No.102
SEPARATION REPORT

TOSOH

Packed columns for reversed-phase chromatography TSKgel ODS-100V and 100Z

Contents

Tosoh corporation

1. Introduction

Of the various separation modes for high-performance liquid chromatography, reversed-phase chromatography (RPC) is the most frequently utilized mode because of the high resolution (various components can be separated in a relatively short period of time); wide range of analyzable compounds (from hydrophilic to hydrophobic), and ease of operation. The octadecyl-silyl (ODS or C18) column is most often utilized in RPC, in which the column is packed with ODS gel, with the stationary phase (C18) chemically bonded to the porous silica gel. However, because unreacted silanol groups exist on the C18-bonded surface, if the pH of the mobile phase is about 4 or higher, these silanol groups dissociate, allowing for secondary interactions with positively charged samples and thus hindering separation and quantification. We have developed two types of ODS with different surface properties utilizing a highly efficient end-capping procedure. In this article, the properties of ODS packing materials and its chromatographic performance are shown along with application data.

2. Specifications and characteristics

Table 1 shows the product lineup for the TSKgel ODS-100V and TSKgel ODS-100Z columns. The 4.6 mm I.D. columns are used for general analysis, while the 2 mm I.D. columns are used for LC-MS (/MS) or microanalysis.

The characteristics of the TSKgel ODS-100V and TSKgel ODS-100Z columns are shown below:

- Common characteristics for both columns
  - 5 µm particle size.
  - Two types of ODS made from the same base silica but with different surface modifications
  - Basic separation properties are the same, but selectivity is different
  - Superior peak shapes for not only neutral compounds but also basic and acidic ones.
  - Minimal lot-to-lot variability
  - Method-development support (columns from three different lots are available)
  - A gel-batch test report (Certificate of Analysis) is given with the columns
  - USP L1 category
  - Available worldwide
- Characteristics of the TSKgel ODS-100V
  - Modest carbon content (15%) and high surface polarity
    → High retention of hydrophilic compounds
- Characteristics of the TSKgel ODS-100Z
  - High carbon content (20%) and low surface polarity
    → High retention of moderately and highly hydrophobic compounds

Table 1. Specifications for TSKgel ODS-100V and TSKgel ODS-100Z

Analysis columns

Product name	Product code	Particle size	Column size
TSKgel ODS-100Z	21461	5 µm	4.6mmI.D. × 15cm
TSKgel ODS-100Z	21462	5 µm	4.6mmI.D. × 25cm
TSKgel ODS-100Z	21460	5 µm	2.0mmI.D. × 5cm
TSKgel ODS-100Z	21459	5 µm	2.0mmI.D. × 15cm
TSKgel ODS-100V	21455	5 µm	4.6mmI.D. × 15cm
TSKgel ODS-100V	21456	5 µm	4.6mmI.D. × 25cm
TSKgel ODS-100V	21457	5 µm	2.0mmI.D. × 5cm
TSKgel ODS-100V	21458	5 µm	2.0mmI.D. × 15cm

Guard columns

Product name	Product code	Packaging
TSKguardcolumnODS-100Z	21454	3 columns
TSKguardcolumnODS-100V	21453	3 columns
Cartridge holder	19018	—

Table 2. Properties of packing material

Packing material	Particle size (µm)	Pore size (A)	Specific surface area (m^2/g)	Functional group	Carbon content (%)	Binding structure
TSKgel ODS-100V	5	100	450	C18	15	Monolayer
TSKgel ODS-100Z	5	100	450	C18	20	Monolayer

3. Properties of packing material

Table 2 shows the properties of the TSKgel ODS-100V and TSKgel ODS-100Z columns. Both TSKgel ODS-100V and TSKgel ODS-100Z contain reversed-phase packing materials with a particle size of 5 µm and a pore size of 100 A, in which C18 is chemically bonded on high-purity silica gel. The specific surface area of the base silica, 450 m^2/g, is larger than that of common commercially available reversed phase columns that have a surface area of 175 - 350 m^2/g. The high surface area enables synthesis of ODS with high retention. The carbon content of ODS-100Z is 20 %, the hydrophobicity of the stationary phase is high, and the retention of common low-molecular-weight organic compounds is high. The carbon content of ODS-100V is 15%, and as a reversed-phase packing material, the polarity of the stationary phase is high, and the retention of highly polar low-molecular-weight organic compounds is relatively high.

The chromatographic properties, column efficiency (theoretical plate number and asymmetry factor) and pressure-flow characteristics, which are related to the base silica, are the same for ODS-100V and ODS-100Z. Retention, steric selectivity and selectivity, which are related to the stationary phase (C18), vary between the two columns. The ion exchange activity, an indicator for silanol activity, is comparable between the ODS-100V and ODS-100Z columns because of high end-capping efficiency.

Figure 1 shows the relationship between hydrophobicity and surface polarity parameters for ODS-100V, ODS-100Z and other commercial ODS columns. The hydrophobicity of ODS-100Z is relatively high, and its surface polarity is moderate. On the other hand, the hydrophobicity of ODS-100V is low, and its surface polarity is high.

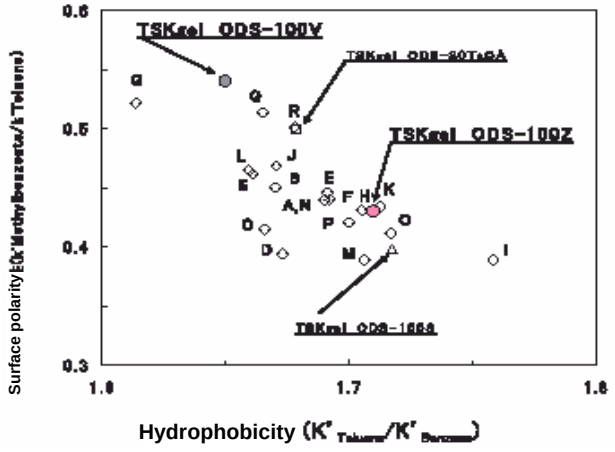

Figure 1. Hydrophobicity and surface polarity parameters of various ODS columns

A-T: Commercial ODS columns

4. Chromatographic properties

4-1. Column efficiency

Figure 2 compares the theoretical plate numbers for naphthalene among the various ODS columns. The theoretical plate numbers for ODS-100V and ODS-100Z are among the best of the other ODS columns we studied.

