Characteristics of Novel Three Micron ODS Columns for Analysis of Small MW Compounds

Roy Eksteen - Tosoh Bioscience LLC, Montgomeryville, PA, USA
Hiroyuki Yamasaki and Tsukasa Oguma - Tosoh Corporation, Shu-nan, Japan

HPLC 2006, San Francisco, CA, USA

Abstract

Octadecylsilyl-bonded silica (ODS) columns are commonly used for product development and quality control of active ingredients in pharmaceutical preparations.

We recently introduced two ODS packings that vary in the density of the C18 ligand, as well as their overall polarities. TSKgel ODS-100V and TSKgel ODS-100Z are prepared using difunctional C18 silane reagents under anhydrous conditions to obtain a carbon load of 15% and 20% respectively. Both phases are exhaustively reacted with various endcapping reagents to limit accessibility of residual silanol groups with basic, acidic and chelating compounds.

In this study we report on the properties of these new materials as well as on the results obtained during our evaluation with the basic and chelating compounds contained in Standard Reference Material 870 (SRM 870).

We will show the results of stringent tests aimed at ensuring peak performance when challenging the columns with compounds that have proven difficult to chromatograph on commercial C18 columns under high speed and LCMS conditions.

Experimental

HPLC system

HPLC measurements were performed on a TOSOH HPLC system equipped with a DP-8020 pump, UV-8020 UV-Vis detector, CO-8020 column oven and AS-8021 autosampler, all from Tosoh (Tokyo, Japan).

Chemicals

All chemicals were purchased from Wako (Osaka, Japan).

Software

Log P values were calculated with KowWin software from Syracuse Research Corporation. (http://www.syrres.com/eSc/est_kowdemo.htm)

Table 1 Properties of TSK-GEL ODS Columns

Column	Particle size (µm)	Pore size (nm)	Specific surface area (m^2/g)	Pore volume (mL/g)	Functional Group	Carbon content* (%)	Bonded phase structure
TSKgel ODS-100V	3	10	450	1.10	C18	15	Monolayer
TSKgel ODS-100Z	3	10	450	1.10	C18	20	Monolayer
TSKgel ODS-100V	5	10	450	1.10	C18	15	Monolayer
TSKgel ODS-100Z	5	10	450	1.10	C18	20	Monolayer

*Measured by quantitative elemental analysis. Typical ligand densities are: ODS-100V 1.8µmol/m^2 [1.4µmol/m^2 before endcapping]; ODS-100Z 2.6µmol/m^2 [2.4µmol/m^2 before endcapping].

TOSOH

Table 2 Chromatographic parameters

TSKgel column	Capacity factor	Steric selectivity	Hydrogen bonding	Hydro-phobicity	Surface polarity	Ion-exchange factor: Basic			Ion-exchange factor: Acidic		Chelating factor	
	$k'_{Naphthalene}$	k'_{TP}/k'_{oP}	k'_{Ca}/k'_{Ph}	k'_{T}/k'_{B}	k'_{MB}/k'_{T}	AF (Des) pH 7.0	k'_{Ami}/k'_{T}	AF (Ami)	k'_{For}/k'_{Ac}	AF (For)	k'_{Quin}/k'_{T}	AF (Quin)
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
ODS-100Z 3μm	2.41	1.32	0.40	1.72	0.44	1.78	2.42	1.21	0.44	1.72	1.80	1.10
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
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

