

Introduction

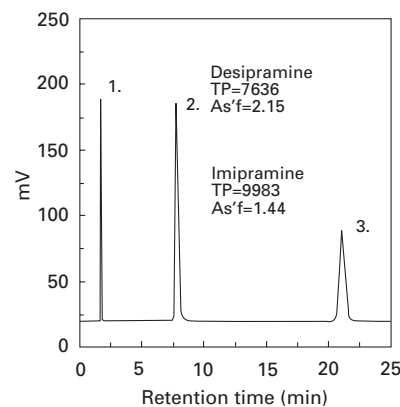
In recent years trends in the release of stationary phases have shifted towards base deactivated surfaces, stronger retention of polar compounds and the capability to operate in pure aqueous solvents. Rarely have new ODS column types provided a universal solution to the multitude of applications being performed. With the introduction of two new Reversed-Phase column types TSK[®]gel ODS-100V and TSKgel ODS-100Z excellent chromatographic performance and selectivity can be achieved for a wide range of sample types.

Especially the analysis of water and lipid soluble vitamins requires both, hydrophilic and hydrophobic retention capabilities of the stationary phase. Also, compatibility to pure aqueous as well as pure organic solvents must be given. 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.

Physical Properties

Both stationary phases consist of the same ultra-pure base silica with 5 µm particle size and 100 Å pores, functionalized with C18 groups. This results in the same chromatographic characteristics for both columns, especially with regard to symmetrical peak shapes for strongly basic and acidic compounds.

Figure 1: Chromatogram of basic compounds



Column: TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
Eluent: 50 mmol/L P.B. (pH 7.0)/MeOH (30/70)
Sample: 1. Uracil, 2. Desipramine, 3. Imipramine
Flow: 1.0 mL/min
Inj. Vol.: 10 µL
Temp.: 40 °C
Detection: 254 nm

Figure 1 shows the separation of Desipramine and Imipramine on the TSKgel ODS-100V with good resolution and asymmetry factors below 1.5 after more than 1500 runs, demonstrating the absence of accessible, residual silanol groups and metal-ion impurities on the silica surface.

Differences in selectivity are achieved by varying the carbon content and the surface optimization. For polar compounds, the TSKgel ODS-100V column with a carbon content of 15 % provides optimal surface properties. The TSKgel ODS-100Z column exhibits a higher carbon content of 20 % providing better results for more hydrophobic molecules.

Table 1 summarizes consistencies and differences. However, both column types are compatible to pure aqueous and pure organic eluents.

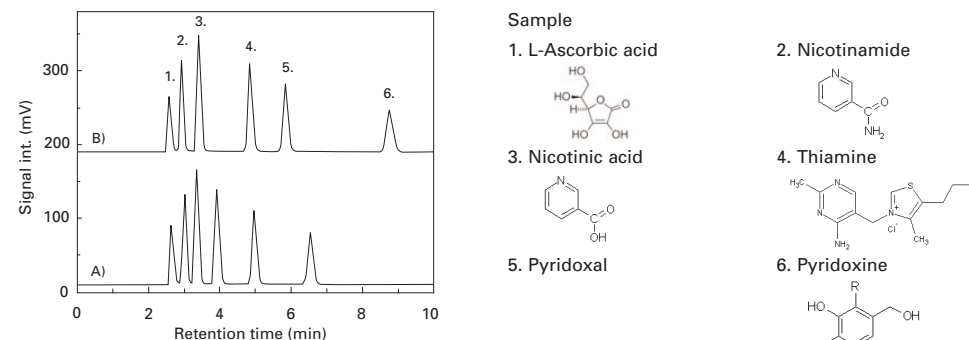
Table 1: Physical properties of TSKgel ODS-100V and TSKgel ODS-100Z

	TSKgel ODS-100V	TSKgel ODS-100Z
Matrix	Ultra-pure silica	Ultra-pure silica
Particle Size	5 µm	5 µm
Pore Size	100 Å	100 Å
Specific Surface Area	450 m ² /g	450 m ² /g
Functional Group	C ₁₈	C ₁₈
Carbon Content	15 %	20 %
Bonding Structure	Monolayer	Monolayer
End-Capping	Yes	Yes
Sample Type	Polar	Hydrophobic

Analysis of Water Soluble Vitamins

A mixture of six water soluble vitamins was analyzed on the TSKgel ODS-100V and TSKgel ODS-100Z under isocratic conditions with 99 % water, and 1 % acetonitril, acidified with 0.1 % TFA to a pH of 2.0. The temperature was optimized to 40 °C, showing good separation of all compounds in an analysis time less than 10 minutes (see figure 2).

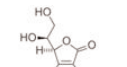
Figure 2: Isocratic separation of water soluble vitamins with TSKgel ODS-100V and TSKgel ODS-100Z



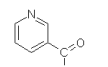
Column: A) TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
B) TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Eluent: H₂O/CH₃CN(99/1) + 0.1 % TFA
Temp.: 40 °C