4-2. Retention

Figure 3 compares naphthalene retention among the various ODS columns under the same conditions of **Figure 2**. The retention of neutral compounds, such as naphthalene, is mainly dependent on carbon content. Since the carbon content of ODS-100Z is high, at approximately 20%, it retains naphthalene reasonably strongly. On the other hand, the carbon content of ODS-100V is approximately 15%, and its naphthalene retention is moderate among the ODS columns tested.

4-3. Column pressure drops

Figure 4 compares column pressure drop with the mobile phase (water/methanol) used in **Figure 2**. The column pressure drop of ODS-100V and ODS-100Z is moderate when compared to the other ODS columns, confirming easy operation for these two columns. The fact that ODS-100V and ODS-100Z exhibit a high number of theoretical plates (see **Figure 2**) but lower column pressure drops compared to other ODS columns, suggests that the narrow particle size distribution results in a homogenously packed bed, with superior pressure-flow characteristics.

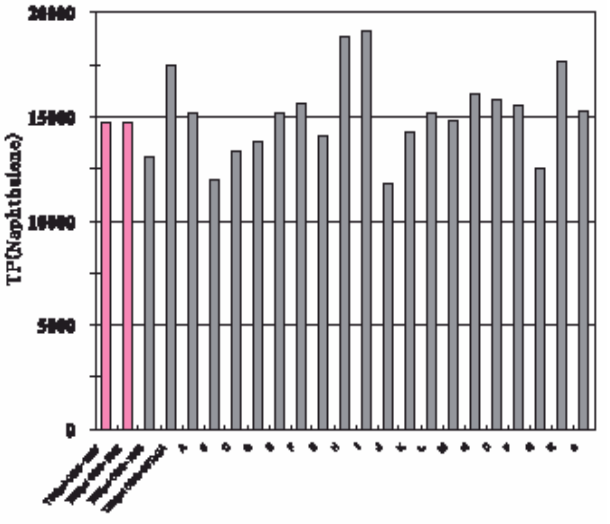

Figure 2. Comparison of theoretical plates number

Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	H_2O/MeOH (30/70)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Sample:	Naphthalene

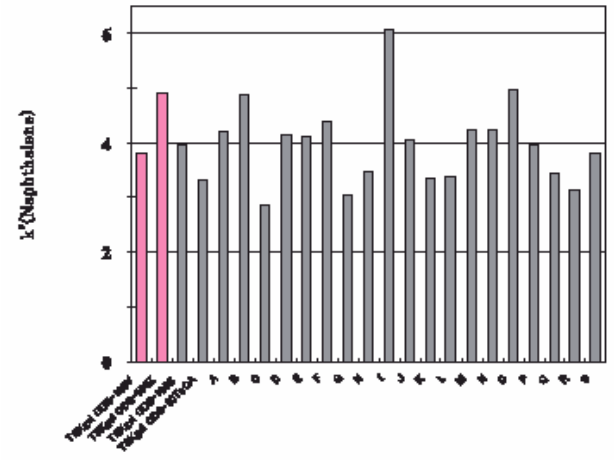

Figure 3. Comparison of retention
Test conditions: The same as Figure 2.

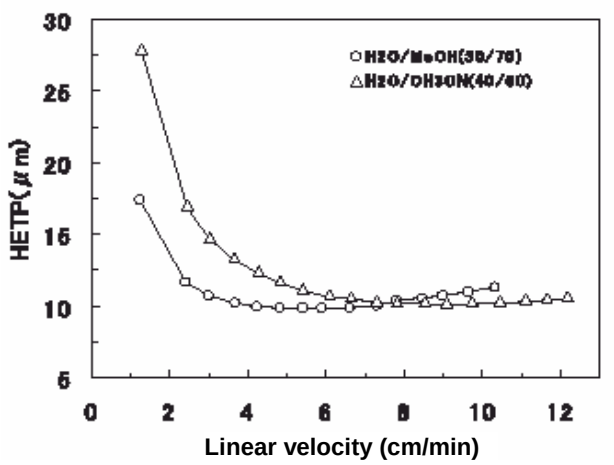

Figure 5. Relationship between HETP and linear velocity
Column: TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Mobile phases: H_2O/MeOH (30/70)
H_2O/CH_3CN (40/60)
Temperature: 40 ˚C
Injection volume: 10 µL
Sample: Naphthalene

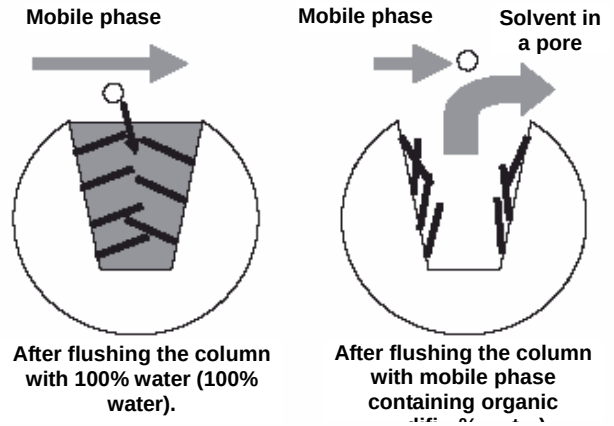

Figure 7. Schematic diagrams to illustrate loss of retention when using mobile phase without organic solvent

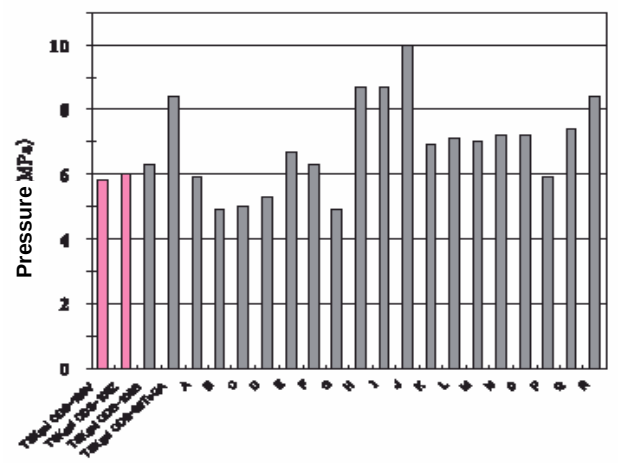

Figure 4. Comparison of column pressure drop
Test conditions: The same as Figure 2.

