



LC/MS Application of new 2.3micron ODS Column in High Throughput Pharmaceutical Analysis

Cara TOMASEK¹, Lin ZHANG²

¹TOSOH Bioscience LLC, ²TOSOH (SHANGHAI) CO., LTD.

www.tosohbioscience.com



Introduction

- Reversed phase liquid chromatography (RPC) is the most widely used analytical technique used in R&D and QC departments within the pharmaceutical industry. The need to obtain higher speed, while maintaining or improving resolution, has been recognized in this and other industries.
- Sub-two micron ODS reversed phase columns have been introduced in recent years to address the need for speed and efficiency. Such columns are commonly referred to by the name of Ultra-HPLC, or uHPLC columns. uHPLC columns cannot be operated at optimum performance in standard HPLC instrumentation.
- TSK-GEL ODS-140HTP, 2.3 μ m columns were introduced by Tosoh in 2007 to provide a combination of high speed and high resolution, while operating at modest pressure, thus making these columns compatible with conventional HPLC equipment.
- In this poster, we discuss the fundamental properties of TSK-GEL ODS-140HTP, 2.3 μ m columns and show separations of traditional Chinese medicine.



Experimental

Fundamental Studies

- **Columns – Tosoh Corp.**

TSKgel ODS-140HTP, 2.3 μ m, 2.1mm ID x 5 or 10cm, Tosoh (Japan)

TSKgel ODS-100V, 3 μ m, 2.0 or 4.6mm ID x 15cm, Tosoh (Japan)

