Separation Report No. 104

3µm Reversed Phase Chromatography: TSKgel ODS-100V Columns

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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 samples, has high separation performance and is robust. Reversed phase octadecyl (C18)-bonded silica gel is widely used as a packing material for the separation of low molecular weight compounds such as pharmaceuticals.

TSKgel ODS-100V, 3µm columns contain a smaller particle size packing material than that of the earlier introduced TSKgel ODS-100V, 5µm columns; see Separation Report No. 102 for a detailed discussion of these columns. To ensure that these TSKgel ODS-100V, 3µm columns maintain the same chemical properties as 5 micron TSKgel ODS-100V columns, they were prepared using the same reagents and procedures. TSKgel ODS-100V, 3µm columns share many of the same features as TSKgel ODS-100V, 5µm columns, such as superior end-capping of residual silanol groups, favorable peak shapes for basic and acidic compounds, and the ability to be used continuously in 100% aqueous mobile phases. This article discusses the properties of TSKgel ODS-100V, 3µm columns and presents some applications.

2. Properties of TSKgel ODS-100V, 3µm columns

2-1. Properties of the packing material

Table 1 compares the fundamental characteristics of TSKgel ODS-100V, 3µm, TSKgel ODS-100V, 5µm and TSKgel ODS-100Z, 5µm columns. 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 columns share the same surface modification, i.e., functional group bonding and residual silanol group end-capping reactions. As shown in Table 2, most of the HPLC values are comparable for the two particle sizes. Furthermore, the carbon content of TSKgel ODS-100V, 3µm has been adjusted to achieve comparable retention factors to that of TSKgel ODS-100V, 5µm columns. Therefore, the separation properties of both columns are comparable.

Figure 1 shows chromatograms of a test mixture containing the same components as NIST SRM 870, run on a TSKgel ODS-100V, 3µm and a TSKgel ODS-100V, 5µm column. The retention of each peak and the asymmetry factors for amitriptyline (a basic compound) and quinizarin (a metal chelating compound) are nearly identical on both columns.

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 a TSKgel ODS-100V, 3µm and a TSKgel ODS-100Z (3µm) column. Compared to TSKgel ODS-100Z, 3µm, with a high carbon content (20%) and low packing material surface polarity, the retention of compounds with low log P values (hydrophilic compounds) was higher for the TSKgel ODS-100V, 3µm column (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 columns than compared to TSKgel ODS-100Z, 3µm columns.

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

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 = $1x10^{-1}$nm

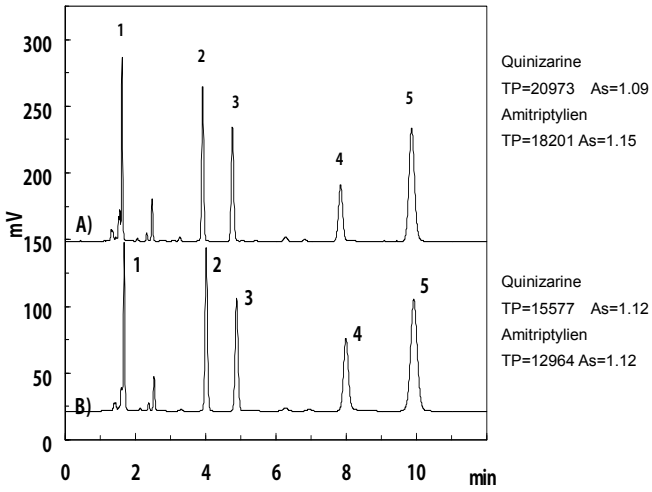

Figure 1 Standard sample chromatograms

Columns: A: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
B: TSKgel ODS-100V, 5µm, 4.6mm ID x 15cm
Eluent: 20mmol/L phosphate buffer, pH 7.0 /CH_3OH = 20/80
Flow rate: 1.0mL/min
Detection: UV@254nm
Injection vol.: 10µL
Samples: 1.uracil 2.toluene 3.ethyl benzene 4.quinizarine 5.amitriptyline

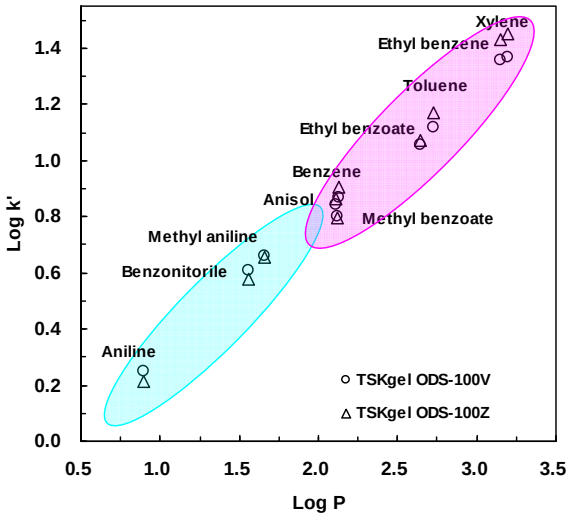

Figure 2 Relationship between hydrophobicity parameter (log P) and retention (log k')

