



# LC/MS applications of newly developed, high performance ODS columns in pharmaceutical analysis

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# Introduction

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Three micron TSK-GEL ODS-100V reversed phase columns were developed by Tosoh Corporation for use in LC/MS applications, in particular for the analysis of active pharmaceutical ingredients and their metabolites. The surface polarity of these ODS columns is higher than that of conventional ODS columns with 15% carbon content. Accessible residual silanol groups have been endcapped to promote efficient, symmetrical peak shapes when analyzing basic compounds at neutral pH. The behavior of these new columns in LC/MS applications was studied, including the effects of mobile phase components, buffer concentration and pH.



# Preliminary Results

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As expected, a 4.6mm ID TSKgel ODS-100V, 3 $\mu$ m column was found to show higher column efficiency at a wide range of linear velocities, when compared with a TSKgel ODS-100V, 5 $\mu$ m column of the same column size, while both columns were found to have the same characteristics for retention, selectivity and ionic properties for acidic and basic analytes. The TSK-GEL ODS-100V, 3 $\mu$ m columns showed relatively strong retention of polar compounds compared to conventional ODS columns. Faster separations of standard analytes, along with higher resolution, were shown and good peak shapes for basic compounds were obtained, even at neutral pH. Under LC/MS-compatible mobile phase conditions, such as a solution containing a low concentration of ammonium formate, a 2.0mm ID TSKgel ODS-100V, 3 $\mu$ m column was found to show symmetrical peak shapes with the analysis of a basic drug, while a comparable 3 $\mu$ m commercial ODS column showed peak tailing. These and other results suggest that 3 $\mu$ m TSK-GEL ODS-100V columns are well-suited for LC/MS applications.



# Experimental Conditions

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TSK-GEL ODS-100V, 3 $\mu$ m reversed phase columns were developed by Tosoh Corporation.

4.6mm ID columns were used for fundamental experiments such as testing for mobile phase components, buffer concentration and pH. A Tosoh HLC-8020 series LC system was used with UV detection at 254nm.

For LC/MS analyses, 2.0mm ID columns were coupled to a Q TRAP<sup>®</sup> mass spectrometer from Applied Biosystems. These columns were tested in various LC/MS applications for the analysis of basic pharmaceuticals, pesticides and environmental substances.

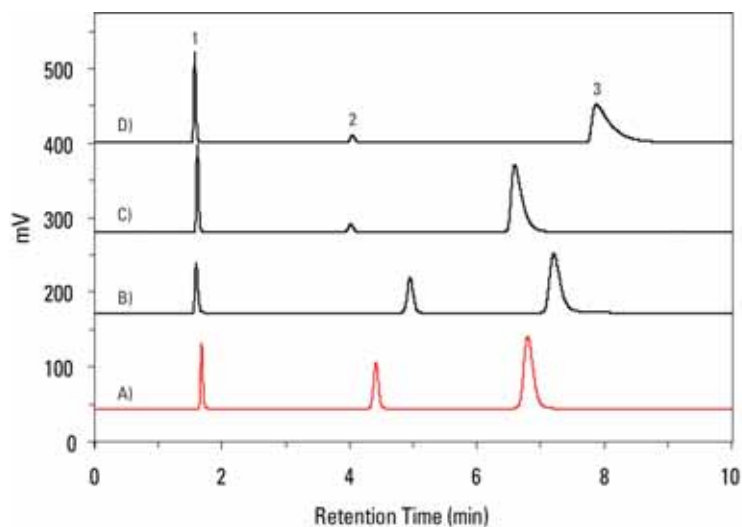


**Table 1: Basic Properties of TSK-GEL ODS-100V, 3 $\mu$ m**

| <b>TSK-GEL ODS-100V, 3<math>\mu</math>m</b> |                 |
|---|-----------------|
| Particle size ( $\mu$ m)                    | 3               |
| Pore size (nm)                              | 10              |
| Surface area (m <sup>2</sup> /g)            | 450             |
| Functionality                               | Octadecyl group |
| Carbon content (%)                          | 15              |
| Bonding structure                           | Monolayer       |