- **Columns – Other uHPLC columns**

Acquity BEH C18, 1.7 μ m, 2.1mm ID x 5cm, Waters

Zorbax Eclipse C18, 1.8 μ m, 2.1mm ID x 5cm, Agilent

Hypersil Gold C18, 1.9 μ m, 2.1mm ID x 5 cm, Thermo-Fischer

- **Instrumentation**

HLC-8020 & CCP series from Tosoh, Japan

ACQUITY UPLC[®] system from Waters, USA

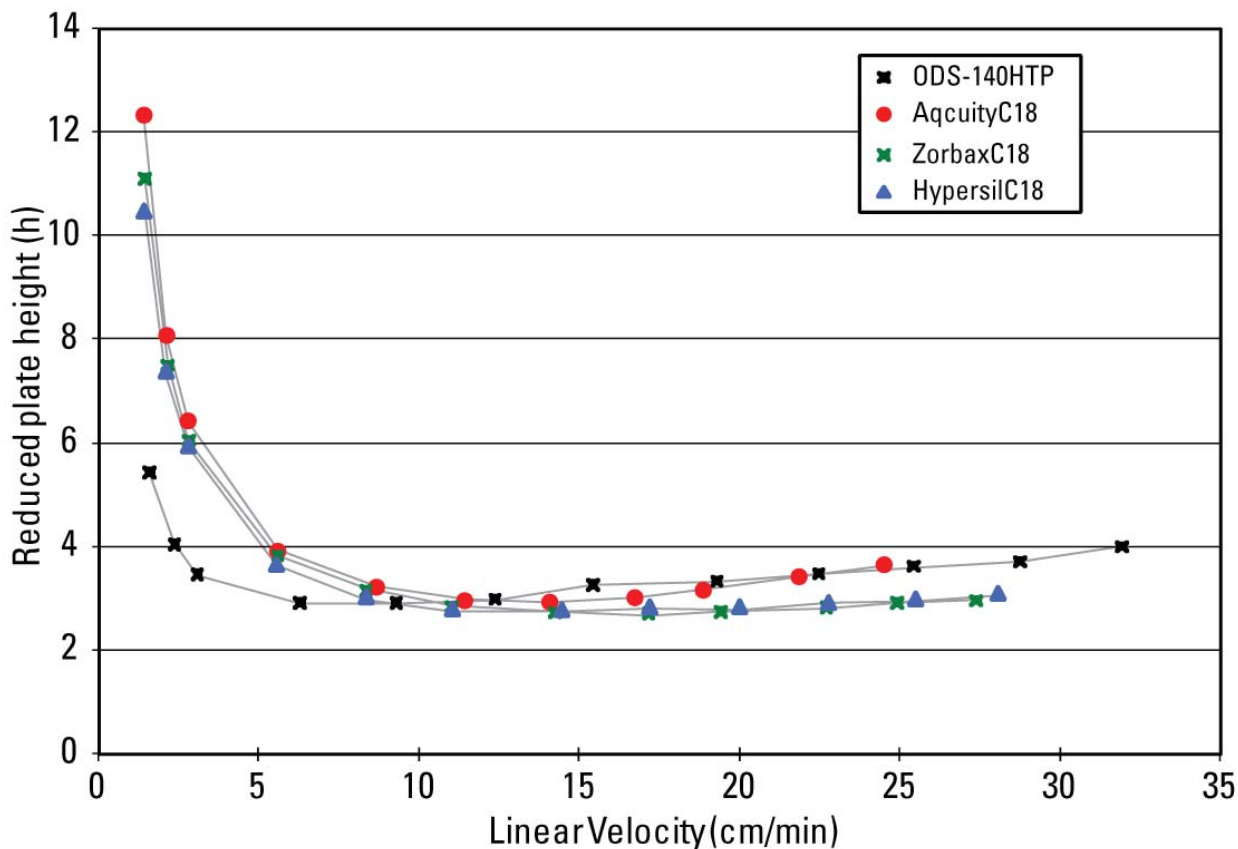


Basic properties of TSK-GEL ODS-140HTP, 2.3 μ m Columns

Properties	TSKgel ODS-140HTP, 2.3μm
Particle size	2.3 μ m
Pore size	14nm
Specific surface area	100 (m ² /g)
Functionality	C18
Carbon content	8%
Bonding chemistry	polylayer



Figure 1: Comparison of Knox curves: TSK-GEL ODS-140HTP, 2.3 μ m and sub-2 μ m ODS Columns



Columns:

TSKgel ODS-140HTP, 2.3 μ m, 2.0mm ID x 5cm

Acquity BEH C18, 1.7 μ m, 2.1mmID x 5cm

Zorbax Eclipse C18, 1.8 μ m, 2.1mm ID x 5cm

Hypersil Gold C18, 1.9 μ m, 2.1mm ID x 5cm

Eluent: H₂O/ACN=50/50

Detection: UV@254nm

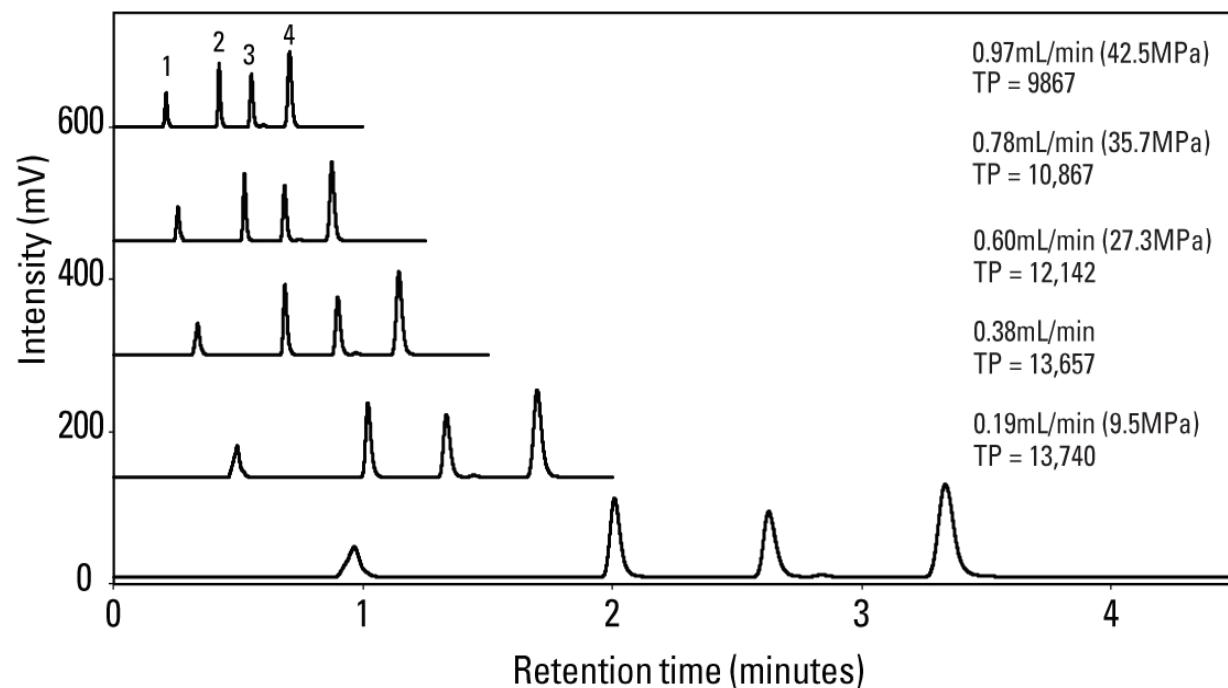
Temperature: 25°C

Injection vol.: 2 μ L

Sample: naphthalene



Figure 2: Comparison of Chromatograms at Various Flow Rates



Column:
TSKgel ODS-140HTP, 2.3 μ m, 2.0mm ID x 10cm

Eluent: H₂O/ACN =50/50

Detection: UV@254nm

Temperature: 25°C

Injection vol.: 2 μ L

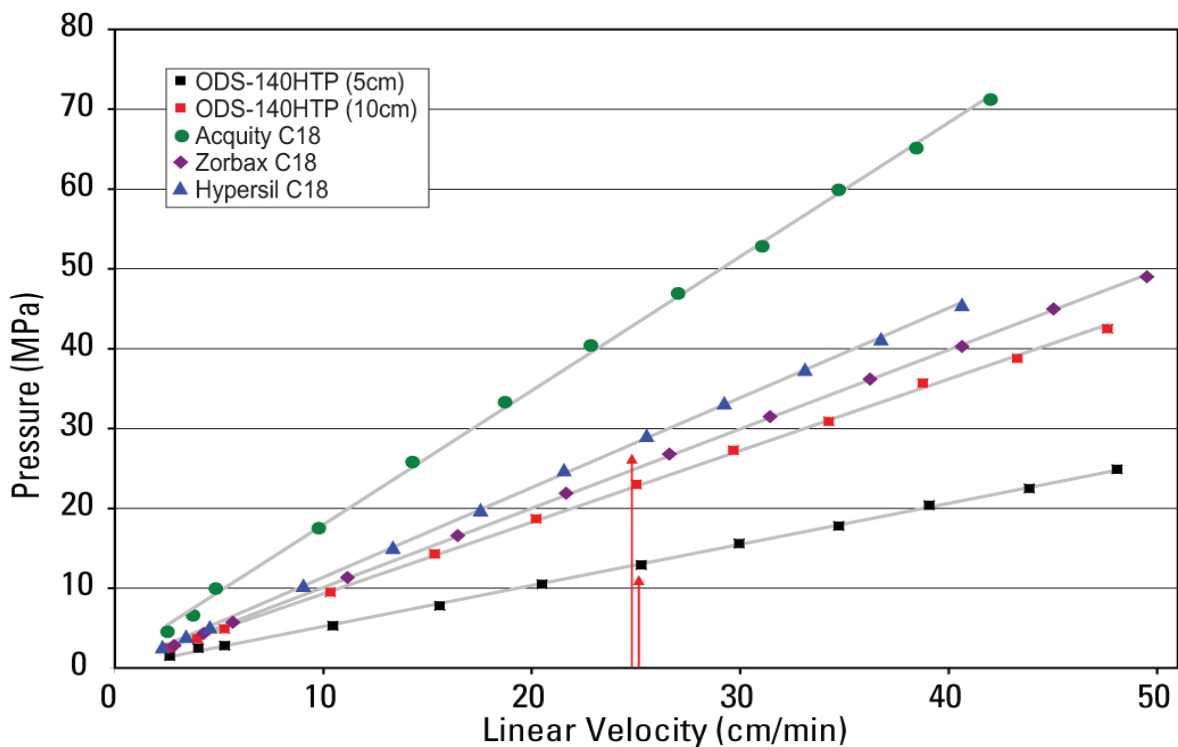
Samples:

1. uracil
2. benzene
3. toluene
4. naphthalene

Only slight reduction of theoretical plates, even at higher flow rates, with use of TSK-GEL ODS-140HTP, 2.3 μ m, 2.1mm ID x 10cm column. The pressure remained relatively low, at 43MPa, even at 1mL/min (5 times the normal flow rate).