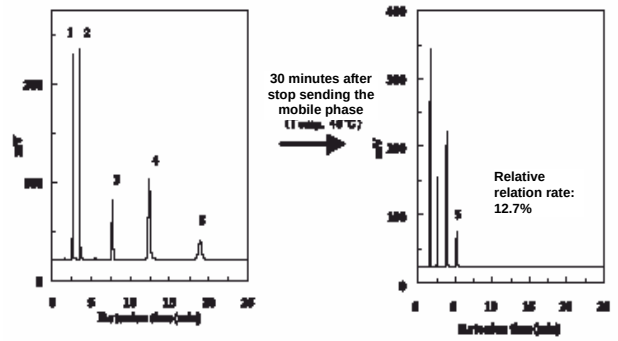

Figure 6. Retention decreases when using a buffer without organic solvent as mobile phase (typical ODS column)
Column: ODS (pore size: 100A)
Size: 4.6 mm I.D. x 15 cm
Mobile phase: 10 mmol/L phosphate buffer, pH 6.8
Flow rate: 1.0 mL/min
Temperature: 40 ˚C
Detection: UV 260 nm
Injection volume: 10 µL
Samples: 1. Cytosine, 2. Uracil, 3. Uridine, 4. Guanosine, 5. Adenine

4-4. H/u curve (van Deemter curve)

Figure 5 shows the relationship between flow rate (linear velocity) and the height equivalent to a theoretical plate (HETP) using ODS-100Z. When using a methanol-containing mobile phase (O), column efficiency was high, with a low HETP, at linear velocities of approximately 4-6 cm/min (flow rate of approximately 0.7-1.0 mL/min). On the other hand, when using an acetonitrile-containing mobile phase, which is less viscous than a methanol solution, (△), column efficiency was high at higher linear velocities of 7-11 cm/min (1.2-1.8 mL/min). Furthermore, the H/u curve was shallower; in comparison to the methanol solution, HETP was less affected over a wider range of flow rates. As such, an aqueous acetonitrile solution is preferred over an aqueous methanol solution as the mobile phase. In practice, the flow rate is usually selected based on the column back pressure and the recommended flow rate range as stated in the instruction manual.

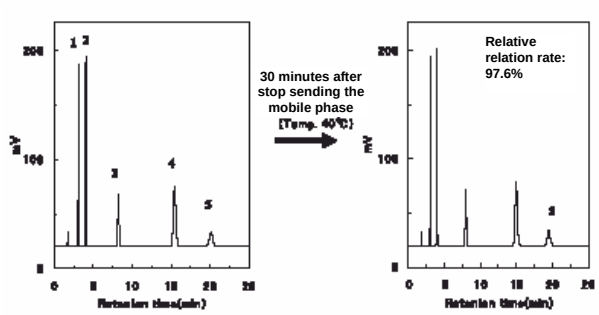

Figure 8. Minimal retention loss when using a mobile phase without an organic modifier (ODS-100V)

Column:	TSKgel ODS-100V
Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	10 mmol/L phosphate buffer, pH 6.8
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Detection:	UV 260 nm
Injection volume:	10 µL
Samples:	1. Cytosine, 2. Uracil, 3. Uridine, 4. Adenine, 5. Guanosine

4-5. Retention using 100% water as the mobile phase (ODS-100V)

In reversed-phase chromatography using a porous packing material, samples are partitioned between the stationary phase and mobile phase and retained in pores depending on hydrophobicity of samples. However, when the concentration of organic solvent in the mobile phase is very low or is zero, sample retention decreases as shown in **Figure 6**. This phenomenon has been explained as follows: When the concentration of organic solvent in the mobile phase is low (or when polarity is high), the stationary phase cannot easily disperse into the mobile phase, and the C18 alkyl chains are more likely to adsorb onto each other by hydrophobic interaction. Also, when there is no organic solvent, water or buffer is pushed out of pores, inhibiting samples from entering the pores and being retained on the column (**Figure 7**). For an experiment conducted under the conditions shown in **Figure 6** using ODS-100V, sample retention barely decreased as is shown in **Figure 8**. This demonstrates that ODS-100V can be used with mobile phases that do not organic solvent, making this column type very suitable for analysis of hydrophilic compounds.

4-6. Ion exchange activity

1) Effects on basic compounds

ODS is generally synthesized by chemically bonding of C18 alkyl chains to silica gel. After the reaction has been completed, , a relatively large number of silanol groups remain unreacted on the silica gel surface due to steric reasons. These unreacted silanol groups affect sample retention and peak shape. **Figure 9** shows the changes in the retention of neutral (benzene) and basic (desipramine) compounds on ODS-100V at various mobile phase pH levels. The retention of benzene was barely affected because its chemical properties do not change regardless of mobile phase pH. However, the retention of desipramine was affected, because the hydrophobicity of this compound increases at higher pH due to reduced dissociation of amino groups. Next, using ODS columns prepared by different endcapping procedures,

changes in the retention and peak shape of desipramine were compared at various mobile phase pH levels (**Figures 10 and 11**). At pH ≥5, retention gradually increased for all columns, with a marked increase for ODS-80Ts QA. In comparison with ODS-100V or ODS-100Z, the end-capping efficiency of ODS-80TsQA is poor and the number of residual silanol groups is relatively high. Thus, when the pH of the mobile phase increased, the electrostatic interaction between the dissociated silanol groups and the amino groups of desipramine became stronger. Clearly, the TSKgel ODS-100V and TSKgel ODS-100Z columns are among the best performing ODS columns for the analysis of basic compounds at low and neutral pH.

