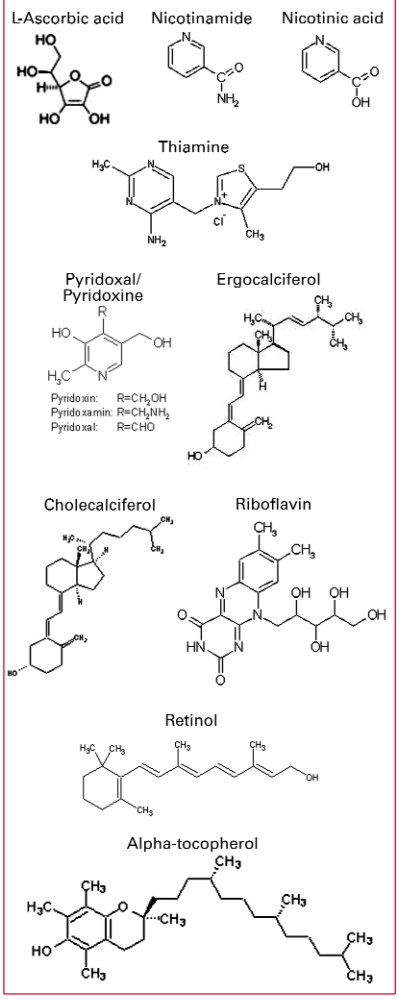
Unique Surface Treatment of Novel TSKgel ODS Columns Provide Separation of Aqueous and Fat Soluble Vitamins

TSKgel
APPLICATION NOTE

Introduction

With the introduction of two new reversed-phase column types, TSKgel® ODS-100V and TSKgel ODS-100Z, excellent chromatographic performance and selectivity can be achieved for a wide range of sample types. TSKgel ODS-100Z and TSKgel ODS-100V are based on a spherical, five micron, silica gel with 100 angstrom pores; each material is derivatized with difunctional octadecylmethylsilane, followed by an exhaustive endcapping reaction.TSKgel ODS-100Z contains approximately 20% carbon, while TSKgel ODS-100V has a lower ligand density resulting in about 15% carbon, which in turn leads to stronger retention of polar compounds.

Structures of vitamins separated on TSKgel ODS-100V and TSKgel ODS-100Z

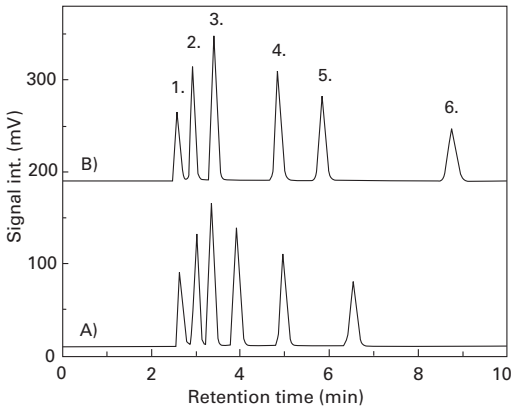


The analysis of water- and lipid-soluble vitamins requires both hydrophilic and hydrophobic retention capabilities of the stationary phase. Also, the composition of the stationary phase must allow for stable retention times in the absence of organic solvents in the mobile phase. As demonstrated, simple and fast analysis methods for the determination water and lipid soluble vitamins are possible on the TSKgel ODS-100V and the TSKgel ODS-100Z columns.

Analysis of Water Soluble Vitamins

A mixture of six water soluble vitamins was analyzed on TSKgel ODS-100V and TSKgel ODS-100Z under isocratic conditions with 99% water-1% acetonitrile, acidified with 0.1% TFA to a pH of 2.0. At an optimized temperature of 40°C good separation was obtained for all compounds in less than 10 minutes (see *Figure 1*).
Both columns managed to separate all vitamins by baseline. Thiamine, pyridoxal and pyridoxine were better separated on the more polar TSKgel ODS-100V than on the ODS-100Z column. Also, separation of nicotinamide and nicotinic acid was enhanced on the more polar column.