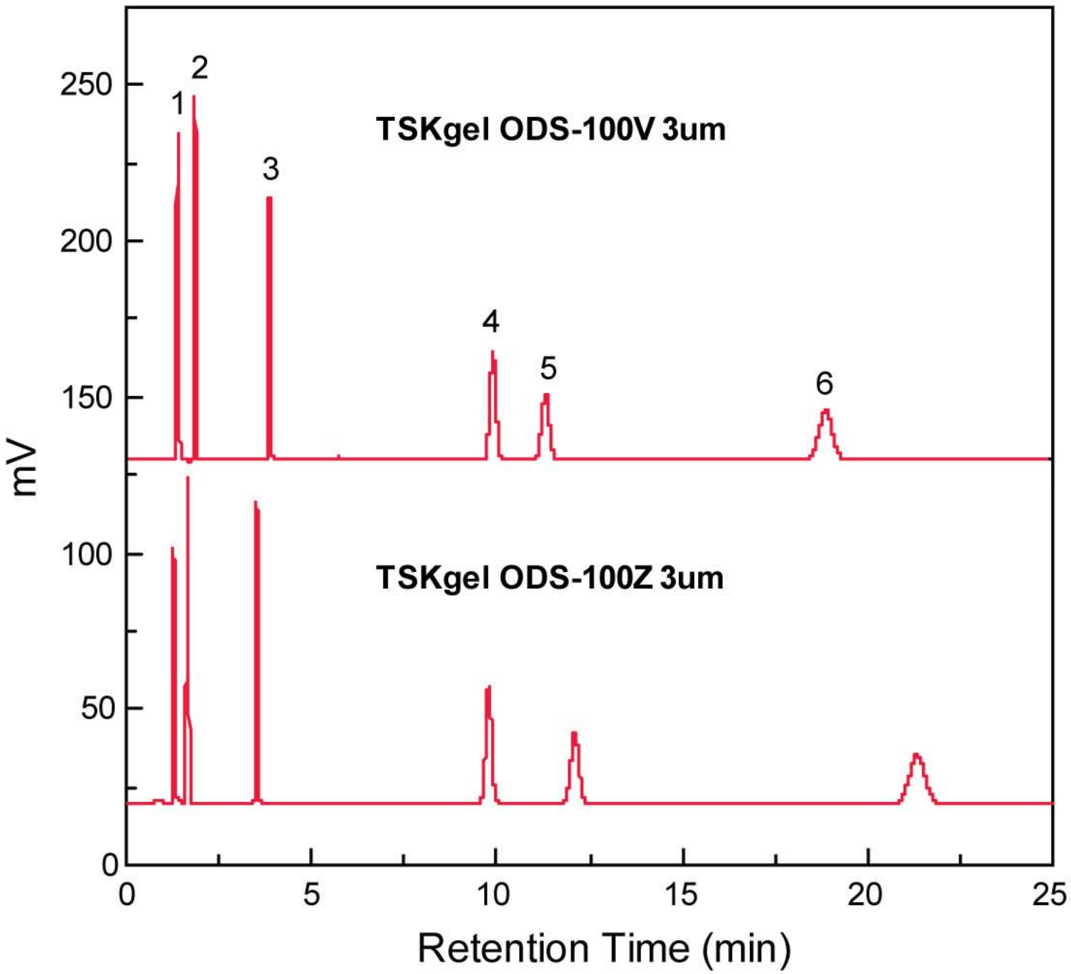
Chromatographic parameters

1. Capacity factor, k' (naphthalene)
2. Steric selectivity α, k' (triphenylene)/k' (o-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. Ion-exchange factor
   a) AF (desipramine) (pH 7.0)
   b) k' (amitriptyline)/k' (toluene)
   c) AF (amitriptyline)
   d) k' (formic acid)/k' (acetic acid)
   e) AF (formic acid)

7) Chelating factor
   a) k' (quinizarin)/k' (toluene)
   b) AF (quinizarin)

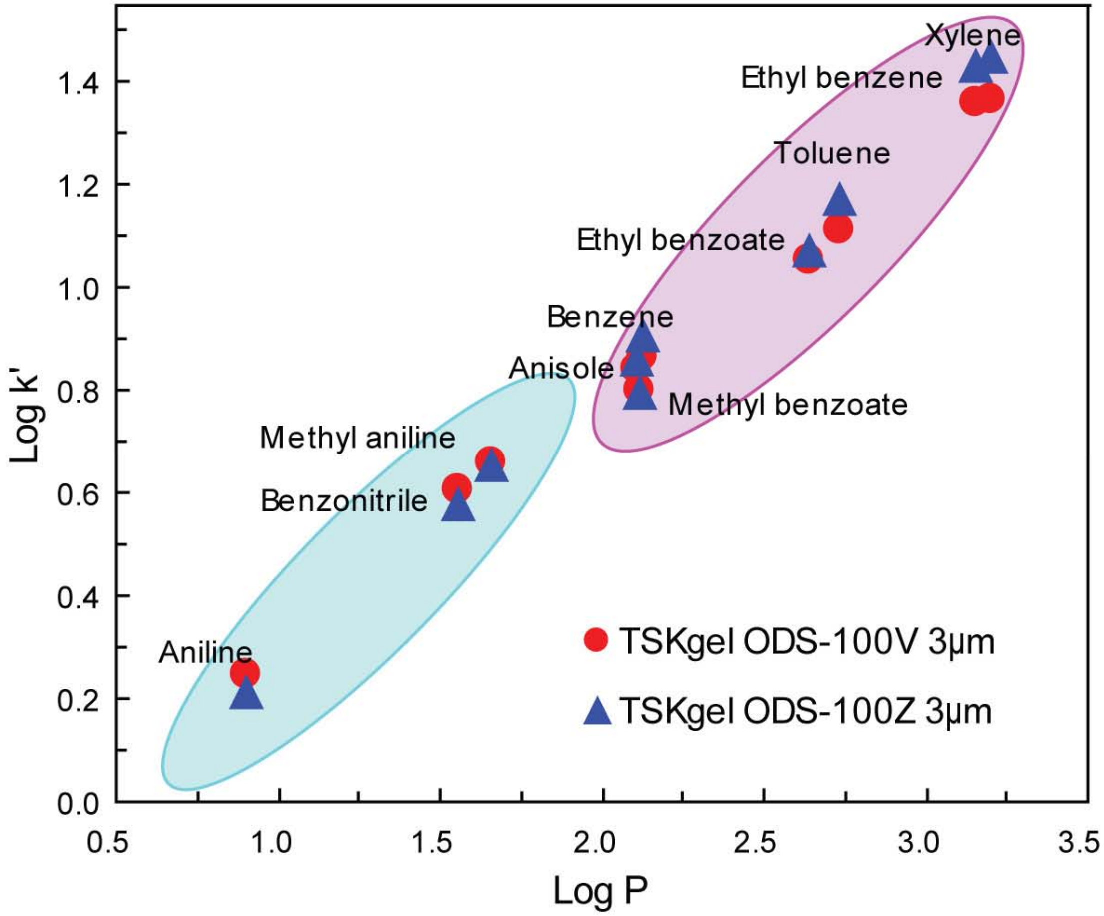
Fig. 1 Elution Profiles for a Standard Test Mixture on ODS-100V and ODS-100Z



