TFA solution was supplied to the column for sufficient replacement, the column was sealed and left for a predetermined time at a **room temperature of 15 to 20 °C**. After a predetermined time, the retention time of the anthracene was measured, and compared with the initial retention time.

Column：　Mightysil RP-18GP Ⅱ　150-4.6 (5μm)
　　Mightysil RP-18GP 150-4.6 (5μm)
Eluent：　Acetonitrile/Water=75/25
Flow rate：　1.0mL/min
Temp.：　40℃
Sample：　Anthracene

Difference of stereoselectivity

Comparison of RP-18GP Ⅱ and RP-18GP

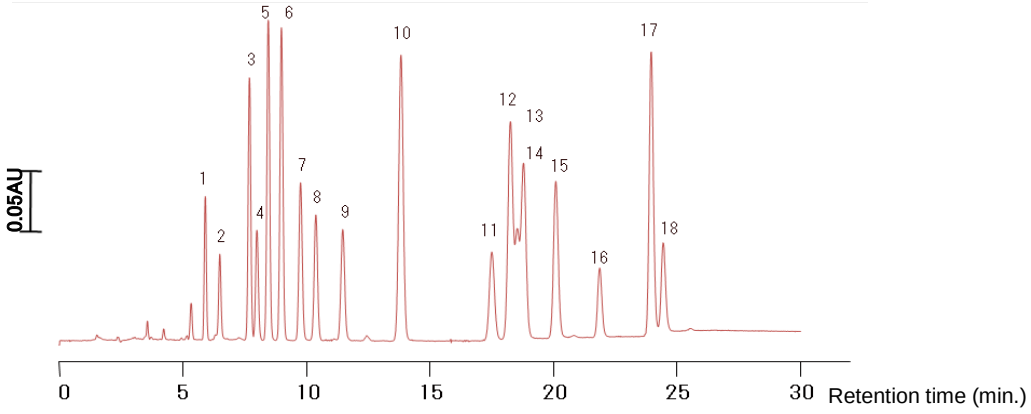
Mightysil RP-18GP Ⅱ	Mightysil RP-18GP



Column: 250-4.6(5μm)
Eluent: Acetonitrile / Water = 75 / 25
Flow rate: 1.0mL / min
Temp: 40℃
Detection: UV254nm

Application DATA (Gradient analysis)

■Analysis of PAHs



Column: Mightysil RP-18GP Ⅱ 250-4.6 (5μm)
Eluent: Acetonitrile/Water=75/25（20min.）→90/10（10min.）
Flow rate: 1.0mL/min
Temp.: 40℃
Detection: UV254nm
Inj. Vol.: 10μL

Samples:	1.Naphthalene	0.4μg/mL	2.Acenaphthylene	0.4μg/mL
	3.Fluorene	0.2μg/mL	4.Acenaphthene	1.0μg/mL
	5.Phenanthrene	0.1μg/mL	6.Anthracene	0.1μg/mL
	7.Fluoranthene	0.2μg/mL	8.Benzo[a]anthracene	0.2μg/mL
	9.Pyrene	0.2μg/mL	10.Chrysene	0.2μg/mL
	11.Benzo[j]fluoranthene	0.2μg/mL	12.Benzo[b]fluoranthene	0.2μg/mL
	13.Benzo[k]fluoranthene	0.2μg/mL	14.Benzo[e]pyrene	0.2μg/mL
	15.Benzo[a]pyrene	0.2μg/mL	16.Dibenz[a,h]anthracene	0.2μg/mL
	17.Indeno[1,2,3-cd]pyrene	0.2μg/mL	18.Benzo[g,h,i]perylene	0.2μg/mL

Mightysil RP-18 GP Aqua

ODS filler which is analyzed by a 100% water

The *"Mightysil RP-18 GP Aqua"* is a ODS filler which was realized high stability and reproducibility with 100% eluent by controlling water repellent phenomenon of surface of filler. It can be used at any organic solvent mixing ratio which is contained 100% water therefore it can be used reliably for separation of extensive samples from separation of hydrophobic compound to hydrophilic compound.

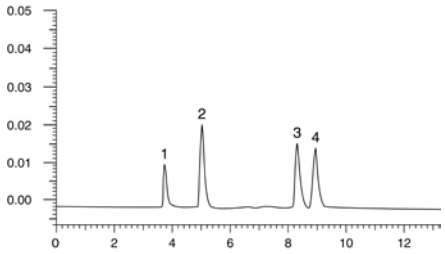
▼Water repellent phenomenon of filler

Since a common ODS filler has a high level of hydrophobicity which is a characteristic of the ligands, there are cases where the water repellency of the surface becomes a problem when water passes through. Although a stable retention time can be acquired in the beginning when water starts passing through, a sharp deterioration of the retention ability is observed with the progression of time.

Separation of hydrophilic compound

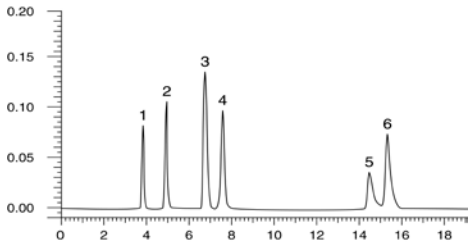
【Mightysil RP-18 GP Aqua 250-4.6(5μm)】

Separation of amino acid



Eluent; Water 100%
Flow rate; 1.0mL/min
Detection RI
Temp; 35℃
Inj. Vol; 20μL
Sample; 1.Alanine (0.1mg/mL) 2.Valine (0.2mg/mL)
3.Isoleucine (0.2mg/mL) 4.Leucine (0.2mg/mL)

Separation of nucleic acid



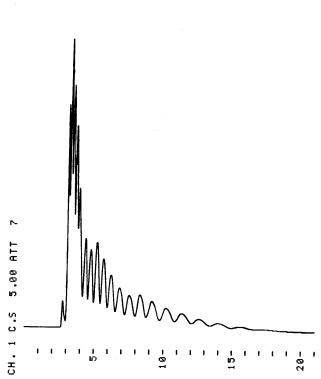
Eluent; 3mmol/L KH_2PO_4
Flow rate; 1.0mL/min
Detection; UV254nm
Temp; 35℃
Inj. Vol; 10μL
Sample; CMP, UMP, GMP, IMP, TMP□ AMP each 0.05mg/mL

Analysis of glucose oligomer

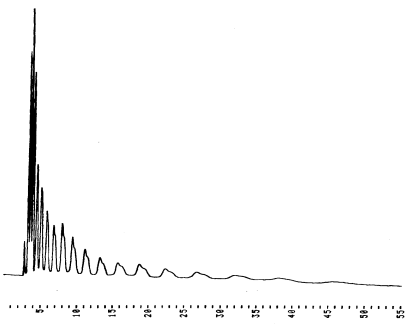
Flow rate; 1.0mL / min.
Detection; RI
Temp.; Room Temp.(24 ℃)
Inj. Vol.; 20μL
Sample; Glucose Oligomer Mixtures(DP4～20)
Commercial Product 25mg/mL

The retention time could be adjusted by slightly changing the acetonitrile concentration. The retention decreased along with the increase in the concentration of acetonitrile, and it was clear that separation was performed with the reversed-phase distribution mode.

① Eluent Water / Acetonitrile ＝ 100 / 2.5



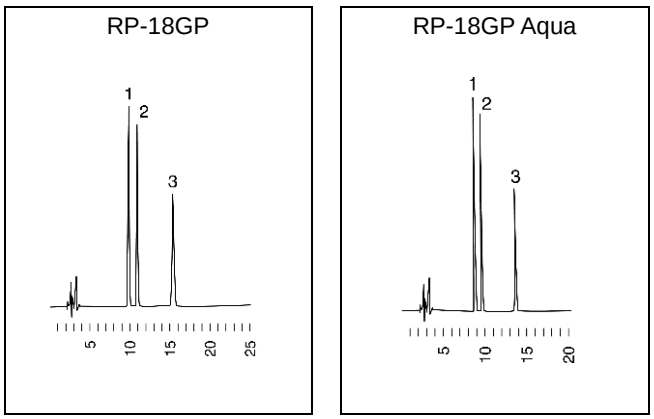
② Eluent Water / Acetonitrile ＝ 100 / 2



Correlation between RP-18GP-Elution of steroids

Equivalent selectivity could be demonstrated, by standardizing the modified ligands and the secondary processing method (end capping) after modification with the conventional "Mightysil RP-18 GP.

Eluent;	Acetonitrile/ 0.05mmol/L KH_2PO_4=30/70
Flow rate;	1.0mL/min
Detection;	UV245nm
Temp;	Ambient (22℃)
Inj. Vol;	20μL
Sample;	1. Cortisol
	2. Cortisone
	3. 6-α-Methylprednisolone
Column;	250-4.6mm, 5μm

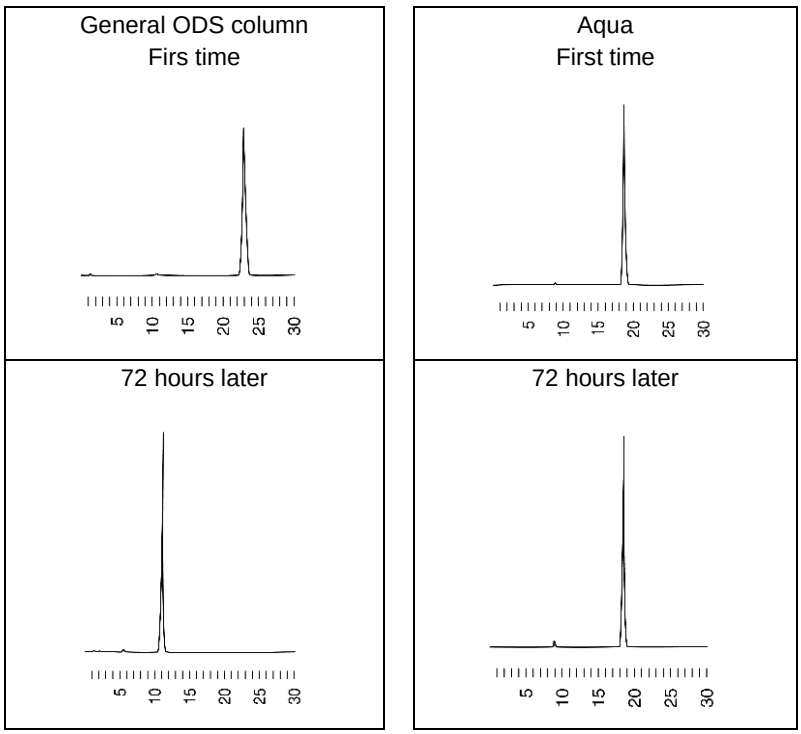


Stability

(1)Separation of dopamine

More stable retention could be demonstrated in a continuous liquid supply test where dopamine was used as a sample, by an eluent with high water content.