# Figure 1: Comparison of Basic Compounds on TSK-GEL ODS-100V, 3 $\mu$ m and Commercial ODS, 3 $\mu$ m Columns



CAPCELL PAK C18 MG, 3 $\mu$ m

L-Column ODS, 3 $\mu$ m

Inertsil ODS-3, 3 $\mu$ m

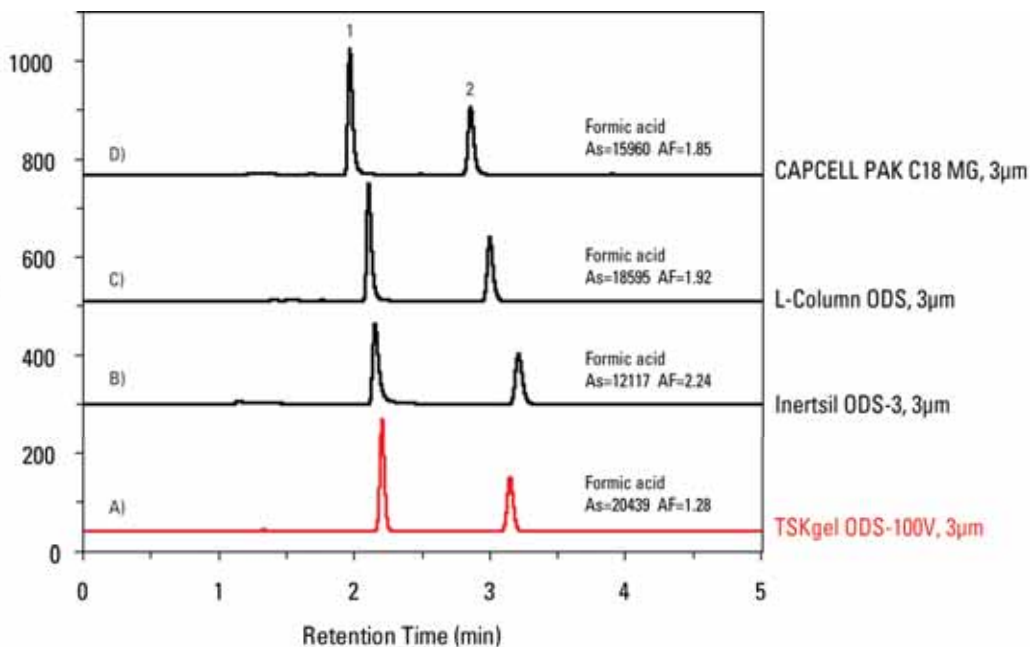
TSKgel ODS-100V, 3 $\mu$ m

Columns: A) TSK-GEL ODS-100V, 3 $\mu$ m (4.6mm ID x 15cm)  
B) Inertsil ODS-3, 3 $\mu$ m (4.6mm ID x 15cm)  
C) L-Column ODS, 3 $\mu$ m (4.6mm ID x 15cm)  
D) CAPCELL PAK C18 MG, 3 $\mu$ m (4.6mm ID x 15cm)

Eluent: 50mmol/L phosphate buffer (pH 7.0)/MeOH(30/70)  
Flow Rate: 1.0mL/min  
Detection: UV@254nm  
Temp.: 40°C  
Inj. Vol.: 10 $\mu$ L  
Samples: 1.uracil 2.benzene 3.desipramine