Columns: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
TSKgel ODS-100Z, 3µm, 4.6mm ID x 15cm
Eluent: H_2O/ACN = 60/40
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Samples: aniline, benzonitrile, methyl aniline, anisole, methyl benzoate, benzene, ethyl benzoate, toluene, ethyl benzene, xylene

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

Column	Retention factor	Stereo-selectivity	Hydrogen bonding	Hydro-phobicity	Surface polarity	Ionization					Coordinate linkage		Retention loss (%)
						Basic			Acidic				
	k'	α	α	α	α	AF (Des)	A (k'Ami/k'EB)	AF (Ami)	A (k'For/k'Ac)	AF (For)	A (k'Quini/k'EB)	AF (Quini)	RT2 Ade/ RT1 Ade
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 factor: k'(Naphthalene)
2. Stereoselectiivty: α=k'(Triphenylene)k'(σ-Terphenyl)
3. Hydrogen bonding: α=k'(Caffeine)/k'(Phenol)
4. Hydrophobicity: α=k'(Toluene)/k'(Benzene)
5. Surface polarity: : α=k'(Methyl benzoate)/k'(Toluene)
6. Ionization
   1) AF (Des) = AF (Desipramine) (pH 7.0)
   2) A (k'Ami/k'EB) = α=k'(Amitriptyline)/k'(Ethyl benzene)
   3) AF (Ami) = As'f(Amitriptyline)
   4) A (k'For/k'Ac) = α=k'(Formic acid)/k'(Acetic acid)
   5) AF (For) = AF (Formic acid)
7. Coordinate linkage
   1) A (k'Quini/k'EB) = α=k'(Quinizarin)/k'(Ethyl benzene)
   2) AF (Quini) = AF (Quinizarin)
8. Retention loss: (%)
   RT1 Ade: Initial elution time for adenine
   RT2 Ade: Elution time for adenine at 30 minutes after injection

2-2. H-u curve (Van Deemter curve)

Figure 3 shows the relationship between a measure of column efficiency (height equivalent to a theoretical plate, HETP) and linear velocity for a TSKgel ODS-100V, 3µm and TSKgel ODS-100V, 5µm column. Because the particle size of the former is smaller than that of the latter, its HETP is smaller (higher column efficiency). Also, for the TSKgel ODS-100V, 5µm column, the smallest HETP and high column efficiency are obtained at a linear velocity of 4 - 6cm/min, but with the TSKgel ODS-100V, 3µm column, which has a smaller particle size, column efficiency is the highest at a greater linear velocity of ≥ 6cm/min. Furthermore, for the TSKgel ODS-100V, 5µm column, a greater linear velocity (≥ 6cm/min) leads to lower column efficiency, while for the TSKgel ODS-100V, 3µm column, column efficiency does not decrease much at high linear velocity and high column efficiency is maintained over a wide range of velocities (6 - 10cm/min; about 1.0 - 1.7mL/min for a column with an internal diameter of 4.6mm). Therefore, compared to a TSKgel ODS-100V, 5µm column, 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 2.0mm ID column with either methanol or acetonitrile as the organic component of the mobile phase. Since acetonitrile is less viscous, maximum column efficiency occurs at a higher linear velocity, and high column efficiency is maintained over a broader range of linear velocities. Figure 5 compares chromatograms obtained using methanol and acetonitrile at flow rates yielding favorable column efficiency (0.20 and 0.50mL/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, Figure 6 shows the durability of a TSKgel ODS-100V, 3µm column using 60% acetonitrile/40% water at a flow rate of 0.50mL/min. During more than 1,000 hours we could not detect any significant change in the column performance parameters (theoretical plate number and asymmetry factor). Thus, TSKgel ODS-100V, 3µm columns provide high efficiency and short analysis times when using a low viscosity solution as a mobile phase and a moderately high flow rate.

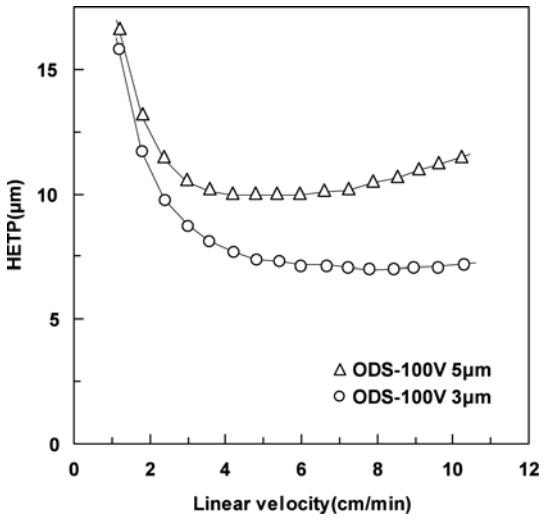

Figure 3 Effect of linear velocity on HETP

Columns: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
TSKgel ODS-100V, 5µm, 4.6mm ID x 15cm
Eluent: H_2O/CH_3OH = 30/70
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: naphthalene

