Development and application of different octadecylsilane core-shell columns in reversed phase high performance liquid chromatography

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INTRODUCTION

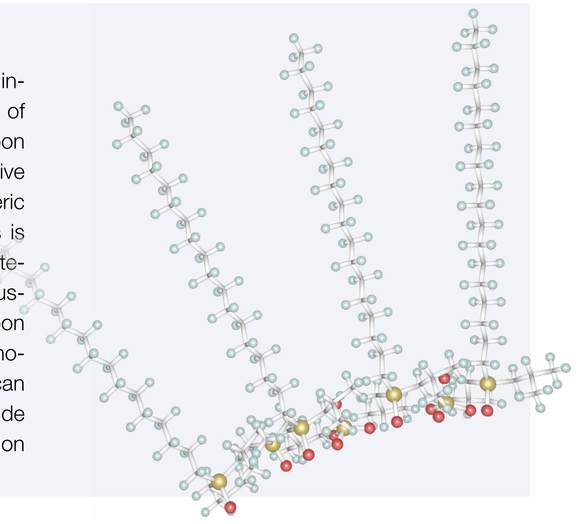
Octadecylsilane (ODS) modified stationary phases are by far the most common high performance liquid chromatography (HPLC) column packing materials, when it comes to daily routine analysis. In particular, superficially porous particles (SPP), also called core-shell particles, are well known to provide the best chromatographic results, regarding the inherent HPLC tradeoff between backpressure and performance. Due to the recent and still ongoing research carried out by column manufacturers, there are plenty of similar and dissimilar ODS modifications available on the market. In this work we point out distinct application fields of two ODS core-shell columns which differ in their underlying surface chemistry.

PRODUCT OVERVIEW

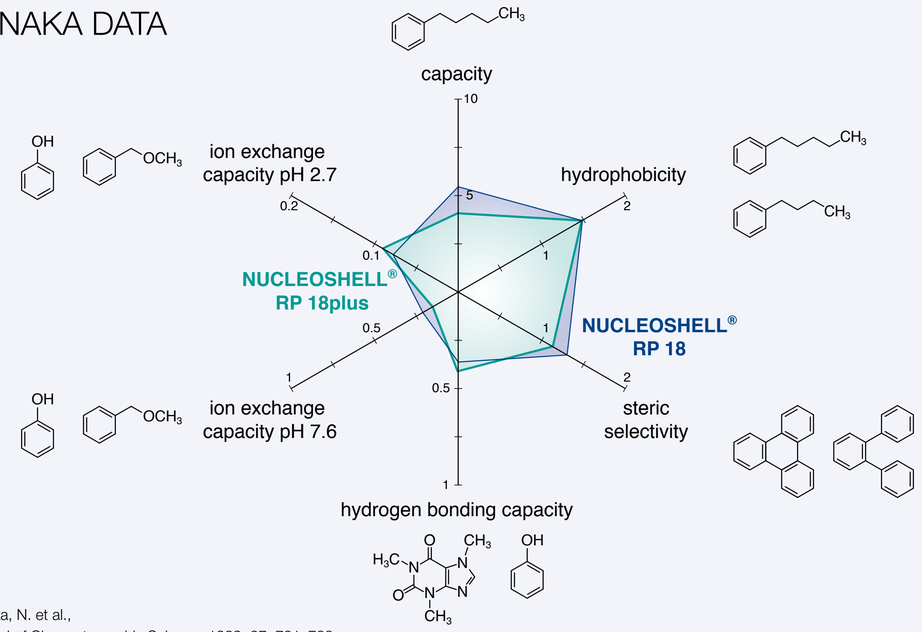
Stationary phase	Pore size	Surface area	Particle size	Carbon load
NUCLEOSHELL® RP 18plus	90 Å	130 m^2/g	2.7 µm	5.7 %
	90 Å	90 m^2/g	5 µm	4.4 %
NUCLEOSHELL® RP 18	90 Å	130 m^2/g	2.7 µm	7.8 %
	90 Å	90 m^2/g	5 µm	6.1 %