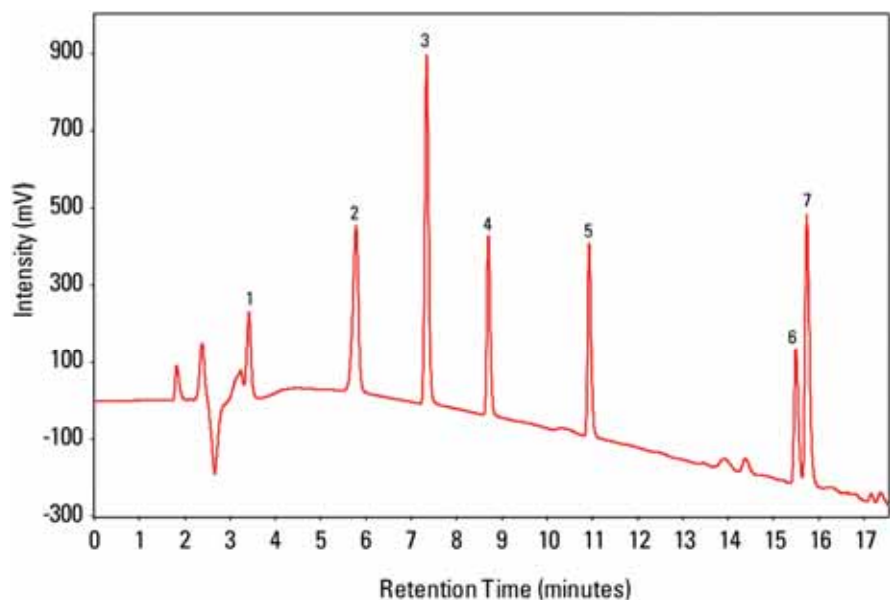
# Figure 2: Comparison of Acidic Compounds on TSK-GEL ODS-100V, 3 $\mu$ m and Commercial ODS 3 $\mu$ m Columns



Column: TSKgel ODS-100V, 3 $\mu$ m (4.6mm ID x 15cm)  
Eluent: H<sub>2</sub>O/ACN(98/2) + 0.1% H<sub>3</sub>PO<sub>4</sub>  
Flow Rate: 1.0mL/min  
Detection: UV@254nm  
Temp.: 40°C  
Inj. Vol.: 10 $\mu$ L  
Samples: 1.formic acid 2.acetic acid



