TOSOH

SEPARATION REPORT NO. 104

3 MICRON REVERSED-PHASE CHROMATOGRAPHY COLUMN: TSKgel ODS-100V 3 µM

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1. Introduction

Reversed-phase chromatography (RPC) is the most frequently employed separation mode in high-performance liquid chromatography, because it can be used for many different types of sample and has superior operability and separation performance. Reversed-phase octadecyl (C18)-bonded silica gel is widely used as a packing material for examination of low-molecular-weight compounds such as pharmaceuticals.

Tosoh has introduced the TSKgel ODS-100V 3µm column, containing packing material with a smaller particle size than that of the previous model, TSKgel ODS-100V 5µm (see Separation Report No. 102). To ensure that the new column maintains the separation properties of TSKgel ODS-100V 5µm, it contains monolayer octadecyl-bonded (C18) silica gel with a particle size of 3µm. TSKgel ODS-100V 3µm shares many of the same features as TSKgel ODS-100V 5µm, such as superior end-capping efficiency of residual silanol groups, favorable peak shapes for basic and acidic compounds, and the possibility of using mobile phases free from organic solvents. This article discusses the fundamental properties of TSKgel ODS-100V 3µm and presents some of applications.

2. Fundamental properties of TSKgel ODS-100V 3µm

2.1. Properties of the packing materials

Table 1 compares the fundamental characteristics of TSKgel ODS-100V 3µm, TSKgel ODS-100V 5µm and TSKgel ODS-100Z 5µm. Apart from particle size, the base silica gels of TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm have the same fundamental properties. Also, TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm share the same surface modification (functional group bonding and residual silanol group end-capping), and, as shown in Table 2, most of the HPLC values are comparable. Furthermore, the carbon content of TSKgel ODS-100V 3µm has been adjusted to achieve a retention comparable to that of TSKgel ODS-100V 5µm. Therefore, the separation properties of the both ncolumns are comparable.

Figure 1 shows chromatograms of test mixture which contains the same component as NIST SRM870, obtained using TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm. The retention of each peak and the asymmetry factors for amitriptyline (a basic compound) and quinizarine (a mtal chelating compound) are nearly the same as each other.

Figure 2 shows the relationship between the hydrophobicity of common low-molecular-weight compounds (hydrophobicity parameter: log P) and retention (retention factor: log k') using TSKgel ODS-100V 3µm and TSKgel ODS-100Z (3µm prototype). Compared to TSKgel ODS-100Z 3µm, with high carbon content (20%) and low packing material surface polarity, the retention of compounds with low log P values (hydrophilic compounds) was higher for TSKgel ODS-100V 3µm (oval region in the lower left corner). Thus, due to the higher surface polarity of the packing material, hydrophilic compounds are retained more strongly by TSKgel ODS-100V 3µm when compared to TSKgel ODS-100Z 3µm.

Table 1: Fundamental properties of TSKgel ODS-100V and ODS-100Z

Column	Particle size (µm)	Pore size (Å)	Specific surface area (m^2/g)	Pore volume (mL/g)	Functional group	Carbon content*) (%)	Phase structure
TSKgel ODS-100V 3µm	3	100	450	1.10	C18	15	Monolayer
TSKgel ODS-100V 5µm	5	100	450	1.10	C18	15	Monolayer
TSKgel ODS-100Z 5µm	5	100	450	1.10	C18	20	Monolayer

*) Measured by quantitative elemental analysis
Å = 1 × 10-1nm

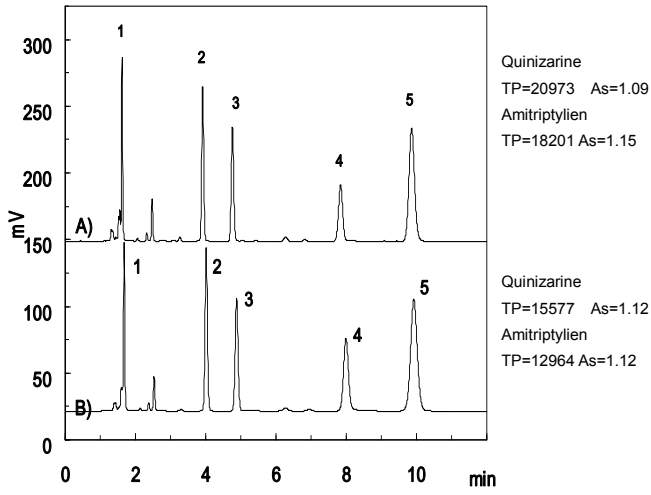


Figure 1: Standard sample chromatograms

Column: A) TSKgel ODS-100V 3µm (4.6 mm I.D. ×15 cm)
B) TSKgel ODS-100V 5µm (4.6 mm I.D. ×15 cm)
Eluent: 20 mmol/L Phosphate Buffer (pH 7.0) /CH_3OH = 20 / 80
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Sample: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarine, 5. Amitriptyline
Inj. volume: 10µL

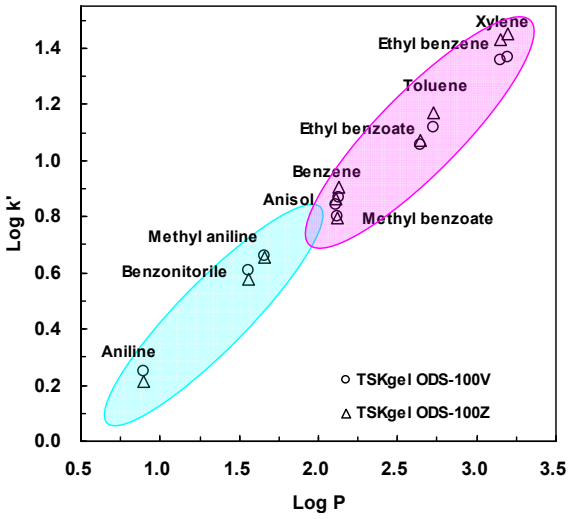


Figure 2 Relationship between hydrophobicity parameter (log P) and retention (log k')

