Isocratic Analysis of Tetracyclines

Application Note

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Introduction

Macroporous, rigid polystyrene/DVB based PLRP-S 100Å columns were designed specifically for the analysis of small molecules by reversed phase chromatography. Polymeric columns have several advantages for the pharmaceutical industry - the pH operating range of 0-14 opens up possibilities not achievable using silicas. As interaction is directly with the surface of the polymer, apparent hydrophobicity is greater than for even high load carbon materials; hydrophilic and hydrophobic species can be chromatographed in aqueous eluents more conducive to sample solubility.

Tetracyclines are broad spectrum antibiotics produced by the growth of certain strains of streptomyces. Quantification of tetracycline (TC) degradation products, eg 4-epianhydrotetracycline (EATC), 4-epitetracycline (ETC) and anhydrotetracycline (ATC), is difficult to achieve on silica columns. Interaction with residual silanols causes incomplete resolution. At slightly alkaline pH and elevated temperature, resolution is improved but degradation of the silica stationary phase becomes unacceptable. Using polymeric PLRP-S columns, a simple isocratic method has been developed which permits quantitation of tetracycline and its degradation products.

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Conditions

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 20.5% Acetonitrile, 79.5% Water, 1 mM Sodium edetate, 0.1 M Tetrabutylammonium hydrogen sulphate, 0.25 M Phosphate buffer, pH 9.0
Flow Rate: 1.0 mL/min
Detection: UV, 254 nm

Results and Discussion

The normal degradation products in a sample of crude tetracycline are shown in Figure 1. Although not shown here, an impurity, 2-acetyl-2-decarboxyamidotetracycline (ACTC), never previously resolved from crude TC, has also been detected using a PLRP-S column. Figure 2 is a sample of pure oxytetracycline. Oxytetracycline is closely related to TC but is less likely to form degradation products.

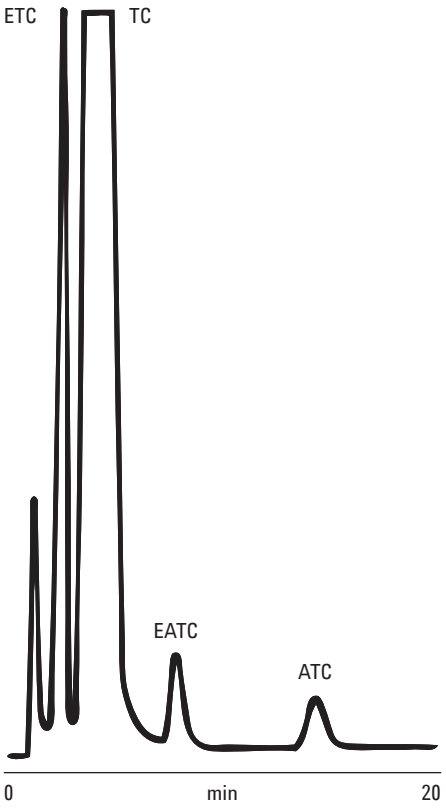

Figure 1. Tetracycline separation revealing the presence of degradation products (courtesy of J Hoogmartens, E Roets & H Vanderhaeghe, Katholieke Universiteit Leuven, Laboratorium voor Farmaceutische Chemie, Leuven, Belgium).

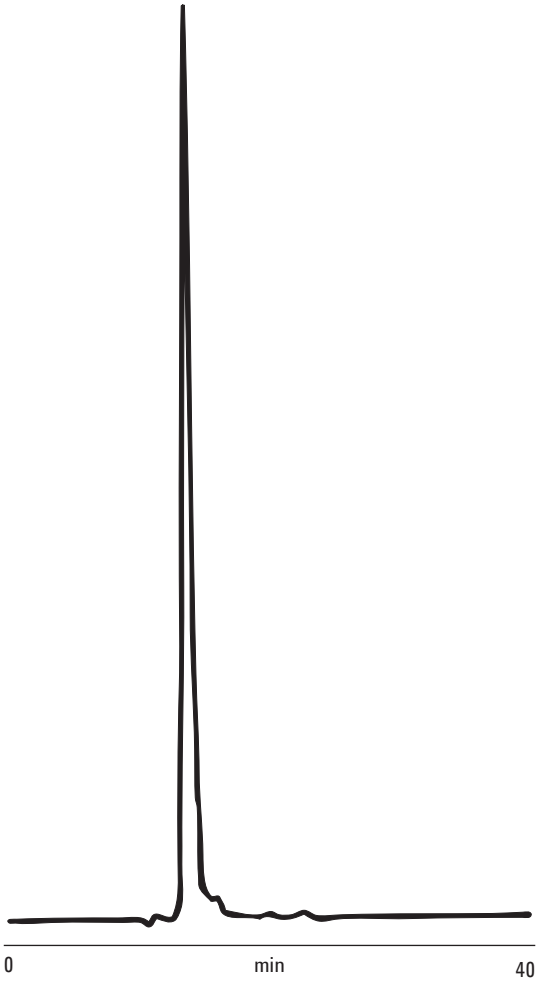

Figure 2. Pure oxytetracycline. Note the absence of degradation products.

Conclusion

The effectiveness of PLRP-S in resolving small molecules is demonstrated in the analysis of tetracyclines. The PLRP-S HPLC phase has outstanding chemical and physical stability. PLRP-S media are inherently hydrophobic and reproducible and do not require a bonded alkyl chain, such as C8 or C18, to confer hydrophobicity. The columns are widely used in separations of synthetic oligomers, synthetic polymer compositional analysis, gigaporous biomolecules, peptides, proteins and oligonucleotides. As a single column, PLRP-S operates across the entire range of HPLC eluents. Because of the stability and physical robustness of PLRP-S, it is possible to switch between organic modifiers such as ACN and tetrahydrofuran, and eluent pH 0 to 14.

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This information is subject to change without notice.

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