Column size;	250-4.6mm, 5μm
	20mM KH_2PO_4/ Methanol=99/1
Eluent;	including of 5mmol/L Sodium pentasulfonate
Flow rate;	1.0mL/min
Detection;	UV230nm
Temp;	40℃
Inj. Vol;	10μL
Sample;	Dopamine HCl 0.1mg/mL



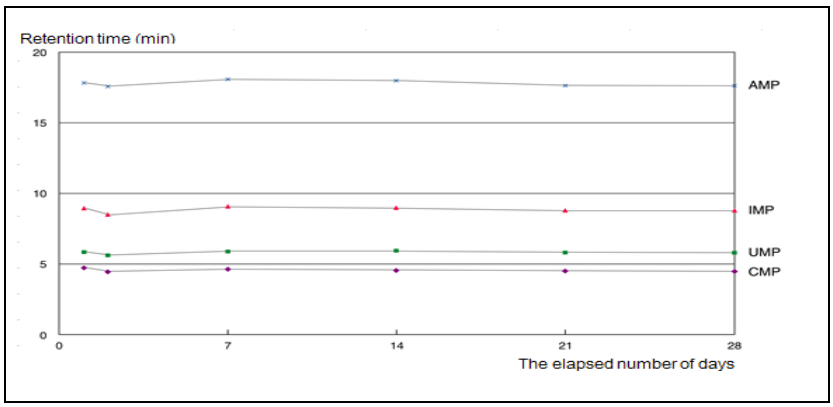
(2)Separation of nucleic acid

Five types of nucleic acids were separated under the conditions of a 100% water eluent, and the variation of the retention time of the "Mightysil RP-18 GP Aqua" was observed.
An initial value of 105.0% to 97.6% could be retained even after 28 days (after 672 hours), and stable separation could be acquired even with a 100% water eluent.

<Analysis condition>

Column;	Mightysil RP-18 GP Aqua 150-4.6mm, 5μm
Eluent;	5mM KH_2PO_4 buffer
Flow rate;	0.5mL/min
Detection;	UV254nm
Temp;	35℃
Inj. Vol;	5L
Sample;	Nucleotide each 0.05mg/mL

<Conditioning of column>
Acetonitrile/Water=50/50 60mL→Eluent 60mL



Mightysil NH_2

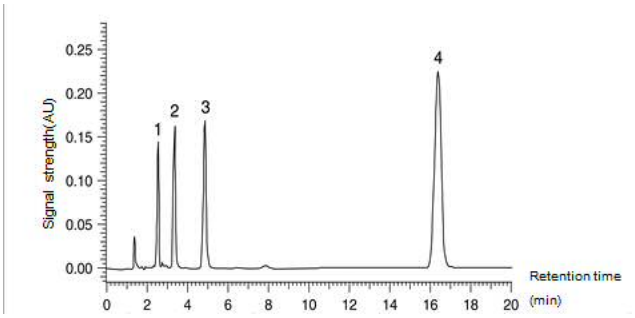
Aminopropylsilane silica gel filler

「Mightysil NH_2」 is filler which monomeric modified aminopropylsilyl based on a high purity silicagel. Aminopropyl type filler is often used in the analysis of the saccharide in particular, but is also applied as the weak anion exchange filler and the reverse phase separation which used specific selectivity. This filler uses high purity silicagel therefore it can be used in various fields without concerning about the nonspecific interaction by metal impurities.

Application

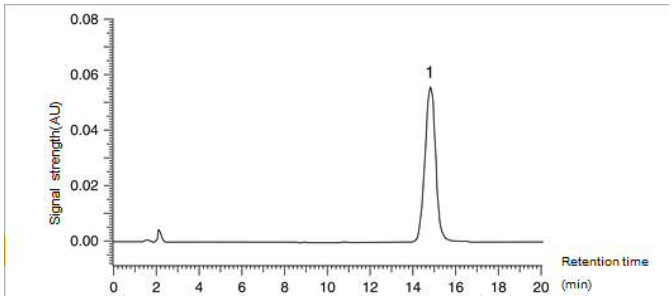
Separation of water soluble vitamin

Column ; Mightysil NH_2 150-4.6, 5μm
Eluent ; Acetonitrile/50mM $NH_4H_2PO_4$=70/30
Flow rate ; 1.0mL/min Detec ion ; UV210nm
Temp ; 40℃ Inj. Vol ; 10μL
Sample; 1.Riboflavine 20μg/mL 2.Pyridoxine HCl 20μg/mL
3.Nicotinic acid 20μg/mL 4.Folic acid 80μg/mL



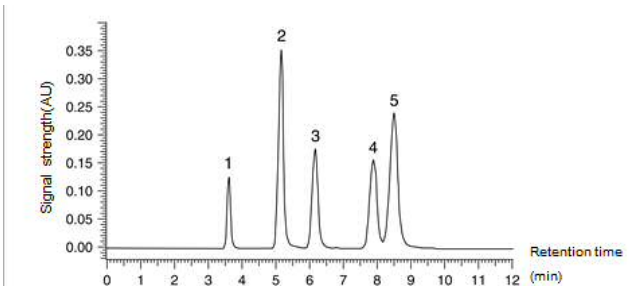
Separation of stevioside

Column ; Mightysil NH_2 150-4.6, 5μm
Eluent ; Acetonitirile/ Water= 80/ 20
Flow rat ; 1.0mL/min Detection UV210nm
Temp. ; 40℃
Inj. Vol ; 10μL
Sample ; 1.Stevioside 0.5mg



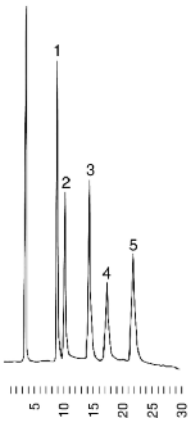
Separation of nucleotides

Column ; Mightysil NH_2 150-4.6, 5μm
Eluent ; 50mM Phosphate buffer (pH3 5)
Flow rate ; 1.0mL/min Detection ; UV254nm
Temp ; 40℃ Inj. Vol ; 10μL
Sample ; 1. 5'-CMP, 2. 5'-AMP, 3. 5'-UMP, 4. 5'-IMP, 5. 5'-GMP each 0.1mg/mL
*pH adjust; add Phosphric acid to 50mmol/L KH_2PO_4
(For buffer solution 100% of eluate, please note about handling of column)



Separation of saccharide

Column; Mightysil NH_2 250-4.6, 5μm
Eluent; Acetonitrile/ Water =75/25
Flow rate; 1.0ml/min
Detection; RI
Inj. Vol; 10μL
Sample : 1.Fructose
2.Glucose
3.Sucrose
4.Maltose
5.Lactose
each 10mg/mL



Handling of Aminopropyl type filler(NH_2)

, NH_2 filler is chemical modified by reactive aminopropyl group, therefore please note the following matters for the handling because of its nature.

(1) Type of organic solvent

The amino group is easily oxidized and reacts with aldehyde ketone. Therefore please do not use solvent including the peroxide and ketone solvent (acetone etc.).

(2) Using water

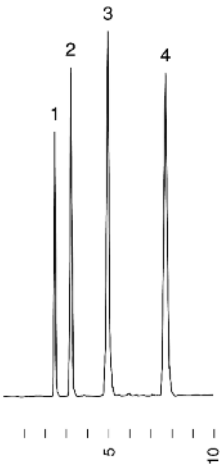
Water content in eluent is up to 30% because aqueous suspension of NH_2 filler shows weak basicity and using 100% water shortens the life of the filler. The durability may be improved by keeping pH of the eluent in the buffer solution the acidity under the condition of over 30% of water content, but we recommend to use it condition of less than 30% of water content to extend column life. Use it in the range of pH 2 ~ 7.5, and buffer solution concentration 50 ~ 200mmol/L is suitable.

Mightysil Si60

Silica gel 60 filler

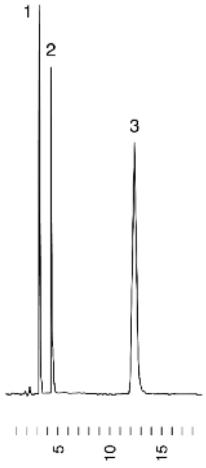
"Mightysil Si60" is a normal phase column using purity over 99.99% of high purity Silica gels. There are extremely low amount of metal impurities in fillers and there is no nonspecific interaction with the sample therefore it can be used reliably for every analysis.

Application



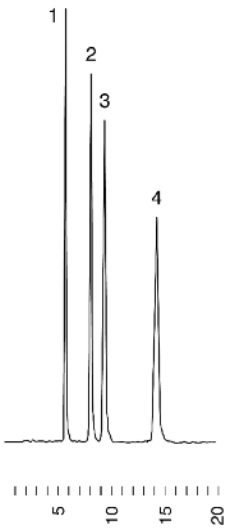
Separation of phthalate ester

Column;	Mightysil Si60 150-4.6, 5µm
Eluent;	n-Heptane / 2-Propanol = 99 / 1
Flow rate;	1.0mL/min
Detection;	UV254nm
Range;	0.32AUFS
Temp;	40℃
Inj. Vol;	5µL
Sample;	1.Dioctyl phthalate 2.Di-n-butyl phthalate 3.Dirth 1 phthalate 4.Dimethyl phthalate each 0.1% in heptane



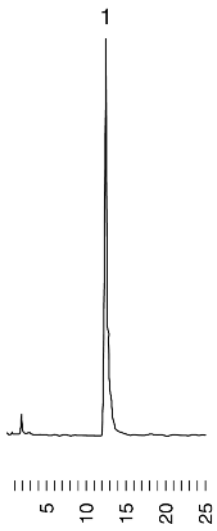
Separation of steroid

Column;	Mightysil Si60 150-4.6, 5µm
Eluent;	n-Hexane /Diethylether / 2-Propanol = 5 / 4 / 1
Flow rate;	1.0mL/min
Detection;	UV240nm
Range;	0.16AUFS
Temp;	40℃
Inj. Vol;	10µL
Sample;	1.Pr gesterone 0.02mg/L 2.Testosterone 0.02mg/L 3.Hydrocortison 0.1 mg/L



Separation of Vitamin E isomer

Column;	Mightysil Si60 150-4.6, 5µm
Eluent;	2,2,4-Trimethylpentane / Ethylacetate = 95 / 5
Flow ate;	1.0mL/min
Detection;	UV 94nm
Range;	0.08AUFS
Temp;	40℃
Inj. Vol;	10µL
Sample;	1.α-Tocopherol 2.β-Tocopherol 3.χ-Tocopherol 4.δ-Tocopherol each 0.1% in Trimethylpentane



Separation of catechin

Column;	Mightysil Si60 150-4.6, 5µm
Eluent;	n-Hexane / Ethylacetate= 37 / 63
Flow rate;	1. mL/min
Detection;	UV280nm
Range;	0.04AUFS
Temp;	40℃
Inj. Vol;	20µL
Sample;	1.Catechin 0.05mg/ml

Handling of silica gel filler

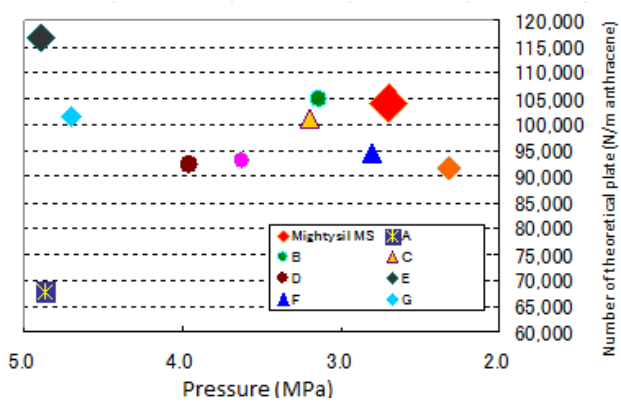
Since the relative retention in the separation by silica gel changes greatly with the moisture content in an organic solvent, it is necessary to pay attention to the water content in organic solvents to acquire separation with high repeatability. There is a possibility that eluents containing polar solvents, such as water, methanol and etc. deactivate the silanol groups on the surface of the silica gel irreversibly, and the column may not revert to its initial state in some cases. Therefore, we recommend that a dedicated silica gel column be prepared for each nonpolar solvent and polar solvent.