Column: TSKgel ODS-100V 3µm (4.6 mm I.D. ×15 cm)
TSKgel ODS-100Z (3 µm: prototype) (4.6 mm I.D. ×15 cm)
Eluent: H_2O/CH_3CN = 60 / 40
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temperature 40□
Sample: Aniline, Benzonitorile, Methyl aniline, Anisol, Methyl benzoate, Benzene, Ethyl benzoate, Toluene, Ethyl benzene, Xylen

Table 2. HPLC properties of TSKgel ODS-100V and ODS-100Z

Column	Retention coefficient k'	Stereoselectivity α	Hydrogen binding α	Hydrophobicity α	Surface polarity α	Ionization					Coordinate linkage		Retention reduction
						Basic			Acidic				
						As'f(Des)	α(k'Ami/k'EB)	As'f(Ami)	α(k'For/k'Ac)	As'f(For)	α(k'Quini/k'EB)	As'f(Quini)	(RT2Adn/RT1Adn)
TSKgel ODS-100V 3µm	1,78	1,24	0,47	1,64	0,54	1,62	2,60	1,08	0,48	1,29	1,98	1,02	97,8
TSKgel ODS-100V 5µm	1,80	1,25	0,45	1,64	0,53	1,59	2,60	1,21	0,48	1,32	1,98	1,16	99,0
TSKgel ODS-100Z 5µm	2,42	1,31	0,40	1,72	0,43	1,62	2,38	1,07	0,44	1,41	1,77	1,20	-

1.Retention coefficient: k'(Naphthalene)
2.Stereoselectivity: α=k'(Triphenylene)/k'(o-Terphenyl)
3.Hydrogen binding: α= k'(Caffeine)/k'(Phenol)
4.Hydrophobicity: α= k'(Toluene)/k'(Benzene)
5.Surface polarity: α= k'(Methyl benzoate)/k'(toluene)

6.Ionization
1) As'f(Des)=As'f(Desipramine) (pH 7.0)
2) α(k'Ami/k'EB) α=k'(Amitriptyline)/k'(Ethyl Benzene)
3) As'f(Ami)=As'f(Amitriptyline)
4) α(k'For/k'Ac) α=k'(Formic acid)/k'(Acetic acid)
5 As'f(For)=As'f(Formic acid)
7.Coordinate linkage
1) α(k'Quini/k'EB) α=k'(Qunizarine)/k'(Ethyl Benzene)
2) As'f(Quini)=As'f (Qunizarine)
8.Retention: %
RT1Adn: Initial elution time for adenine
RT2Adn: Elution time for adenine at 30 minutes after injection
*As: asymmetry coefficient

2-2. H – u curve (Van Deemter curve)

Figure 3 shows the relationship between height equivalent to a theoretical plate (HETP) and linear velocity for TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm. Because the particle size of the former is smaller than that of the latter, its HETP is smaller (higher column efficiency). Also, for TSKgel ODS-100V 5µm, the smallest HETP and high column efficiency are obtained at a linear velocity of 4 - 6 cm/min, but with TSKgel ODS-100V 3µm, which has a smaller particle size, column efficiency is the highest at a greater linear velocity of ≥ 6 cm/min. Furthermore, for TSKgel ODS-100V 5µm, a greater linear velocity (≥ 6 cm/min) leads to lower column efficiency, while for TSKgel ODS-100V 3µm, column efficiency does not decrease much at high linear velocity, and high column efficiency is maintained over a wide range of velocities (6 - 10 cm/min; about 1.0 - 1.7 mL/min for a column with an internal diameter of 4.6 mm). Therefore, compared to TSKgel ODS-100V 5µm, chromatography can be performed with higher resolution and higher velocity.

Various organic solvents can be used as RPC mobile phases, but the range of optimal linear velocity varies for different solvents. Figure 4 shows an H - u curve obtained using a 2.0-mm I.D. column with either methanol or acetonitrile as a mobile phase. Since acetonitrile is less viscous, maximum column efficiency occurs at a higher linear velocity, and high column efficiency is maintained over a greater range of linear velocity. Figure 5 compares chromatograms obtained using methanol and acetonitrile at flow rates yielding favorable column efficiency (0.20 and 0.50 mL/min, respectively). This figure shows that using acetonitrile, RPC can be performed in about 2/5 of the time taken for methanol without compromising column efficiency. In addition, Fig. 6 shows the durability of TSKgel ODS-100V 3µm column using acetonitrile at a flow rate of 0.50 mL/min. After 1,000 hours, the theoretical plate number had not decreased, and favorable column performance (theoretical plate number and asymmetry factor) was obtained. In this manner, without compromising column efficiency, RPC can be performed in a short time by using a low-viscosity solution as a mobile phase and increasing flow rate.