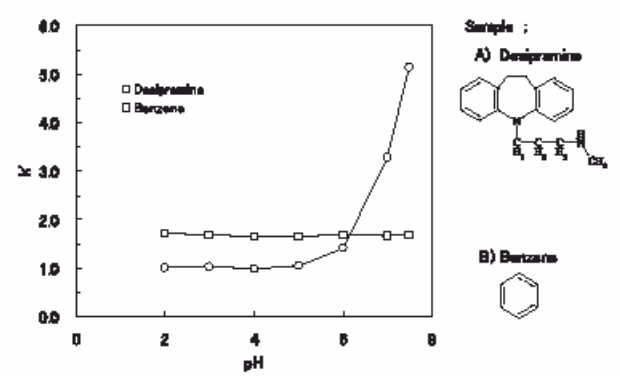

Figure 9. Relationship between retention and mobile phase pH (comparison between neutral and basic compounds)

Column:	TSKgel ODS-100V
Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	50 mmol/L phosphate buffer/MeOH (30/70)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	A) Desipramine B) Benzene

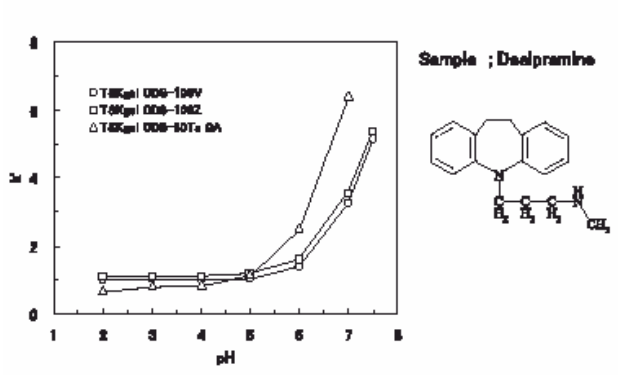

Figure 10. Relationship between basic compound retention and mobile phase pH

Columns:	TSKgel ODS-100V, TSKgel ODS-100Z and TSKgel ODS-80TsQA
Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	50 mmol/L phosphate buffer/MeOH (30/70)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Sample:	Desipramine

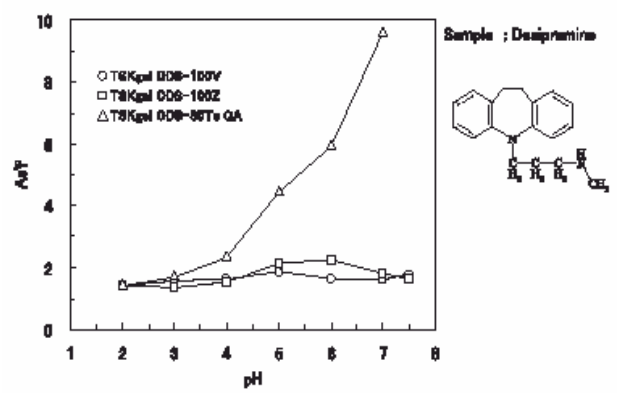

Figure 11. Relationship between the asymmetry factor of a basic compound and pH of mobile phase

Test conditions: The same as given in Figure 10.

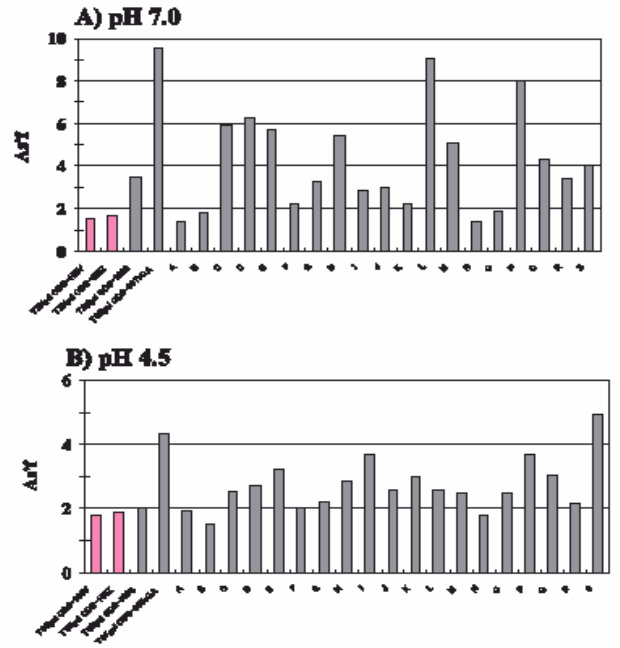

Figure 12. Comparison of the asymmetry factor of basic compounds

Column size:	4.6 mm I.D. x 15 cm
Mobile phases:	A) 50 mmol/L phosphate buffer (pH 7.0)/MeOH (30/70) B) 50 mmol/L NaH_2PO_4/MeOH (50/50)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	A) Desipramine B) Nortriptyline

Figure 11 shows the changes in asymmetry factor under the same conditions as given in Figure 10. With ODS-80Ts QA, the higher the mobile phase pH, the greater the asymmetry factor for the desipramine peak. However, with ODS-100V or ODS-100Z, there were no distinct changes in asymmetry factor, and, irrespective of mobile phase pH, there was minimal peak tailing. The reason for this is that the endcapping efficiency of ODS-100V and ODS-100Z is very high, reducing the number of residual silanol groups. Because there is very little peak tailing for basic compounds with both ODS-100V and ODS-100Z when using a neutral mobile phase, it is possible to analyze basic compounds even when these compounds are strongly retained. **Figure 12** compares the asymmetry factor of basic compounds using a neutral or moderately acidic mobile phase among the various ODS columns. In comparison with the other ODS columns, the asymmetry factor of ODS-100V and ODS-100Z is very small (low peak tailing). **Figure 13** compares chromatograms of basic compounds between ODS-100V and commercial AQ-type ODS columns. In contrast to commercial AQ-type ODS columns, the ODS-100V column showed no peak tailing for both desipramine and imipramine, and gave very symmetrical peak shapes for these compounds.

