FUJI SILYSIA CHEMICAL

CHROMATOREX

Technical Bulletin

ARG SILICA

For HILIC compounds

INTRODUCTION

For Hydrophilic Interaction Chromatography compounds

Hydrophilic compounds have been separated in reversed-phased (RP) mode by using media such as C18 (ODS) silica gel in combination with aqueous solvent mixtures. However, there are still many high hydrophilic compounds which cannot be separated using typical RP mode.

Recently, a technique of Hydrophilic Interaction Chromatography (HILIC) has been developed and it is possible to separate high hydrophilic compounds.

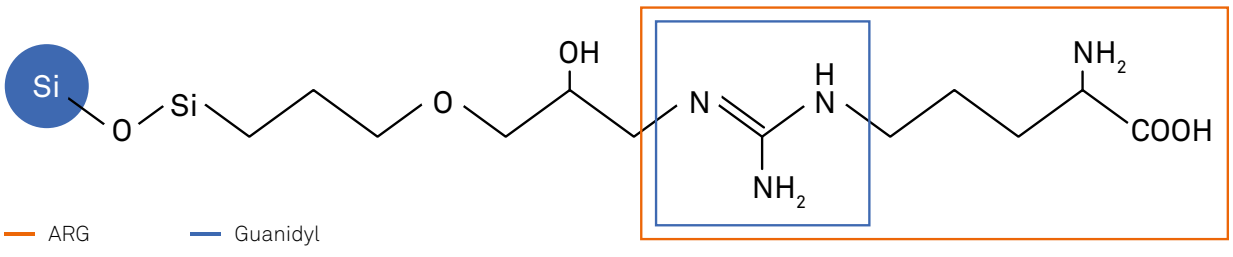
Fuji Silysia Chemical Ltd. (FSC) developed "ARG Silica" for HILIC mode (Patent applied in Japan). ARG Silica can separate hydrophilic compounds such as amino acid, peptide, vitamin and nucleic acid.

Various particle size of ARG Silica are available for analysis and large scale purification. ARG Silica is dedicated to the separation of various hydrophilic compounds.

ARG SILICA

ARG Silica is based on a chemical surface modification with arginine amino acid. ARG Silica has strong affinity to hydrophilic compounds and generates high separation performance and different selectivity compared with other grades.

Surface functional group of ARG Silica



ARG Silica has a **"guanidyl"** function, improving the hydrophilicity of the media. In HILIC mode, mainly acetonitrile/water mixtures are first choice for mobile phase. High polarity elutes are strongly retained to ARG Silica by hydrophilic interaction. As water content increases, elution time is getting shorter. Thus, separation pattern of ARG Silica is opposite of RP mode where retention time is getting longer as water content increases.

HOW TO USE

It is necessary to flush the column by 70 % acetonitrile / water or mobile phase for 10 times higher than column volume to equilibrate column separation layer for preparation before using. If separation was operated with insufficient condition, separation property would be unstable.

Recommendable water content in mobile phase is less than 30 %.

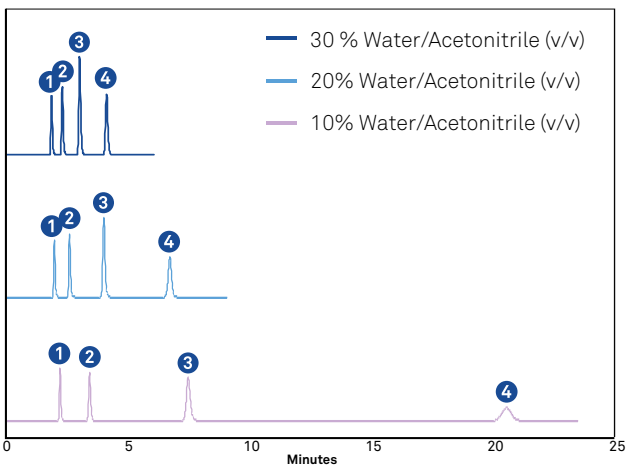
Separation layer would be unstable if higher water content in mobile phase is used. Also if water content between injection solution and mobile phase has big difference, separation peak pattern would be degraded. Thus, the smallest difference of water content between mobile phase and injection solution is recommended.