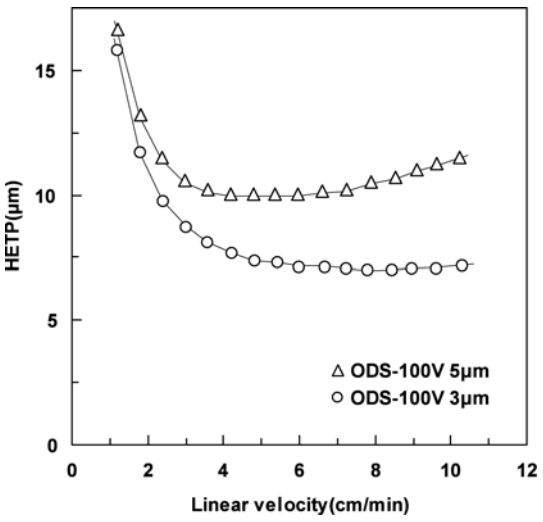


Figure 3 Effects of linear velocity on height equivalent to a theoretical plate (HETP)
Column: TSKgel ODS-100V 3µm (4.6 mm I.D.x 15 cm)
TSKgel ODS-100V 5µm (4.6 mm I.D.□15 cm)
Eluent: H_2O/CH_3OH = 30/70
Detection: UV 254 nm
Temp.: 40 °C
Sample: Naphthalene
Inj. volume: 10µL

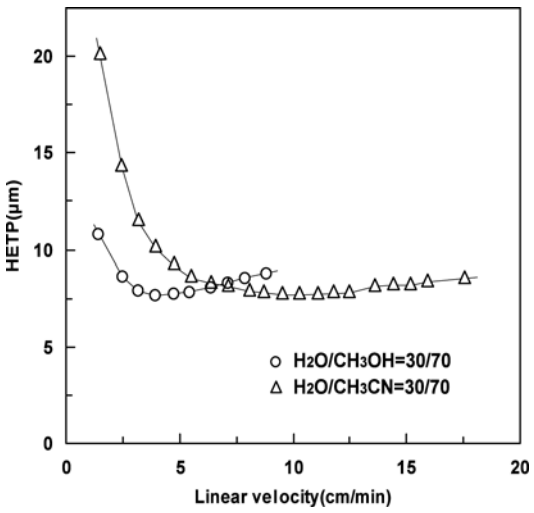


Figure 4 Effects of linear velocity on height equivalent to a theoretical plate: Effect of organic solvent
Column: TSKgel ODS-100V 3µm (2.0 mm I.D.x 15 cm)
Eluent: H_2O/CH_3OH = 30/70
H_2O/CH_3CN = 40/60
Detection: UV 254 nm
Temp.: 25 °C
Sample: Naphthalene
Inj. volume: 2µL

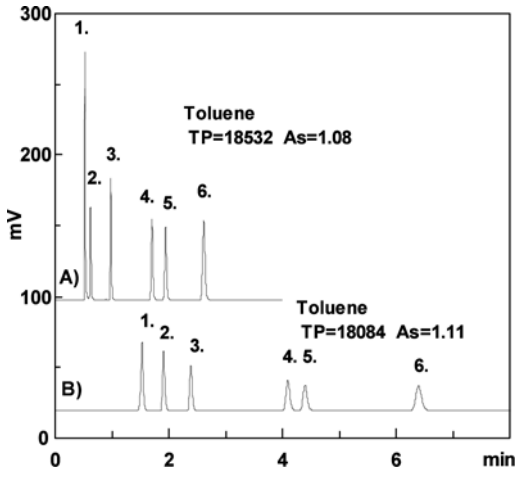


Figure 5 **Comparison of chromatograms for standard chemicals**

Column: TSKgel ODS-100V 3µm (2.0 mmI.D.x 15 cm)
Eluent: A) H_2O/CH_3CN = 40/60
B) H_2O/CH_3OH = 30/70
Flow rate: A) 0.50 mL/min
B) 0.20 mL/min
Detection: UV 254 nm
Temp.: 25 °C
Sample: 1. Uracil, 2. Caffeine
3. Phenol 4. Methyl benzoate
5. Benzene 6. Toluene
Inj. volume: 2µL

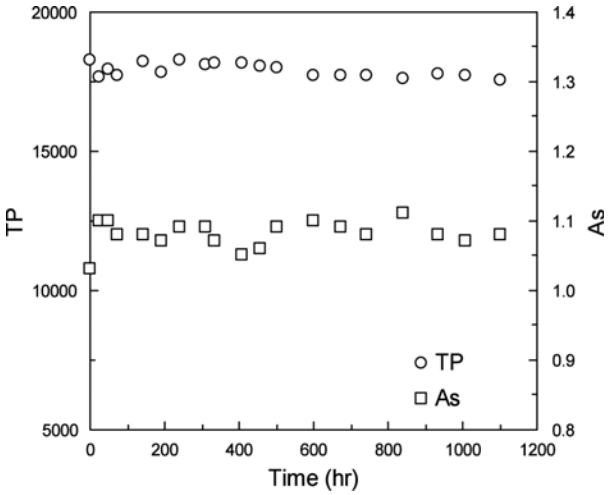


Figure 6 **Durability under long term flushing with eluent**

Column: TSKgel ODS-100V 3µm (2.0 mmI.D.x 15 cm)
Eluent: H_2O/CH_3CN = 40/60
Flow rate: 0.50 mL/min
Detection: UV 254 nm
Temp.: 25 °C
Sample: Toluene
Inj. volume: 2µL

2-3 Residual ion exchange activity

In ODS packing materials (C18-bonded silica gel), residual silanol groups affect the retention and peak shape of basic compounds. Similarly to TSKgel ODS-100V 5µm, end-capping of residual silanol groups is efficient for TSKgel ODS-100V 3µm. Chromatograms for desipramine (basic) and benzene (neutral) before and after end-capping of residual silanol groups in TSKgel ODS-100V 3µm were compared (Fig. 7). No marked changes were observed in the retention and peak shape of benzene before and after end-capping (Peak 3). Because desipramine (Peak 2) has electrostatic interactions with residual silanol groups, the chromatogram on TSKgel ODS-100V 3µm without end-capping exhibited strong retention and peak tailing (Chromatogram B); in contrast, when TSKgel ODS-100V 3µm with end-capping was used, elution and peak shapes were normal (Chromatogram A).