Figure 3: Comparison of Flow Rate and Pressure Drop: TSKgel ODS-140HTP, 2.3 μ m and sub-2 μ m ODS Columns



Columns:

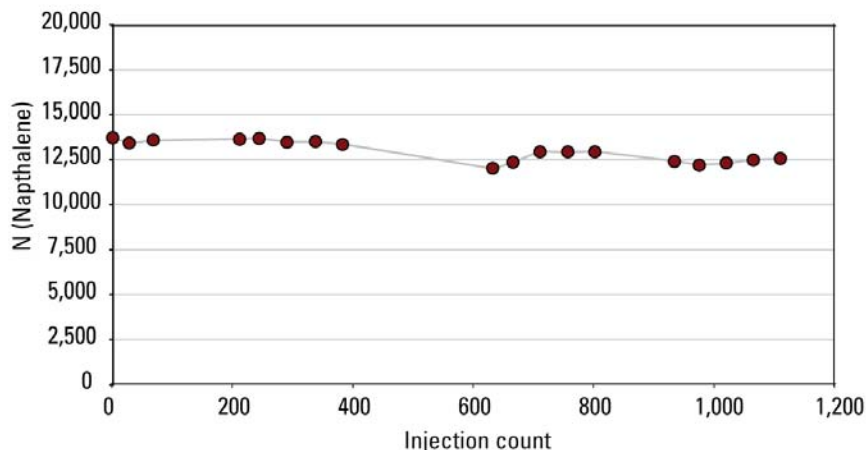
- TSKgel ODS-140HTP, 2.3 μ m, 2.0mm ID x 5cm
- TSKgel ODS-140HTP, 2.3 μ m, 2.0mm ID x 10cm
- Acquity BEH C18, 1.7 μ m, 2.1mm ID x 5cm
- Zorbax Eclipse C18, 1.8 μ m, 2.1mm ID x 5cm
- Hypersil Gold C18, 1.9 μ m, 2.1mm ID x 5cm

- Eluent: H₂O/ACN=50/50
- Detection: UV@254nm
- Temperature: 25°C
- Injection vol.: 2 μ L
- Sample: naphthalene

TSK-GEL ODS-140HTP, 2.3 μ m columns operate at lower pressure than competitive sub-2 μ m columns. The pressure drop of a 5cm TSKgel ODS-140HTP, 2 μ m column at 25cm/min is more than 50% lower than that for smaller particle size competitive columns. Not surprisingly, the pressure drop over a 10cm TSKgel ODS-140HTP, 2 μ m column was still lower than any of the competitive 5cm columns.



Figure 4: Column Stability under Step Gradient Conditions



Running conditions (No sample injection)

Column: TSKgel ODS-140HTP, 2.3 μ m, 2.1mm ID x 10cm

Eluent: A) H₂O/MeOH(90/10) Flow rate: 0.6mL/min

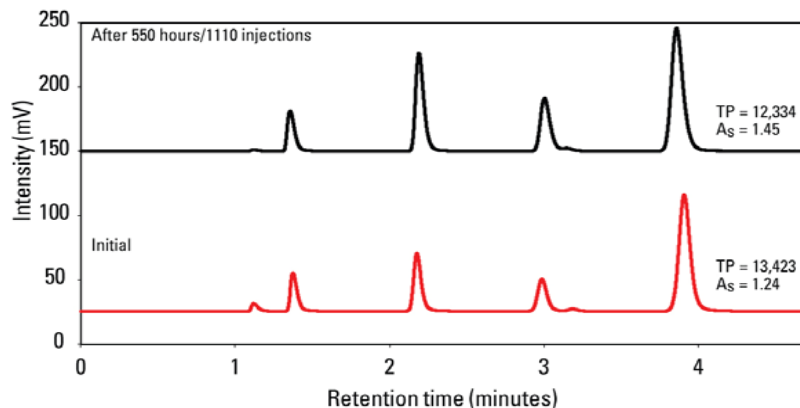
B) H₂O/MeOH(50/50) Temp.: 25°C

C) MeOH

Step gradient: A, B, C for 5min each step, 30min interval

Pressure: A) 45MPa B) 59MPa C) 32MPa

The number of theoretical plates remained basically unchanged for a TSKgel ODS-140HTP, 2.3 μ m column during 110 gradient cycles consisting of five minute step gradients from 10% to 50% and from 50% to 100% methanol at 0.6mL/min. During each cycle, pressure fluctuated between 30 and 60MPa as a result of changes in mobile phase viscosity.