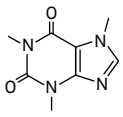
MOBILE PHASE

More than 4 % water content in mobile phase is recommended to take advantage of HILIC's separation property. Acetonitrile, Isopropanol, Methanol and Water can be applied as mobile phase. As shown in below chromatogram, elution power of acetonitrile is weak, while elution power of water is strong. In general, acetonitrile / water system is used as mobile phase. Effective range of pH for mobile phase is pH 2.0 ~ 9.0.

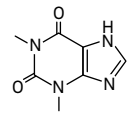
COMPOSITION OF MOBILE PHASE

Influence of water in retention time when using ARG Silica in HILIC mode

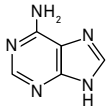




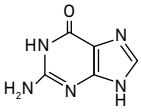
1 Caffeine



2 Theophylline



3 Adenine



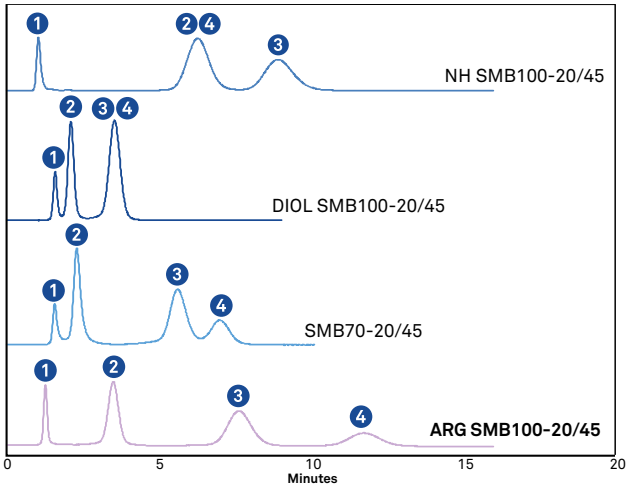
4 Guanine

Media ARG SPS100-5
Column Φ4.6 mm x 150 mm
Flow rate 1 ml/min
Detector UV254 nm

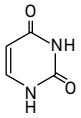
COMPARATIVE DATA BETWEEN ARG SILICA, NH, DIOL AND BARE SILICA

ARG Silica is more effective for the separation of nucleobases and purine bases than NH Silica, Diol Silica and bare Silica. The added guanidyl function improves the hydrophilicity of ARG Silica.

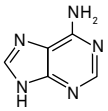
Nucleobases Separation



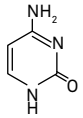
1 Toluene (t_0)



2 Uracil



3 Adenine

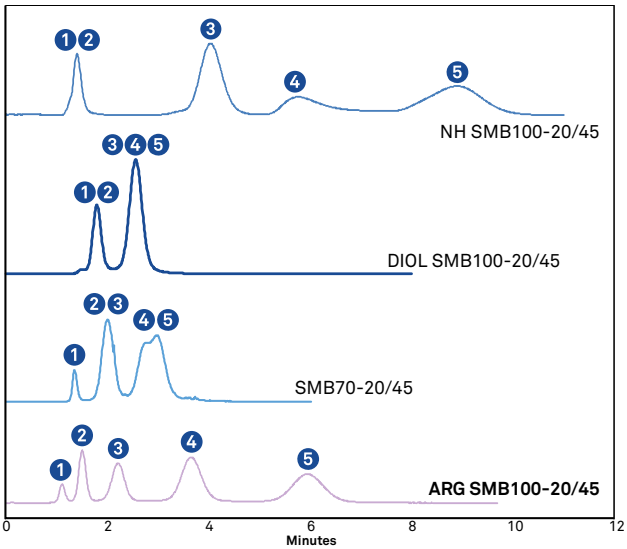


4 Cytosine

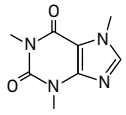
Column size Φ28 mm x 100 mm
Particle size 30 µm
Mobile Phase Acetonitrile / Water (90:10) (v / v)