Sample

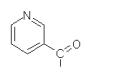
1. L-Ascorbic acid



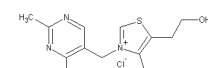
2. Nicotinamide



3. Nicotinic acid



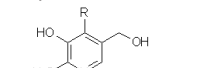
4. Thiamine



5. Pyridoxal



6. Pyridoxine



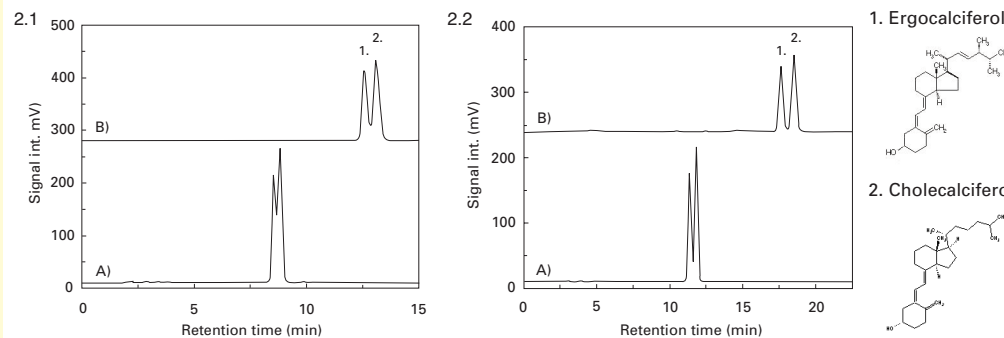
Inj. Vol.: 5 µL
Detection: UV @ 280 nm
Flow Rate: 1.0 mL/min

Both columns managed to separate all vitamins by baseline. Retention of Thiamin, Pyridoxal and Pyridoxin was higher on the more polar TSKgel ODS-100V than on the ODS-100Z. Also, separation of Nicotinamide and Nicotinic acid was enhanced on the more polar column.

Analysis of Lipid Soluble Vitamins

Analysis of vitamin D₂ (Ergocalciferol) and D₃ (Cholecalciferol) is critical because they differ only in one methyl-group and one double bonding. As shown in Figure 3 separation was achieved under isocratic conditions with 100 % acetonitrile at a flow rate of 1.0 mL/min. The influence of temperature on resolution was evaluated at 25 °C and 40 °C.

Figure 3: Separation of vitamin D with TSKgel ODS-100V and with TSKgel ODS-100Z



Column 2.1: TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
Elution conditions for both columns:

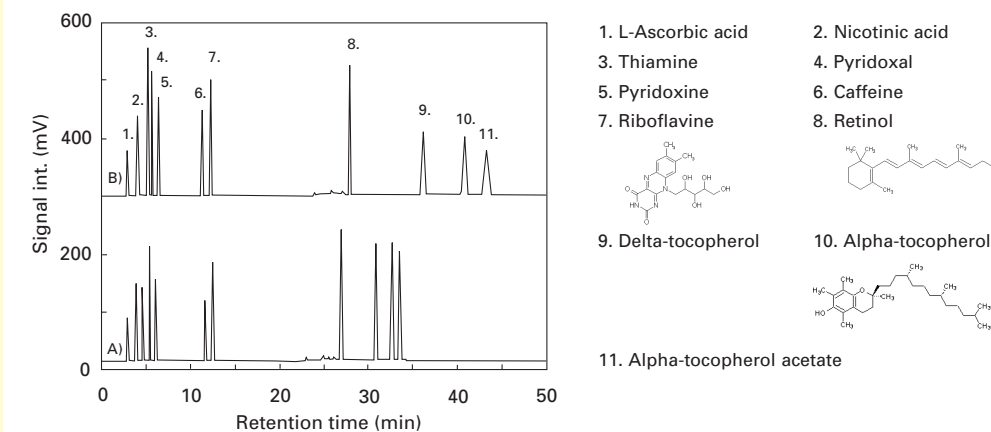
Column 2.2: TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Eluent: CH₃CN Detection: UV @ 280 nm
Flow Rate: 1.0 mL/min Temp.: A) 40 °C, B) 25 °C

Here the TSKgel ODS-100Z column with the higher carbon content provides better resolution. Ergocalciferol and Cholecalciferol elute nearly baseline separated. 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 could be separated in one single run on TSKgel ODS-100V and TSKgel ODS-100Z as demonstrated in figure 4. To separate the mixture of six water soluble vitamins, caffeine, and four fat soluble vitamins, a gradient of water containing 0.1 % TFA (eluent A) and acetonitrile containing 0.1 % TFA (eluent B) was applied. Flow rate was 1 mL/min, at a temperature of 40 °C. To elute the polar vitamins, 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.

Figure 4: Separation of water and fat soluble vitamins on ODS-100V and ODS-100Z in a single run



Column: A) TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
B) TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Eluent: A) 0.1 % TFA in H₂O
B) 0.1 % TFA in CH₃CN
Gradient: 0 min (B: 0 %) 20 min (B: 40 %) 22 min (B: 100 %) 50 min (B: 100 %)
Inj. Vol.: 5 µL
Detection: UV @ 280 nm
Flow Rate: 1.0 mL/min
Temp.: 40 °C

Both columns were capable to separate the ten vitamins and caffeine. According 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-100Z column, took nearly 45 minutes with broader peaks, but with higher resolution of the Tocopherols.

Conclusion

TSKgel ODS-100V and TSKgel ODS-100Z provide

- Good peak shape for both, acidic and basic analyte due to optimized endcapping
- Compatible to 100 % aqueous and to pure organic eluent
- Different selectivity due to its respective surface properties
- Simultaneous separation of water and fat soluble vitamins

TSKgel ODS-100V exhibits

- High surface polarity
- Higher retention of water soluble vitamins

TSKgel ODS-100Z exhibits

- Higher hydrophobicity
- Higher separation power of fat soluble vitamins