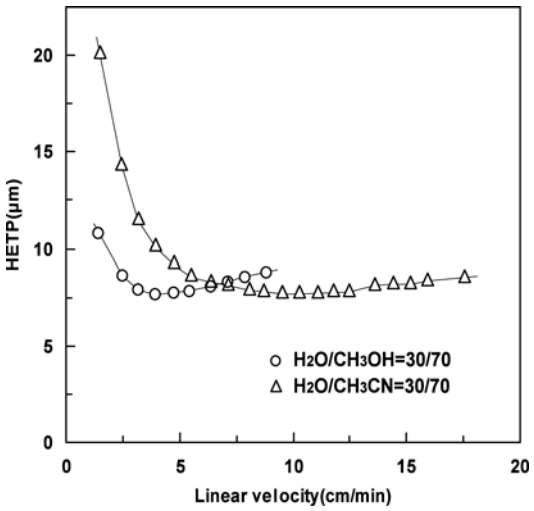

Figure 4 Efficiency as a function of linear velocity: Effect of organic solvent

Column: TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Eluent: H_2O/CH_3OH = 30/70
H_2O/ACN = 40/60
Detection: UV@254nm
Temperature: 25°C
Injection vol.: 2µL
Sample: naphthalene

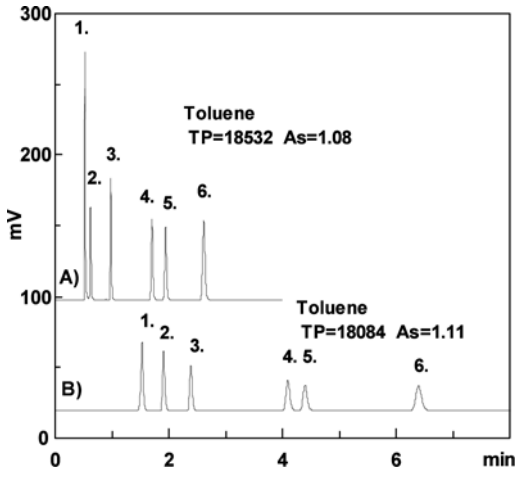

Figure 5 **Comparison of chromatograms for standard chemicals**

Column: TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Eluent: A) H_2O/ACN = 40/60
B) H_2O/CH_3OH = 30/70
Flow rate: A) 0.50mL/min
B) 0.20mL/min
Detection: UV@254nm
Temperature: 25°C
Injection vol.: 2µL
Samples: 1. uracil
2. caffeine
3. phenol
4. methyl benzoate
5. benzene
6. toluene

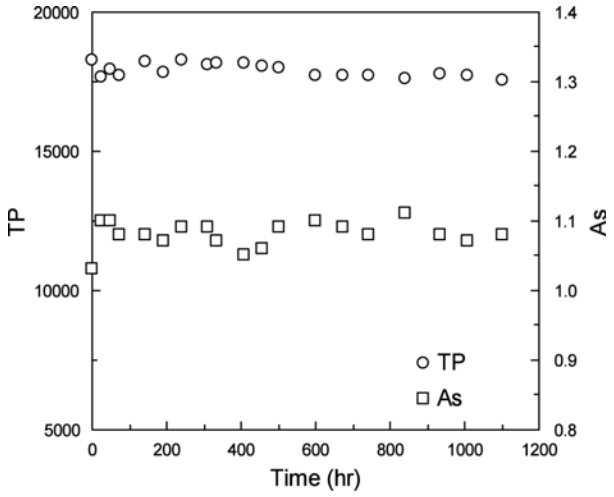

Figure 6 **Durability under long term flushing with eluent**

Column: TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Eluent: H_2O/ACN = 40/60
Flow rate: 0.50mL/min
Detection: UV@254nm
Temperature: 25°C
Injection vol.: 2µL
Sample: toluene

2-3 Residual ion exchange activity

In ODS packing materials (C18-bonded silica gel), residual and accessible silanol groups affect the retention and peak shape of basic compounds even when the C18 bonding reaction was followed by an exhaustive endcapping procedure. As with 5 micron TSKgel ODS-100V columns, we used a novel procedure to end-cap residual silanol groups on TSKgel ODS-100V, 3µm columns. In Figure 7 we compare chromatograms for desipramine (basic) and benzene (neutral) before and after end-capping of residual silanol groups in a TSKgel ODS-100V, 3µm column. No marked changes were observed in the retention and peak shape of benzene before and after end-capping (Peak 3). Because desipramine (peak 2) is positively charged under the employed mobile phase conditions it can interact with negatively charged residual silanol groups. The chromatogram obtained on the ODS-100V, 3µm column before end-capping exhibited strong retention and peak tailing (chromatogram B). In contrast, when an endcapped TSKgel ODS-100V, 3µm column was used, elution and peak shapes were normal (chromatogram A).