<u>Conditions</u>

Columns:	TSKgel ODS-100V 3µm (4.6mmID x 15cm) TSKgel ODS-100Z 3µm (4.6mmID x 15cm)
Eluent:	H_2O/CH_3CN (60/40)
Flow:	1.0mL/min
Detect:	UV 254nm
Temp:	40°C
Inj. volume:	10µL
Sample:	1. Uracil 2. Caffeine 3. Phenol 4. Methyl benzoate 5. Benzene 6. Toluene

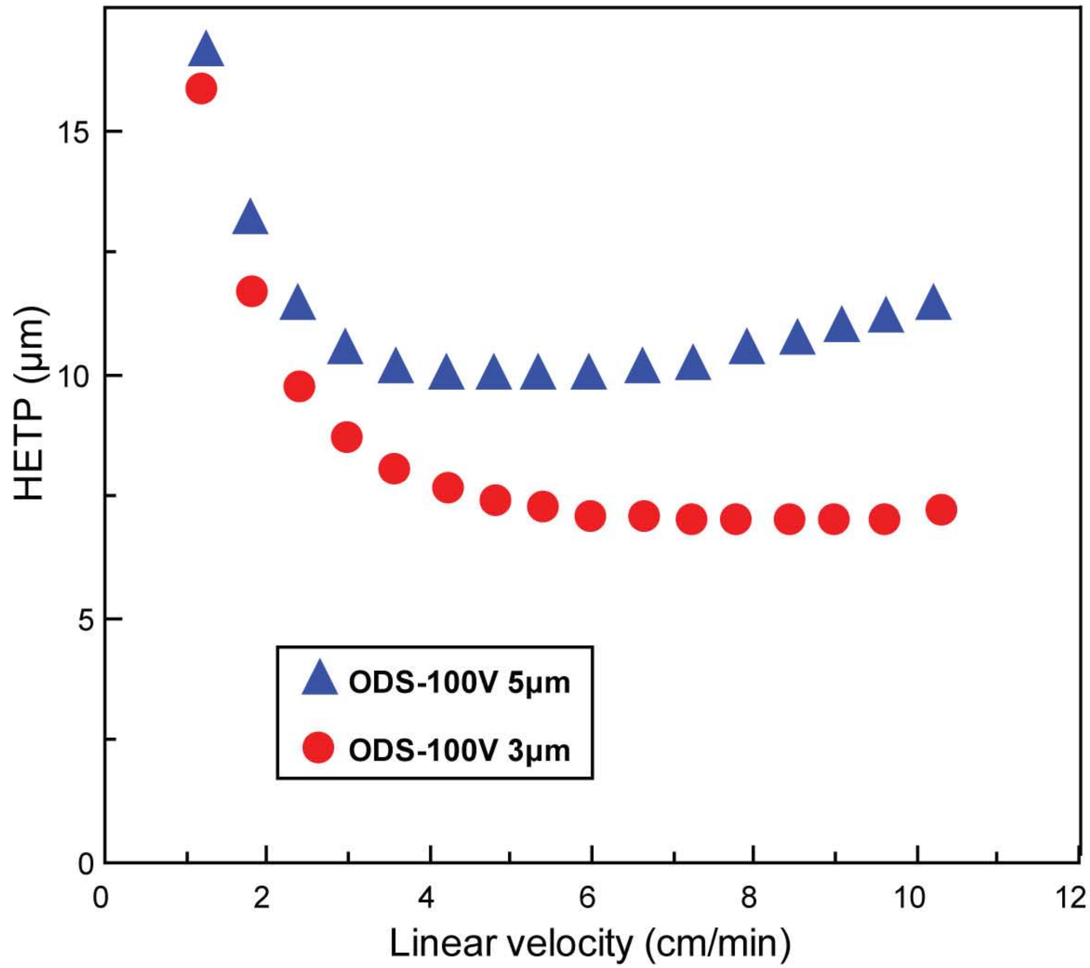
Fig. 2 Effect of Endcapping on Retention of Polar Compounds



Conditions

Columns:	TSKgel ODS-100V 3µm (4.6mmID x 15cm)
	TSKgel ODS-100Z 3µm (4.6mmID x 15cm)
Eluent:	H_2O/CH_3CN (60/40)
Flow:	1.0mL/min
Detect:	UV 254nm
Temp:	40°C
Inj. volume:	10µL

Fig. 3 Relationship Between HETP and Linear Velocity as a Function of Particle Size



Conditions

Columns: TSKgel ODS-100V 5μm (4.6mmID x 15cm)
TSKgel ODS-100V 3μm (4.6mmID x 15cm)

Eluent: H_2O/MeOH (30/70)