Figure 8 shows the retention of benzene and desipramine using TSKgel ODS-100V 3µm with mobile phases of various pH. The retention of benzene, a neutral compound, was mostly stable regardless of mobile phase pH; in contrast, the retention of desipramine, a basic compound, increased with pH due to decreasing amino group dissociation and higher hydrophobicity. Figures 9 and 10 show the retention and asymmetry factors of desipramine using TSKgel ODS-100V 3µm and an ODS column with insufficient end-capping at various mobile phase pH values. In general, as shown in Fig. 9, differences in residual silanol group end-capping affect the retention in neutral mobile phases; retention of desipramine was higher for the packing material with insufficient end-capping. Also, as shown in Fig. 10, while use of a packing material with insufficient end-capping in a neutral mobile phase resulted in poor peak shapes (tailing) for desipramine, TSKgel ODS-100V 3µm yielded favorable peak shapes with minimal tailing regardless of mobile phase pH.

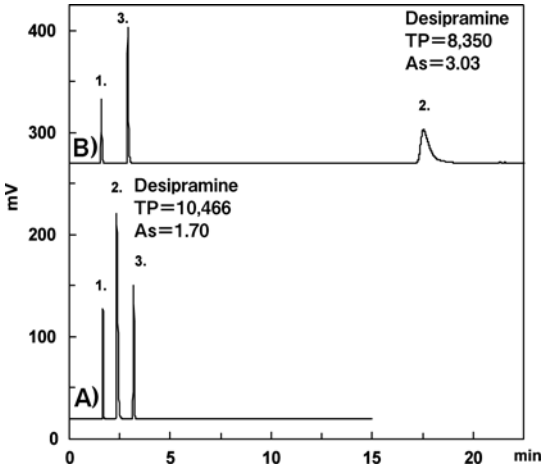


Figure 7 Comparison of chromatograms for basic compounds (desipramine): Effect of end-capping

Column: A) TSKgel ODS-100V 3 µm (4.6 mm x 15 cm)
B) TSKgel ODS-100V 3 µm (4.6 mm x 15 cm) (Not endcapped)
Eluent: 5 mmol/L $HCOONH_4$/ CH_3OH = 20/80
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: 1. Uracil 2. Desipramine (52 mg/L) 3. Benzene
Inj. volume: 10µL

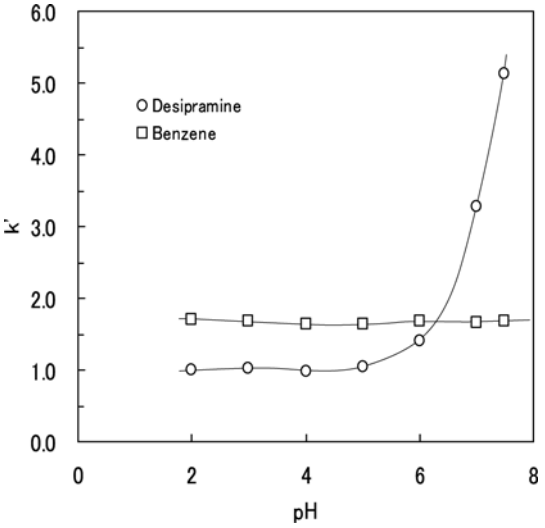
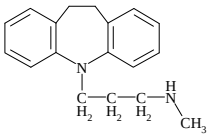


Figure 8 Relationship between mobile phase pH and retention: Comparison of basic and neutral compounds

Column: TSKgel ODS-100V 3µm (4.6 mm □ 15 cm)
Eluent: 50 mmol/L phosphate buffer (pH 2-75) /CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: Desipramine, Benzene
Inj. volume: 10µL

Desipramine



Benzen

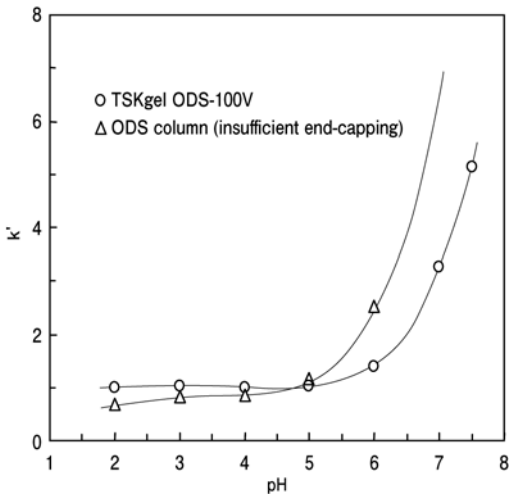
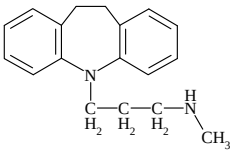


Figure 9 **Relationship between mobile phase pH and retention for a basic compound (desipramine)**

Column: TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm) ODS column (4.6 mm I.D. x 15 cm) (insufficient end-capping)
Eluent: 50mmol/L phosphate buffer (pH 2-75) /CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: Desipramine
Inj. volume: 10µL

Desipramine



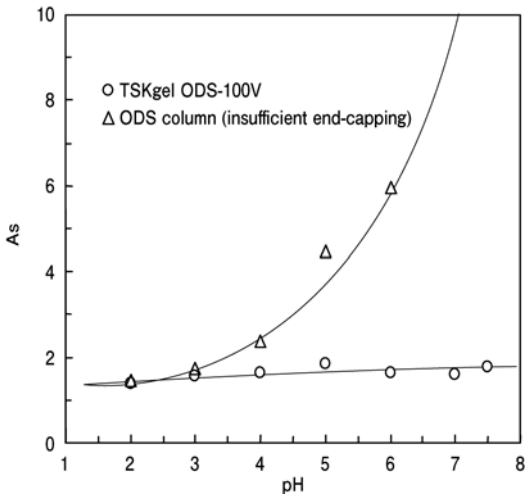
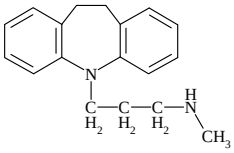