Figure 8 shows the retention of benzene and desipramine using a TSKgel ODS-100V, 3µm column as a function of mobile phase pH. The retention of benzene, a neutral compound, was essentially 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 the resulting increase in hydrophobicity. Figures 9 and 10 show the retention and asymmetry factors of desipramine using a TSKgel ODS-100V, 3µm column and an ODS column with insufficient endcapping at various mobile phase pH values. In general, as shown in Figure 9, differences in the endcapping of residual silanol groups affected the retention of desipramine under neutral mobile phase conditions; retention of desipramine was higher for the packing material with insufficient endcapping. Also, as shown in Figure 10, while use of a packing material with insufficient endcapping in a neutral mobile phase resulted in poor peak shape (tailing) for desipramine, the TSKgel ODS-100V, 3µm column yielded favorable peak shapes with minimal tailing regardless of mobile phase pH.

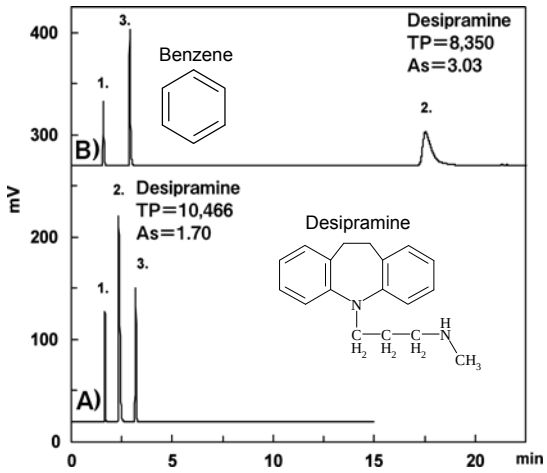

Figure 7 **Comparison of chromatograms for basic compounds (desipramine): effect of end-capping**

Columns: A: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
B: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm (*not endcapped*)
Eluent: 5mmol/L $HCOONH_4$/ CH_3OH = 20/80
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Samples: 1. uracil
2. desipramine (52 mg/L)
3. benzene

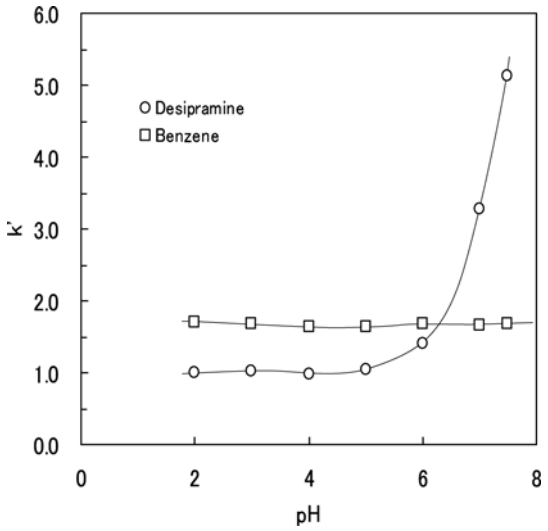

Figure 8 **Relationship between mobile phase pH and retention: comparison of basic and neutral compounds**

Column: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Eluent: 50mmol/L phosphate buffer, pH 2-7.5 /CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Samples: desipramine, benzene

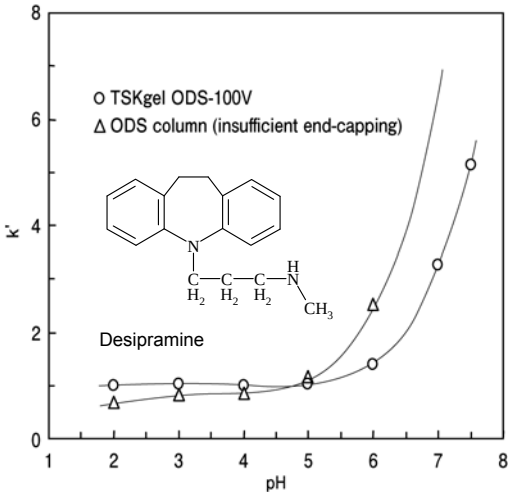

Figure 9 **Relationship between mobile phase pH and retention for desipramine**

Columns: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
ODS column, 4.6mm ID x 15cm (insufficient end-capping)
Eluent: 50mmol/L phosphate buffer, pH 2-7.5 /CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: desipramine