Flow rate 28 ml/min
Temperature 35°C
Detector UV 254 nm

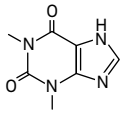
Purines Bases Separation



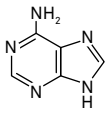
1 Toluene (t_0)



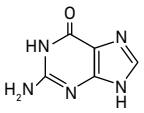
2 Caffeine



3 Theophylline



4 Adenine



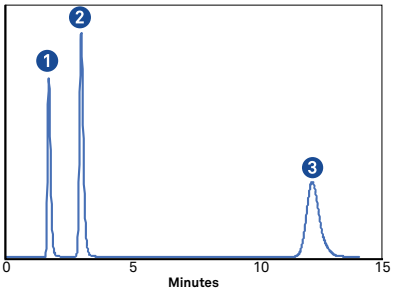
5 Guanine

Column Φ28 mm x 100 mm
Particle size 30 µm
Mobile phase Acetonitrile / Water (80 : 20) (v / v)

Flow rate 28 ml/min
Temperature 35°C
Detector UV254 nm

SEPARATION OF HYDROPHILIC COMPOUNDS

1. Separation of polypeptides



1. Angiotensin Ⅲ
2. Bradykinin
3. Angiotensin Ⅰ

Amino acid sequence

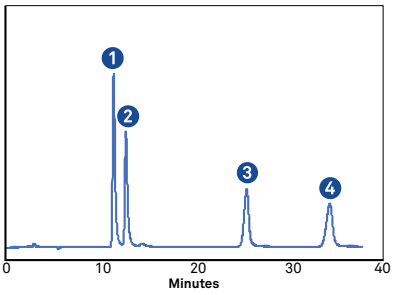
1. (Arg-Val-Tyr-Ile-His-Pro-Phe)
2. (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)
3. (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe)

Media ARG SPS100-5
Column Φ4.6 mm x 250 mm

Mobile phase
Methanol / Acetonitrile /
50mM Ammonium Acetate
pH6.8 (60:20:20) (v/v)

Flow rate 1 ml/min
Temperature 35°C
Detector UV220 nm

2. Separation of oligopeptides



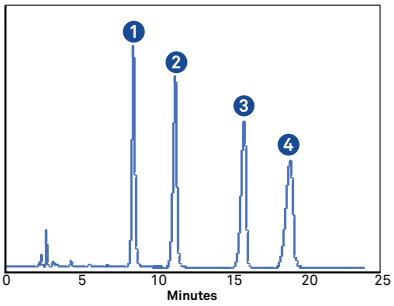
1. Gly-Phe
2. Gly-Leu
3. Gly-Gly
4. Gly-Gly-Gly

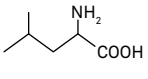
Media ARG SPS100-5
Column Φ4.6 mm x 250 mm

Mobile Phase
Acetonitrile / 50mM Tris-HCl
pH=8.5 (70:30) (v/v)

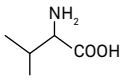
Flow Rate 1 ml/min
Temperature 35°C
Detector UV220 nm

3. Separation of amino acids





1. L- Leucine



2. L- Valine

3. L - Alanine

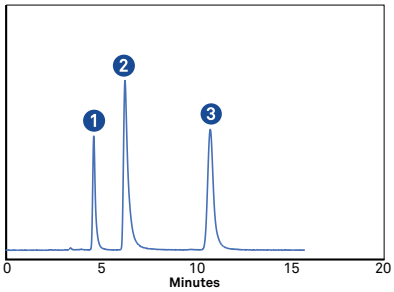
4. Glycine

Media ARG SPS100-5
Column Φ4.6 mm x 250 mm

Mobile Phase
Acetonitrile / Water
(75:25) (v/v)