# Figure 3: Separation of Six Cold Products, Including Phenylephrine, using a TSKgel ODS-100V, 3 $\mu$ m Column

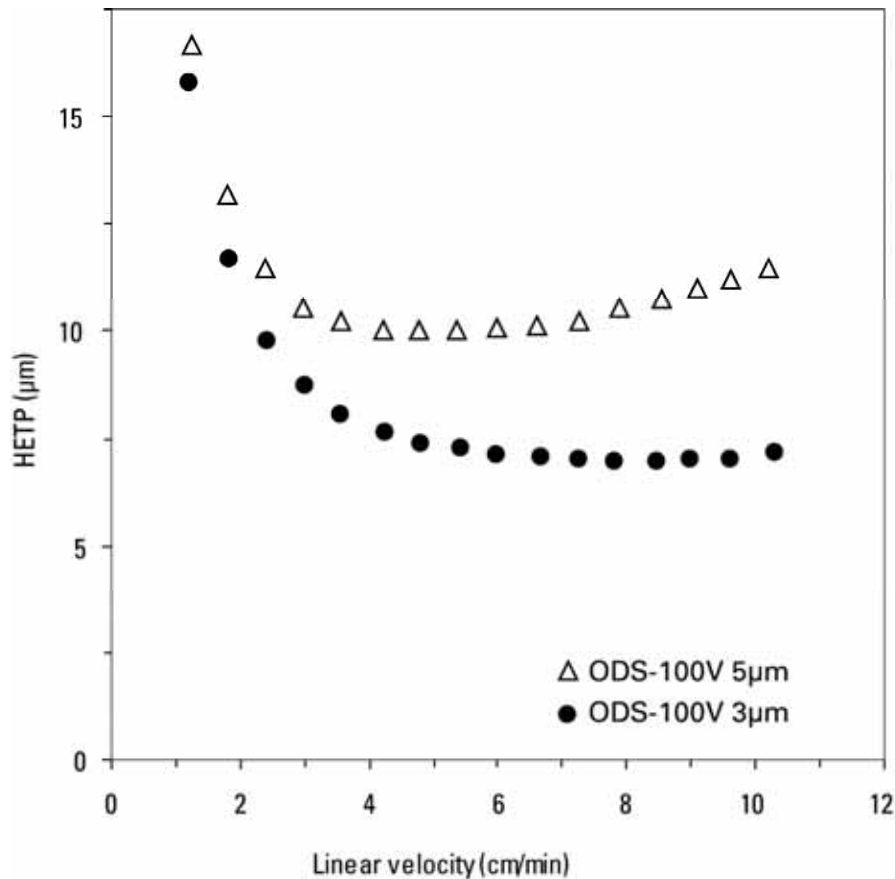


**Column:** TSKgel ODS-100V, 3 $\mu$ m (4.6mm ID x 15cm)  
**Eluent:** A: 0.15% TFA in H<sub>2</sub>O  
B: 0.02% TFA in ACN/MeOH (75/25)  
**Gradient:** 0min (A 96%) $\rightarrow$  15min (A 40%) $\rightarrow$  17min (A 40%)  
**Flow rate:** 1.0mL/min  
**Detection:** UV@210nm  
**Temperature:** 40°C  
**Injection volume:** 20 $\mu$ L  
**Concentration:** 80 $\mu$ g/mL  
**Diluent:** MeOH/ H<sub>2</sub>O (50/50)  
**Samples:**  
1. maleate  
2. phenylephrine  
3. acetaminophen  
4. doxylamine  
5. chlorpheniramine  
6. dextromethorphan  
7. diphenhydramine





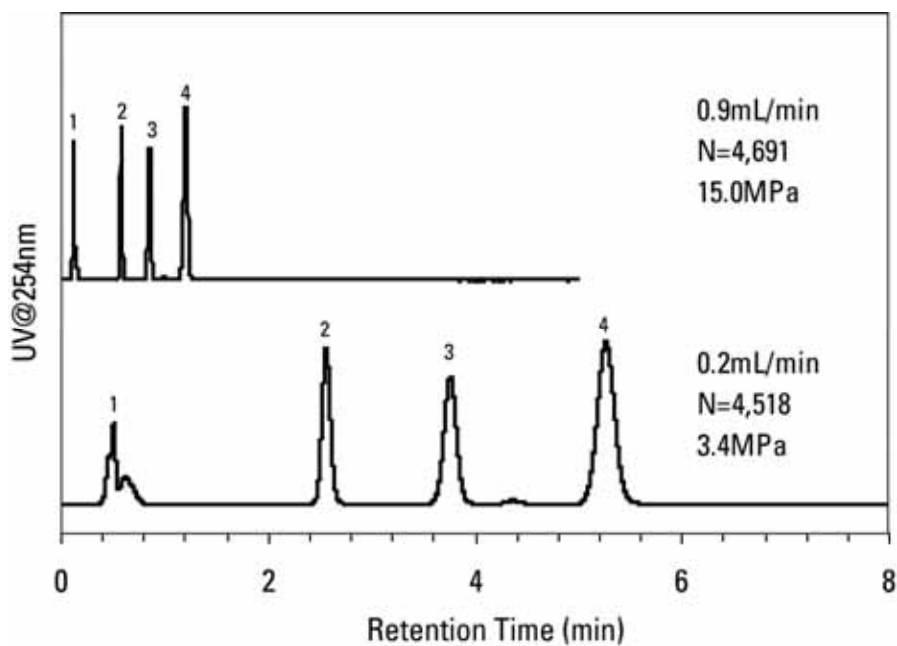
# Figure 4: Comparison of H/u Curves on 3 $\mu$ m and 5 $\mu$ m TSK-GEL ODS-100V Columns



Columns: ( $\Delta$ ) TSKgel ODS-100V, 3 $\mu$ m (4.6mm ID x 15cm)  
( $\bullet$ ) TSKgel ODS-100V, 5 $\mu$ m (4.6mm ID x 15cm)  
Eluent: H<sub>2</sub>O/MeOH (30/70)  
Detection: UV@254 nm  
Temp.: 40°C  
Inj. vol.: 10 $\mu$ L  
Sample: naphthalene



