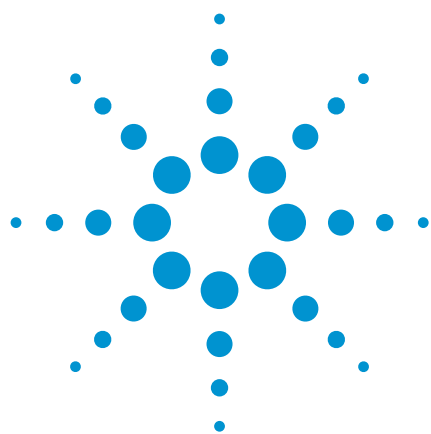




Analysis of Flavonoids

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 - 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.

Flavonoids are receiving articular interest in their role as antioxidants. They are polyphenolic compounds, occuring naturally in a great many vegetables, fruit and beverages. Flavonoids are known to inhibit oxidation of low density lipoproteins in-vitro.

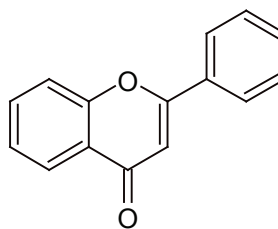


Figure 1. Flavone backbone (2-phenyl-1,4-benzopyrone).

Quantification of flavonoid content of foodstuffs is complicated by the need to hydrolyze the flavonoid glycosides to the parent flavonoid.



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PLRP-S polymeric reversed phase columns are particularly suited to the analysis of these types of compound because they benefit from excellent physical and chemical stability over the complete pH range. These features may be exploited by reducing the number of steps required to prepare a sample for analysis. Any strongly retained contaminants can be effectively removed by rigorous sanitization without detrimentally affecting the column.

Conditions

Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent A: 25 mM KH₂PO₄, pH 2.5
Eluent B: 100 % ACN
Gradient: 30-80 % B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

Results and Discussion

Peak Identification

1. Chrysin 5,7-dihydroxyflavone
2. Apigenin 4',5,7-trihydroxyflavone
3. Kaempferol 3,4',5,7-tetrahydroxyflavone
4. Quercetin 3,3',4',5,7-pentahydroxyflavone
5. Myricetin 3,3',4',5,5',7-hexahydroxyflavone

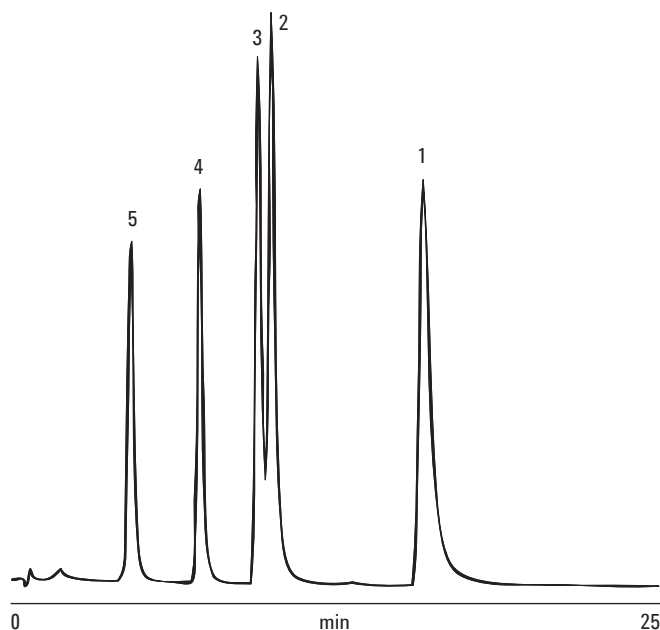


Figure 2. Separation of five flavonoids by PLRP-S.

Conclusion

The effectiveness of PLRP-S in resolving small molecules is demonstrated in the analysis of flavonoids. 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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