Mightysil RP-18MS

LC/MS support　Silica gel base ODS column

We suggest the HPLC column "Mightysil MS" which support becoming high sensitivity LC/MS analysis and is used reliably. We improve the conventional "Mightysil GP" series and realize lower back pressure with keeping high number of theoretical plates. It also reduces an effluent as much as possible which is seen at the beginning of use of column by reviewing a washing process and the filling method of the filler.

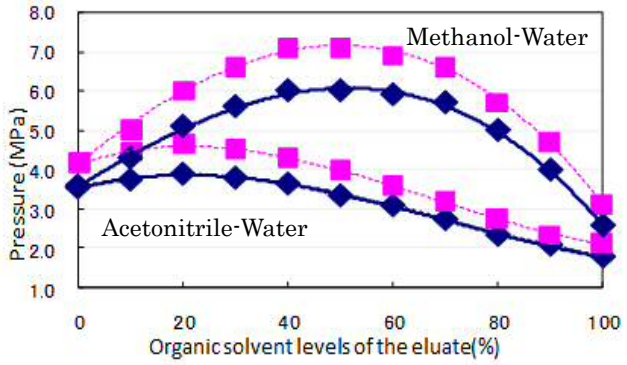
Number of theoretical plate･pressure　Compare with marketed



It has good balance of low back pressure and high number of theoretical plates and it is possible to analyze various analysis objects efficiently.

Column size: 150-4.6 (5μm)
Eluent: Acetonitrile/Water=75/25
Flow rate: 1.0mL/min
Temp. : 40℃
Detector: UV254nm
Inj. Vol.: 5μL
Sample: Anthracene 5μg/mL

Column back pressure fluctuation

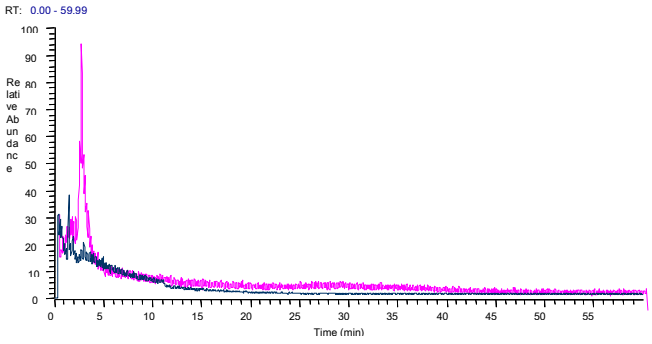


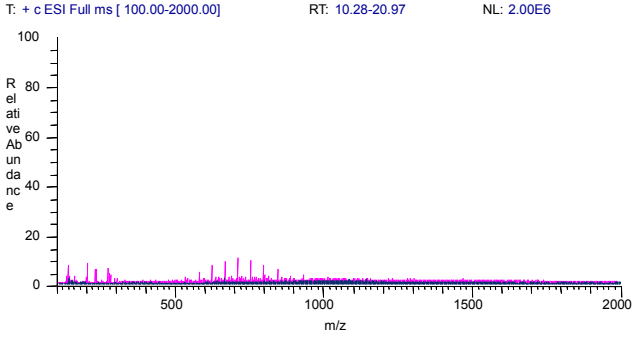
We succeeded in reducing 10% of column back pressure in using acetonitrile / water type compared with the GP series by improving classification accuracy of the "Mightysil GP" series. It also can be reliably used for the methanol / water type gradient analysis that cared back pressure.

Column: Mightysil RP-18MS 150-2 (5μm) ◆
Mightysil RP-18GP 150-2 (5μm) ■
Flow rate: 0.2mL/min
Temp.: 40℃

Test of effluent just after disclose a product

Apply washing and filling technology of the filler which was cultivated in the "Mightysil GP" series and designed optimization in consideration of LC/MS analysis. It reduce to elute dispersants used at column filling to the MS detecting parts as much as possible by solution sending just after the product is opened. It can analyze after opening the product soon without concerning about the pollution of the column.

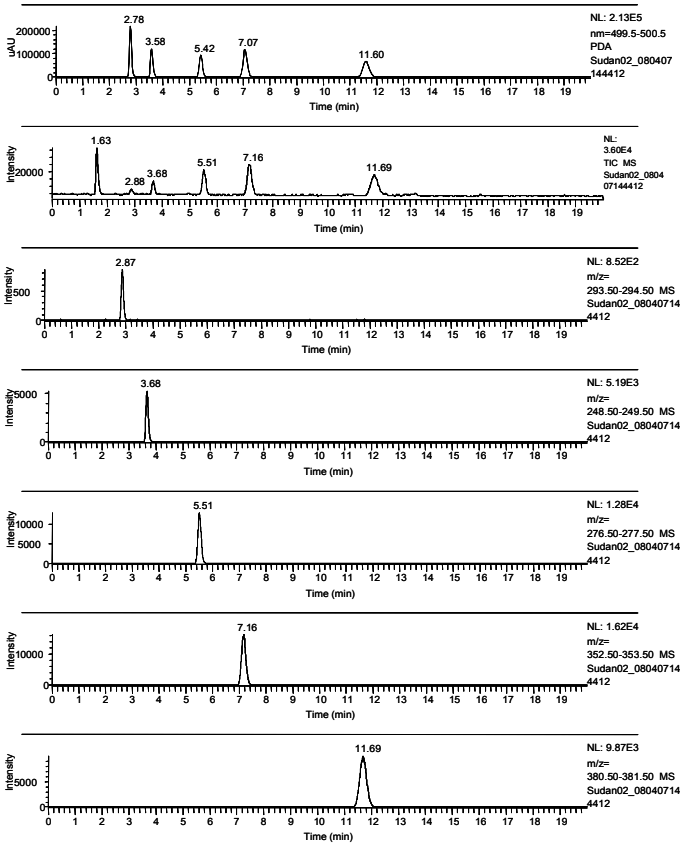




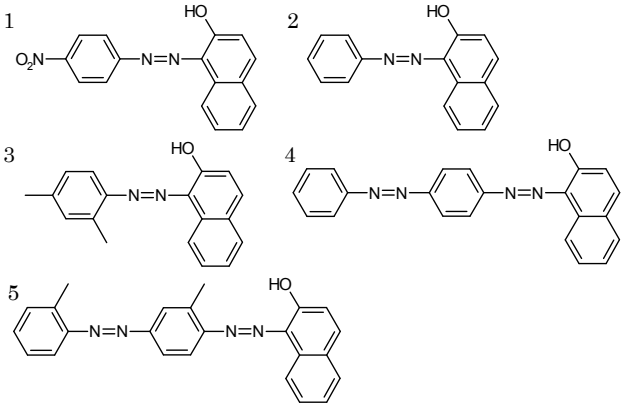
Column size: 150-4.6 (5μm)　Eluent: Acetonitrile 100%　—Purple : Mightysil GP
Flow rate: 1.0mL/min　Column temp.: 40℃　—Blue : Mightysil MS

Application

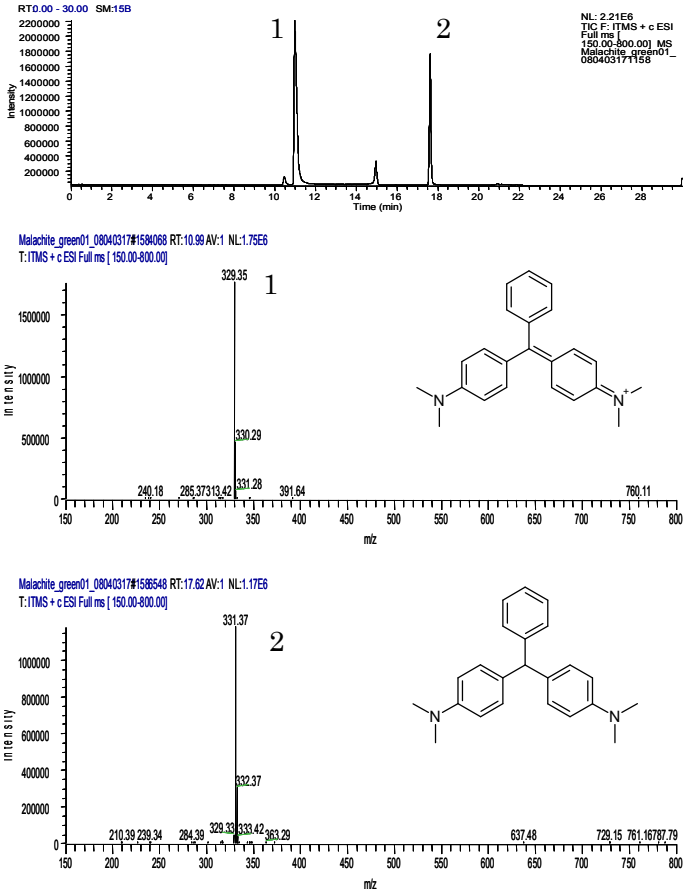
■Example of Sudan dyes analysis



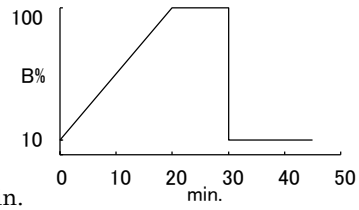
Column: Mightysil RP-18MS 150-2.0 (5μm)
Eluent: Acetonitrile/0.1%(v/v)Formic acid=90/10
Flow rate: 0.2mL/min
Detection: MS ESI+
Temperature: 40℃
Sample Vol.: 10μL
Samples: 1. Para red
2. Sudan Ⅰ
3. Sudan Ⅱ
4. Sudan Ⅲ
5. Sudan Ⅳ Each 5μg/mL



■Example of malachite green analysis



Column: Mightysil RP-18MS 150-2.0 (5μm)
Eluent: A.0.01mol/L Ammonium formate
B. Acetonitrile



Flow rate: 0.2mL/min.
Detection: MS ESI+
Temp.: 40℃
Inj. Vol.: 2μL
Samples: 1. Malachite green 0.05mg/mL
2. Leucomalachite green 0.05mg/mL

Mightysil RP-18 PA

Silica-based ODS column for pesticide analysis

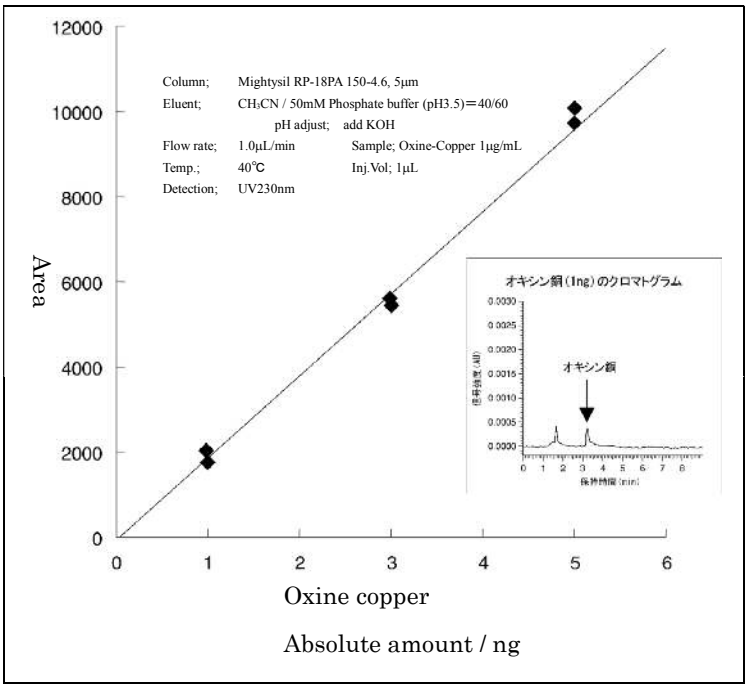
In recent years, the addition of the provisional instruction guidance to affect the setting of the pesticide residue standards, the revision of water quality standards, a golf course pesticide is carried out, and the importance of the pesticide analysis increases more and more. HPLC methods are used many as an analysis, but aggravation of quantitative caused by the nonspecific adsorption to the filler becomes the problem when analyzing silica-based ODS column in the analysis of oxine copper which is a golf course pesticide. "Mightysil RP-18 PA" is ODS column for pesticide analysis that controlled nonspecific adsorption of oxine copper in an extremely low level by reviewing the inactivating method of the residual silanol group. Of course you can use it as normal ODS column and you can confidence for other pesticide analysis. To have further patronage; thanking you in advance.