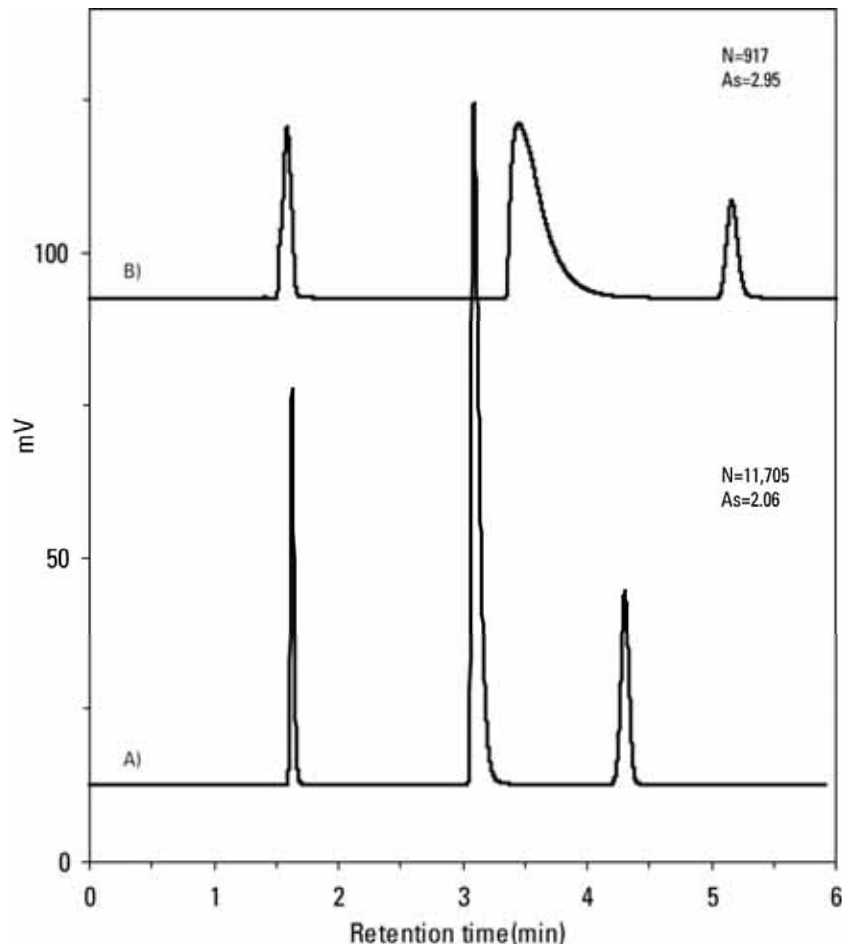
# Figure 5: High Speed Analysis on a 2mm ID TSKgel ODS-100V, 3 $\mu$ m Column



Column: TSKgel ODS-100V, 3 $\mu$ m (2.0mm ID x 5cm)  
Eluent: H<sub>2</sub>O/ACN=50/50  
Flow rate: 0.9mL/min (upper)  
0.2mL/min (lower)  
Detection: UV@254 nm  
Temp.: 25°C  
Inj. vol.: 2 $\mu$ L  
Samples: 1. uracil 2. benzene  
3. toluene 4. naphthalene



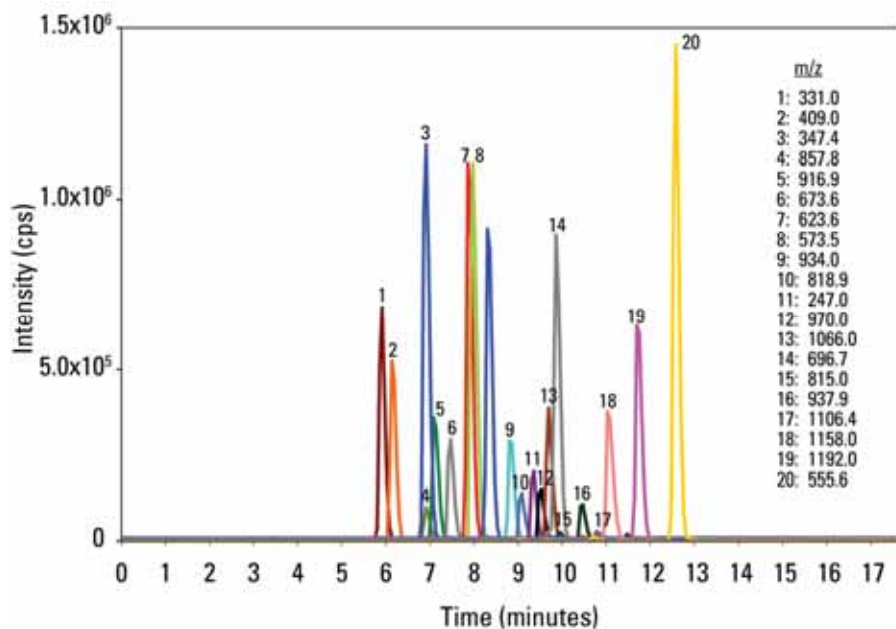
# Figure 6: Comparison of Basic Compound Separation on TSKgel ODS-100V, 3 $\mu$ m and Commercial ODS, 3 $\mu$ m Column with LC/MS Compatible Mobile Phase