Figure 1. Isocratic separation of water soluble vitamins with TSKgel ODS-100V and TSKgel ODS-100Z



Columns:	A) TSKgel ODS-100V, 4.6mmID x 15cm B) TSKgel ODS-100Z, 4.6mmID x 15cm
Eluent:	H_2O/CH_3CN (99/1) + 0.1% TFA
Temp.:	40°C
Inj. volume:	5µL
Detection:	UV @ 280nm
Flow rate:	1.0mL/min.
Samples:	1. L-Ascorbic acid, 2. Nicotinamide, 3. Nicotinic acid, 4. Thiamine, 5. Pyridoxal, 6. Pyridoxine

Analysis of Lipid Soluble Vitamins

Analysis of vitamin D2 (ergocalciferol) and D3 (cholecalciferol) is critical because they differ only in one methylene group and one double bond. As shown in *Figure 2*, separation was achieved under isocratic conditions in 100% acetonitrile at a flow rate of 1.0mL/min. The influence of temperature on resolution was evaluated at 25°C and 40°C.

For this separation TSKgel ODS-100Z, which has higher % carbon than TSKgel ODS-100V, provides better resolution. Ergocalciferol and cholecalciferol elute from both columns with near baseline separation. On the TSKgel ODS-100V column, hydrophobic retention is not high enough for a baseline separation of both vitamin D compounds. On both columns, separation is enhanced at lower temperature (25°C).

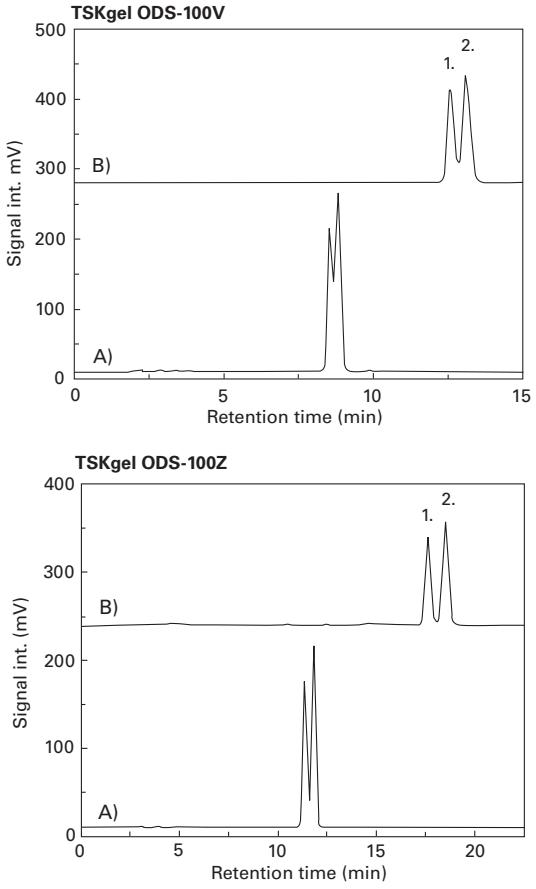
Simultaneous Separation of Water and Lipid Soluble Vitamins

Water and lipid soluble vitamins were separated in one single run on TSKgel ODS-100V and TSKgel ODS-100Z as demonstrated in *Figure 3*. To separate the mixture of six water-soluble vitamins, caffeine, and four

fat-soluble vitamins, required a gradient from water containing 0.1% TFA (eluent A) to acetonitrile containing 0.1% TFA (eluent B). Flow rate was 1mL/min, at a temperature of 40°C. To elute the polar vitamins, the content of acetonitrile in the eluent was gradually increased up to 40% within 20 minutes, then, within 2 minutes, up to 100%, enabling elution of the less polar vitamins within another 27 minutes.

Both columns were capable to separate the ten vitamins and caffeine. Due to the lower hydrophobicity of the ODS-100V column, elution was possible within 35 minutes, with very sharp peaks. Elution on the more hydrophobic TSKgel ODS-100V column, required nearly 45 minutes with broader peaks, but with higher resolution of the tocopherols.