Flow Rate 1 ml/min
Temperature 35°C
Detector UV210 nm

4. Separation of vitamins



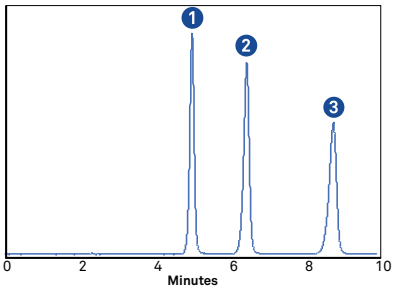
1 Riboflavin (Vitamin B_2) 2 Thiamine (Vitamin B_1) 3 Cyanocobalamine (Vitamin B_{12})

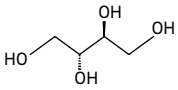
Media ARG SPS100-5
Column Φ4.6 mm x 250 mm

Mobile Phase
Acetonitrile / 50mM Tris-HCl pH=8.5 (70:30) (v/v)

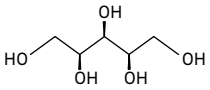
Flow Rate 1 ml/min
Temperature 35°C
Detector UV254 nm

5. Separation of sugar alcohols

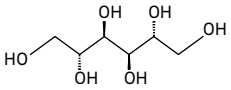




1 Erythlitol



2 Xyritol



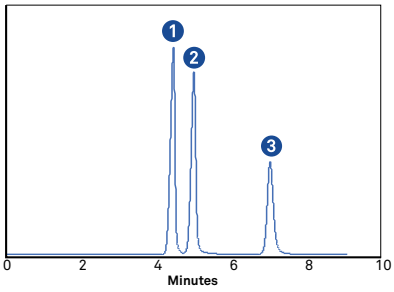
3 Mannitol

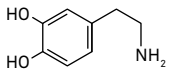
Media ARG SPS100-5
Column Φ4.6 mm x 150 mm

Mobile Phase
Acetonitrile / Water (80:20) (v/v)

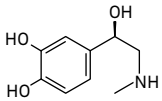
Flow Rate 1 ml/min
Temperature 35°C
Detector ELSD

6. Separation of catecholamines

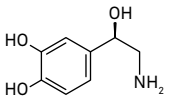




1 Dopamine



2 Adrenaline



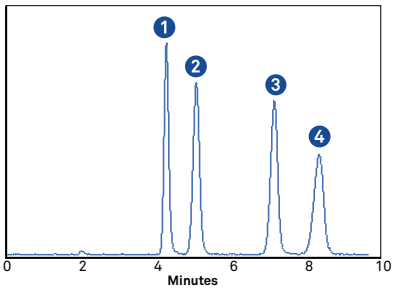
3 Noradrenaline

Media ARG SPS100-5
Column Φ4.6 mm x 150 mm

Mobile Phase
Acetonitrile / 50mM Ammonium formate pH3.5 (85:15) (v/v)

Flow Rate 1 ml/min
Temperature 35°C
Detector UV270nm

7. Separation of monosaccarides and disaccharides



1 Fructose 2 Glucose 3 Sucrose 4 Maltose

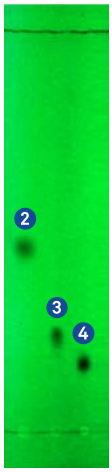
Media ARG SPS100-5
Column Φ4.6 mm x 150 mm

Mobile Phase
Acetonitrile / Water (75:25) (v/v)