Columns: A) TSKgel ODS-100V, 3 $\mu$ m (4.6mm ID x 15cm)  
B) Inertsil ODS-3, 3 $\mu$ m (4.6mm ID x 15cm)  
Eluent: 5 mmol/L HCOONH<sub>4</sub>/MeOH (20/80)  
Flow rate: 1.0 mL/min  
Detection: UV@254 nm  
Temp.: 40°C  
Inj. vol.: 10 $\mu$ L  
Samples: 1. uracil 2. desipramine (26ug/mL)  
3. benzene



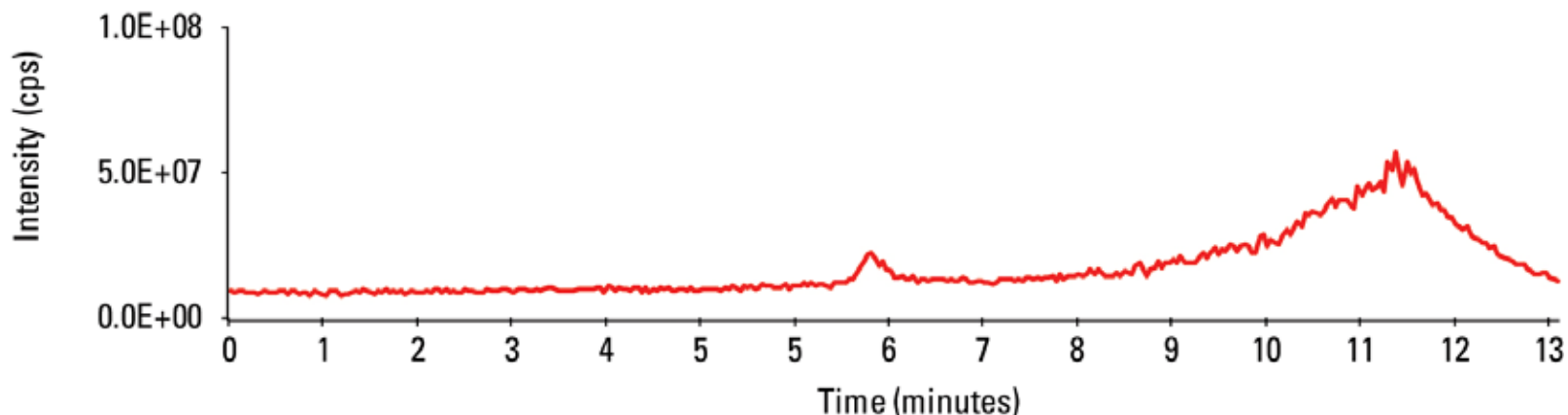
## Figure 7: Rapid Identification of 20 Peptides



Column: TSKgel ODS-100V, 3 $\mu$ m (2.0mm ID x 15cm)  
Eluent: A: 0.1% TFA in H<sub>2</sub>O, B: 0.1% TFA in ACN  
Gradient: 0min (B 10%) $\rightarrow$  15min (B 70%) $\rightarrow$  17min (B 70%)  
Flow rate: 0.2mL/min  
Injection volume: 2 $\mu$ L  
Sample:  $\beta$ -lactoglobulin tryptic digest  
Instrument: Q TRAP<sup>®</sup> (Applied Biosystems), ESI+