SURFACE MODIFICATION

Both stationary phases being compared in this work involve ODS surface modification techniques. In case of NUCLEOSHELL® RP18, a high-density polymeric carbon layer covers the silica surface in a bulky and protective way. This results in excellent pH stability and good steric selectivity. By contrast, NUCLEOSHELL® RP 18plus is produced by use of a monomeric ODS agent with sterically demanding side chains as indicated in the illustration next to this text. This leads to a reduced carbon load with enhanced polar selectivity for less hydrophobic compounds. The decrease in C18-chain density can be explained by steric hindrance due to the bulky side groups, allowing for increased silica-analyte interaction and therefore greater retention of polar compounds.



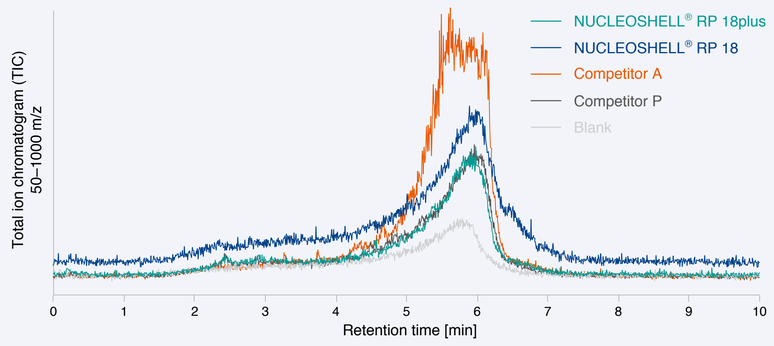
TANAKA DATA



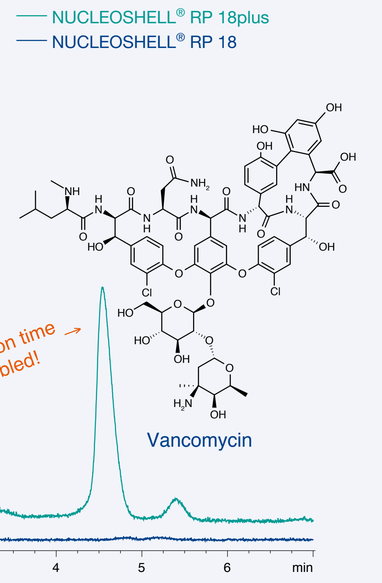
Tanaka, N. et al.,
Journal of Chromatographic Science, 1989, 27, 721–728

MS BLEEDING CHARACTERISTICS

Columns: 50 x 2 mm
Eluent: A) water + 0.1 % formic acid, B) acetonitrile + 0.1 % formic acid; 95 % A → 5 % A in 4.5 min (0.5 min) → 95 % A in 0.5 min (4.5 min)
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: ESI(+), 50–1000 m/z



SEPARATION EXAMPLES

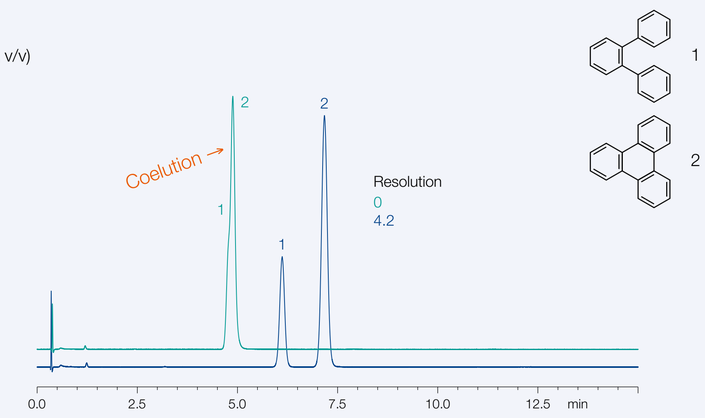


Polar selectivity shown for vancomycin

Columns: 50 x 3 mm
Eluent: water – methanol – acetonitrile – glacial acetic acid adjusted to pH 3.2 with NaOH (100:8:2:0.3, v/v/v/v)
Flow rate: 0.9 mL/min
Temperature: 35 °C
Detection: UV, 240 nm
Injection: 10 µL, 10 µg/mL vancomycin