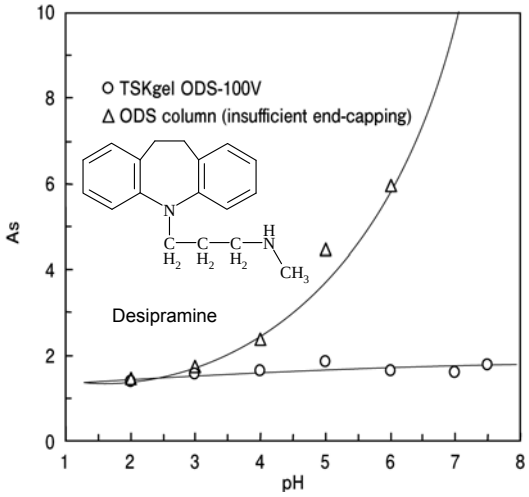

Figure 10 **Relationship between mobile phase pH and peak shape for desipramine**

Columns: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
ODS column, 4.6mm ID x 15cm (insufficient end-capping)
Eluent: 50mmol/L phosphate buffer, pH 2-7.5 /CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: desipramine

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

In RPC, a 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 with an asymmetry factor As (10%) close to 1 at buffer concentrations of 5 - 50mmol/L independent 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 low ionization efficiency and the possibility of ion source contamination. In general, the mobile phase buffer concentration is maintained at ≤ 10mmol/L.

Figure 12 shows the chromatograms obtained by subjecting desipramine (basic compound, peak 2) to RPC using a TSKgel ODS-100V, 3µm column and a competitor's ODS, 3µm column with 5mmol/L ammonium formate as a mobile phase. For the TSKgel ODS-100V, 3µm column, we obtained reasonable peak shape (at As ~ 2.0) at 5mmol/L ammonium formate concentration.

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) for a TSKgel ODS-100V, 3µm column using a phosphate or ammonium formate buffer in the mobile phase. When low ionic strength ammonium formate is used at 5mmol/L concentration, a higher sample concentration for desipramine results in more peak tailing compared to the use of high ionic strength phosphate buffer at the same 5mmol/L buffer concentration. Figure 14 shows the relationship between sample concentration and peak shape (asymmetry factor) for desipramine using a TSKgel ODS-100V, 3µm column and a competitor's ODS, 3µm column with low concentration (5mmol/L) ammonium formate in the mobile phase. When using the competitor's ODS, 3µm column, marked peak tailing was observed, even at low sample concentrations (see also Figure 12). The data shown in Figures 11-14 demonstrates that the TSKgel ODS-100V, 3µm column exhibits excellent properties under frequently used LC/MS(/MS) mobile phase conditions.

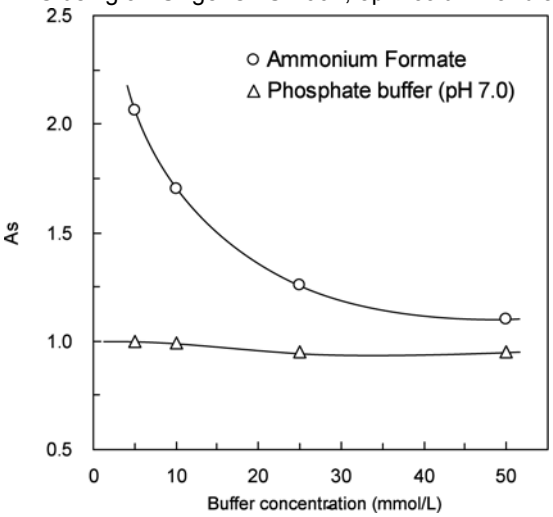

Figure 11 Relationship between mobile phase salt concentration and peak symmetry: effect of salt type

Column: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Eluent: 5-50mmol/L $HCOONH_4/CH_3OH$ = 30/70
5-50mmol/L phosphate buffer, pH 7.0 /CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: desipramine

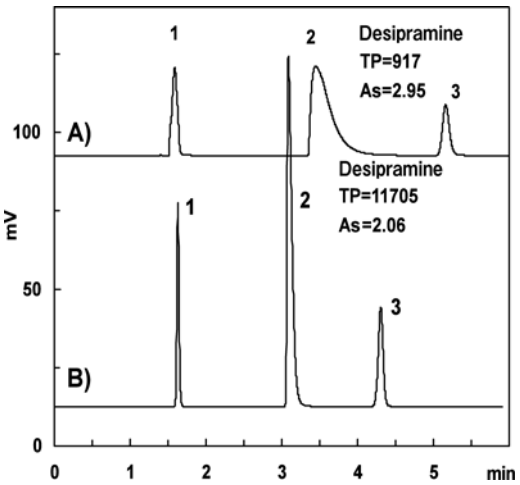

Figure 12 Comparison of chromatograms of desipramine obtained using different columns

Columns: A: Competitor's ODS, 3µm, 4.6mm ID x 15cm
B: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Eluent: 5mmol/L $HCOONH_4$/ CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Samples: 1. uracil 2. desipramine (26µg/mL) 3. benzene