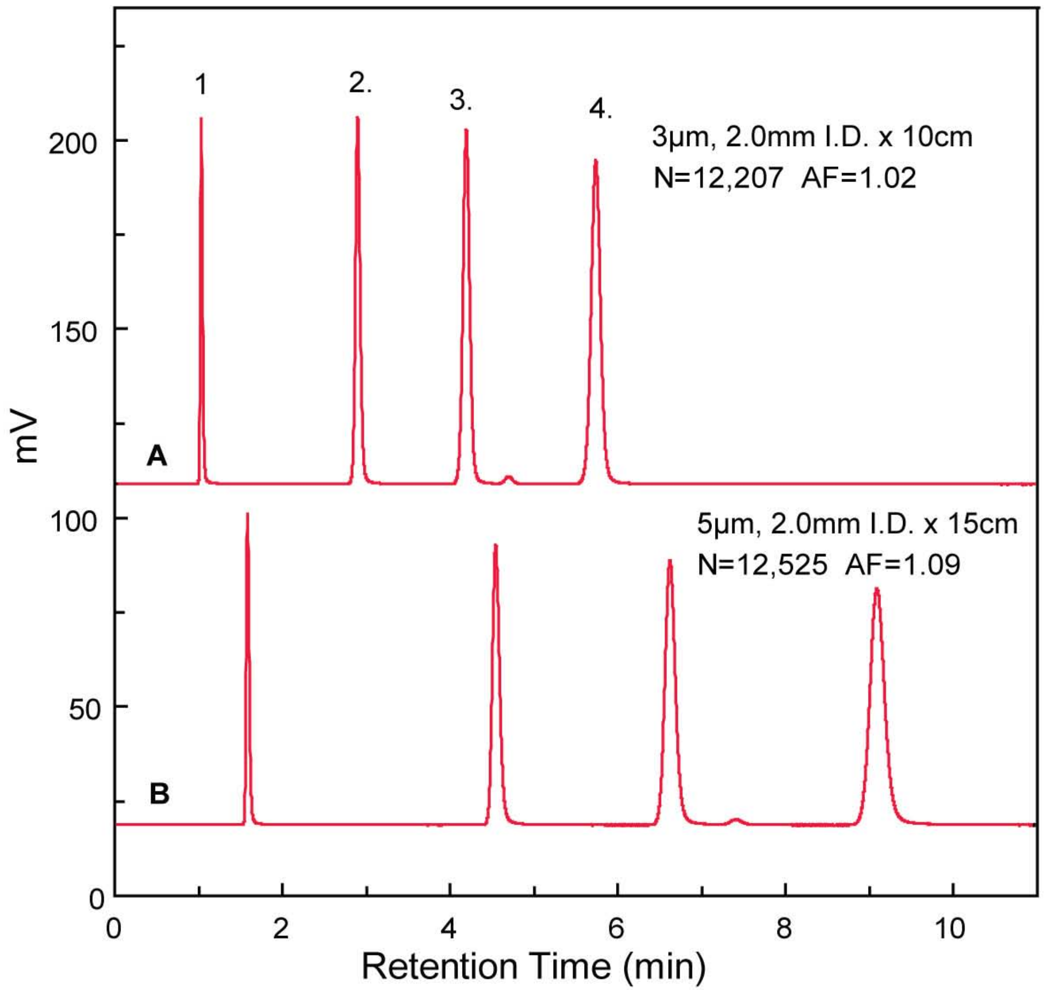
Detect: UV 254nm

Temp: 40°C

Inj. volume: 10μL

Sample: Naphthalene

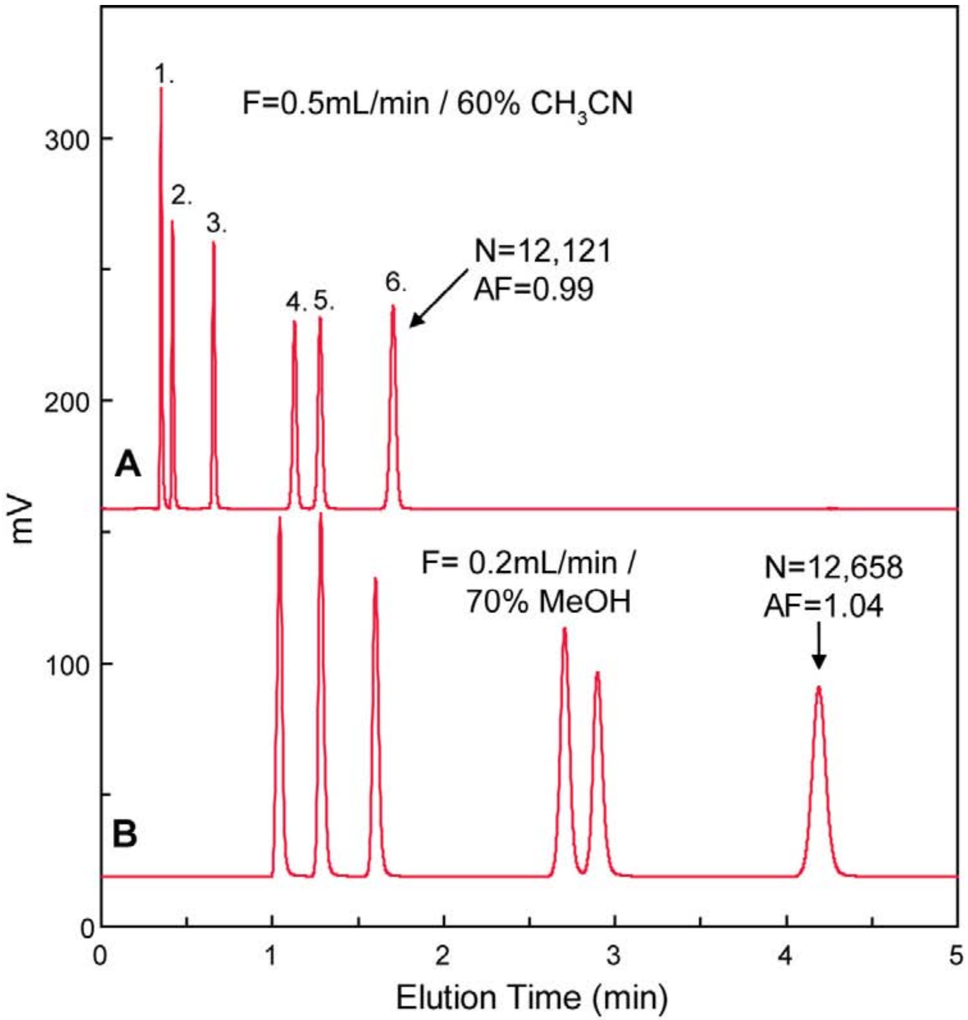
Fig. 4 Efficiency and peak symmetry of aromatics on 2mm ID ODS-100V columns