High quantitative

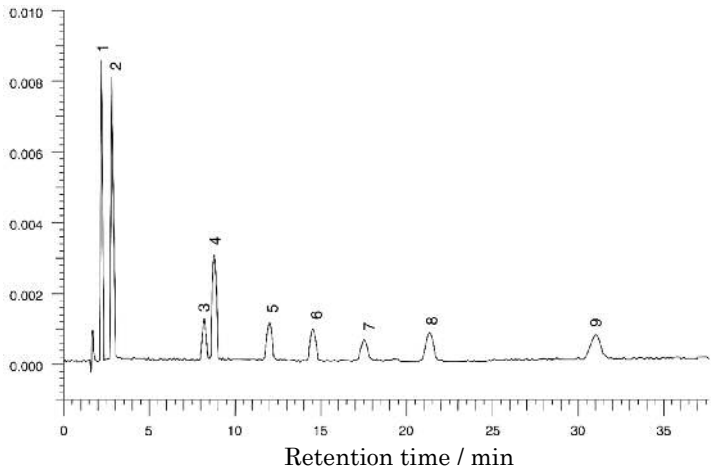
Low-concentrated oxine copper of absolute amount less than 1ng of the standard value is eluted well without adding EDTA to the elution. The metal impurities of filler or the adsorption of a coordination-related compound caused by column materials was being a problem in the silica base ODS column conventionally. These problems were solved by enough inactivation of the filler in "Mightysil RP-18 PA".

Great operability

In the analysis of the golf course pesticide, it is not necessary to add EDTA to the eluent. Not only save trouble of equilibration, but also other pesticide analyses can use it willingly. In addition, "Mightysil RP-18 PA" shows stable maintenance behavior under the eluent of 100% water, and even in gradient from 100% water in the simultaneous analysis can also get a stable result.



Simultaneous analysis of 9 golf course pesticide component (EDTA-free)
– Use Pesticides standard solution 24 (Cat.No.34023-96) –



Column; Mightysil RP-18 PA 150-4.6, 5μm
Eluent; CH_3CN/25mM Phosphate buffer(pH3.30)=7/13
Flow rate; 1.0mL/min
Temp; 40℃
Detection; UV 270nm(0～2.5min)
240nm(2.5～5.0min) 230nm(5.0～13.0min)
240nm(13.0～16.0min) 255nm(16.0～19.5min)
245nm(19.5～25.0min)
Inj. Vol; 5μL
Sample; 1. Asuluam 2. Oxine-Cupper 3. Triclopyr
4. Thiram 5. Mecoprop 6. Flazasulfron,
7. Siduron 8. Halosulfuron-methyl 9. Azoxystrobin
each 1μg/mL

＊Repeatability Guarantee

The repeatability between high column lots and between filler batches could be achieved for agricultural chemicals overall including oxine copper, by deactivating the residual silanol groups with sufficient repeatability. We perform tests on the elution behavior of agricultural chemicals including oxine copper for each filler batch, and guarantee repeatability so that the product can be used reliably for continuous monitoring.

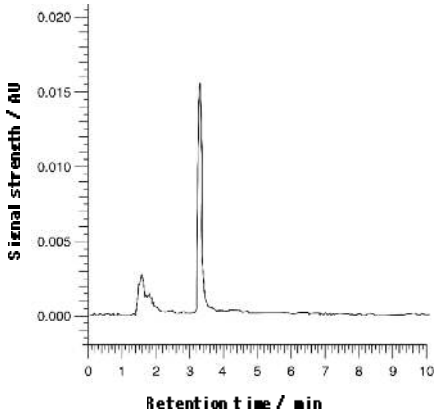
We also perform tests and confirmation of each filler batch regarding the characteristics (level of hydrophobicity, level of end capping achieved and the elution behavior of acidic substances) which a general purpose ODS column must be capable of.

For pH dependence of Oxine copper

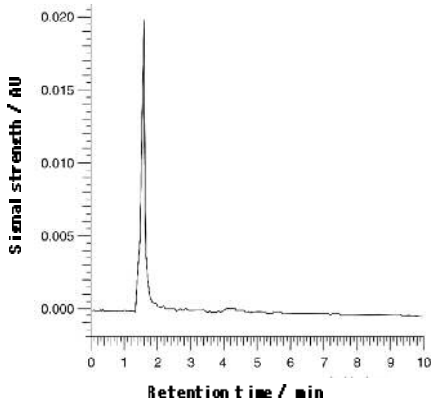
As for the coordination-related compounds such as oxine copper, a dissociation state is changed by pH of the solution in the property; as a result, pH of the eluent is also influence on peak shape. "Mightysil RP-18 PA" has a high inactivation of the residual silanol group, and, as a result, a change of the elution behavior of oxine copper by eluent pH is kept to a minimum.

Change of the peak shape of oxine copper by pH of the eluent

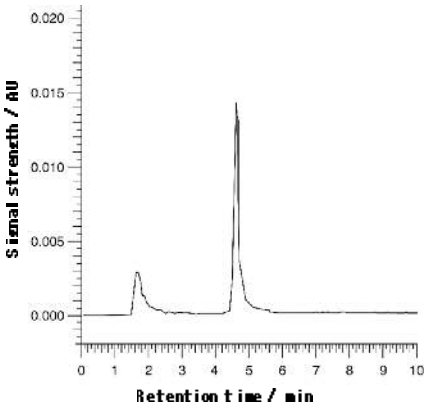
pH 2.1



pH 3.5



pH 4.5



Application

Analysis of Food analysis mixed standard solution 1 (Cat. No.16246-96)

-Analysis of nine kinds of sulfa drug -

Column; Mightysil RP-18 PA 150-4.6, 5μm

Eluent A; Acetonitrile/ Water/ Acetic acid = 10/ 90/ 0.3

B, Acetonitrile/ Water/ Acetic acid = 60/ 40/ 0.3

Gradient;

Min	A(%)	B(%)
0	100	0
25	7	25
30	75	25

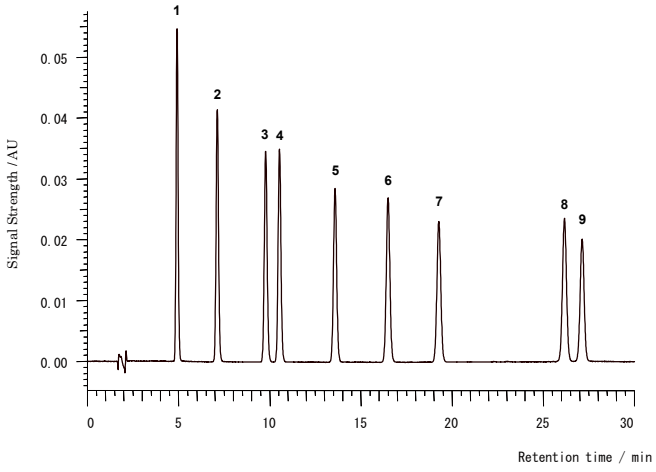
Flow rate; 1.0mL/min

Temp; 40℃

Detection; UV265nm

Inj. Vol; 5μL

Sample; 1. Sulfadiazine, 2. Sulfamerazine, 3. Sulfadimidine,
4. Sulfamethoxypyridazine, 5. Sulfamonomethoxine, 6. Sulfamethoxazole,
7. Sulfisoxazole, 8. Sulfadimethoxine, 9. Sulfaquinoxaline sodium salt
each 0.01mg/mL in Methanol



Analysis of Pesticides standard solution 43 (Cat.No.34042-96)

-7 kinds of food analysis LC subject -

Column; Mightysil RP-18 PA 150-4.6, 5μm

Eluent; Acetonitrile/ Water = 70/ 30

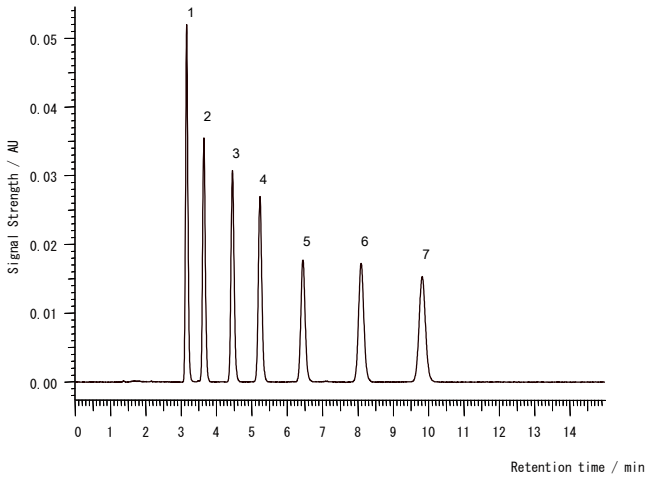
Flow rate; 1.0mL/min

Temp; 40℃

Detection; UV250nm

Inj. Vol; 5μL

Sample; 1. Diflubenzuron, 2. Tebfenozide, 3. Hexaflumuron, 4. Teflubenzuron,
5. Lufenuron, 6. Flufenoxuron, 7, Chlorfluazuron
each 10mg/L in Acetonitrile



Mightysil DNPH

Column of analyses Aldehyde for DNPH

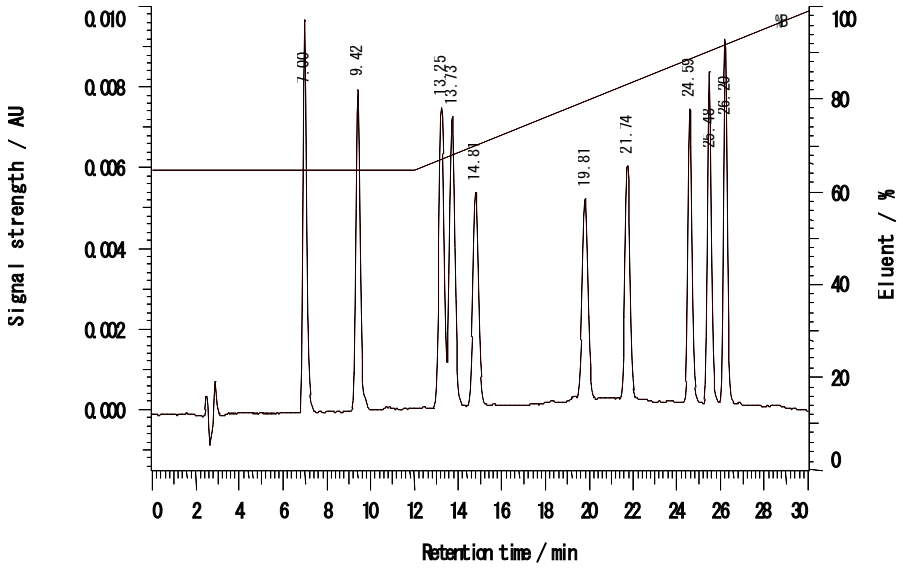
There are several types of aldehydes listed, as the chemical substance in the guidelines concerning indoor air pollution, as the specific offensive odor substances in the hazardous air pollutants and in the laws and ordinances and guidelines of the specific offensive odor substances. In the analysis of these aldehydes, the aldehyde is collected by a 2,4-DNPH impregnated cartridge adsorbent, and is derivatized into a DNPH compound. Subsequently, a separation analysis is performed on the derivatized aldehyde by gas chromatography or liquid chromatography.