Figure 10 **Relationship between mobile phase pH and peak shape for a basic compound (desipramine)**

Column: TSKgel ODS-100V 3 µm (4.6 mm I.D x 15 cm) ODS column (4.6 mm I.D. x 15 cm) (insufficient end-capping)
Eluent: 50 mmol/L phosphate buffer (pH 2-75) /CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: Desipramine
Inj. volume: 10µL

Desipramine



2-4 Effects of mobile phase on LC/MS(/MS) analysis

In RPC, phosphate buffer is generally used to adjust the pH of the mobile phase. However, in LC/MS(/MS), the use of phosphate buffer, which is nonvolatile, lowers ionization efficiency and contaminates the MS detector; for this reason, it is necessary to use a low-concentration volatile buffer such as formic acid, ammonium formate or ammonium acetate. Figure 11 shows the effect of mobile phase salt concentration (phosphate and ammonium formate) on the peak shape of desipramine, a basic compound. When using phosphate buffer with high ionic strength as a mobile phase, peak shapes are generally favorable, or the asymmetry factor As is close to 1 at buffer concentrations of 5 - 50 mmol/L, independently of buffer concentration, but when ammonium formate, with low ionic strength, was used as a mobile phase, lower buffer concentrations resulted in larger As values and greater peak tailing. When using a UV detector in actual testing, favorable peak shapes are obtained by increasing the salt concentration of the mobile phase, but in LC/MS(/MS), the salt concentration of the mobile phase must be low to avoid poor ionization efficiency and the possibility of ion source contamination. In general, the mobile phase concentration is set at ≤ 10 mmol/L.

Figure 12 shows the chromatograms obtained by subjecting desipramine (basic compound, Peak 2) to RPC using TSKgel ODS-100V 3µm and a competitor's ODS (3 µm) with 5 mmol/L ammonium formate as a mobile phase. For TSKgel ODS-100V 3µm, favorable peak shapes were obtained even when the concentration of ammonium formate was low.

When analyzing a basic compound using low-concentration formic acid or ammonium formate as a mobile phase, the sample concentration has a marked effect on peak shape. Figure 13 shows the relationships between sample concentration and peak shape (asymmetry factor) obtained by carrying out RPC using TSKgel ODS-100V 3µm and phosphate buffer or ammonium formate as a mobile phase. When ammonium formate (low ionic strength) is used, a higher sample concentration (for desipramine) results in greater peak tailing compared to the use of phosphate buffer (high ionic strength). Figure 14 shows the relationship between sample concentration and peak shape (asymmetry factor) for desipramine using TSKgel ODS-100V 3 µm and a competitor's ODS (3 µm), with low-concentration (5 mmol/L) ammonium formate as a mobile phase. When the competitor's ODS (3 µm) was used, marked peak tailing was seen, starting at the low-concentration area (see Fig. 12). As mentioned above, TSKgel ODS-100V 3µm exhibits superior properties under the conditions most frequently used in LC/MS(/MS).

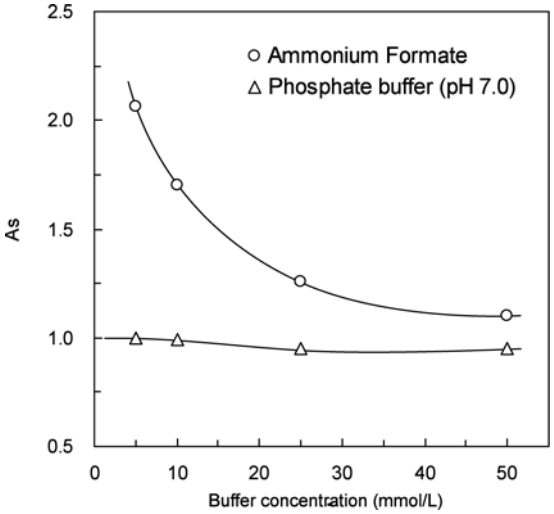
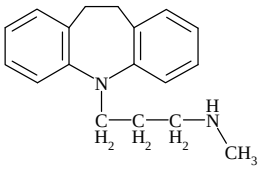


Figure 11 Relationship between mobile phase salt concentration and peak shape: Effect of salt type

Column: TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm)
Eluent: 5-50 mmol/L $HCOONH_4/CH_3OH$ = 30/70
5-50 mmol/L Phosphate buffer (pH 7.0) / CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: Desipramine
Inj. volume: 10µL

Desipramine



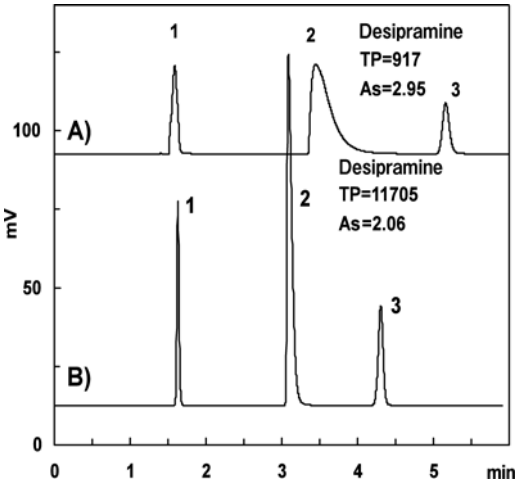


Figure 12 Comparison of chromatograms of desipramine obtained using different columns

