

## Efficient Reversed Phase Columns for High Throughput Analyses

### Introduction

It is well known that columns packed with 3 micron particles are more efficient at the expense of higher back pressure compared to columns packed with 5 micron particles. History suggests that the gain in efficiency is traded off by reducing column length, which in turn results in shorter analysis times and a lowering of column back pressure. Recently, Tosoh Bioscience introduced 3 micron TSKgel® ODS-100V and ODS-100Z columns. The benefits of the new columns have been described in terms of more symmetrical peaks for basic, acidic and chelating compounds<sup>(1)</sup>. These advantages were attributed to the inertness of the silica and the innovative bonding chemistries used to prepare the endcapped C18 bonded phases<sup>(2)</sup>. This Performance Data sheet discusses how column efficiency, flow rate and mobile phase composition can be used to increase sample throughput.

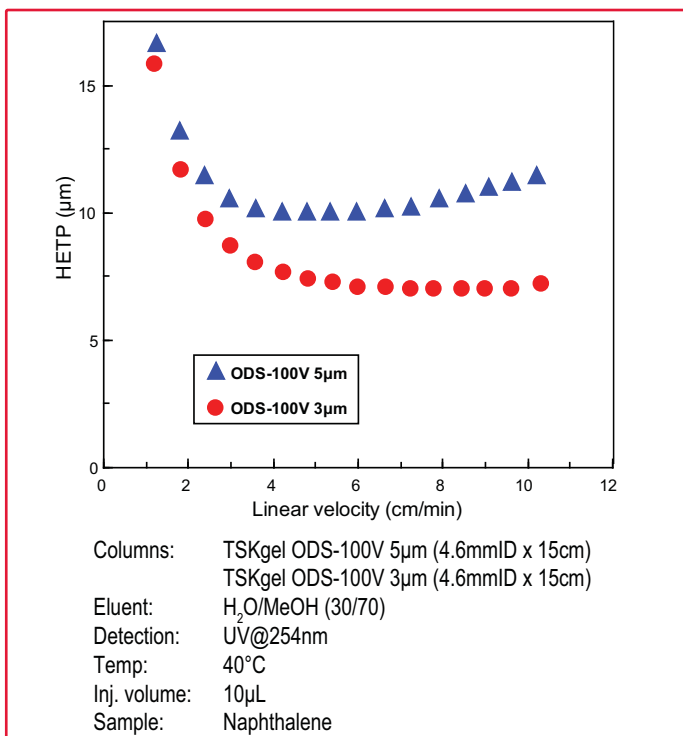
### Results

Three micron packed columns are used in high throughput analyses on conventional HPLC instruments. The data in *Figure 1* shows that a column packed with 3 micron particles has lower HETP values, i.e., higher plate numbers, than a 5 micron packed column. In addition, the benefit of 3 micron particles becomes more pronounced at higher flow rates or linear velocities. While methanol and acetonitrile are the most common organic modifiers used in reversed phase HPLC, the lower viscosity of acetonitrile and its higher solvent strength in RPLC result in lower back pressure, which either allows the use of higher flow rates, or, at the same flow rate, reduces wear and tear on the HPLC system.

*Figure 2* shows that a 2mm ID x 15cm column packed with 3 micron ODS-100V generates less than 20MPa pressure in 60% ACN at 0.4mL/min. At this or higher flow rates, high efficiency separations of relatively complex sample mixtures can be performed within minutes.

*Figure 3* shows the baseline separation of 6 aromatic compounds at 0.5mL/min with room to spare for the separation of other compounds with similar retention properties. Based on the retention of uracil, a void volume marker, the last compound elutes with a capacity factor of 3.7 and the analysis is completed in about 100 seconds, which for this separation would allow more than 1,000 analyses per day.