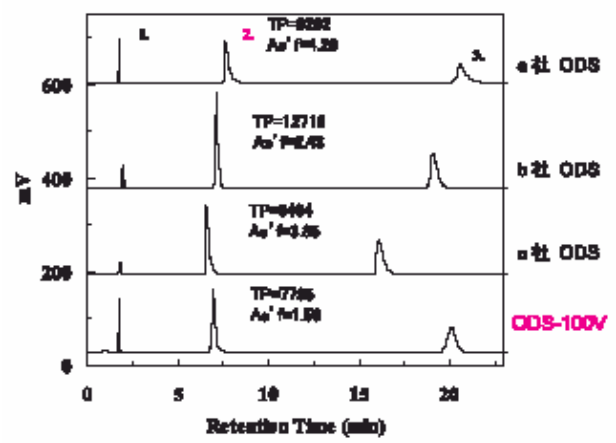

Figure 13. Comparison of chromatograms of basic compounds (ODS-100V and commercial AQ type ODS columns)

Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	50 mmol/L phosphate buffer (pH 7.0)/MeOH (30/70)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Detection:	UV (254 nm)
Injection volume:	10 µL
Samples:	1. Uracil, 2. Desipramine, 3. Imipramine

2) Effects on acidic compounds

Figure 14 compares the peak shape (asymmetry factor) of an acidic compound (formic acid) using an acidic mobile phase. The peak shape of acidic compounds is generally symmetric with an acidic mobile phase, but as shown in **Figure 14**, peak tailing was found on many ODS columns. The asymmetry factor for both ODS-100V and ODS-100Z was very small, indicating symmetrical peak shapes. **Figure 15** compares chromatograms of acidic compounds on ODS-100V and commercial AQ-type ODS columns. With ODS-100V, the peaks for formic acid and acetic acid were symmetrical.

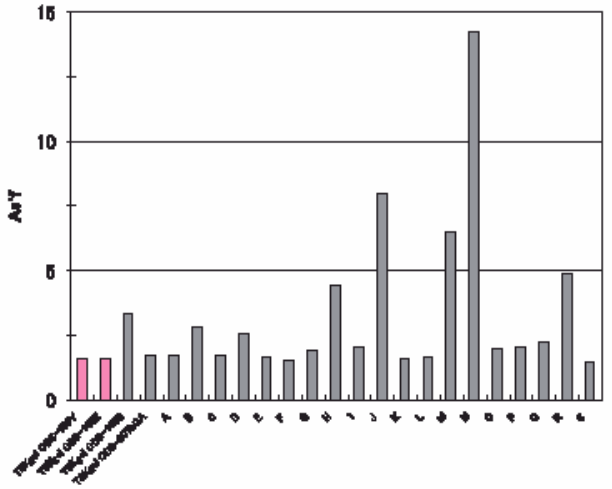

Figure 14. Comparison of the asymmetry factor of an acidic compound

Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	H_2O/CN_3CN (98/2) + 0.1% H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Sample:	Formic acid

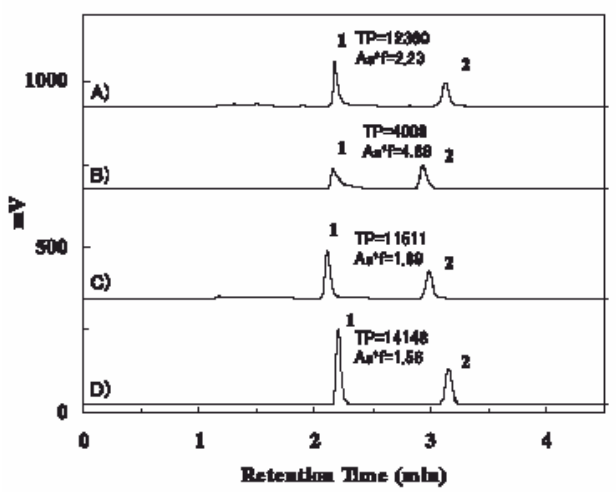

Figure 15. Comparison of chromatograms for acidic compounds

Columns:	A)-C): Commercial AQ type ODS columns D): TSKgel ODS-100V
Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	H_2O/CN_3CN (98/2) + 0.1% H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	1. Formic acid, 2. Acetic acid

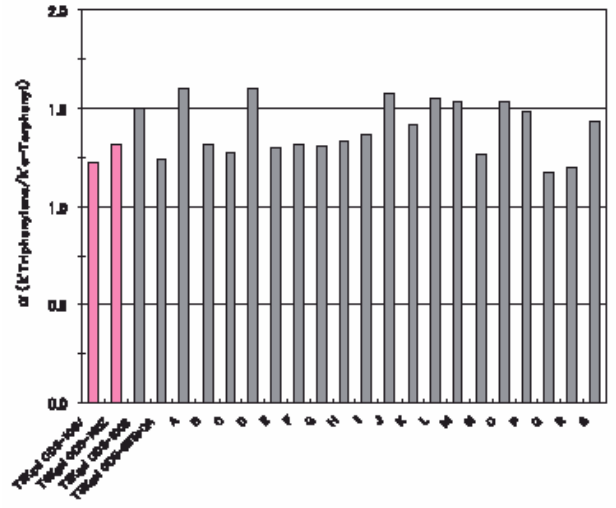

Figure 16. Comparison of steric selectivity

Column size:	4.6 mm I.D. x 15 cm
Mobile phase:	H_2O/MeOH (30/70)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	Triphenylene, *o*-Terphenyl

4-7. Steric selectivity

Figure 16 compares the steric selectivity of the various ODS columns. In general, the steric selectivity of polymeric stationary phases having a high surface density of alkyl chains is superior to that of monomeric stationary phases. In **Figure 16**, columns with a separation factor (α) for triphenylene and *o*-terphenyl of 1.5 or higher are expected to contain polymeric stationary phases. Both ODS-100V and ODS-100Z have monomeric stationary phases, and because the carbon content of ODS-100Z is higher than that of ODS-100V (approximately 20% and 15%, respectively), the steric selectivity of ODS-100Z is higher (**Figure 17**) and as such is better suited for the analysis of heterocyclic compounds