In recent years, dedicated columns according to the separation application are being used, and the "Mightysil DNPH" also guarantees a performance specialized in the separation of DNPH aldehydes. The following introduces an example of an analysis when methanol is used as the eluent. In various official analytical methods, acetonitrile is used as an eluent however methanol can also be used to satisfactorily separate substances. Please use in combination with an aldehyde-DNPH standard stock solution to experience the performance of this product. We are looking forward to your patronage in the future.

Separation of aldehydes for DNPH

Column ; Mightysil DNPH 250-4.6
Eluent ; A : Water
B : Methanol
Flow rate ; 1mL/min
Detection ; UV360nm
Temp. ; 40℃
Standard; Aldehydes-2,4-DNPH standard stock solution 2 (Cat.No.01106-96)
Sample Conc. ; 1ppm
Sample Vol. ; 10μL

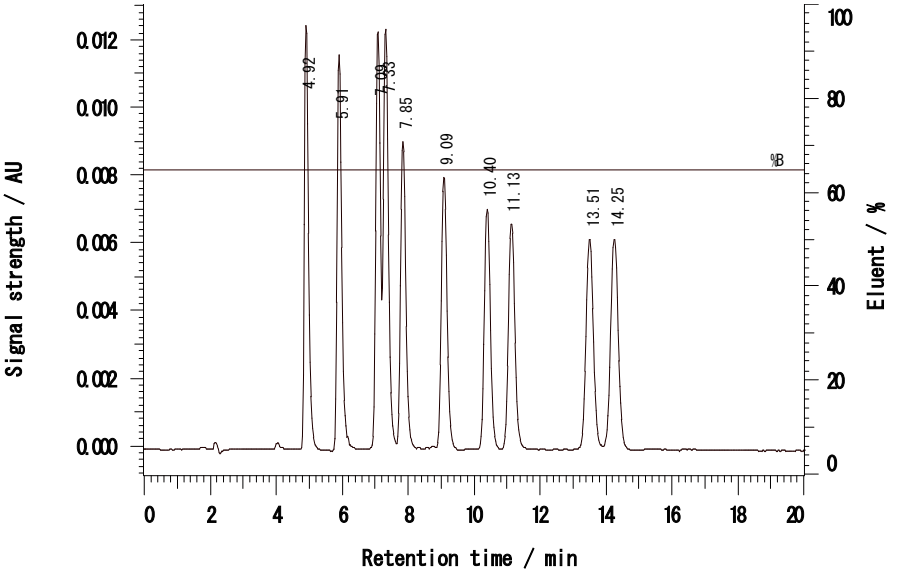
1.	Formaldehyde-DNPH	(7.00min)
2.	Acetaldehyde-DNPH	(9.42min)
3.	Acrolein-DNPH	(13.25min)
4.	Acetone-DNPH	(13.75min)
5.	Propionaldehyde-DNPH	(14.81min)
6.	Crotonaldehyde-DNPH	(19.81min)
7.	Butylaldehyde-DNPH	(21.74min)
8.	Benzaldehyde-DNPH	(24.59min)
9.	*iso*-Valeraldehyde-DNPH	(25.40min)
10.	Valeraldehyde-DNPH	(28.20min)



Analysis case in the official method

Column ; Mightysil DNPH 250-4.6
Eluent ; A : Water
B : Acetonitrile
Flow rate ; 1mL/min
Detection ; UV360nm
Temp. ; 40℃
Standard ; Aldehydes-2,4-DNPH standard stock solution 2 (Cat.No.01106-96)
Sample Conc. ; 1ppm
Sample Vol. ; 10μL

1.	Formaldehyde-DNPH	(4.82min)
2.	Acetaldehyde-DNPH	(5.81min)
3.	Acrolein-DNPH	(7.09min)
4.	Acetone-DNPH	(7.33min)
5.	Propionaldehyde-DNPH	(7.85min)
6.	Crotonaldehyde-DNPH	(9.09min)
7.	Butylaldehyde-DNPH	(10.40min)
8.	Benzaldehyde-DNPH	(11.13min)
9.	*iso*- Valeraldehyde-DNPH	(13.51min)
10.	Valeraldehyde-DNPH	(14.25min)



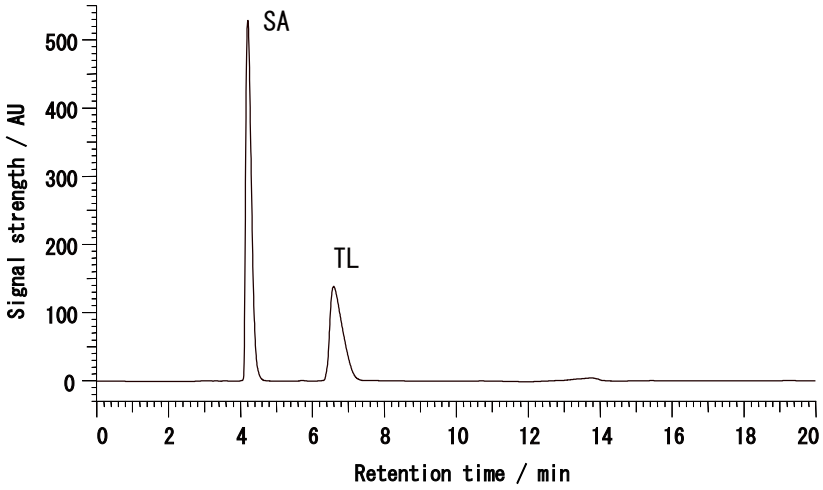
＊ We recommend that a 150 mm size be used for the analysis of formaldehyde and acetaldehyde, and a 250 mm size be used for the analysis of other various aldehydes.

Mightysil Si60 BDF

Column for oil product testing

Since 1997, an additional inspection item is set that regardless of including or not including BDF (Bio Diesel Fuel) on quality inspection of light oil. And the content of Fatty acid methyl esters (FAME) and triglyceride (TG) is carried out by HPLC method. "Mightysil Si60 BDF" is confirmed the separation performance by Methyl stearate (SA) and by trilinolein (TL)

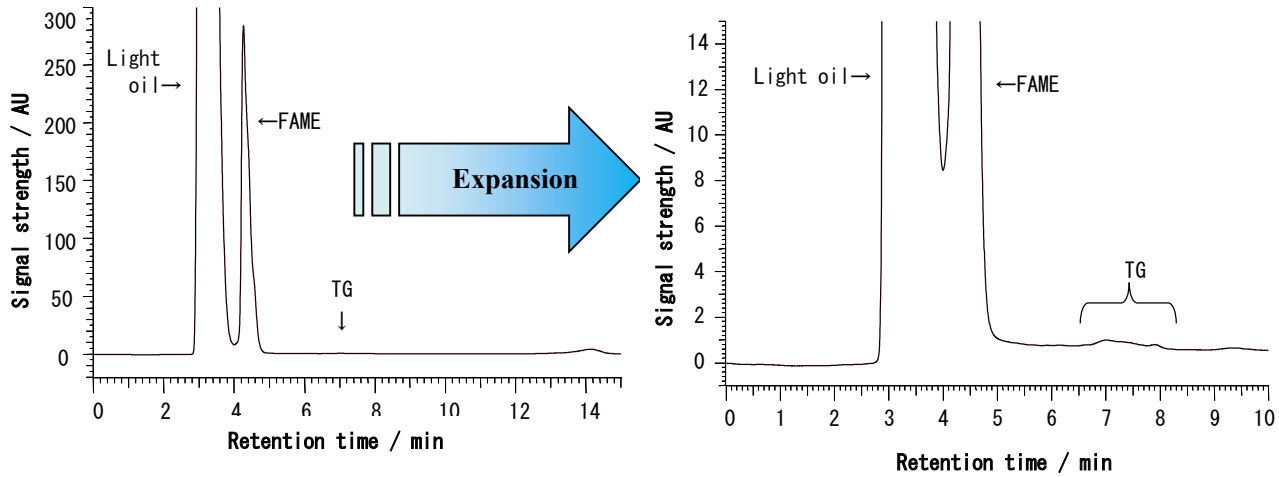
Additional inspection item	Light oil (BDF in)	Light oil (BDF out)	Analysis test method
Fatty acid methyl esters	Max. 5.0w%	Mas. 0.1w%	HPLC
Triglyceride	Max. 0.01w%	Mas. 0.01w%	



Column; Mightysil Si60 BDF 250-4.6, 5□m
Eluent; 0.4vol% 2-Propanol/Hexane solution (Cat.No.32459-79)
Flow rate; 1.0mL/min
Temp.; 40℃
Detection; RI
Inj. Vol.; 10□L
Sample; Methyl stearate (SA) 5g/50mL
Trilinolein (TL) 0.05g/50mL

Standard item	specification
Retention time of Methyl stearate (min)	Min. 3.5
Resolution of Methyl stearate and Trilinolein	Min. 3

Application



Column ;Mightysil Si60 BDF 250-4.6, 5□m
Eluent ;0.4vol% 2-Propanol/ Hexane solution (Cat.No.32459-79)
Flow rate ;1.0mL/min Temp. ;40℃
Detection ;RI Inj. Vol. ;10□L
Sample ;Light oil added 5% BDF

Handling of Mightysil

1. Inspection of column efficiency

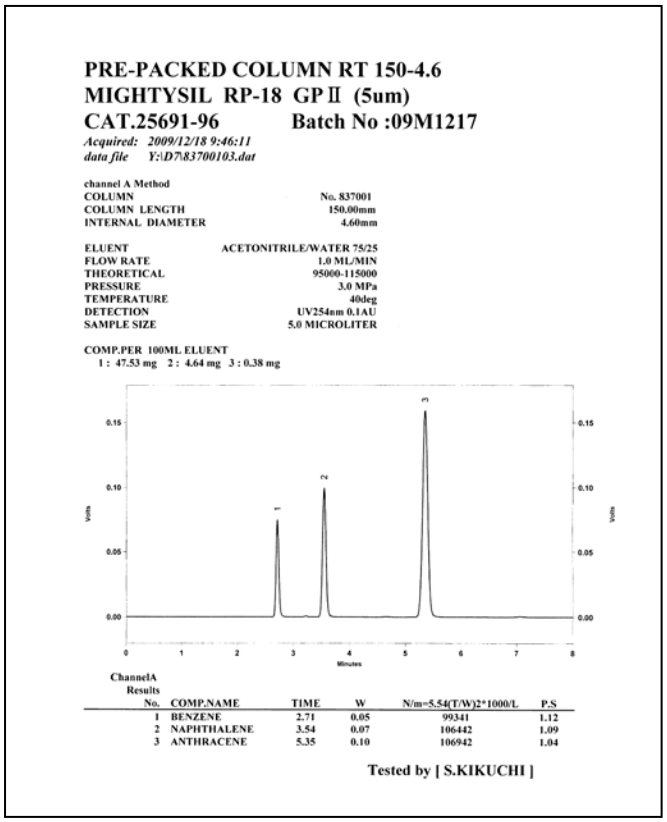
To take occasionally performance test among analysis, it is possible to validate whether the column will demonstrate expected performance. Two ways are considered

a) By shipping test condition

If the column is used for various methods, we recommend you validating the initial column efficiency under the condition of our shipping test method shown on the certificate included in the package. When you encounter loss of performance, by re-evaluating under the same test after sufficient washing of column – it is possible to find whether the problem was caused by degradation of column or not.

b) By system suitability test

When a column is used for one method only in the quality control activities, etc. of pharmaceuticals, we recommend that the column performance be evaluated by the system conformity test indicated in the standard operation procedure.