Column performance test conditions

Column: TSKgel ODS-140HTP, 2.3 μ m, 2.1mm ID x 10cm

Eluent: H₂O/MeOH(30/70) Inj. vol.: 2 μ L

Flow rate: 0.2mL/min Temp.: 25°C

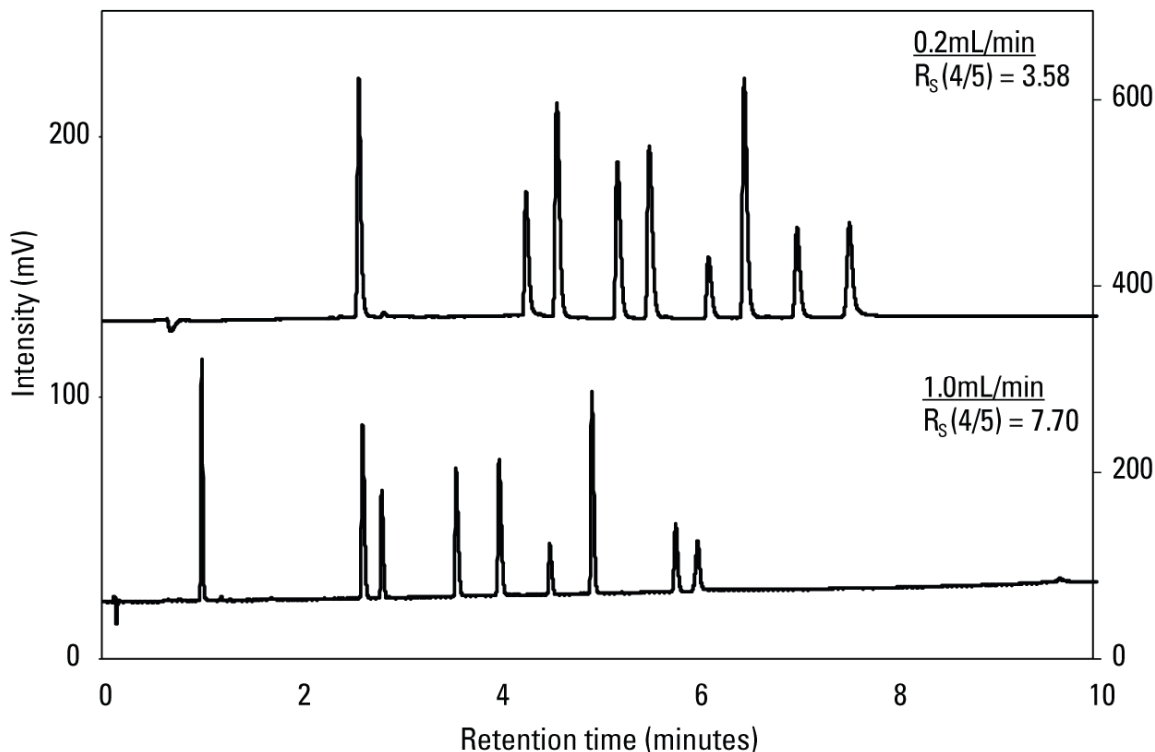
Detection: UV@254nm

Samples: 1. phenol 2. benzene 3. toluene 4. naphthalene

TSK-GEL ODS-140HTP, 2.3 μ m columns are durable when operated at high flow rate and high pressure.