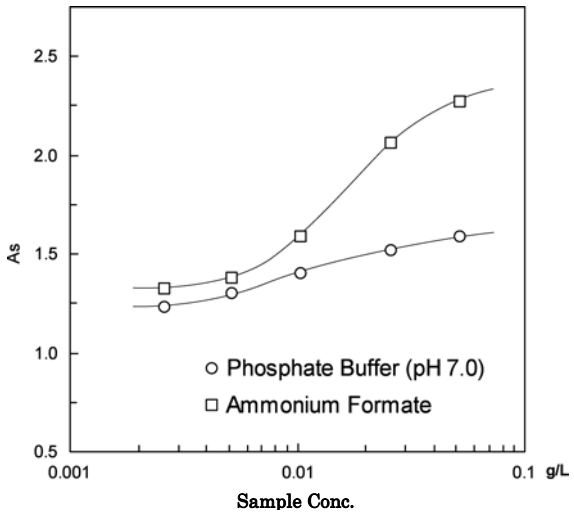

Figure 13 **Relationship between sample concentration and peak symmetry: effect of salt type**

Column: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Eluent: 5mmol/L $HCOONH_4$/ CH_3OH = 30/70
5mmol/L phosphate buffer, pH 7.0
/CH_3OH =30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: desipramine

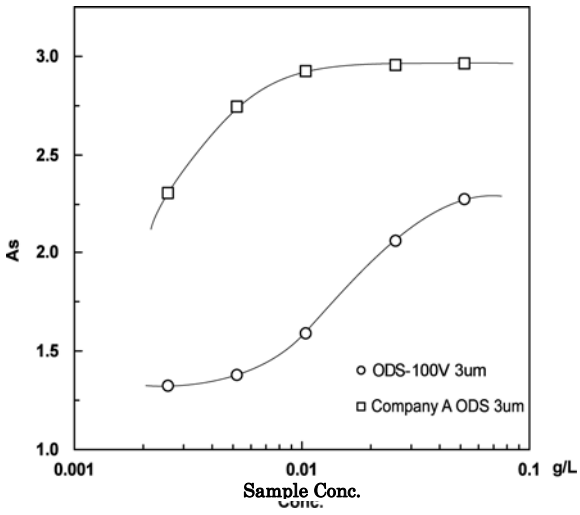

Figure 14 **Relationship between sample concentration and peak symmetry: comparison with competitor's ODS column**

Columns: A: TSKgel ODS-100V, 3µm,
4.6mm ID x 15cm
B: Competitor's ODS, 3µm,
4.6mm ID x 15cm
Eluent: 5mmol/L $HCOONH_4$/ CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Sample: desipramine

3. Applications

Figures 15 and 16 compare the performance of TSKgel ODS-100V, 3µm and TSKgel ODS-100V, 5µm columns, while Figures 17 - 19 show LC/MS chromatograms obtained using a TSKgel ODS-100V, 3µm column.

Figures 15 and 16 show chromatograms of acidic and basic compounds on TSKgel ODS-100V, 3µm and TSKgel ODS-100V, 5µm columns. Because the TSKgel ODS-100V, 3µm column has a higher theoretical plate number, narrower peaks were obtained. Also, the retention for all sample components was comparable regardless of the particle size of the packing material and no peak tailing was observed on either the TSKgel ODS-100V, 3µm or TSKgel ODS-100V, 5µm column.

Figure 17 shows SIM (single ion monitoring) chromatograms obtained for amino glycoside antibiotics by LC/MS using a TSKgel ODS-100V, 3µm column. Because aminoglycoside antibiotics are very hydrophilic, the compounds were retained by adding an ion pair reagent to the mobile phase. Needless to say, when using an MS detector, the ion pair reagent must be volatile. The ion-pair reagent heptafluorobutyric acid (HFBA) provided acceptable peak shapes for all five aminoglycoside antibiotics.

Figure 18 shows an SIM chromatogram obtained by subjecting microcystin to LC/MS using a TSKgel ODS-100V, 3µm column. Microcystin is a hepatotoxin which is synthesized in algal blooms formed due to eutrophication of lakes. Because microcystin also acts as a carcinogen promoter it is often monitored 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. As the chromatogram was obtained at the maximum residue level (MRL), separation and detection were acceptable.

Figure 19 shows SIM chromatograms of sulfonamides obtained by LC/MS using a TSKgel ODS-100V, 3µm column. 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.