PRE-PACKED COLUMN RT 150-4.6
MIGHTYSIL RP-18 GP Ⅱ (5um)
CAT.25691-96 Batch No :09M1217
Acquired: 2009/12/18 9:46:11
data file Y:\D7\83700103.dat

channel A Method

COLUMN	No. 837001
COLUMN LENGTH	150.00mm
INTERNAL DIAMETER	4.60mm
ELUENT	ACETONITRILE/WATER 75/25
FLOW RATE	1.0 ML/MIN
THEORETICAL	95000-115000
PRESSURE	3.0 MPa
TEMPERATURE	40deg
DETECTION	UV254nm 0.1AU
SAMPLE SIZE	5.0 MICROLITER

COMP.PER 100ML ELUENT
1 : 47.53 mg 2 : 4.64 mg 3 : 0.38 mg

ChannelA
Results

No.	COMP.NAME	TIME	W	N/m=5.54(T/W)2*1000/L	P.S
1	BENZENE	2.71	0.05	99341	1.12
2	NAPHTHALENE	3.54	0.07	106442	1.09
3	ANTHRACENE	5.35	0.10	106942	1.04

Tested by [S.KIKUCHI]

1-1. The column is shipped with the following solvent.

	RP-18/RP-8 DNPH、(H)、(L)	RP-4	Si60/NH_2
Analytical (I.D. 1mm～3mm)	Acetonitrile / Water =75/25	Acetonitrile / Water =60/40	n-Heptane
Analytical (I.D. 4.6mm、6mm)		Acetonitrile / Water =75/25	
Preparative (I.D. 10mm～50mm)	Methanol / Water =80/20		

2. Starting Separation

2-1. Equilibrating

Generally 10times of column volume solvent is needed for each equilibration step, and takes it finished by gaining a stable base line or uniform retention of a standard sample.

2-1-1. Reversed Phase

a) Organic solvent/water	Consider if the mobile phase you use is miscible with solvent in the column, then purge the column until the base line is stabilized. If you want the organic component to change to something different (e.g. Acetonitrile→Methanol, etc.), it is advised to place organic 100% step in equilibration and subsequent stepwise increasing of water is followed. e.g.) Acetonitrile/Water=75/25 → Methanol 100% → Methanol/Water =60/40
b) Organic solvent/buffer	Basically same to a), in addition, the last step before the mobile phase should consist of organic/water, which should have the same ratio of eluent. Equilibration takes more time when the salt concentration is low (5～10mmol/L or less). e.g.) Acetonitrile/Water=75/25 → ～ → Methanol/Water=60/40 → Methanol/Buffer=60/40
c) Mobile phase of water or buffer is 100%, or of higher water content	The surface of RP-18 is highly hydrophobic so water 100% mobile phase naturally has a lower affinity to stationary phase. If the mobile phase you use has higher water content, we recommend gradually changing to the mobile phase by stepwise increases of water through organic solvent100%, to gain stable separation. In case of separation at water of 100%, it is advised to use "RP-18GP Aqua", which exhibits more stable retention in water at the 100% mobile phase. e.g.) ～ → Acetonitrile 100% → Acetonitrile/Water=50/50 → Water 100% → Buffer 100%
d) Mobile phase containing ion-pair reagent	Equilibration of the mobile phase containing ion-pair reagent requires amore longer time. In general, it often takes solvent over 100 ~ 200 times of column volume until completion. The procedure is as same as C). e.g.)Acetonitrile/Water =75/25→Acetonitrile/Water=90/10→Acetonitrile/Buffer(Ionpair) = 90/10

2-1-2. Normal phase (Silica)

Organic solvent	After confirmation that the present solvent in the column is miscible with the preferred mobile phase, purge until you gain a stable base line. e.g.) Heptane → Hexane/2-Propanol

2-1-3. NH_2

Organic solvent or Organic solvent/ Aqueous solvent	In case of non-polar solvent use only, equilibration is the same as silica. If you use the mobile phase with water or polar solvent like methanol, be aware that these solvents may not be miscible with the shipped one, you should place an intermediate step to avoid failure of equilibration. e.g.) Heptane → Ethyl acetate → Acetonitrile → Acetonitrile/Water=75/25 → Acetonitrile/Buffer=75/25

2-2. Caring for the column

<Mobile phase>

pH	Mightysil is a silica based material, so use between pH 2.0~ 7.5.
Quality of the solvent	HPLC grade solvents should be used and high purity reagents are recommended for buffer or ion-pair. Always filter the mobile phase through membrane filters of 0.45μm or under .
Degas	Always degas the mobile phase. Often, It is not sufficient to only use the online-degasser equipped with HPLC system.
Salt concentration	Salt concentration is preferable under 0.2mol/L. Confirm solubility of salts at changing mobile phases to prevent precipitation of buffer.

<Handling>

Prevention of channeling	Physical shock or extremely rapid change in pressure when starting to pump or changing to different mobile phases, will lead to channeling in the column and result in irreversible performance loss.
Pressure and flow velocity	Use the column under pressure of 20MPa (About 200kgf/cm^2 or 2900psi). Higher flow rate leads to void the column and result in performance loss.
Temperature	Do not exceed 80°C.

3. Washing or regenerating of the column

Generally 10 times of column volume solvent is used at each washing step, if not sufficient, wash until you gain a stable base line to complete elution of the contaminant.

3-1. Reversed phase (DNPH)

General	Use acetonitrile. For heavy contamination, Tetrahydrofuran can be used.
RP-18 GP Aqua	If 100% water is used, the contaminant of mobile phase is well adsorbed to stationary phase, so changing of retention or loss of performance tends to occur. In this case washing by acetonitrile will recover performance.
Column used with ion-pair reagent	Generally washing takes a long time to eliminate ion-pair reagent from the surface of the stationary phase. Follow these washing steps: to wash out the buffer purge the column 100 ~ 200times of column volume of solvent, desalt from the mobile phase, then rinse the column with 100% acetonitrile until a stable base line is obtained. e.g.) Acetonitrile/Buffer(Ion pair)=10/90 → Acetonitrile/Water= 10/90 → Acetonitrile 100%

3-2. Silica gel

	Wash with more polar solvent than actual used in the mobile phase. To avoid continuously deactivating silanol, the column used for the non-polar solvent system should not be rinsed with methanol except in special cases. e.g.) Heptane → Ethyl acetate → Acetone → cleaning solvent (2-Propanol) After washing, the column is re-equilibrated to the actually used mobile phase at procedure of 2-1-2 in reverse order, 15~20 times of column volume are purged at each step.

3-3. NH_2

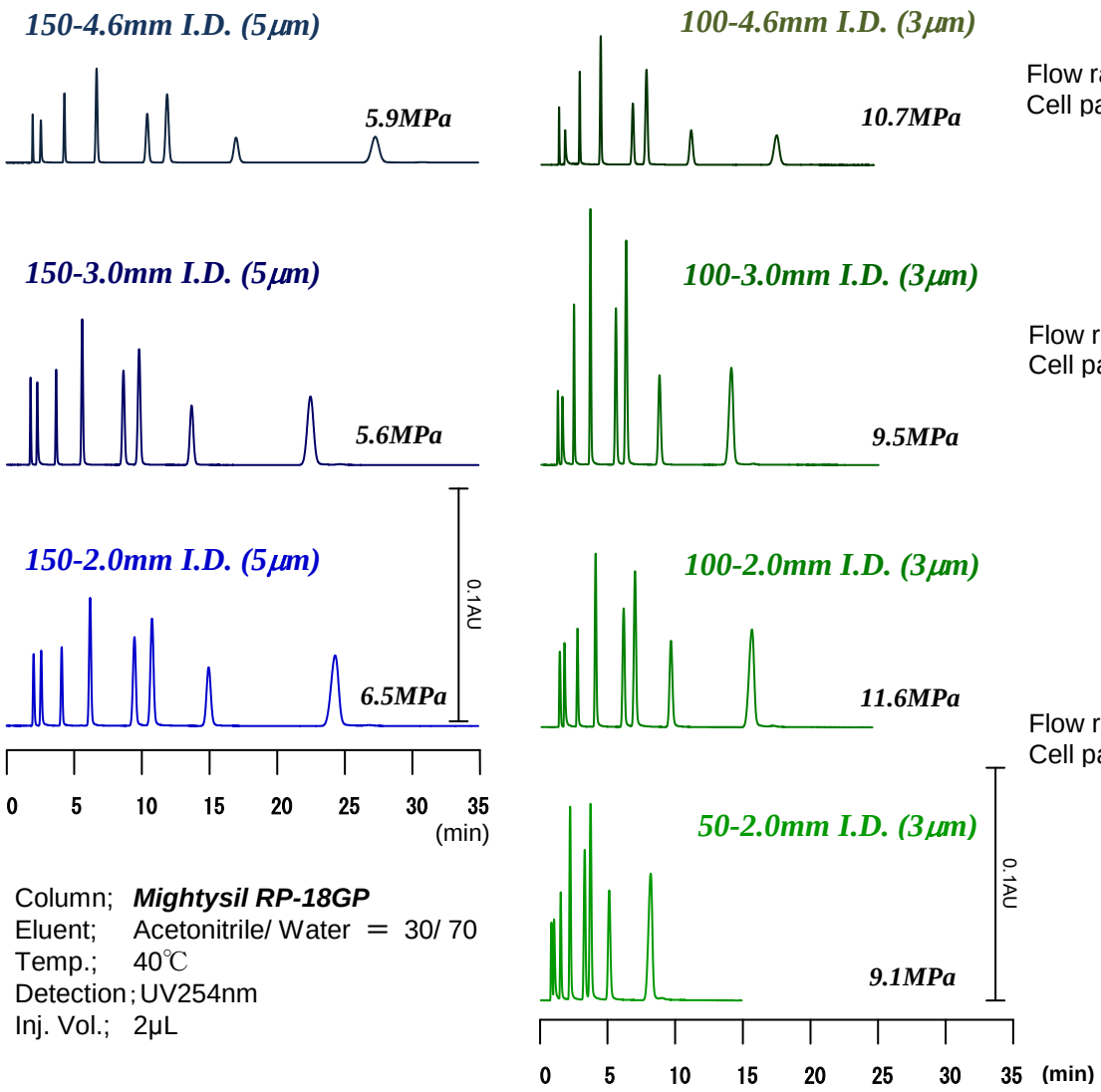
Normal phase solvent	Wash with polar solvent considering miscibility.
	e.g.) Heptane → Ethanol → washing solvent (Acetonitrile or Methanol)
Reversed phase solvent	If you need to wash with water, the content should be below 30%. The stationary phase will be damaged at over 30%. Complete washing within as short a time as possible if water above 30% is needed.
	e.g.) Acetonitrile/Buffer = 90/10 →Acetonitrile/Water = 90/10 → washing solvent (Acetonitrile or Methanol) → washing solvent (Acetonitrile/Water = 70/30) [→Acetonitrile/Water =50/50 or Water100% and etc.]