<u>Conditions</u>

Columns:	A.) TSKgel ODS-100V 3µm (2.0mmID x 10cm)
	B.) TSKgel ODS-100V 5µm (2.0mmID x 15cm)
Eluent:	H_2O/MeOH (30/70)
Flow rate:	0.20mL/min
Detection:	UV 254nm
Temp:	25°C
Inj. volume:	2µL
Sample:	1. Uracil
	2. Benzene
	3. Toluene
	4. Naphthalene

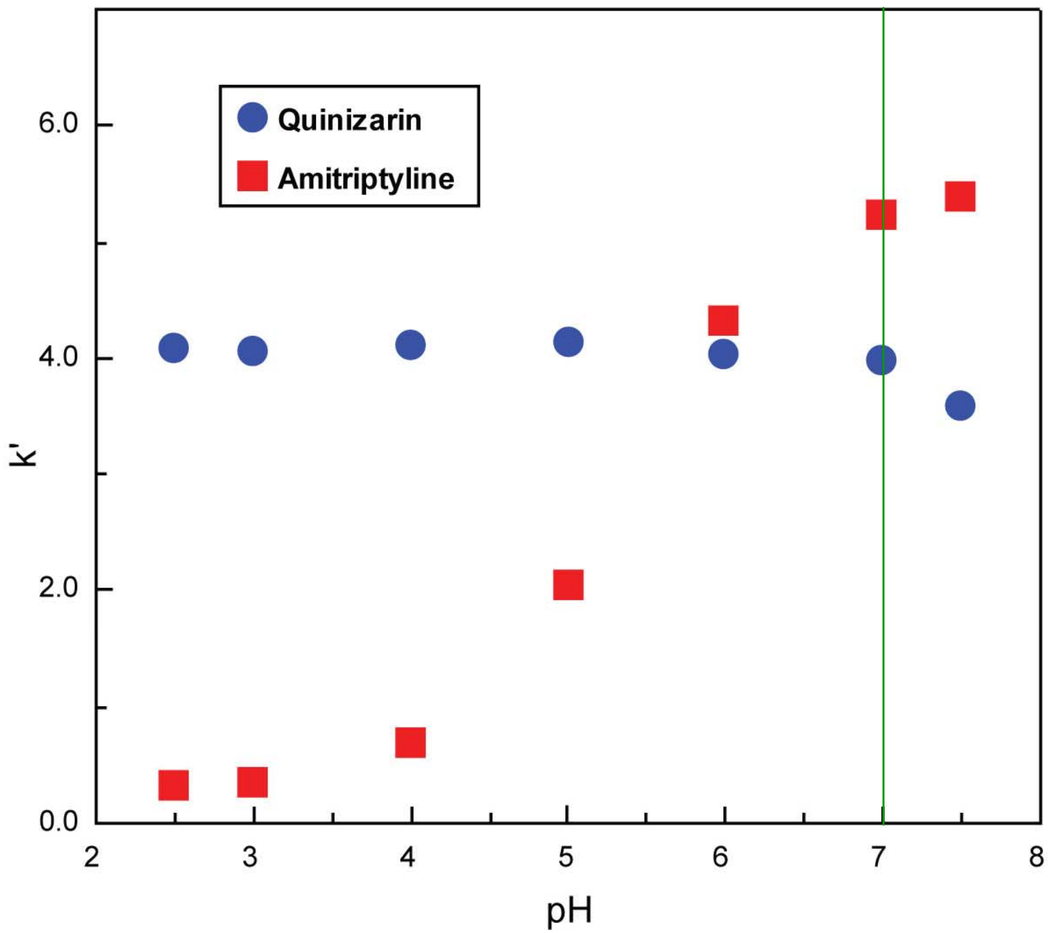
Fig. 5 Fast Analysis on 2mm ID 3 micron ODS-100V Column