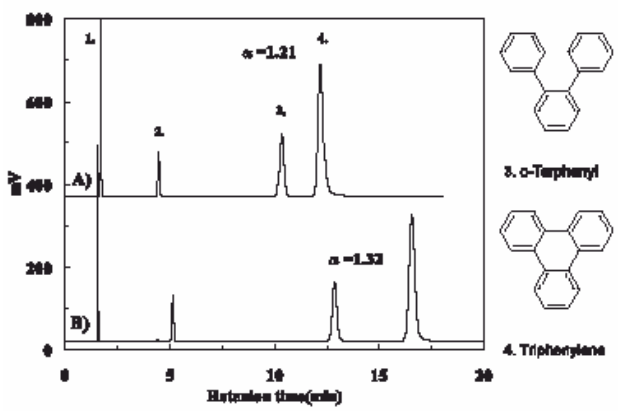

Figure 17. Comparison of steric selectivity between ODS-100V and ODS-100Z

Columns:	A) TSKgel ODS-100V (4.6 mm I.D. x 15 cm) B) TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Mobile phase:	H_2O/MeOH (20/80)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	1. Uracil, 2. Benzene, 3. *o*-Terphenyl, 4. Triphenylene

4-8. Durability

In general, ODS columns gradually deteriorate if a low pH acidic mobile phase is continuously used. To test this effect, a commonly utilized acidic mobile phase (50% methanol solution containing 0.1% TFA) was used in chromatography with ODS-100Z over a long period, and the chromatograms taken before, during, and after were compared (**Figure 18**). There was minimal change in the retention time, theoretical plate number and asymmetry factor for amitriptyline (a basic compound) before and after using the acidic mobile phase.

4-9. Effects of organic solvent concentration in sample solution

In reversed phase chromatography, the lower the polarity of the mobile phase (the higher the concentration of the organic solvent), the greater the proportion of sample that exists in the mobile phase, giving lower retention.

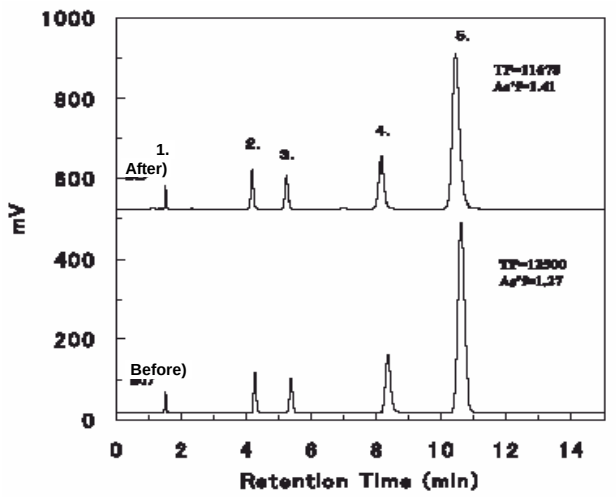

Figure 18. Comparison of chromatograms before and after flushing the column with an acidic mobile phase

Column: TSKgel ODS-100Z (4.6 mm I.D. × 15 cm)
Mobile phase: 20 mmol/L phosphate buffer (pH 7.0)/MeOH (20/80)
Flow rate: 1.0 mL/min
Detection: 254 nm
Temperature: 40 ˚C
Injection volume: 2 µL
Samples: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarine, 5. Amitriptyline
Acidic mobile phase passage
Mobile phase: H_2O/MeOH(50/50)+0.1% TFA
Flow rate: 1.0 mL/min
Flushing volume: 50 L

When the concentration of an organic solvent in a sample solution is higher than that in the mobile phase, the sample partitioning between mobile phase and stationary phase is affected, resulting in a broadened peak. Using p-hydroxy benzoate esters, the effects of organic solvent concentration in sample solution on peak shape (HETP) were investigated (**Figure 19**). When the acetonitrile concentration of the mobile phase was 40% and methyl hydroxy benzoate was used as the sample, HETP increased above an organic solvent concentration of 50%; in other words, the peak shape became broader. Also, as the hydrophobicity of the samples increased, the organic solvent concentration at which the broad peak was obtained became greater. The more hydrophilic the sample , the greater is the effect of the organic solvent concentration in the sample solution. To avoid this effect, it is desirable to lower the organic solvent concentration in the sample solution as much as possible (i.e., by diluting with a high polarity solvent such as water) or to lower the injection volume.

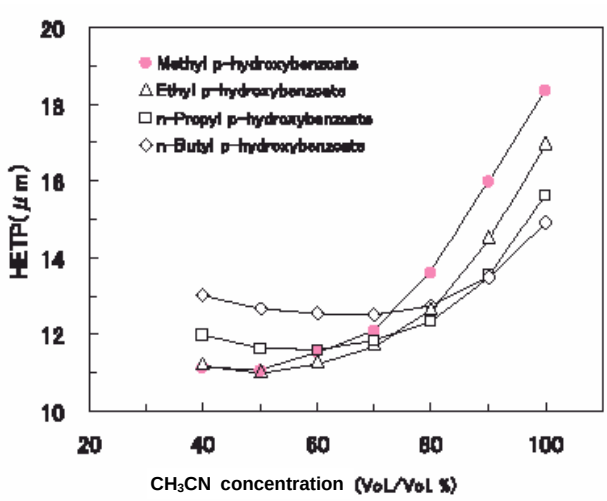

Figure 19. Effects of organic solvent concentration in sample solution on peak shape

Column: TSKgel ODS-100V (4.6 mm I.D. × 15 cm)
Mobile phase: H_2O/acetonitrile (60/40)
Flow rate: 1.0 mL/min
Temperature: 40 ˚C
Injection volume: 10 µL
Samples: (Methyl, Ethyl, n-Propyl and n-Butyl)-p-hydroxy benzoate esters

4-10. Lot-to-lot variability

Figure 20 shows the chromatograms for SRM870 of lots using different packing material and base silica. There were no marked differences among the chromatograms, confirming that lot-to-lot variability is very small and that the consistency of the manufactured packing material is high.