4. Storage

The column should be purged of the following organic solvents if you plan on storing it for a long period of time. Before storage, use the washing procedure. Be sure the end plugs are firmly in place, never let the column dry out. If the column is partially dried, purge with degassed solvent to recover performance of column.

Example of Reducing Diameter of a Column for General-purpose Analysis Columns (150-4.6, 5 μm)

When reducing the diameter of a column, the following chromatogram can be acquired by optimizing the flow rate and cell capacity of a detector.



Column; ***Mightysil RP-18GP***
Eluent; Acetonitrile/ Water ＝ 30/ 70
Temp.; 40℃
Detection; UV254nm
Inj. Vol.; 2μL

Samples;

1. Caffeine	35μg/mL	5. *N*-Methylaniline	50μg/mL
2. Pyridine	35μg/mL	6. 2-Ethylaniline	310μg/mL
3. Aniline	85μg/mL	7. 3-Nitroanisole	280μg/mL
4. *o*-Toluidine	250μg/mL	8. *N,N*-Dimethylaniline	70μg/mL

in Eluent

When you use the column of 1mm I.D. , 2mm I.D. , including the cell volume of the detector, it's required optimization of the flow path system.

Column Size	150-4.6mm I.D. (5μm)	150-3mm I.D. (5μm)	150-2mm I.D. (5μm)	100-4.6mm I.D. (3μm)	100-3mm I.D. (3μm)	100-2mm I.D. (3μm)	50-2mm I.D. (3μm)
Sensitivity	1	2	5	1	2	3～5*	3～5*
Time	1	1	1	2/3	2/3	2/3	1/3
Solvent consumption	**1**	**1/2**	**1/5**	**2/3**	**1/3**	**2/15**	**1/15**
Efficiency	1	1	1	1	1	1	1/2

＊;Sensitivity is when detector is used the cells of the order of the optical path length 3mm .

Scale up of Analytical to Preparative

Preparative (I.D. 10mm~50mm)

	Length-I.D. (mm)	Flow rate * (mL/min)	injection volume (μL)	Load (mg)
Analytical column	250-4.6	1	1～15	0.05～1
	250-6	1～2	10～25	0.1～2
Preparative column	250-10	4～6	20～150	1～20
	250-20	15～20	50～1000	10～100
	250-50	100～120	500～2500	100～2000

Other information

***The calculation of the flow rate ***

Because linear velocity (cm/min) is showed as (1), when under the conditions of the I.D. 4.6mm, flow rate 1.0mL/min is adapt to I.D. 6mm, the flow rate at time of the combined linear velocity is 1.70 mL/min by (2)~(4).

Linear velocity (cm/min)＝Flow rate (cm^3/min) / Cross section (cm^2)..... (1)

Linear velocity $_{I.D.4.6mm}$＝1(cm^3/min) / 0.166(cm^2)＝6.02(cm/min) ..... (2)

6.02(cm/min) ＝Flow rate $_{I.D.\ 6mm}$ (cm^3/min) / 0.2826(cm^2)..... (3)

Flow rate $_{I.D.\ 6mm}$ ＝6.02(cm/min)×0.2826(cm^2)＝1.701(cm^3/min)..... (4)

Relations of the I.D. and cross-section and flow

Using the upper expression, the change of flow rate is shown by the change of I.D. to the right chart. The solvent consumption can reduce by reduction of the flow rate due to the smaller diameter.

I.D. (mm)	Cross section (cm^2)	Flow rate (cm^3/min)
2	0.03	0.19
3	0.07	0.43
4.6	0.17	1
6	0.28	1.70
10	0.79	4.73
20	3.14	18.90

Relations of the particle diameter and the number of theoretical plates and column pressure

It shows the ratio of the respective values of the particle diameter 3μm when the number of theoretical plates and column pressure of particle diameter 5μm is set to 1 to the right table.

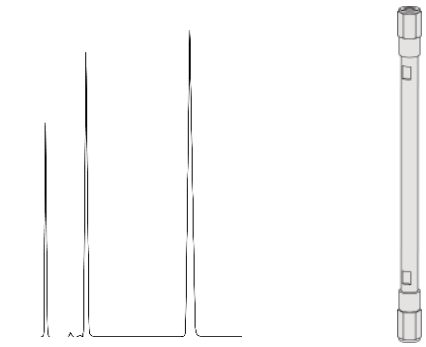
Particle diameter (μm)	The number of theoretical	Column pressure
5	1	1
3	1.7	2.8

Guard column system

It is recommended to use a Guard columns in order to extend the life of the column. Avoid back pressure, ghost peaks, and broad peaks caused by contamination, eg., from biological extraction samples. KANTO offers convenient column guard systems:

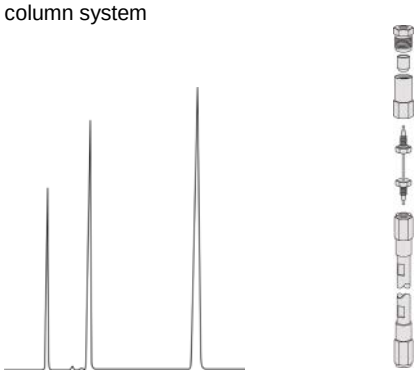
- ●Easy-to-use and economical cartridge type
- ●Small dead-volume type; a high performance column guard with smaller dead volume that protects the resolution of the main column.

Only Analytical column



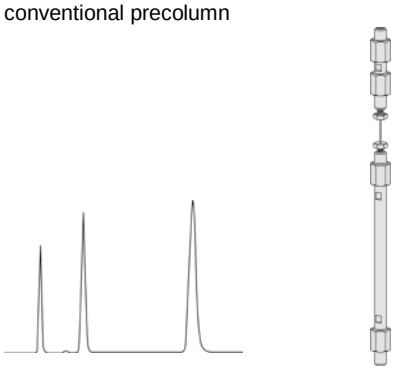
Decrease in Theoretical Plate Height 0%

Equipped with Mightysil Guard column system



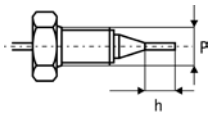
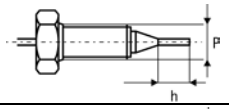
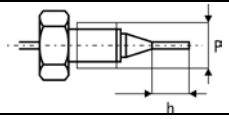
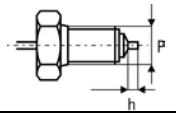
Decrease in Theoretical Plate Height 8%

Equipped with conventional precolumn



Decrease in Theoretical Plate Height 60%

Type of connection

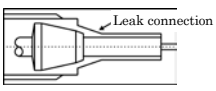
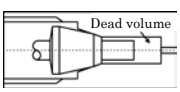
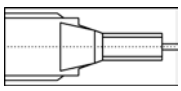
Maker	Connection Type		Length of "h"	type
KANTO/Merck		No.10-32UNF P=4.8mm	Abt. 2.0mm	A
Waters			Abt. 3.5mm	
TOSOH			Abt. 4.5mm	
Shimadzu（LC6A or later）			Abt. 2.3mm	
Hitachi（L6500 or later）			Abt. 2.9mm	
Jasco（800 or later）			Abt. 2.5mm	
Old-Jasco (BIP,TRI ROTAR or earlier)		No.10-32UNF P=4.8mm	Abt. 4.5mm	B
Old-Hitachi (655 or earlier)		M6 P=6.0mm	Abt. 4.7mm	C
Old-Shimadzu u(LC5A or earlier)		No.10-32UNF P=4.8mm	Abt. 1.4mm	D

"h" is the measured approximate value. Consider this value as a reference value.

Most manufacturers use Type A in the above table for the connecting parts of an HPLC column (thread and ferrule specifications). However, even though these specifications are the same, each company uses a different length of ferrule tip (h), so pay attention when replacing the column. The column can be connected reliably without concerns of these differences, by using the handy connector.

Product name	Description	Packing	Cat. No.
Handy Connectors	One-piece PEEK finger tight fitting For 1/16"capillary tube	5 pieces	18685-96

●Effects of the length of "h"

Metal ferrule and Nut	Handy Connectors
The length of "h" is fixed, it is caused a leak connection and a dead volume.	●Leak free connection ●Minimizes dead volume
 	

Product List

Mightysil RP-18GP II (5μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25685-96
10	20	1(G)	25686-96
250	10	1	25687-96
10	10	2(G)	25688-96
150	6	1	25689-96
250	4.6	1	25690-96
150	4.6	1	25691-96
75	4.6	1	25692-96
5	4.6	10(G)	25693-96
250	3	1	25694-96
150	3	1	25695-96
250	2	1	25696-96
150	2	1	25697-96
50	2	1	25698-96
5	2	2(G)	25699-96

Mightysil RP-18GP II (3μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25606-96
150	4.6	1	25607-96
100	4.6	1	25608-96
75	4.6	1	25609-96
50	4.6	1	25610-96
5	4.6	2(G)	25611-96
250	3	1	25612-96
150	3	1	25613-96
150	2	1	25614-96
100	2	1	25615-96
50	2	1	25616-96
5	2	2(G)	25617-96

Mightysil RP-18GP (5μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25480-96
50	20	1	25649-96
10	20	1(G)	25481-96
250	10	1	25478-96
10	10	2(G)	25479-96
250	6	1	25476-96
150	6	1	25477-96
250	4.6	1	25415-96
150	4.6	1	25416-96
100	4.6	1	26060-96
75	4.6	1	25417-96
50	4.6	1	26061-96
5	4.6	10(G)	25418-96
250	4	1	25448-96
150	4	1	25449-96
250	3	1	25438-96
150	3	1	25437-96
100	3	1	25655-96
75	3	1	25435-96
50	3	1	25656-96
250	2	1	25470-96
150	2	1	25471-96
100	2	1	25542-96
50	2	1	25543-96
5	2	2(G)	25474-96

Mightysil RP-18(H)GP series (5μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25551-96
150	4.6	1	25552-96
75	4.6	1	25553-96
5	4.6	10(G)	25554-96