## Figure 8: TIC on TSKgel ODS-100V, 3 $\mu$ m with LC/ESI-MS Under Low pH Mobile Phase Condition



Column: TSKgel ODS-100V, 3 $\mu$ m (2.0mm ID x 15cm)

Eluent: A; 0.1% HCOOH in H<sub>2</sub>O  
B; 0.1% HCOOH in ACN

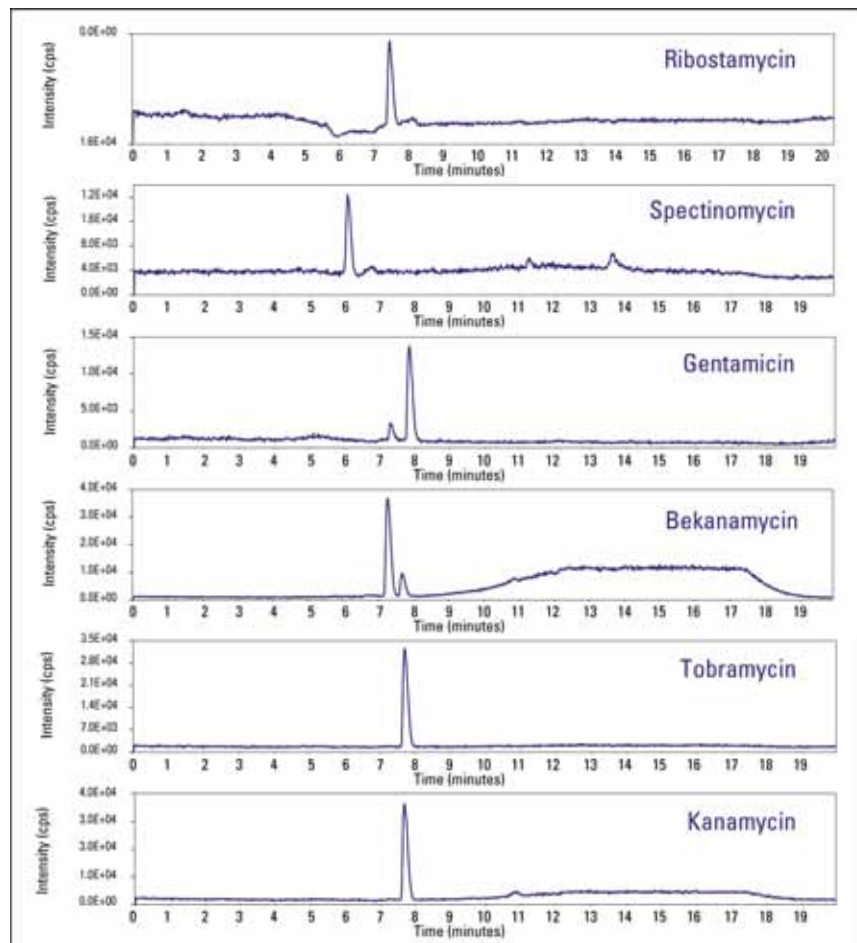
Gradient: 0min (B 5%) → 10min (B 100%) → 13min (B 100%) → 15min (B 5%)

Flow rate: 0.2mL/min

Detector: ESI+, TIC (Range: 50-1000)