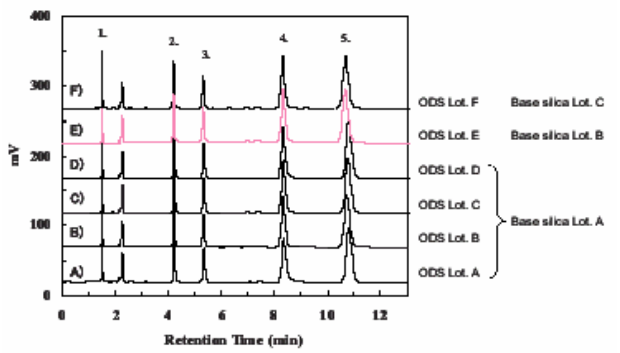

Figure 20. Lot-to-lot variability for gel and base silica (ODS-100Z)

Columns:	TSKgel ODS-100Z (4.6 mm I.D. x 15 cm) A)-D): Base silica lot A E): Base silica lot B F): Base silica lot C
Mobile phase:	20 mmol/L phosphate buffer (pH 7.0)/MeOH (20/80)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Detection:	UV (254 nm)
Injection volume:	10 µL
Samples:	SRM870, 1. Uracil, 2. Methyl benzene, 3. Toluene, 4. Quinizarine, 5. Amitriptyline

5. Application data

Figures 21-25 shows Application data of ODS-100V and ODS-100Z.

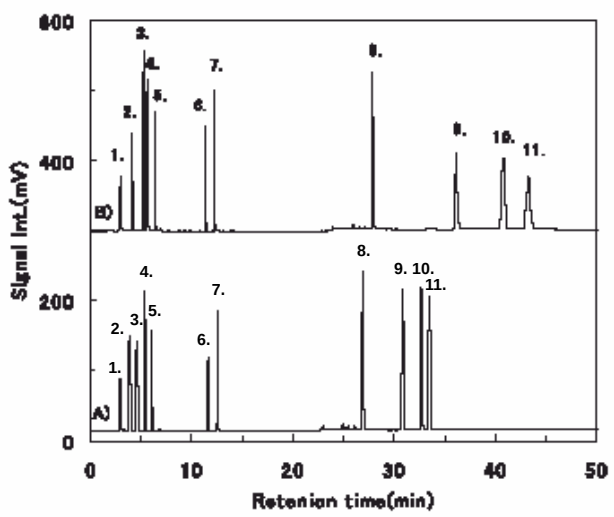

Figure 21. Chromatograms for vitamins (comparison between ODS-100V and ODS-100Z)

Columns:	A) TSKgel ODS-100V (4.6 mm I.D. x 15 cm) B) TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Mobile phases:	A) 0.1% TFA in H_2O B) 0.1% TFA in CH_3CN
Gradient:	0 min (B: 0%) → 20 min (B: 40%) → 22 min (B: 100%)→ 50 min (B: 100%)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Detection:	UV (280 nm)
Injection volume:	5 µL
Samples:	1. L-Ascorbic acid, 2. Nicotinic acid, 3. Thiamine, 4. Pyridoxal, 5. Pyridoxine, 6. Caffeine, 7. Riboflavin, 8. Retinol, 9. δ-Tocopherol, 10. α-Tocopherol, 11. α-Tocopherol acetate

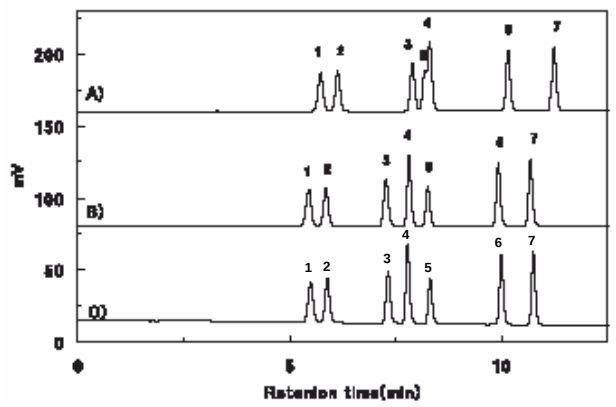

Figure 22. Chromatograms of catechins

Columns:	A) TSKgel ODS-100V B) TSKgel ODS-100Z C) TSKgel ODS-80TsQA
Column size:	4.6 mm I.D. x 15 cm
Mobile phases:	A) 10 mmol/L KH_2PO_4 B) CH_3CN
Gradient:	0 min (B: 18%) → 15 min (B: 60%) → 20 min (B: 60%) → 21 min (B: 18%)
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	5 µL
Samples:	1. (-)-Epigallocatechin, 2. (-)-Catechin, 3. (-)-Epigallocatechin gallate, 4. Caffeine, 5. (+)-Epicatechin, 6. (-)-Epicatechin gallate, 7. (-)-Catechin gallate

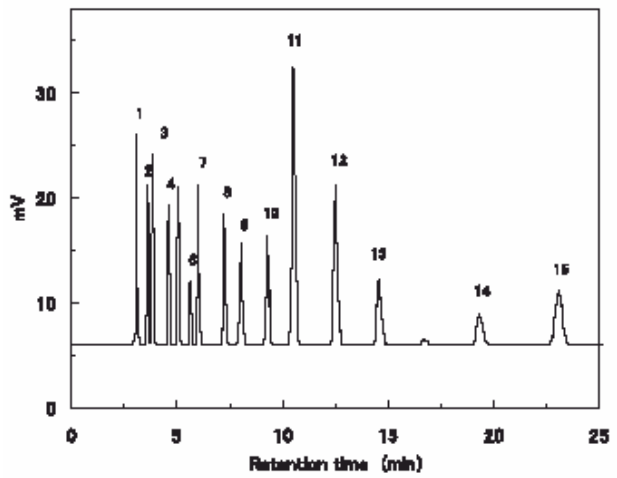

Figure 23. Chromatograms for organic acids

Column:	TSKgel ODS-100V (4.6 mm I.D. x 25 cm)
Mobile phase:	0.1% H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	40 ˚C
Injection volume:	10 µL
Samples:	1. Oxalic acid (0.1 g/L), 2. L-Tartaric acid (0.5), 3. Formic acid (1.0), 4. L-Malic acid (1.0), 5. L-Ascorbic acid (0.1), 6. Lactic acid (1.0), 7. Acetic acid (1.0), 8. Maleic acid (0.01), 9. Citric acid (1.0), 10. Succinic acid (1.0), 11. Fumaric acid (0.025), 12. Acrylic acid (0.1), 13. Propionic acid (2.0), 14. Glutaric acid (1.0), 15. Itaconic acid (0.025)