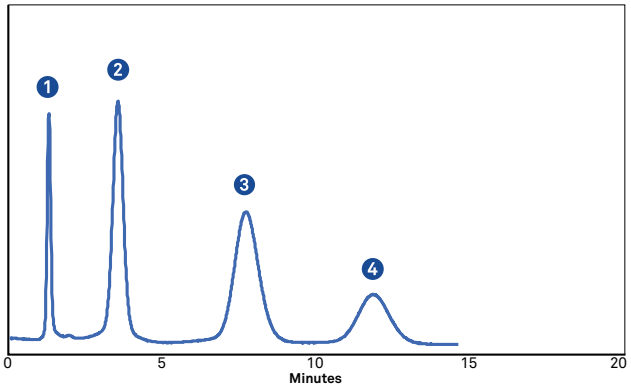
Flow Rate 1 ml/min
Temperature 60°C
Detector ELSD

Separation of nucleic acid bases by TLC

ARG TLC
UV254 nm



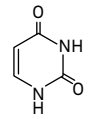
Acetonitrile / Water
(90:10) (v/v)



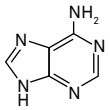
Media ARG SMB100-20/45
Column Φ28 mm x 100 mm
Mobile Phase Acetonitrile / Water (90:10) (v/v)

Flow Rate 28 ml/min
Temperature 35°C
Detector UV254 nm

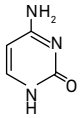
1 Toluene (t_0)



2 Uracil



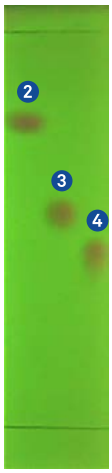
3 Adenine



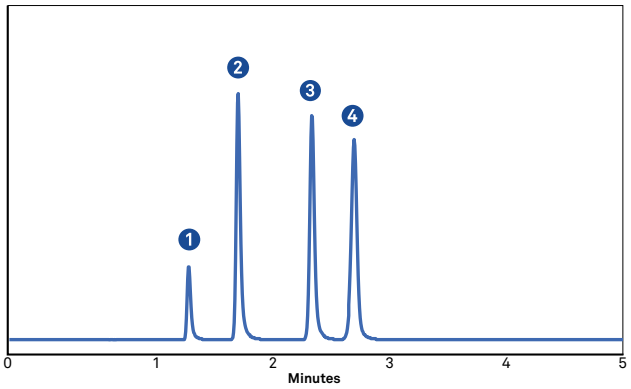
4 Cytosine

Separation of Heterocyclic compounds by TLC

ARG TLC
UV254 nm



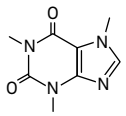
Acetonitrile / Water
(90:10) (v/v)



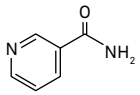
Media ARG SPS100-3
Column Φ3.0 mm x 150 mm
Mobile Phase Acetonitrile / Water (90:10) (v/v)

Flow Rate 0.50 ml/min
Temperature 35°C
Detector UV254 nm

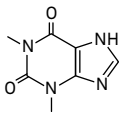
1 Toluene (t_0)



2 Caffeine



3 Nicotinamide



4 Theophyline

PRODUCT INFORMATION

Bare Silica :
10nm (100 Å), with 3, 5, 10, 20/45, and 40/75 µm
Packaging 100g, 1kg, 20kg

TLC Plates :
Glass Plates 20 x 20 cm
Packaging 10 pieces/box

Fuji Silysia

FUJI SILYSIA CHEMICAL LTD.
Head Office
2-1846 Kozoji-cho,
Kasugai-shi,
Aichi-Ken
Japan 487-0013

FUJI SILYSIA CHEMICAL LTD.
Sales Office
23rd floor, Nagoya Intl. Center Bldg.
1-47-1 Nagono,
Nakamura-Ku,
Nagoya-shi,
Aichi-Ken
Japan 450-0001

Phone: +81 52 587 0451
Fax: +81 52 587 0455
E-mail: chromato-int@fuji-silysia.co.jp

FUJI SILYSIA CHEMICAL S.A.
International Chromatography Center
En Budron E9
CH-1052 Le Mont-sur-Lausanne
Switzerland

Phone: +41 21 652 3436
Fax: +41 21 652 4737
E-mail: info@chromatorex.com

www.chromatorex.com

10/2020