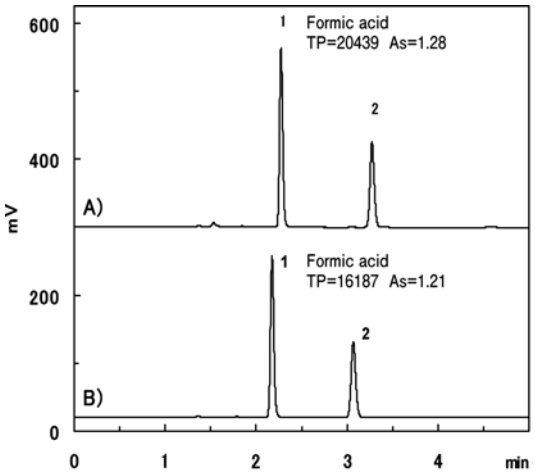

Figure 15 Chromatograms of organic acids

Columns: A: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
B: TSKgel ODS-100V, 5µm, 4.6mm ID x 15cm
Eluent: H_2O/CH_3OH = 98/2 + 0.1% H_3PO_4
Flow rate: 1.0mL/min
Detection: UV@210nm
Temperature: 40°C
Injection vol.: 10µL
Samples: 1. formic acid 2. acetic acid

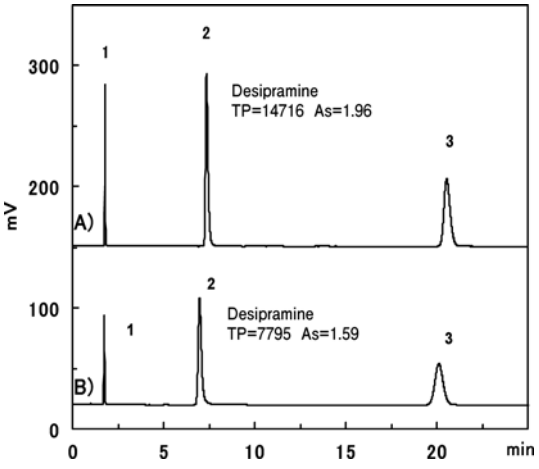

Figure 16 Chromatograms of basic compounds

Columns: A: TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
B: TSKgel ODS-100V, 5µm, 4.6mm ID x 15cm
Eluent: 50mmol/L phosphate buffer, pH 7.0 /CH_3OH = 30/70
Flow rate: 1.0mL/min
Detection: UV@254nm
Temperature: 40°C
Injection vol.: 10µL
Samples: 1. uracil 2. desipramine 3.imipramine

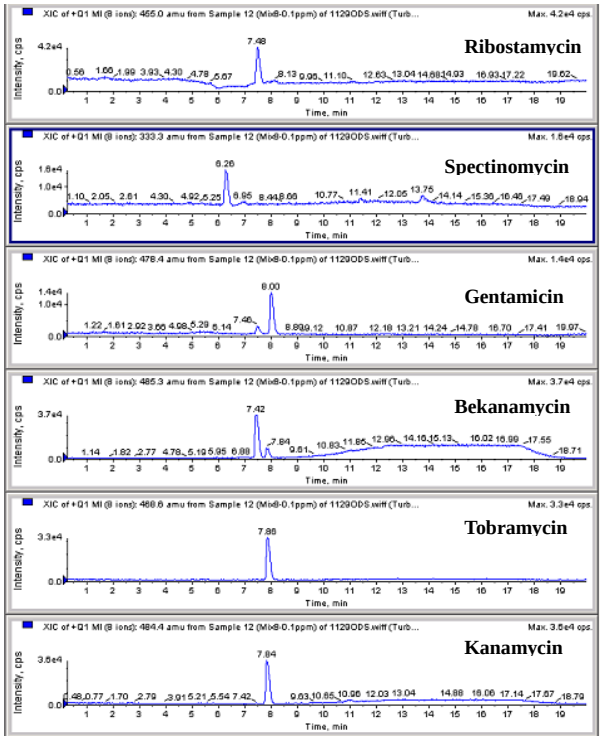

Figure 17 **LC/MS analysis of aminoglycoside antibiotics**

Column: TSKgel ODS-100V, 3µm
2.0mm ID x 15cm
Eluent: A: 5mmol/L HFBA
B: ACN
Gradient: 0 min (10% B)
10 min (60% B)
15 min (60% B)
Flow rate: 0.2mL/min
Detection: MS QTRAP (Applied Biosystems)
Ion source: ESI
Polarity: Positive
Injection vol.: 5µL
Samples: ribostamycin, spectinomycin, gentamicin, bekanamycin, tobramycin, kanamycin

Sample concentration: 0.1ppm each

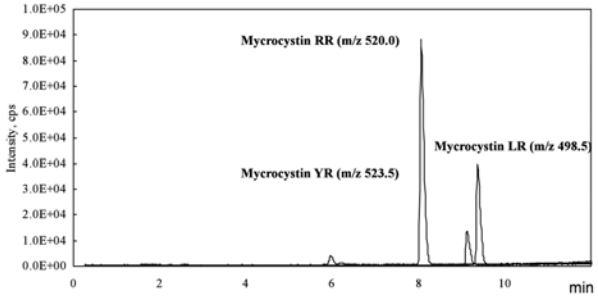

Figure 18 **LC/MS of microcystin**

Column: TSKgel ODS-100V, 3µm,
2.0mm ID x 15cm
Eluent: A: 0.1% HCOOH
B: 0.1% HCOOH in ACN
Gradient: 0 min (10% B)
10 min (60% B)
15 min (60% B)
Flow rate: 0.2mL/min
Detection: MS QTRAP (Applied Biosystems)
Ion source: ESI
Polarity: Positive
Temperature: 40°C
Injection vol.: 5µL
Samples: microcystin RR, YR, LR