Conditions

Column:	TSKgel ODS-100V 3µm (2.0mmID x 10cm)
Eluent:	A) H_2O/CH_3CN (40/60)
	B) H_2O/MeOH (30/70)
Flow rate :	A) 0.50mL/min
	B) 0.20mL/min
Pressure:	A) 17.8MPa
	B) 12.6MPa
Detection:	UV 254nm
Temp:	25°C
Inj. volume:	1µL
Sample:	1. Uracil
	2. Caffeine
	3. Phenol
	4. Methyl benzoate
	5. Benzene
	6. Toluene

Fig. 6 Retention of Basic and Chelating Compounds (SRM 870) as a Function of pH



Conditions

Column: TSKgel ODS-100V 3μm (4.6mmID x 15cm)

Eluent: 20mM Phosphate buffer (at indicated pH) /MeOH (20/80)

Flow rate : 1.0mL/min

Detection: UV 254nm

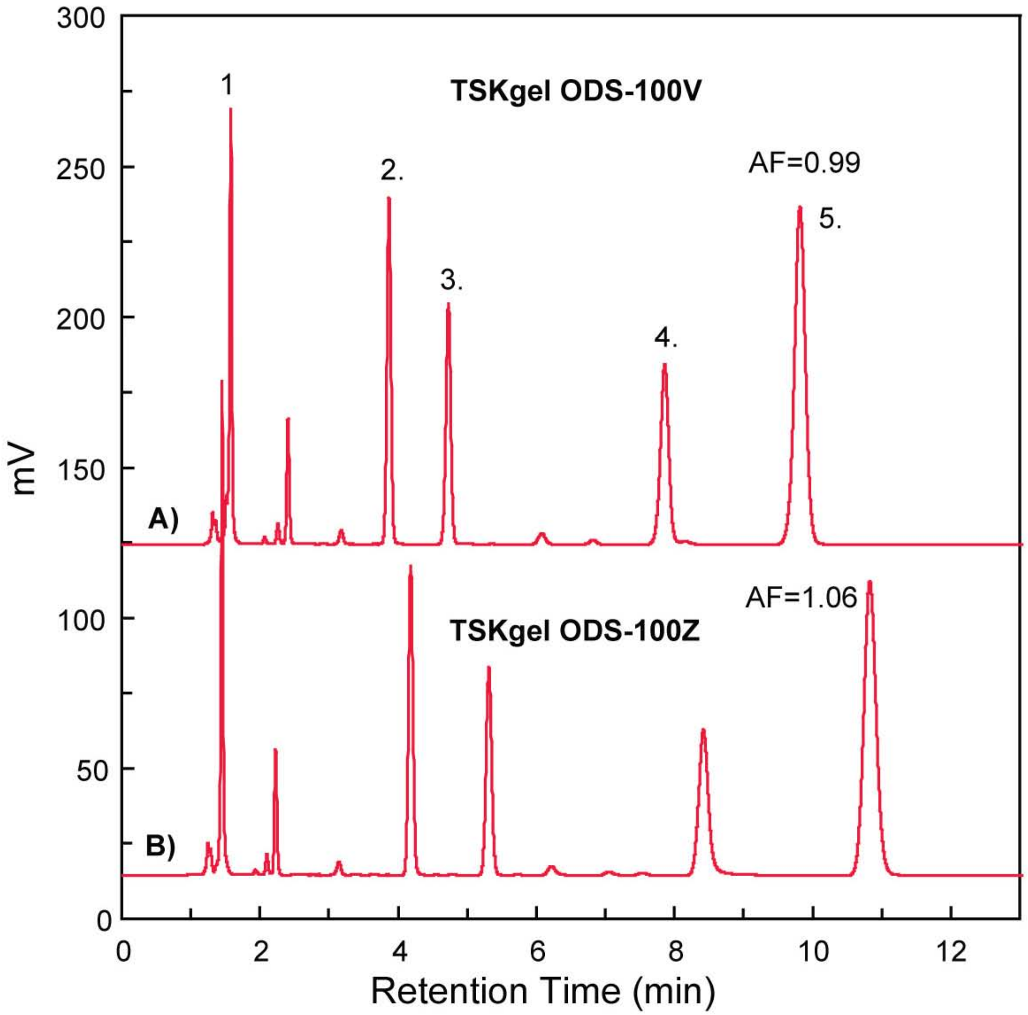
Temp: 40°C

Inj. volume: 10μL

Sample: 1. Quinizarin
2. Amitriptyline

Fig. 7 Column Performance for Basic and Chelating Compounds (SRM 870)