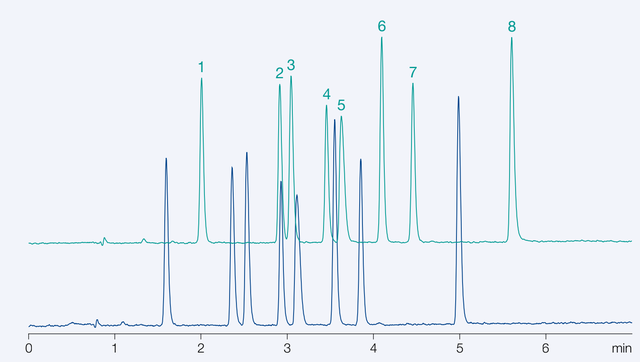
Comparison of steric selectivity

Columns: 50 x 3 mm each
Eluent: methanol – water (70:30, v/v)
Flow rate: 0.56 mL/min
Temperature: 40 °C
Detection: UV, 254 nm
Injection: 2.0 µL
Peaks:
1. *o*-Terphenyl
2. Triphenylene



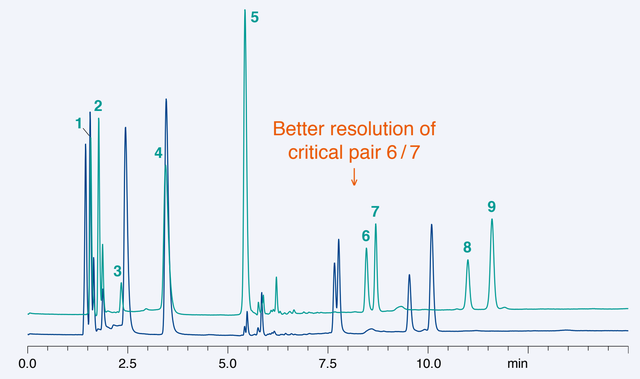
Separation of acidic pharmaceuticals

Columns: 100 x 3 mm
Eluent: A) 25 mmol/L KH_2PO_4 pH 7, B) acetonitrile; 75 % A → 60 % A in 5 min (2 min)
Flow rate: 0.56 mL/min
Temperature: 20 °C
Detection: UV, 278 nm
Injection: 0.5 µL
Peaks:
1. Ketoprofen
2. Fenoprop
3. Fenoprofen
4. Flurbiprofen
5. Ibuprofen
6. Carprofen
7. Diclofenac
8. Meclofenamic acid



Separation of water-soluble vitamins

Columns: 100 x 3 mm
Eluent: A) 20 mmol/L KH_2PO_4 pH 3, B) acetonitrile – methanol (30:70, v/v); 100 % A (2 min) → 70 % A in 1 min (12 min)
Flow rate: 0.25 mL/min
Temperature: 15 °C
Detection: UV, 220 nm
Injection: 1 µL
Peaks:
1. Pyridoxamine (vitamin B_6)
2. Thiamine (vitamin B_1)
3. Ascorbic acid (vitamin C)
4. Pyridoxal (vitamin B_6)
5. Pyridoxine (vitamin B_6)
6. Folic acid
7. Cyanocobalamine (vitamin B_{12})
8. Riboflavin (vitamin B_2)
9. Biotin (vitamin B_7)



CONCLUDING REMARKS

The results from this work illustrate that there is no “universal applicability” of SPP ODS columns, but rather the operator needs to choose from a range of specifically designed surface modifications for the application of interest. Corresponding NUCLEOSHELL® RP columns with different surface chemistries can be utilized for the separation of hydrophobic and hydrophilic sample mixtures while both still being classified as L1 columns in USP terms.

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