Column: A) Competitor's ODS (3 µm) (4.6 mm I.D.x 15 cm)
B) TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm)
Eluent: 5 mmol/L $HCOONH_4$/ CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: 1. Uracil 2. Desipramine (26 µg/mL) 3. Benzene
Inj. volume: 10µL

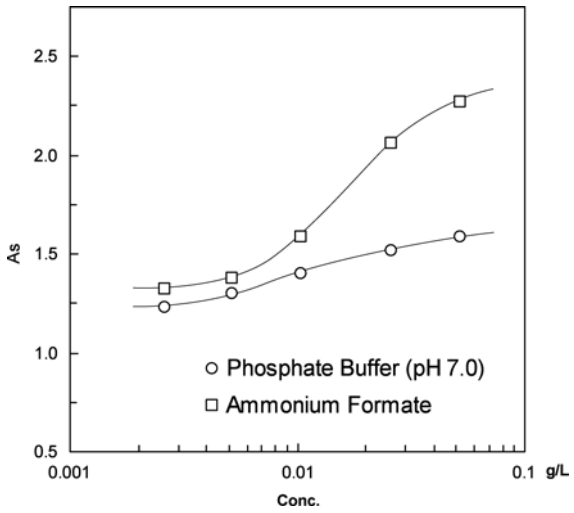


Figure 13 **Relationship between sample concentration and peak shape: Effect of salt type**

Column: TSKgel ODS-100V 3 µm (4.6 mm I.D.x 15 cm)
Eluent: 5 mmol/L $HCOONH_4$/ CH_3OH = 30/70
5 mmol/L Phosphate buffer (pH 7.0) / CH_3OH =30/70
Flow rate: 1.0 mL/min
Detection: UV 254nm
Temp.: 40 °C
Sample: Desipramine
Inj. volume: 10µL

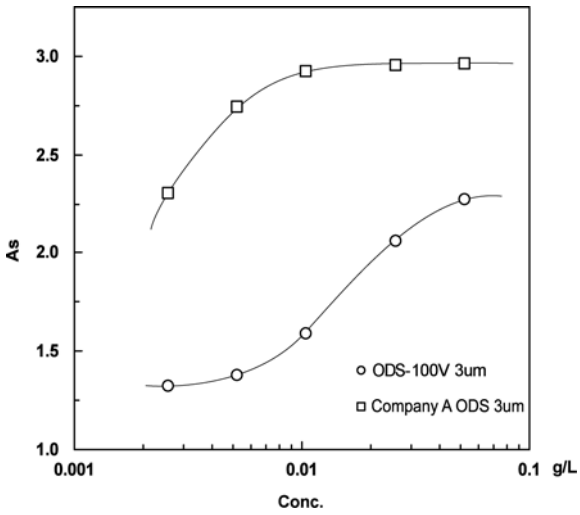


Figure 14 **Relationship between sample concentration and peak shape: Comparison with competitor's ODS column**

Column: A) TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm)
B) Competitor's ODS (3 µm) (4.6 mmI.D. x 15 cm)
Eluent: 5 mmol/L $HCOONH_4$/ CH_3OH = 30/70
Flow rate: 1.0 mL/min
Detection: UV 254 nm
Temp.: 40 °C
Sample: Desipramine
Inj. volume: 10µL

3. Applications

Figures 15 and 16 are comparison data for TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm, while Figs. 17 - 19 show LC/MS chromatograms obtained using TSKgel ODS-100V 3µm.

Figures 15 and 16 show chromatograms of acidic and basic compounds measured using TSKgel ODS-100V 3µm and TSKgel ODS-100V 5µm. Because TSKgel ODS-100V 3µm has a higher theoretical plate number, sharper peaks were obtained. Also, the retentions of both samples were mostly comparable regardless of the particle size of the packing material, and no peak tailing was seen for either TSKgel ODS-100V 3µm or TSKgel ODS-100V 5µm.

Figure 17 shows SIM chromatograms obtained by subjecting aminoglycoside antibiotics to LC/MS using TSKgel ODS-100V 3µm. Because aminoglycoside antibiotics are generally highly hydrophilic, sufficient retention cannot be achieved in RPC, so an ion pair reagent is added to the mobile phase. Furthermore, if using an MS detector, the ion pair reagent must be volatile. When we performed LC/MS with addition of heptafluorobutyric acid (HFBA), the peak shapes for all five aminoglycoside antibiotics were favorable.

Figure 18 shows an SIM chromatogram obtained by subjecting microcystin to LC/MS using TSKgel ODS-100V 3µm. Microcystin is a hepatotoxin which is synthesized in algal blooms formed due to eutrophication of lakes, and it also acts as a carcinogenic promoter. Therefore, it is necessary to monitor this compound in lake water. The 2003 revision of the drinking water test method requires an assay for microcystin-LR, which is produced by various types of algal bloom. Although the chromatogram was obtained by conducting chromatography at the maximum residue limit (MRL), separation and detection are favorable.

Figure 19 shows SIM chromatograms of sulfonamides obtained by LC/MS using TSKgel ODS-100V 3µm. Sulfonamides are synthetic antibiotics which are widely used in veterinary science. The simultaneous analysis methods issued by the Ministry of Health, Labor and Welfare of Japan (Shokuan No. 1129002) “Simultaneous test method (I) for veterinary pharmaceuticals by HPLC (animal husbandry and aquatic products)” lists 16 sulfonamides, and the test method specifies that HPLC be used for quantification and LC/MS(/MS) for confirmation. In this experiment, sharp peaks without tailing were obtained for all sulfonamides.