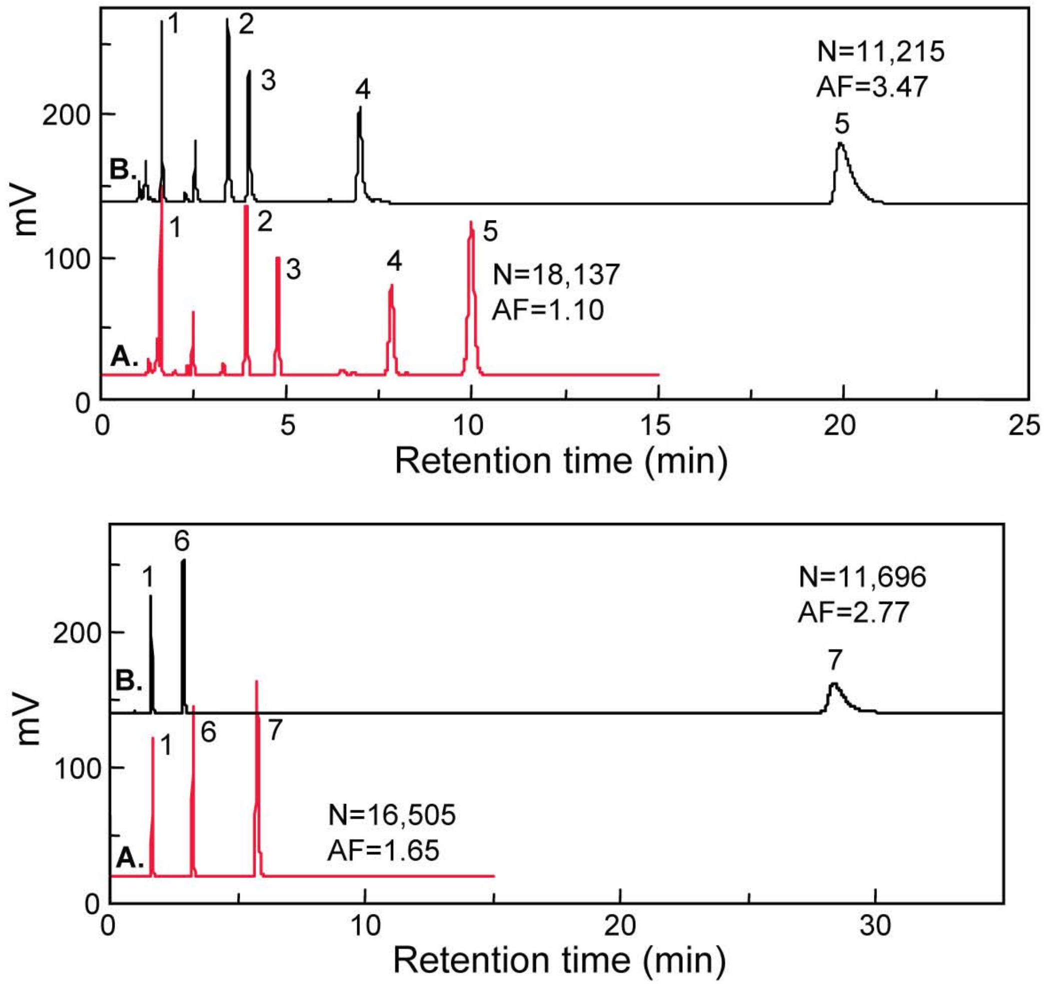
TOSOH



<u>Conditions</u>

Columns:	(A) TSKgel ODS-100V 3μm (4.6mmID x 15cm)
	(B) TSKgel ODS-100Z 3μm (4.6mmID x 15cm)
Eluent:	20mM Phosphate buffer (pH 7.0) /MeOH (20/80)
Flow rate :	1.0mL/min
Detection:	UV 254nm
Temp:	40°C
Inj. volume:	10μL
Sample:	1. Uracil
	2. Toluene
	3. Ethyl benzene
	4. Quinizarin
	5. Amitriptyline

Fig. 8 Efficiency and Peak Symmetry on ODS-100V columns with and without endcapping



Conditions

Columns: (A) TSKgel ODS-100V 3µm (4.6mmID x 15cm)
(B) TSKgel ODS-100V 3µm (not endcapped) (4.6mmID x 15cm)

Eluent: 20mmol/L Phosphate buffer (pH 7.0) /MeOH (20/80)

