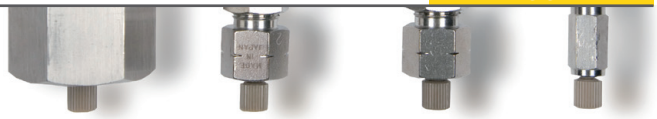

TSKgel® NH2-100 DC

INTRODUCTION

The determination of an active pharmaceutical ingredient (API) and its counterion is an important assay during the drug development process as well as in final product QC. With the introduction of a novel HILIC column, TSKgel NH2-100 DC, the simultaneous separation of an API and its respective counterions can be accomplished easily in conjunction with a reversed phase TSKgel column.

The TSKgel NH2-100 DC column is packed with 3 µm silica particles containing 100 Å pores. Due to a high ligand density and large surface area, this amino-alkyl bonded column shows high retention for hydrophilic compounds/ions. An endfitting enables the direct connection of the TSKgel NH2-100 DC column to the endfitting of a TSKgel reversed phase column (Figure 1). This allows the concurrent separation using a linear gradient of an API and its counterion without the loss of column efficiency normally experienced when connecting two columns with capillary tubing.

PRODUCT HIGHLIGHTS

- High retention for hydrophilic compounds and anions
- Direct connect fittings to any TSKgel column
- Simultaneous separation of API and counterion without loss of column efficiency

Figure 1

APPLICATIONS

Separation of drug and counter ions at pH 7.0

Maleic acid and p-toluene sulfonic acid are commonly used as counter ions in pharmaceutical preparations. Both of these organic acids are hydrophilic and are not retained on a TSKgel ODS-100V reversed phase column at pH 7.0 in 70% methanol eluent (Figure 2).

With the connection of a TSKgel NH2-100 DC column prior to the TSKgel ODS-100V column, the simultaneous determination of maleic acid and the API desipramine becomes possible. Maleic acid is slightly retained on the TSKgel NH2-100 DC column by an anion exchange interaction. Desipramine, on the other hand, does not interact with the protonated amino groups as it is positively charged. The chromatogram in Figure 2 at pH 7.0 is a reasonable compromise between insufficient retention at pH 4.5 and too long retention at pH 2.0, as is shown in Figure 3. At pH 2.0 both acids show higher retention on the TSKgel NH2-100 DC column, but desipramine is actually slightly excluded from the amino column, while its retention on the reversed phase column is minimal at this low pH.

ANALYSIS OF MALEIC ACIDS AND DESIPRAMINE

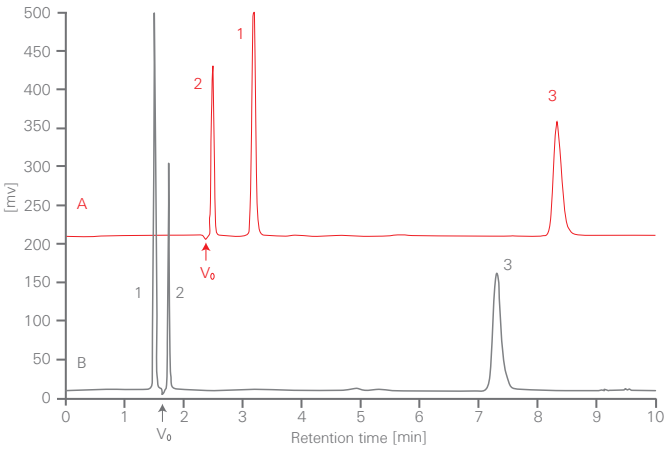

Figure 2

Columns: A: TSKgel NH2-100 DC, 3 µm, 4.6 mm ID x 5 cm L + TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; B: TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; Mobile phase: 50 mmol/L phosphate buffer, pH 7.0 MeOH = 30/70; Flow rate: 1.0 mL/min; Inj. volume: 5 µL; Temp.: 40°C; Detection: UV@210nm; Samples: [1] maleic acid (50 mg/L); [2] p-toluene sulfonic acid (50 mg/L); [3] desipramine (50 mg/L)