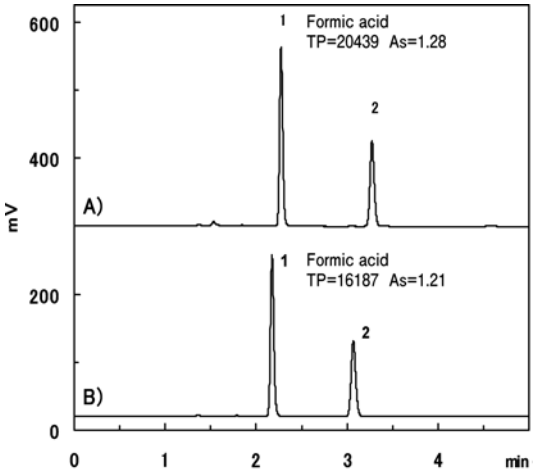


Figure 15 Chromatograms of organic acids

Column:	A) TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm) B) TSKgel ODS-100V 5 µm (4.6 mm I.D. x 15 cm)
Eluent:	H_2O/CH_3OH = 98/2 + 0.1 % H_3PO_4
Flow rate:	1.0 mL/min
Detection:	UV210 nm
Temp.:	40 °C
Sample:	1.Formic acid, 2.Acetic acid
Inj. volume:	10 µL

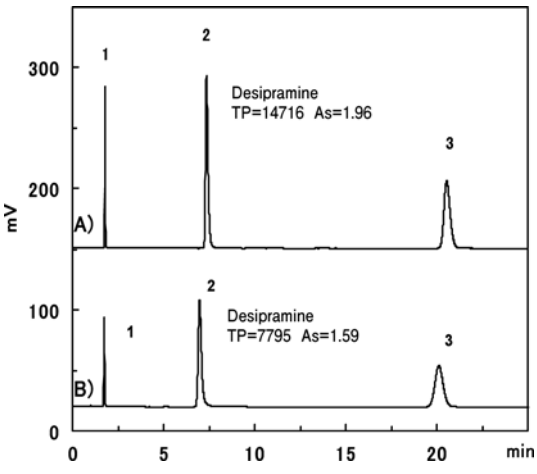
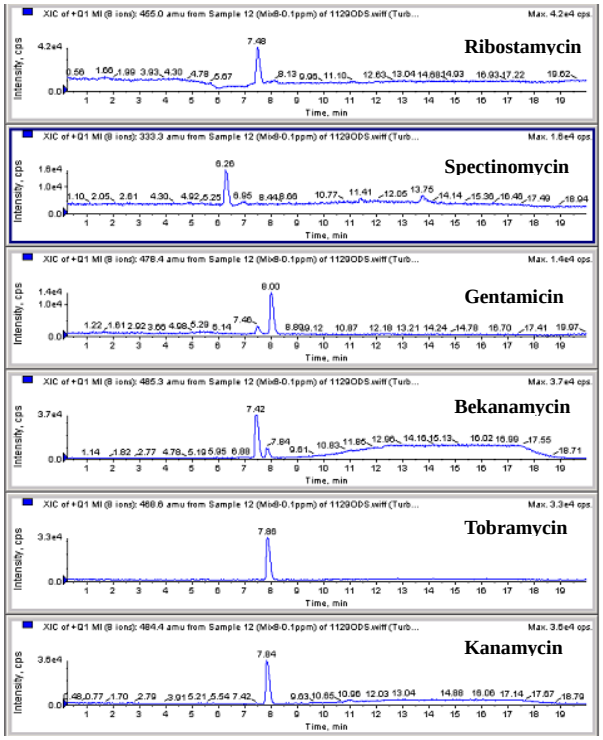


Figure 16 Chromatograms of basic compounds

Column:	A) TSKgel ODS-100V 3 µm (4.6 mm I.D. x 15 cm) B) TSKgel ODS-100V 5 µm (4.6 mm I.D. x 15 cm)
Eluent:	50mmol/L phosphate buffer (pH 7.0) /CH_3OH = 30/70
Flow rate:	1.0 mL/min
Detection:	UV254 nm
Temp.:	40 °C
Sample:	1.Uracil, 2.Desipramine, 3.Imipramine
Inj. volume:	10 µL



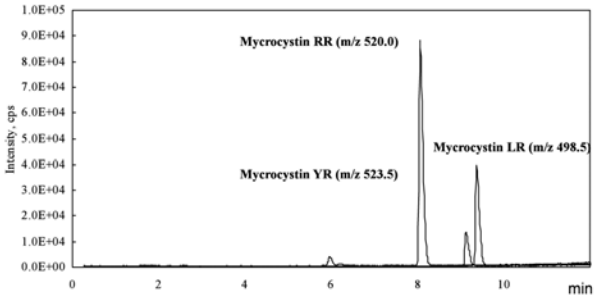


Figure 17 LC/MS analysis of aminoglycoside antibiotics

Column: TSKgel ODS-100V 3µm, (2.0 mm I.D. x 15 cm)
Eluent: A: 5mM HFBA
B: CH_3CN
Gradient: 0 min (B 10%)
10 min (B 60%)
15 min (B 60%)
Flow rate: 0.2 mL/min
Detection: MS QTRAP (Applied Biosystems)
Ion source: ESI
Polarity: Positive
Sample: Ribostamycin, Spectinomycin, Gentamicin, Bekanamycin, Tobramycin, Kanamycin
Inj. volume: 5 µL
Sample concentration: 0.1 ppm each

Figure 18 LC/MS of microcystin

Column: TSKgel ODS-100V 3 µm (2.0 mm I.D. x 15 cm)
Eluent: A: 0.1% HCOOH
B: 0.1% HCOOH in CH_3CN
Gradient: 0 min (B: 10%)
10 min (B: 60%)
15 min (B: 60%)
Flow rate: 0.2 mL/min
Detection: MS Q TRAP (Applied Biosystems)
Ion source: ESI
Polarity: Positive
Temp.: 40 °C
Sample: Mycrocystin RR, YR, LR
Inj. volume: 5µL