Flow rate : 1.0mL/min

Detection: UV 254nm

Temp: 40°C

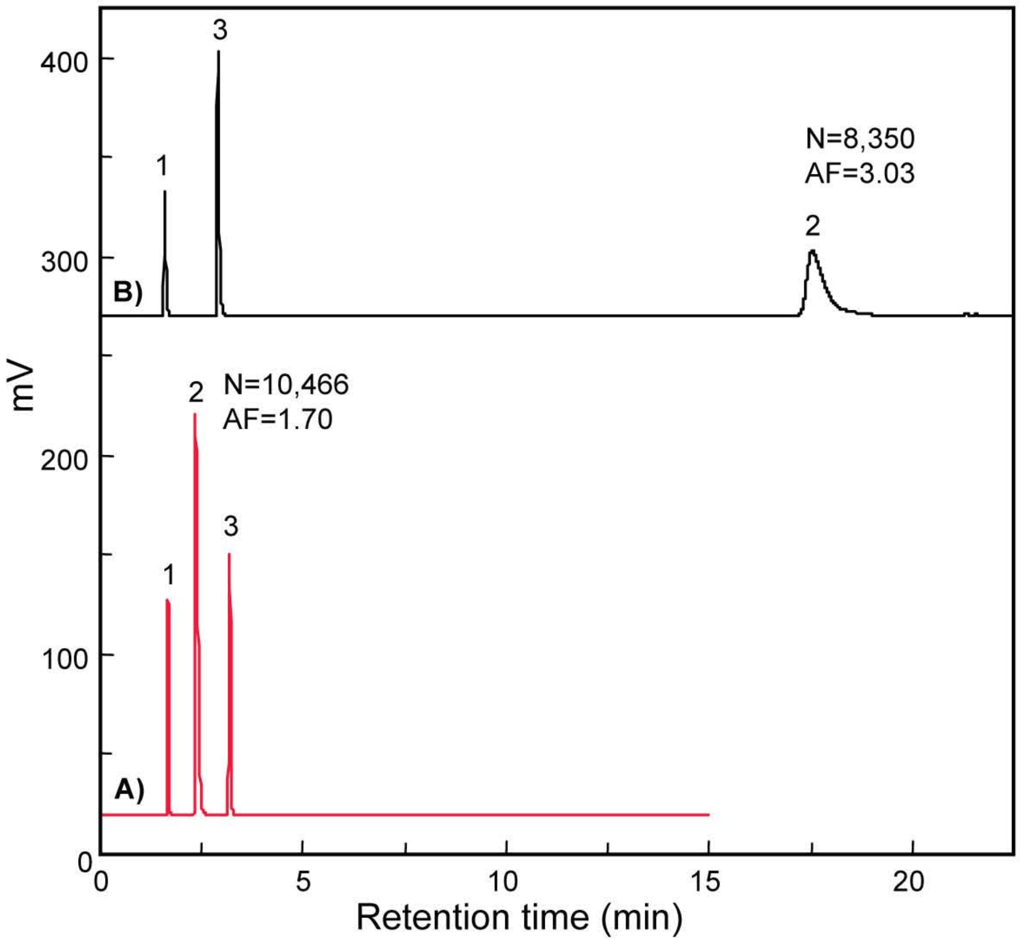
Inj. volume: 10µL

Samples:

Top panel:
1. Uracil
2. Toluene
3. Ethyl benzene
4. Quinizarin
5. Amitriptyline

Bottom panel:
1. Uracil
6. Benzene
7. Desipramine

Fig. 9 Desipramine Peak Shape in 5mM Ammonium Formate as a Function of Endcapping



<u>Conditions</u>

Columns:	(A) TSKgel ODS-100V 3µm (4.6mmID x 15cm)
	(B) TSKgel ODS-100V 3µm (not endcapped) (4.6mmID x 15cm)
Eluent:	5mmol/L $HCOONH_4$ /MeOH (20/80)
Flow rate :	1.0mL/min
Detection:	UV 254nm
Temp:	40°C
Inj. volume:	10µL
Samples:	1. Uracil
	2. Desipramine (52µg/mL)
	3. Benzene

TOSOH

Results & Discussion

TSKgel ODS-100Z has higher C18 ligand density than ODS-100V (Table 2)

- Hydrophobic compounds are stronger retained on ODS-100Z (Fig. 1-2)
- Methylene group selectivity (k'T/k'B) is larger on ODS-100Z
- Full C18 coverage phase provides higher shape selectivity

TSKgel ODS-100V has higher endcapping ligand density (Table 2)

- Polar compounds containing amino or carboxyl groups are stronger retained on ODS-100V (Fig. 1-2)
- Polar group selectivity is larger on ODS-100V (Fig. 1-2)

Efficiency and peak shape for non-polar test compounds (Fig. 3-5)

- Minimum HETP values of ~ 2dp were obtained at the optimum linear velocities for columns packed with 5µm and 3µm particles
- 2mm ID columns can be packed with high column efficiency
- Fast and high efficiency separations can be achieved by increasing flow rate using an acetonitrile mobile phase

Results & Discussion Continued

Efficiency and peak shape for basic and chelating compounds (Fig. 6-9)

- Both the strong base amitriptyline and the chelating compound quinizarin elute with good peak shape from 3 μm ODS-100V and ODS-100Z columns, which demonstrates that both compounds are effectively shielded from interacting with residual silanol groups. (Fig. 6-7)
- The base silica contains very low levels of metal impurities (data not shown). This can be deduced from Fig. 8 [top panel], where quinizarin elutes with only modest tailing from an ODS-100V column that has not been endcapped.
- Amitriptyline elutes with poor peak shape from an ODS-100V column that has not been endcapped. After the endcapping reaction the retention of amitriptyline is reduced due to the absence of secondary retention from the interaction with accessible silanol groups, while the peak shape of amitriptyline becomes symmetrical. (Fig. 8)
- The secondary amine desipramine is even longer retained than amitriptyline on a ODS-100V column that has not been endcapped. As with amitriptyline, desipramine retention reduces drastically and peak shape improves greatly after endcapping. Our studies suggest that desipramine is a very sensitive compound to evaluate interaction with residual and accessible silanol groups on ODS columns. (Fig. 9)

Conclusions

1. TSKgel ODS-100V and TSKgel ODS-100Z columns provide symmetrical peak shapes when analyzing hydrophilic as well as hydrophobic compounds.
2. The low C18 ligand density allows TSKgel ODS-100V to be used under 100% aqueous mobile phase conditions.
3. Three micron, 2mmID TSKgel ODS-100V and TSKgel ODS-100Z columns provide efficient high throughput analyses at high flow rates.
4. 5mmol/L ammonium formate has proven to be a suitable buffer for high performance LCMS analyses of basic pharmaceutical compounds when using TSK-GEL ODS-100V and ODS-100Z columns.