pH DEPENDENCE OF RETENTION

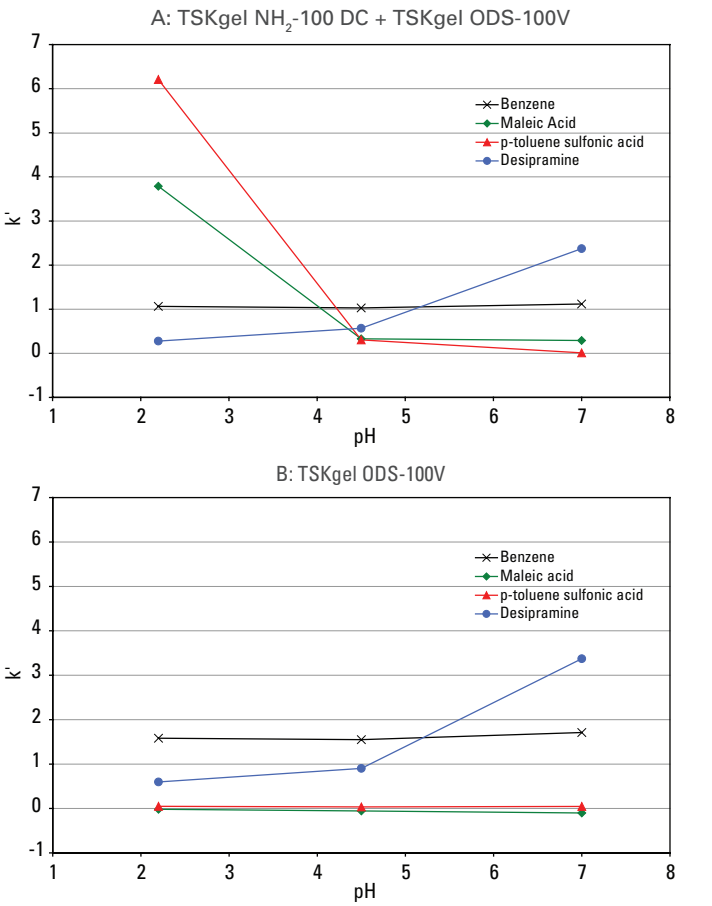

FIGURE 3

Column: A: TSKgel NH2-100 DC, 3 µm, 4.6 mm ID x 5 cm L + TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; B: TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; Mobile phase: H_2O/MeOH/H_3PO_4 = 30/70/0.1; 50 mmol/L NaH_2PO_4, pH 4.5/MeOH = 30/7050mmol/L phosphate buffer, pH 7.0/MeOH = 30/70

Analysis of components in cold medicine

Guaiacol sulfonic acid is an expectorant and is used in some over-the-counter (OTC) cold medicines. As shown in Figure 4, at pH 2.5 guaiacol sulfonic acid is not retained on a reversed phase TSKgel ODS-100V column as it is predominantly present in its dissociated form.

At the same time, other common components in cold medicine are retained under the reversed phase conditions. When a TSKgel NH2-100 DC column is connected ahead of the TSKgel ODS-100V column, guaiacol sulfonate is strongly retained on the polar column while all other components do not interact or interact only minimally with the TSKgel NH2-100 DC column, thus enabling the simultaneous determination of all APIs in a single run.

ANALYSIS OF COLD MEDICINE

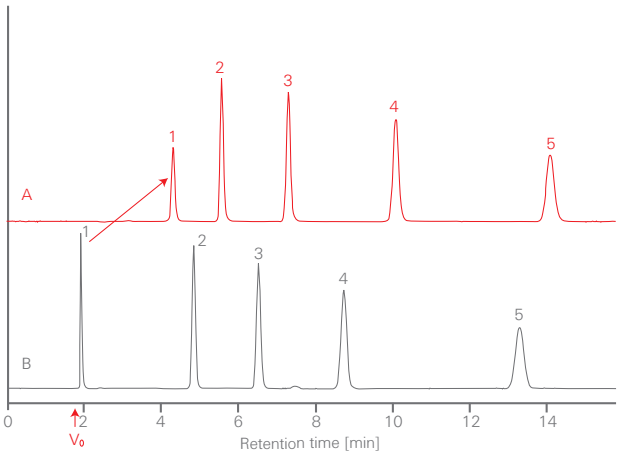

FIGURE 4

Columns: A) TSKgel NH2-100 DC, 3 µm, 4.6 mm ID x 5 cm L + TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; B) TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L; Mobile phase: 50 mmol/L NaH2PO4, pH 2.5/MeOH = 65/35 Flow rate: 1.0 mL/min; Inj. volume: 5 µL; Temp.: 40°C; Detection: UV@280nm Samples: [1] guaiacol sulfonic acid (50 mg/L); [2] anhydrous caffeine (25 mg/L); [3] salicylamide (125 mg/L); [4] aspirin (250 mg/L); [5] ethenzamide (125 mg/L)

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
21999	TSKgel NH2-100 DC, 3 µm	Silica	Stainless steel	4.6 mm ID x 5.0 cm L
21972	Guard Cartridge for 4.6 mm ID columns, 3 pieces/pkg	Silica	Stainless steel	3.2 mm ID x 1.5 cm L
19018	Guard Cartridge Holder for P/N 21972	Silica	Stainless steel	3.2 mm ID x 1.5 cm L