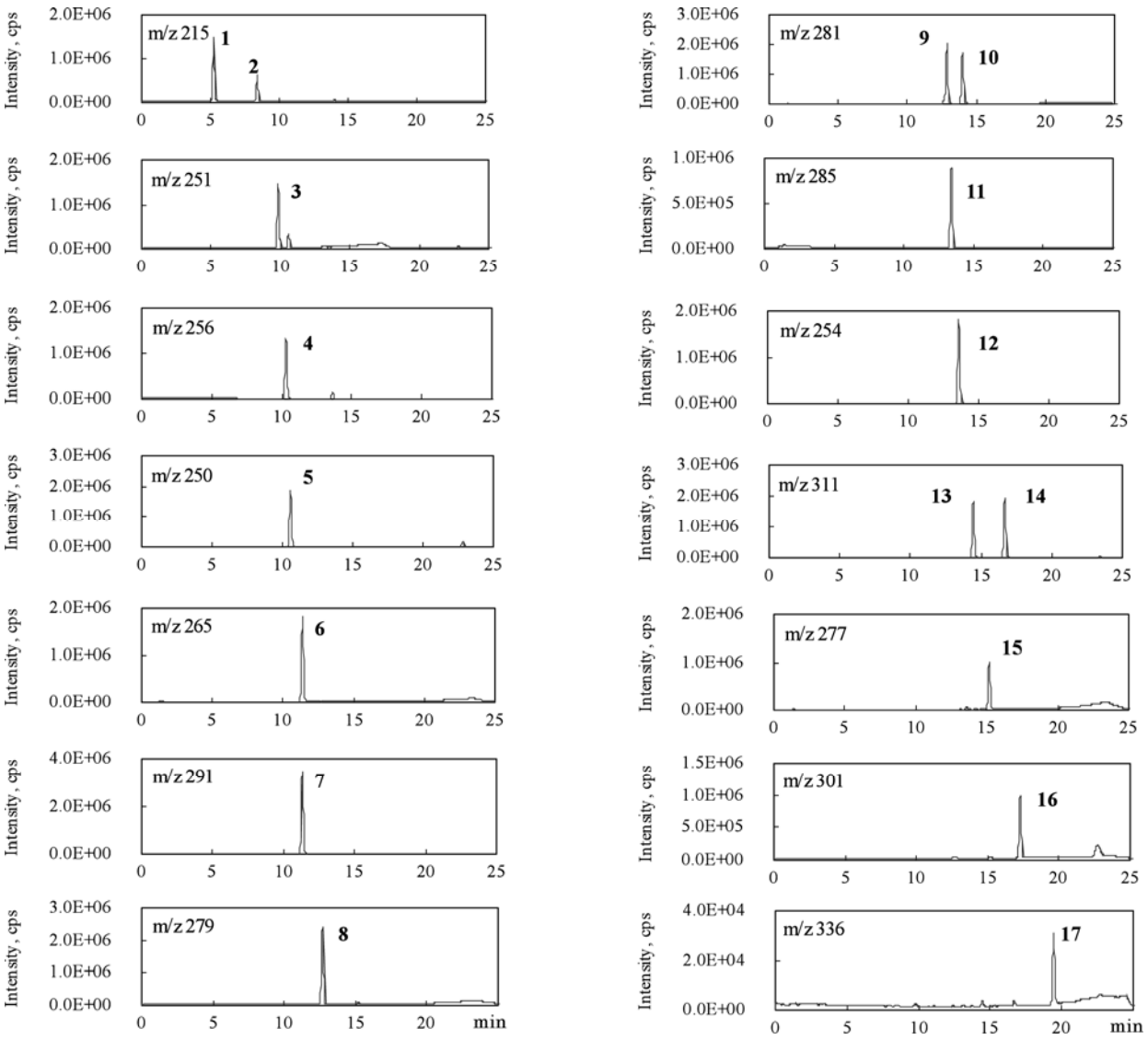


Figure 19 Simultaneous analysis of sulfonamides by LC/MS

Column: TSKgel ODS-100V 3 µm (2.0 mmI.D. x 15 cm)
Eluent: A: 0.1 % HCOOH
B: 0.1 % HCOOH in CH3OH
Gradient: 0min 0 % B
20min 70 % B
22min 70 % B
23min 0 % B
Flow rate: 0.2 mL/min
Detection: MS Q Trap (Applied Biosystems)
Ion source: ESI
Polarity: positive
Mode: SIM
Temp.: 500 °C
Ion spray voltage: 5000V

Temperature: 40 °C
Sample: 1. sulfaguanidine 2. sulfacetamide
3. sulfadiazine 4. sulfathiazole
5. sulfapyridine 6. sulfamerazine
7. trimethoprim 8. sulfadimidin
9. sulfamethoxypyridazine
10. sulfamonomethoxine
11. sulfachloropyridazine
12. sulfamethoxazole
13. sulfadoxine 14. sulfadimethoxine
15. sulfabenzamide 16. sulfaquinoxaline
17. sulfanitranInj.
volume: 2µL

(Q TRAP is a registered trademark of Applied Biosystems/MDS SCIEX)

4. Conclusions

As stated above, TSKgel ODS-100V 3μm possesses the same separation properties as TSKgel ODS-100V 5μm, including high retention of hydrophilic compounds and favorable peak shape for basic compounds. In addition, column efficiency is higher due to the smaller particle size, and high column efficiency can be achieved over a wider range of flow rates, leading to shorter chromatography times. Tosoh has developed various columns with internal diameters ranging from 1.0 to 4.6 mm, including columns for LC/MS(/MS) and microanalysis, allowing selection of the most suitable column size for various applications.