Figure 2. Separation of vitamin D with TSKgel ODS-100V and TSKgel ODS-100Z

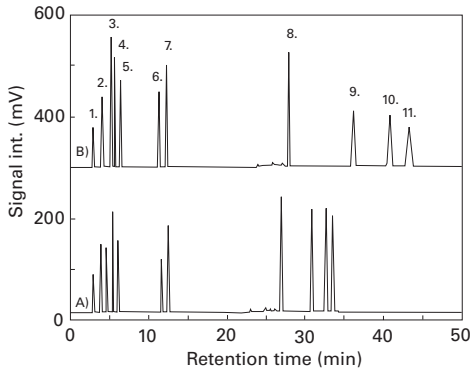


Columns:	A) TSKgel ODS-100V, 4.6mmID x 15cm B) TSKgel ODS-100Z, 4.6mmID x 15cm
Eluent:	CH_3CN
Temp.:	A) 40˚C; B) 25˚C
Detection:	UV @ 280nm
Flow rate:	1.0mL/min.
Samples:	Ergocalciferol, 2. Cholecalciferol

Conclusion

The recently commercialized TSKgel ODS-100V and TSKgel ODS-100Z column set provides a universal solution for most reversed-phase separations.

Figure 3. Separation of water- and fat-soluble vitamins on TSKgel ODS-100V and TSKgel ODS-100Z in a single run



Columns:	A) TSKgel ODS-100V, 4.6mmID x 15cm B) TSKgel ODS-100Z, 4.6mmID x 15cm
Eluent:	A) 0.1% TFA in H_2O B) 0.1% TFA in CH_3CN
Gradient:	0 min (B: 0%) 20 min (B: 40%) 22min (B: 100%) 50min (B: 100%)
Temp.:	40˚C
Inj. volume:	5µL
Detection:	UV @ 280nm
Flow rate:	1.0mL/min.
Samples:	1. L-Ascorbic acid, 2. Nicotinic acid, 3. Thiamine, 4. Pyridoxal, 5. Pyridoxine, 6. Caffeine, 7. Riboflavine, 8. Retinol, 9. Delta-tocopherol, 10. Alpha-tocopherol, 11. Alpha-tocopherol acetate

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Separation of Vitamin A Isomers

Vitamin A, also called retinol, is a fat-soluble vitamin that has several important functions, among them the prevention of night blindness, promoting growth, and enhancing immunity. Although vitamin A is present in animal food products, in vegetables it is present as pro-vitamin A, a precursor of vitamin A, usually in the form of β-carotene. As shown in Figure 1, in addition to the all-trans form, some isomers of vitamin A are also present. This application shows the separation of the all-trans and 9-cis retinol isomers using a high coverage 3 micron packed C18 column. The UV detector wavelength was set at 325nm, the absorption maximum for retinol.

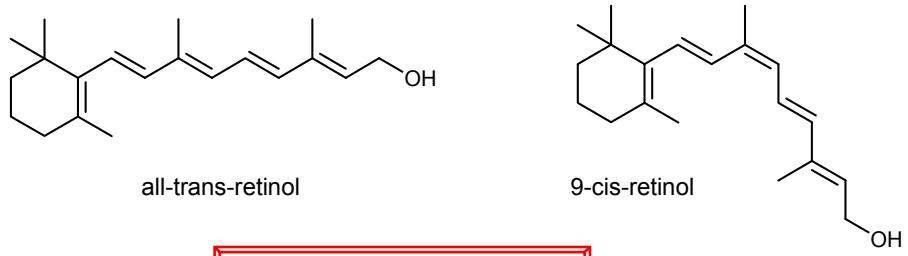

Figure 1. Isomers of vitamin A

Table 1. Conditions

Column:	**TSKgel ODS-100Z, 3µm, 4.6mm ID x 15cm**
Mobile phase:	water/acetonitrile (20/80)
Flow rate:	1.0mL/min
Detection:	UV@325nm
Temperature:	40°C
Injection vol.:	10µL

Figure 2. Chromatogram of vitamin A isomer reference standards (50mg/L each)