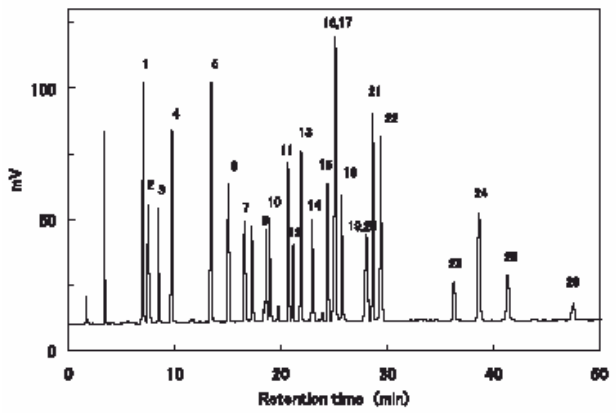

Figure 24. Chromatograms for polymer additives

Column:	TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
Mobile phases:	A) H_2O B) CH_3CN
Gradient:	0 min (B: 60%) → 20 min (B: 100%)
Flow rate:	1.0 mL/min
Temperature:	50 ˚C
Detection:	UV (225 nm)
Injection volume:	10 µL
Concentration:	10 mg/L each
Samples:	1. Cyasorb UV-24, 2. BHA, 3. Ionox 100, 4. Seesorb 101, 5. Tinuvin P, 6. Yoshinox SR, 7. Seesorb 202, 8. BHT, 9. Noclizer M-17, 10. Yoshinox 2246R, 11. Topanol CA, 12. Yoshinox 425, 13. Cyanox 1790, 14. Cyasorb UV-531, 15. Ionox 220, 16. Nonflex CBP, 17. Tinuvin 326, 18. Tinuvin 120, 19. Irganox 3114, 20. Uvtex OB, 21. Tinuvin 327, 22. Tinuvin 328, 23. Irganox 1010, 24. Irganox 1330, 25. Irganox 1076, 26. Irgafos 168

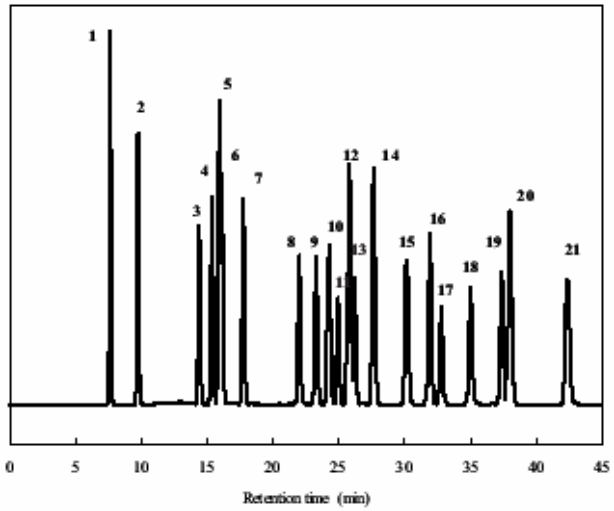

Figure 25. Gradient analysis mono-, di-, and trinucleotides

Column:	TSKgel ODS-100V (4.6 mm I.D. x 25 cm)
Mobile phases:	A) 20 mmol/L t-butylamine + H_3PO_4 (pH 6.8) B) A/CH_3OH (90/10)
Gradient:	0 min (B: 0%) → 35 min (B: 100%)
Flow rate:	1.0 mL/min
Temperature:	25 ˚C
Detection:	UV (260 nm)
Injection volume:	2 µL
Concentration:	0.3 g/L each
Samples:	1. CMP, 2. UMP, 3. CDP, 4. dUMP, 5. GMP, 6. IMP, 7. UDP, 8. CTP, 9. TMP, 10. GDP, 11. IDP, 12. AMP, 13. UTP, 14. dGMP, 15. TDP, 16. GTP, 17. ITP, 18. ADP, 19. TTP, 20. dAMP, 21. ATP

6. Conclusions

As described above, because TSKgel ODS-100V and ODS-100Z are made of the same base silica, they have similar column efficiency (theoretical plate numbers) and pressure-flow characteristics. Also, because of highly efficient endcapping, residual ion-exchange activity of the gel is low, and symmetrical peaks with minimal tailing can be obtained for basic and acidic compounds. Since the surface modification methods for TSKgel ODS-100V and ODS-100Z differ, however, the sample selectivity is different. In general, TSKgel ODS-100V is best suited for the retention and separation of hydrophilic compounds, while TSKgel ODS-100Z is best for the retention and separation of hydrophobic compounds. With these two ODS columns, a wide variety of samples can be optimally separated.

*: Tosoh carefully measured the data presented in this article, but does not guarantee their accuracy. Tosoh recommends that each user gather data and confirm results for the particular test environments, conditions and judgment criteria.

Scientific Instrument Division, Tosoh Corporation

Tokyo Headquarters, Sales Division	3-8-2 Shiba, Minato-ku, Tokyo 105-8623, Telephone: 03-5427-5180
Osaka Branch, Scientific Instrument G	1-2-6 Dojimahama, Kita-ku, Osaka 530-0004, Telephone: 06-6344-3857
Nagoya Branch, Scientific Instrument G	1-17-13 Nishiki, Naka-ku, Nagoya 460-0003, Telephone: 052-211-5730
Fukuoka Branch	1-13-2 Tenjin Chuo-ku, Fukuoka 810-0001, Telephone: 092-781-0481
Sendai Branch	1-11-1 Honmachi, Aoba-ku, Sendai 980-0014, Telephone: 022-266-2341
Yamaguchi office	4560 Kaiseicho, Shunan, Yamaguchi 746-8501, Telephone: 0834-63-9888
Customer support center	2743-1 Hayakawa, Ayase, Kanagawa 252-1123, Telephone: 0467-76-5384
Tosoh Scientific Instrument Division Website:	http://www.tosoh.co.jp/science/
Tosoh HLC database:	http://www2.tosoh.co.jp/hlc/hlcdb.nsf/StartJ?OpenForm
Email contact:	tskgel@tosoh.co.jp