Figure 5: Effect of Flow Rate on Resolution at Constant Gradient Time



Column: TSKgel ODS-140HTP, 2.3 μ m, 2.1mm ID x 5cm

Eluent: A: H₂O/ACN(95/5) + 0.1% TFA
B: H₂O/ACN(50/50) + 0.1% TFA

Flow rate: A) 1.0mL/min, B) 0.2mL/min

Temperature: 40°C

Injection vol.: 10 μ L

Detection: UV@215nm

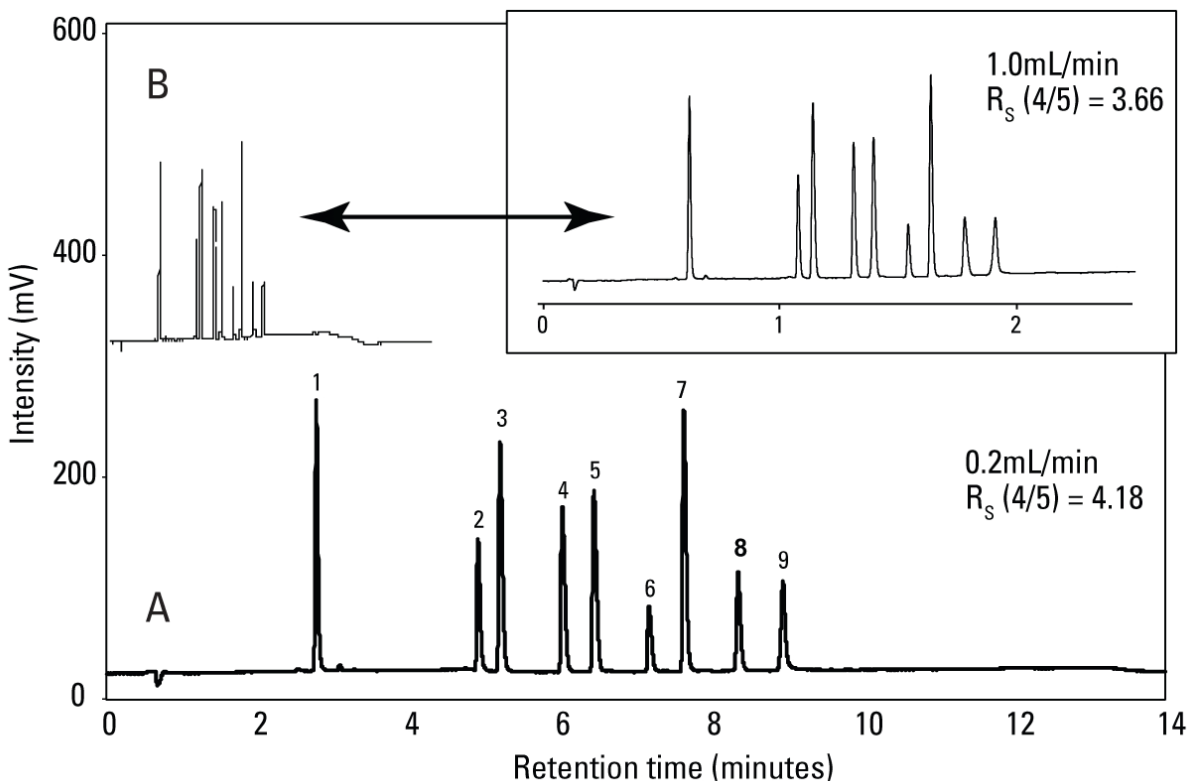
Gradient : 0-100% B (linear gradient)

Gradient time: 10min

Samples: 1. DSIP 2. BAM-12P
3. met-enkephalin 4. angiotensin II
5. (Val⁵)-angiotensin 6. substance P
7. somatostatin 8. calcitonin
9. gastrin



Figure 6: Effect of Flow Rate on Resolution at Constant Gradient Volume



Column: TSKgel ODS-140HTP, 2.3 μ m, 2.1mm ID x 5cm

Eluent: A: H₂O/ACN(95/5) + 0.1% TFA
B: H₂O/ACN(50/50) + 0.1% TFA

Flow rate: A): 0.2mL/min B): 1.0mL/min

Temperature: 40°C

Injection vol.: 10 μ L

Detection: UV@215nm

Gradient: 0-100% B (linear gradient)

Gradient Vol.: 2.5mL

Gradient time: A): 12.5min B): 2.5min

Samples: 1. DSIP 2. BAM-12P
3. met-enkephalin 4. angiotensin
5. (Val⁵)-angiotensin 6. substance P
7. somatostatin 8. calcitonin
9. gastrin