G : Guard column

Mightysil RP-18GP (3μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25513-96
150	4.6	1	25514-96
100	4.6	1	25469-96
75	4.6	1	25515-96
50	4.6	1	25468-96
30	4.6	1	25650-96
5	4.6	2(G)	26057-96
250	3	1	25544-96
150	3	1	25546-96
100	3	1	25652-96
75	3	1	25545-96
50	3	1	25653-96
250	2	1	25516-96
150	2	1	25517-96
100	2	1	25472-96
50	2	1	25473-96
5	2	2(G)	25475-96
100	1	1	25436-96
50	1	1	25434-96

Mightysil RP-18(L)GP series (5μm)

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25547-96
150	4.6	1	25548-96
75	4.6	1	25549-96
5	4.6	10(G)	25550-96
100	2	1	25644-96
50	2	1	25643-96

● *Mightysil RP-18GP Aqua (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25660-96
10	20	1(G)	25661-96
250	10	1	25665-96
10	10	2(G)	25666-96
250	4.6	1	25640-96
150	4.6	1	25641-96
5	4.6	10(G)	25642-96
250	3	1	25667-96
150	3	1	25668-96
250	2	1	25669-96
150	2	1	25670-96
100	2	1	25671-96
50	2	1	25672-96
5	2	2(G)	25673-96

● *Mightysil RP-18GP Aqua (3μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25674-96
150	4.6	1	25675-96
100	4.6	1	25676-96
50	4.6	1	25677-96
5	4.6	2(G)	25678-96
250	3	1	25679-96
150	3	1	25680-96
150	2	1	25681-96
100	2	1	25682-96
50	2	1	25683-96
5	2	2(G)	25684-96

● *Mightysil RP-8GP (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25518-96
10	20	1(G)	25519-96
250	10	1	25520-96
10	10	2(G)	25521-96
250	6	1	25522-96
150	6	1	25523-96
250	4.6	1	25463-96
150	4.6	1	25462-96
75	4.6	1	25461-96
5	4.6	10(G)	25460-96
250	4	1	25450-96
150	4	1	25451-96
250	3	1	25524-96
150	3	1	25525-96
75	3	1	25526-96
250	2	1	25527-96
150	2	1	25528-96
5	2	2(G)	25529-96

● *Mightysil RP-8GP (3μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25439-96
150	4.6	1	25440-96
100	4.6	1	25441-96
5	4.6	2(G)	25442-96
150	2	1	25443-96

● *Mightysil RP-18 (15μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	50	1	25413-96
50	50	1(G)	25414-96

● *Mightysil NH$_2$ (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25662-96
150	4.6	1	25663-96
5	4.6	10(G)	25664-96
250	4	1	25452-96
150	4	1	25453-96
250	3	1	25454-96
150	3	1	25455-96
150	2	1	25456-96

● *Mightysil RP-4GP (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25530-96
10	20	1(G)	25531-96
250	10	1	25532-96
10	10	2(G)	25533-96
250	6	1	25534-96
150	6	1	25535-96
250	4.6	1	25467-96
150	4.6	1	25466-96
75	4.6	1	25465-96
5	4.6	10(G)	25464-96
250	3	1	25536-96
150	3	1	25537-96
75	3	1	25538-96
250	2	1	25539-96
150	2	1	25540-96
5	2	2(G)	25541-96

● *Mightysil NH$_2$ (3μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25489-96
150	4.6	1	25490-96
100	4.6	1	25491-96
5	4.6	2(G)	25492-96
150	2	1	25493-96

G : Guard column

● *Mightysil Si60 (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	20	1	25499-96
10	20	1(G)	25510-96
250	10	1	25497-96
10	10	2(G)	25498-96
250	4.6	1	25482-96
150	4.6	1	25483-96
75	4.6	1	25484-96
5	4.6	10(G)	25485-96
250	4	1	25457-96
150	4	1	25458-96
250	3	1	25486-96
150	3	1	25487-96
75	3	1	25488-96
150	2	1	25459-96

● *Mightysil Si60 BDF (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25555-96

● *Mightysil RP-18PA (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	25419-96
150	4.6	1	25420-96
5	4.6	10(G)	25421-96
250	2	1	26069-96
150	2	1	26070-96
5	2	2(G)	26071-96

G : Guard column

● *Mightysil RP-18MS (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
150	4.6	1	26073-96
5	4.6	10(G)	26074-96
150	2	1	26075-96
50	2	1	26076-96
5	2	2(G)	26077-96

● *Mightysil DNPH (5μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
250	4.6	1	26066-96
150	4.6	1	26067-96
5	4.6	2(G)	26068-96

● *Mightysil RP-18PA (3μm)*

Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
150	4.6	1	26078-96
5	4.6	2(G)	26081-96
150	3	1	26079-96
150	2	1	26080-96
50	2	1	26083-96
5	2	2(G)	26082-96

● *Mightysil* column packing

Product Name	Particle size(μm)	Package size	Catalog No.
Mightysil RP-18 GP	5	10g	26091-43
	3	10g	26092-43

● Guard column holder

Product Name	Length (mm)	I.D. (mm)	Package (No./pack)	Catalog No.
Mightysil guard column holder for 20mm I.D.	10	20	1	25431-96
Mightysil guard column holder for 10mm I.D.	10	10	1	25432-96
Mightysil guard column holder for 3/4.6/6mm I.D.	5	4.6	1	25430-96
Mightysil guard column holder for 2mm I.D.	5	2	1	25433-96

＊Capillary for connecting is attached with Guard column holder.

●Accessory

Product Name	Package (No./pack)	Catalog No.
Handy Connector (Fingertight) For O.D. 1/16 " Capillary, Ferrule/Screw Combined PEEK Connector	5	18685-96

HLC-SOL

Solvent for HPLC

Solvent for HPLC

Product Name	Grade	Package	Catalog No.	Guarantee Items
Acetonitrile -Plus-	for HPLC	1L	01031-1B*	JP reagent
		3L	01031-2B*	
Acetonitrile -Plus-	for HPLC (for medicine analysis)	1L	01858-79*	JP reagent、ACS、Reag.Ph Eur
		3L	01858-76*	
Acetone	for HPLC	1L	01026-1B	
		3L	01026-2B	
Ethanol (99.5)	for HPLC	1L	14033-1B	
		3L	14033-2B	
Chloroform	for HPLC	1L	07278-1B	
		3L	07278-2B	
Ethyl acetate	for HPLC	1L	14029-1B	
		3L	14029-2B	
Carbon tetrachloride	for HPLC	1L	07140-1B	
1,4-Dioxane	for HPLC	1L	10425-1B	
Cyclohexane	for HPLC	1L	07547-1B	
		3L	07547-2B	
1,2-Dichloroethane	for HPLC	1L	10149-1B	
o-Dichlorobenzene	for HPLC	1L	10127-79	
		3L	10127-76	
Dichloromethane	for HPLC	1L	10158-1B	
		3L	10158-2B	
N,N-Dimethylformamide	for HPLC	1L	10344-1B*	JP reagent
		3L	10344-2B*	
Distilled water -Plus-	for HPLC	1L	11307-1B	
		3L	11307-2B	
Tetrahydrofuran, stabilizer free	for HPLC	200mL	40060-3B*	JP reagent
		500mL	40060-5B*	
		1L	40060-1B*	
		3L	40060-2B*	
Tetrahydrofuran, stabilizer free	for HPLC (for medicine analysis)	3L	40060-76*	
Tetrahydrofuran (stabilizer containing)	for HPLC	1L	41120-79	
		3L	41120-76	
2,2,4-Trimethylpentane	for HPLC	1L	31005-1B	
Toluene	for HPLC	1L	40180-1B	
		3L	40180-2B	
1-Butanol	for HPLC	1L	04354-1B	
tert-Butyl methyl ether	for HPLC	1L	04418-1B	
2-Propanol -Plus-	for HPLC	1L	32435-1B*	JP reagent
		3L	32435-2B*	
1,1,1,3,3,3-Hexafluoro-2-propanol	for HPLC	100mL	18529-1B	
		500mL	18529-3B	
Hexane	for HPLC	1L	18041-1B*	JP reagent
		3L	18041-2B*	
Heptane (n-Heptane)	for HPLC	1L	18005-1B	
Benzene	for HPLC	1L	04084-1B	
Methanol -Plus-	for HPLC	1L	25183-1B*	JP reagent
		3L	25183-2B*	
Methanol -Plus-	for HPLC (for medicine analysis)	1L	25190-79*	JP reagent、ACS、Reag.Ph Eur
		3L	25190-76*	
N-Methyl-2-pyrrolidinone	for HPLC	1L	25336-79	

*JP reagent = compliant with [for LC] standard of the Japanese Pharmacopeia

Solvent for LC/MS

Product Name	Grade	Package	Catalog No.
Acetonitrile	for LC/MS	200mL	*01033-23
Acetonitrile -Plus-	for LC/MS	1L	*01033-79
		3L	*01033-76
Distilled water	for LC/MS	200mL	11307-23
Distilled water -Plus-	for LC/MS	1L	11307-79
		3L	11307-76
Methanol	for LC/MS	200mL	*25185-23
Methanol -Plus-	for LC/MS	1L	*25185-79
		3L	*25185-76
2-Propanol	for LC/MS	200mL	*32435-12
2-Propanol -Plus-	for LC/MS	1L	*32435-79
		3L	*32435-76

* = compliant with [for LC] standard of the Japanese Pharmacopeia

Reagent for HPLC

Product Name	Grade	Package	Catalog No.
1mol/L Ammonium acetate solution	for HPLC	100mL	01969-23
1mol/L Ammonium formate solution	for HPLC	100mL	01298-23
Formic acid	for HPLC	1mL(A)×5	16233-96
		25mL(S)	16233-97
Acetic acid	for HPLC	1mL(A)×5	01021-96
		25mL(S)	01021-97
Trifluoroacetic acid	for HPLC	1mL(A)×5	40578-1B
		25mL(A)	40578-2B
Phosphoric acid	for HPLC	25mL(S)	32187-96

A: ampule, S : screw cap

Premix product for HPLC

Product Name	Grade	Package	Catalog No.
0.1vol% Formic acid-acetonitrile	for HPLC	1L	01922-79
		3L	01922-76
0.1vol% Formic acid-distilled water	for HPLC	1L	16245-1B
		3L	16245-2B
0.1vol% Trifluoroacetic acid-acetonitrile	for HPLC	1L	41132-79
		3L	41132-76

Solvent for preparative liquid chromatography

Product Name	Grade	Package	製品番号
Acetonitrile	for preparative liquid chromatography	3L×2	01031-84
		18L	01031-96
Ethanol (99.5)	for preparative liquid chromatography	18L	14033-84
Chloroform	for preparative liquid chromatography	25kg	07278-84
Ethyl acetate	for preparative liquid chromatography	18L	14029-96
Distilled water	for preparative liquid chromatography	18L	11334-96
Distilled water (cubitainer)	for preparative liquid chromatography	20L	11334-92
Hexane	for preparative liquid chromatography	18L	18635-96
Tetrahydrofuran (stabilizer containing)	for preparative liquid chromatography	3L×2	41120-84
Methanol	for preparative liquid chromatography	3L×2	25183-84
		18L	25183-96

Search product, CAS, keyword, ...

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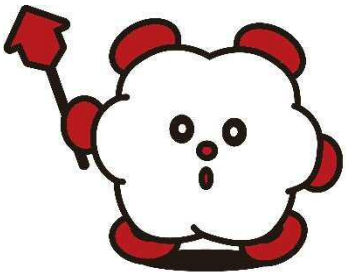

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