Separation of Nucleobases using TSKgel® SuperSW mAb HTP column in HILIC mode

TSKgel
APPLICATION NOTE

Introduction

Hydrophilic Interaction Liquid Chromatography (HILIC) is one of the fastest growing modes of separation, in which any polar chromatographic surface can be used. Chemically bonded diol coated phases, as found in TSKgel SW size exclusion chromatography (SEC) columns, demonstrate high polarity and hydrogen bonding properties and do not contain ionizable groups other than the unreacted residual silanols, making them appropriate for HILIC mode.

For many years, SEC columns have been used to separate various nucleic acid species such as DNA, RNA and tRNA as well as their constituent bases, adenine, guanine, thymine, cytosine, and uracil. In medicine, several primary nucleobases are the basis for the nucleoside analogues and other synthetic analogs which are used as anticancer and antiviral agents. Nucleobase modifications are the basis of oligonucleotide-based therapeutics, making their purification very important.

The TSKgel SuperSW mAb HTP column is a newly introduced SEC column designed for the high throughput separation of monoclonal antibodies from their high and low molecular mass variants. TSKgel SuperSW mAb HTP has a diol coating to minimize secondary interactions which may occur in SEC separations. This note demonstrates the benefits of using a TSKgel SuperSW mAb HTP column in HILIC mode for the superior resolution of four nucleobases, as opposed to using the column in SEC mode or using a HILIC column.

Materials and Methods

Instrumentation:	Agilent 1100 HPLC system run by Chemstation (ver B.04.02)
Columns:	TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm TSKgel Amide-80, 5 µm, 2.0 mm ID × 10 cm
Mobile phase:	A: acetonitrile (HILIC mode) B: 15 mmol/L ammonium bicarbonate, pH 7.4 (HILIC mode)
Mobile phase:	100 mmol/L phosphate/100 mmol/L sodium sulfate, pH 6.7 + 0.05% NaN_3 (SEC mode)
Gradient:	Isocratic
Flow rate:	0.4 mL/min
Detection:	UV @ 280 nm
Injection vol.:	1 µL
Temperature:	ambient
Samples:	uracil (1.5 mg/mL), adenine (1.5 mg/mL), cytosine (1.5 mg/mL), cytidine (1.5 mg/mL) from Sigma Aldrich

Results and Discussion

Figure 1 illustrates the separation of 4 nucleobases using the TSKgel SuperSW mAb HTP column in HILIC mode with 15 mmol/L ammonium bicarbonate, pH 7.4 as mobile phase B. It is important to note that the order of elution of the analytes does not correlate with their molecular mass (as in SEC separations), but instead is based on their relative hydrophilicity.

Figure 1. Separation of Four Nucleobases using TSKgel SuperSW mAb HTP Column in HILIC Mode at pH 7.4

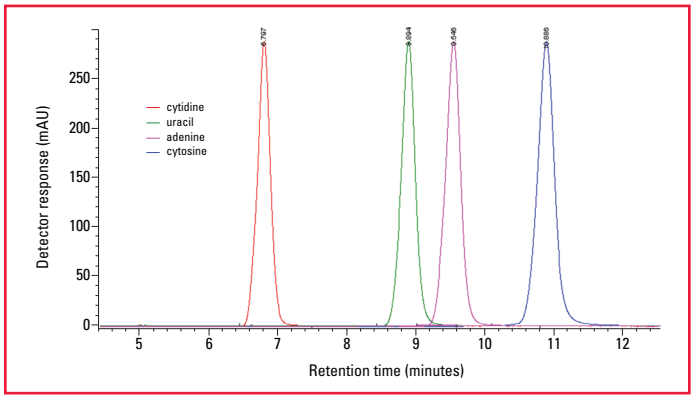
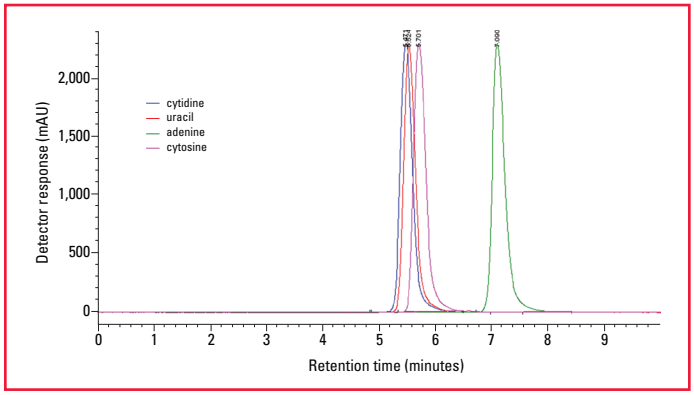


Figure 2 illustrates the separation of the four nucleobases on the TSKgel SuperSW mAb HTP column using conventional SEC conditions. As expected, due to the similarities in molecular masses between the four compounds, significant interference is observed amongst the peaks of interest, particularly the three pyrimidine derivatives, when separated on the TSKgel SuperSW mAb HTP column under SEC conditions. The late elution of adenine (relative to the other 3 compounds) may be attributed to possible interactions between the stationary phase and the derivatized purine compound, leading to a shift towards longer retention time.

Figure 2. Separation of Nucleobases using the TSKgel SuperSW mAb HTP Column under Conventional SEC Conditions



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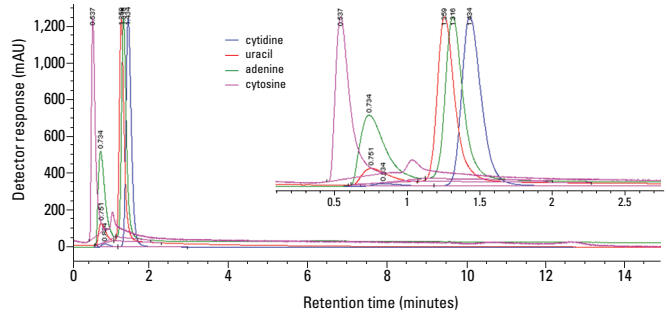
In an effort to explore the novelty of the separation of nucleobases using the TSKgel SuperSW mAb HTP column in HILIC mode, the same separation was carried out using a TSKgel Amide-80 HILIC column. The use of the TSKgel Amide-80 column yields very poor separation of the four nucleobases with virtually no retention of any of the components *(Figure 3)*.

Figure 3. Separation of Nucleobases using the TSKgel Amide-80 HILIC Column



Conclusions

This work illustrates the novelty and utility of the TSKgel SuperSW mAb HTP column as a diol-functionalized HILIC column for the high resolution separation of nucleobases on the basis of their relative hydrophilicity, rather than differences in their relative molecular mass. As shown, markedly different separation profiles are observed with the use of the TSKgel Amide-80 HILIC column under identical chromatographic conditions. Additionally, nucleobase separation using the TSKgel SuperSW mAb HTP under conventional SEC conditions yielded poor resolution of all components, making it an ineffective mode of separation for this application. The TSKgel SuperSW mAb HTP column, while designed for SEC separation of monoclonal antibodies, is an extremely effective tool in HILIC mode that should be considered for the fast separation of nucleobases.

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TOSOH BIOSCIENCE LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Tel: 800-366-4875
email: info.tbl@tosoh.com
www.tosohbioscience.com

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