**Figure 1.** Column Efficiency as a Function of Flow Rate for 3 and 5 micron TSKgel ODS-100V Columns



**Figure 2.** Column Back Pressure as a Function of Solvent and Flow Rate

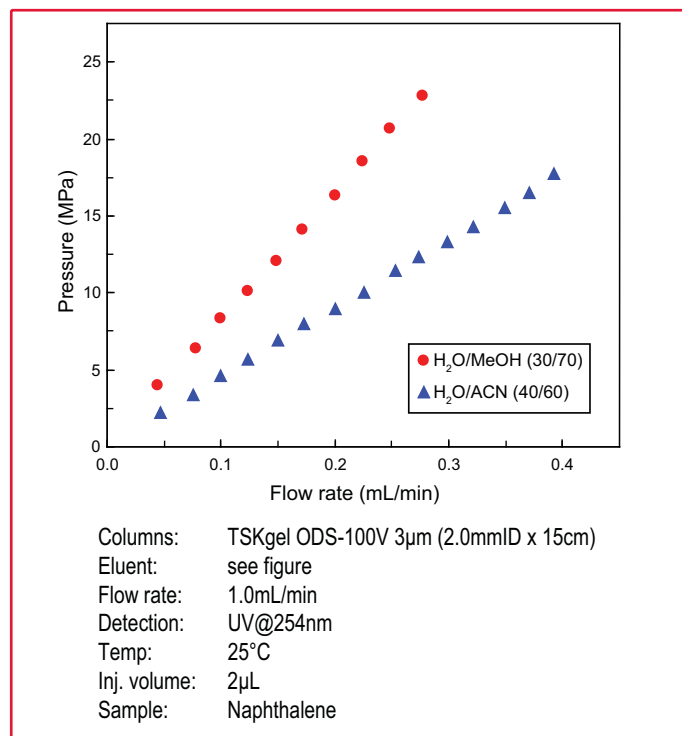
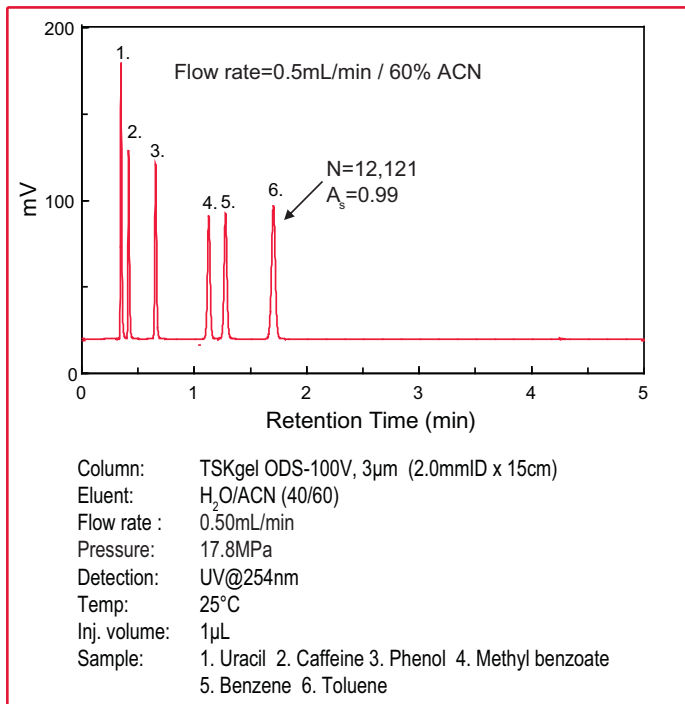


Figure 3. Reducing Analysis Times on 2mm ID Columns



## Conclusion

Three micron packed TSKgel ODS-100V columns are the appropriate tools to increase throughput in high volume applications.

## References

- (1) R. Eksteen, *The Application Notebook, Supplement to LCGC June 2006*, 78 (2006).
- (2) R. Eksteen and H. Tomizawa, *The Application Notebook, Supplement to LCGC February 2006*, 68 (2006).

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## TOSOH BIOSCIENCE

TOSOH Bioscience LLC  
3604 Horizon Drive, Suite 100  
King of Prussia, PA 19406  
Orders & Service: (800) 366-4875  
Fax: (610) 272-3028  
[www.separations.us.tosohbioscience.com](http://www.separations.us.tosohbioscience.com)  
email: [info.tbl@tosoh.com](mailto:info.tbl@tosoh.com)