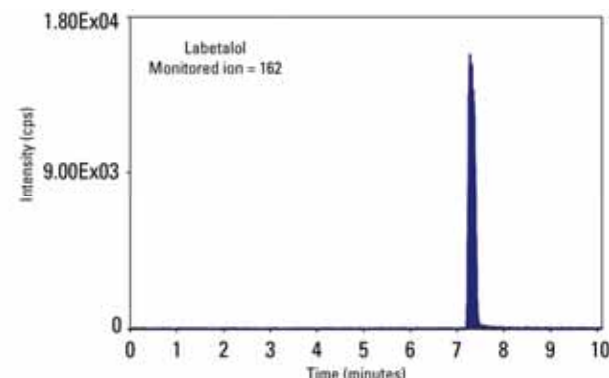
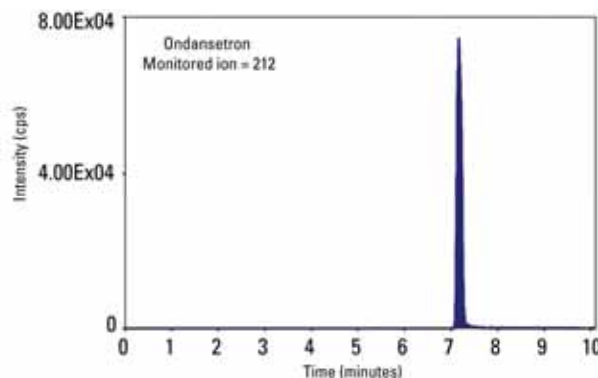
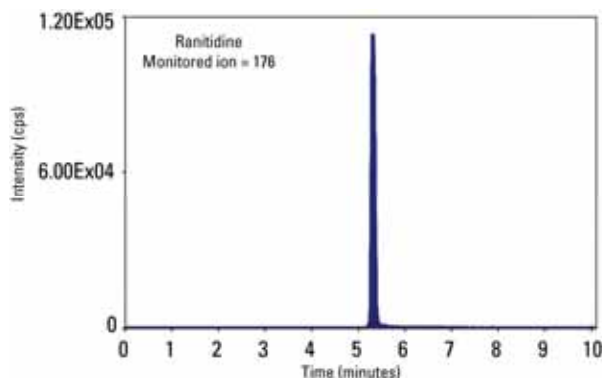
# Figure 9: LC/MS Application of Aminoglycosides Antibiotics on TSKgel ODS-100V, 3 $\mu$ m Column



Instrument: QTRAP (Applied Biosystems)  
Ion source: ESI  
Polarity: Positive  
Column: TSKgel ODS-100V, 3 $\mu$ m (2.0mm ID x 15cm)  
Eluent A: 5mM HFBA in H<sub>2</sub>O  
Eluent B: ACN  
Gradient: 0 min (B 10%) → 10 min (B 60%) → 15 min (B 60%)  
Flow rate: 0.2mL/min  
Inj. vol.: 5 $\mu$ L  
Conc.: 0.1ppm



# Figure 10: Comparing Behavior of Basic Drugs on TSKgel ODS-100V, 3 $\mu$ m Column



Column: TSKgel ODS-100V, 3 $\mu$ m (2.0mm ID x 15cm)  
Eluent: A) 10mM HCOONH<sub>4</sub> (pH3.75)  
B) ACN  
Gradient: 0min (B 0%) $\rightarrow$ 10min (B 80%) $\rightarrow$ 13min (B 80%)  
Flow rate: 0.2mL/min  
Inj. volume: 5 $\mu$ L  
Concentration: 50ng/mL  
Instrument: QTrap (Applied Biosystems), ESI+



# Conclusions

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- (1) TSK-GEL ODS-100V, 3 $\mu$ m columns showed relatively strong retention and provided symmetrical peak shapes for both acidic and basic compounds (even at neutral pH) compared to other commercially available ODS columns. Faster separations of standard analytes, along with higher resolution, were shown.
- (2) A TSKgel ODS-100V, 3 $\mu$ m column showed excellent separation of six drugs commonly found in over the counter cold medicines. Sharp peaks were obtained for both phenylephrine and doxylamine, compounds typically difficult to separate.
- (3) Because of high mechanical stability, TSK-GEL ODS-100V, 3 $\mu$ m, 2mm ID columns enable high speed analysis.
- (4) TSK-GEL ODS-100V, 3 $\mu$ m columns provided symmetrical peaks of a basic drug with buffers containing a low concentration of ammonium formate, as are commonly found in LC/MS applications.
- (5) A low background noise level was observed in the total ion chromatogram (TIC), indicating low bleeding from the column. This data suggests that TSK-GEL ODS-100V, 3 $\mu$ m columns are well suited for LC/MS applications.