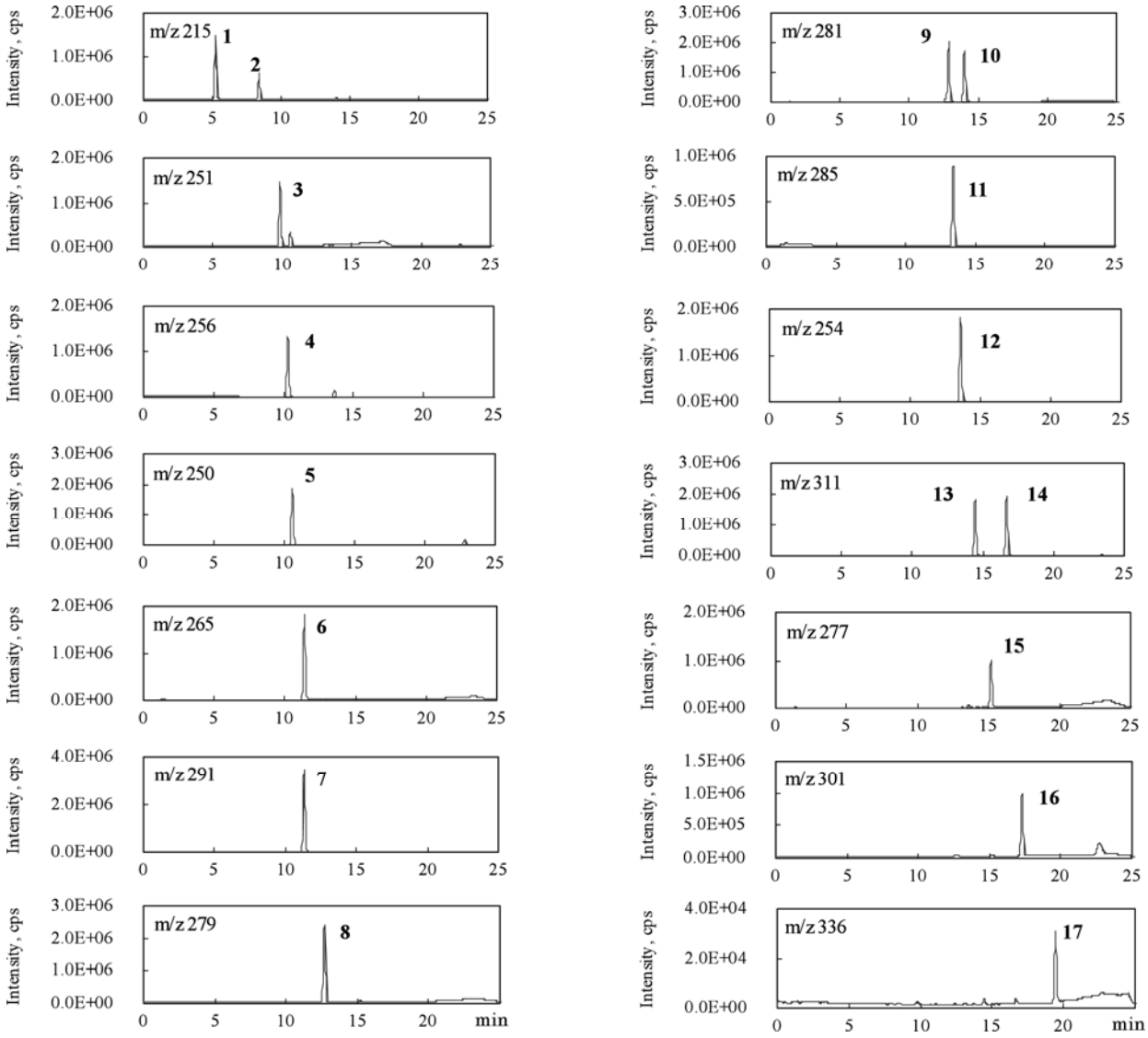

Figure 19 Simultaneous analysis of sulfonamides by LC/MS

Column: TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
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: 0.2mL/min
Detection: MS QTrap (Applied Biosystems)
Ion source: ESI
Polarity: positive
Mode: SIM
Temperature: 500°C
Ion spray voltage: 5000V

Temperature: 40°C
Inj. volume: 2µL
Samples: 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. sulfanitran

4. Conclusions

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

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