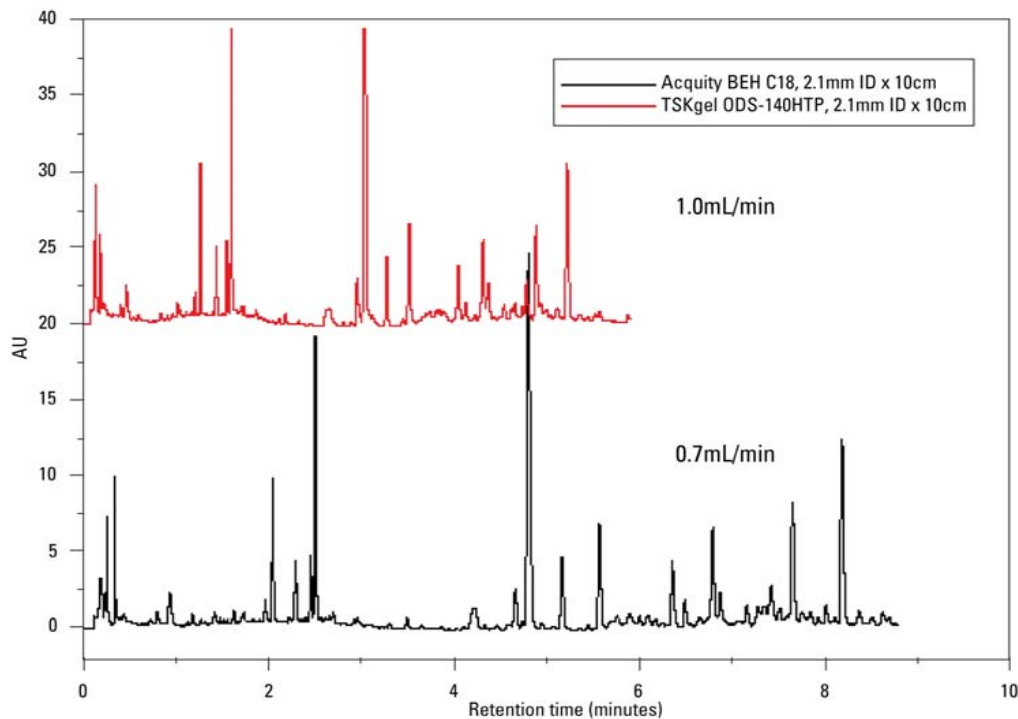
Figure 7: Separation of *C. auriculatum* Royle ex Wight using TSKgel ODS-140HTP, 2.3 μ m Column



***C. auriculatum* Royle ex Wight is a traditional Chinese drug used for its anti-tumor activity and anti-gastric lesion activity.**



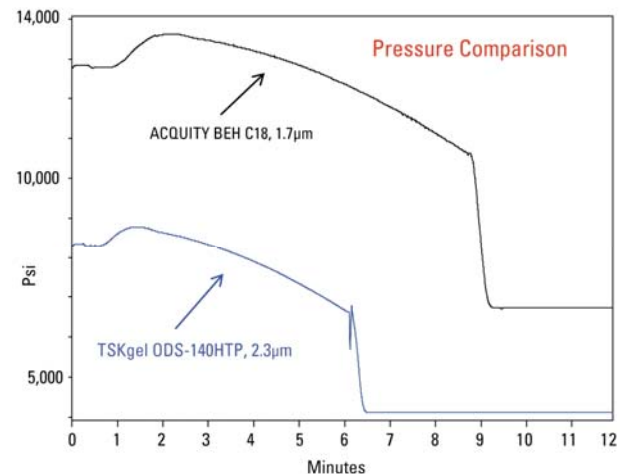
Figure 8: Comparison of Separation of *C. auriculatum* Royle ex Wight



Optimum Gradient for Acquity BEH C18: 0min (5%B) 0.68min (5%B)
2.28min (30%B) 8.57min (68%B) 8.70min (100%B) 20min (100%B)
Column temperature: 40°C

**Optimum Gradient for TSKgel ODS-140HTP: 0min (5%B) 0.48min (5%B)
1.6min (30%B) 6.0min (68%B) 6.1min (100%B) 20min (100%B)
Column temperature: 40°C**

Traditional Chinese medicine [TCM] was kindly provided by Prof. Weidong Zhang from Shanghai Department of Pharmaceutical Analysis, the Second Military Medical University, Shanghai, China.



Columns

TSKgel ODS-140HTP, 2.3 μ m. 2.1mm ID x 10cm
Acquity BEH C18, 1.7 μ m. 2.1mm ID x 10cm

Instrument

Acquity UPLC System with Acquity UPLC TUV Detector

Conditions

Mobile Phase A: Water
Mobile Phase B: ACN
Flow Rate: 1.0mL/min (TSKgel ODS-140HTP, 2.3 μ m)
0.7mL/min (Acquity BEH C18)
Detection: UV@220nm
Temperature: 40°C
Sampling rate: 80Hz
Injection vol.: 1 μ L
Sample: 10g/L extract of *C. auriculatum* Royle ex Wight by 95% ethanol



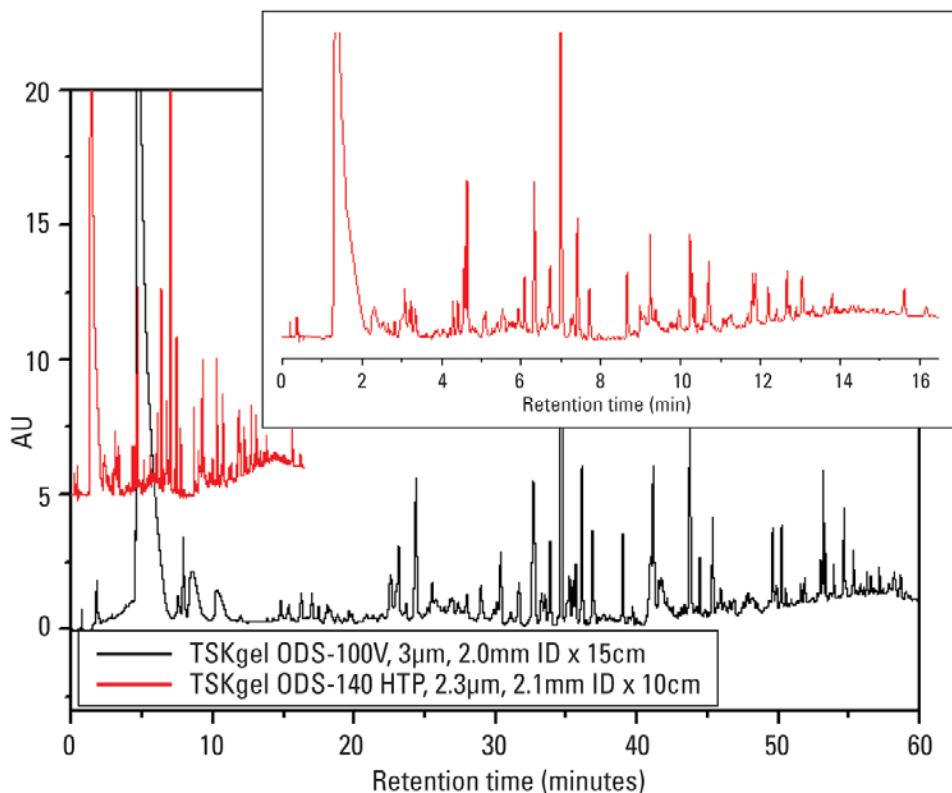
Figure 9: Separation of *Crinum latifolium* L using TSKgel ODS-140HTP, 2.3 μ m Column



***Crinum latifolium* L is a traditional Chinese drug used to invigorate blood circulation**



Figure 10: Comparison of Separation of *Crinum latifolium L*



Columns

TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 10cm

TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm

Instrument

Acquity UPLC System with Acquity UPLC TUV Detector

Conditions

Mobile Phase A: Water

Mobile Phase B: ACN

Flow Rate: 0.523mL/min (TSKgel ODS-140HTP, 2.3µm)

Flow Rate: 1mL/min (TSKgel ODS-100V)

Detection: UV@220 nm

Temperature: 35°C

Sampling rate: 80Hz

Injection vol.: 2µL (TSKgel ODS-140HTP, 2.3µm)

20µL (TSKgel ODS-100V)

Sample: 50g/L extract of *Crinum latifolium L* by 95% ethanol

Optimum Gradient for TSKgel ODS-100V: 0min (5%B) 0.1min (5%B) 30min (40%B) 55min (100%B) 65min (100%B) 65.1min (5%B)

Column temperature: 35°C

Optimum Gradient for TSKgel ODS-140HTP: 0min (5%B) 0.08min (5%B) 7.47min (40%B) 13.66min (100%B) 16.13min (100%B) 16.14min (5%B)

Column temperature: 35°C

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Conclusions

- TSK-GEL ODS-140HTP, 2.3 μ m columns are operated at lower pressure drop than competitive sub-2 μ m ODS columns because of their larger particle size: 50-60% lower than sub-2 μ m ODS columns
 - Allows high-throughput separation with conventional HPLC instrumentation
- 10cm TSKgel ODS-140HTP column provides higher resolution in less time and at lower pressure drop than competitive sub-2 μ m ODS 5cm columns.
- Excellent durability of TSK-GEL ODS-140HTP columns was achieved at high flow rates under gradient elution conditions.
- TSK-GEL ODS-140HTP, 2.3 μ m columns provide high throughput (high speed and high resolution) separation with conventional HPLC instrumentation.
- TSK-GEL ODS-140HTP, 2.3 μ m columns are applicable to various traditional Chinese medicine analysis with high throughput and low pressure.