

Improved GPC/SEC Analysis of Low Molecular Weight Polyphenols with Agilent OligoPore Columns

Application Note

BioPharma

Author

Graham Cleaver
Agilent Technologies, Inc.

Introduction

Oligopin and Vitaflavan are 2 low molecular weight polyphenols found naturally in pine bark and various other plants. They have antioxidant properties and are said to be beneficial in cardiovascular health, in helping to maintain the health and structure of veins and in improving circulation and as anti-aging medicines. Both have complex structures and a large oligomeric component. Analyzing such materials by GPC/SEC can be used to both identify and quantify oligomers, allowing different sources of the materials to be identified. However, such detailed analysis requires high resolution separations.

This application note compares the analysis of Oligopin and Vitaflavan on a traditional combined set of 4 Agilent PLgel 5 μm individual pore size columns typically chosen for this kind of application, and on a high pore volume Agilent OligoPore column set, focused on low molecular weight separations. OligoPore is one of the Agilent PlusPore range of columns specifically designed for high resolution GPC.

Based on the separation obtained, a recommendation for the column set of choice for low molecular weight separations such as these was made.



Materials and Methods

Samples of Oligopin and Vitaflavan (Purextract, Dax Cedex, France) were dissolved in eluent to produce solutions at concentrations of 0.4% (w/v) and 0.2% (w/v). The Oligopin sample was filtered prior to injection.

Conditions

Columns:

Single pore size set: Agilent PLGel 5 μm 500 \AA (p/n PL1110-6525), Agilent PLGel 5 μm 100 \AA (p/n PL1110-6520), and 2 each Agilent PLGel 5 μm 50 \AA (p/n PL1110-6515) (all 7.5 \times 300 mm)

OligoPore set: 3 each Agilent OligoPore, 7.5 \times 300 mm (p/n PL1113-6520)

Calibrants: Agilent polystyrene narrow standards

Eluent: THF

Injection volume: Single pore size set, 200 μL

OligoPore set, 100 μL

Flow rate: 1.0 mL/min

Flow rate marker: Toluene

Temperature: Ambient

Pressure: Single pore size set, 10.9 MPa

OligoPore set, 8.1 MPa

Sample concentration: Single pore size set, 0.4% (w/v)

OligoPore set, 0.2% (w/v)

Detector: UV

Results and Discussion

UV detection showed excellent sensitivity for the samples and so was used for the analyses. Figure 1 shows the 2 polyphenols on the single pore size set.

The chromatograms of the samples obtained using the OligoPore column set contained considerably more oligomeric detail (Figures 2 and 3) than those obtained on the single pore size column set, showing that increased resolution was obtained.

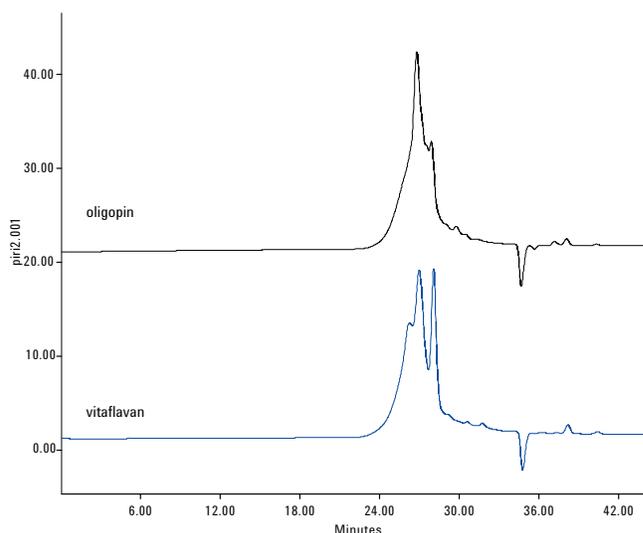


Figure 1. Oligopin and Vitaflavan analyzed on Agilent PLGel 5 μm columns.

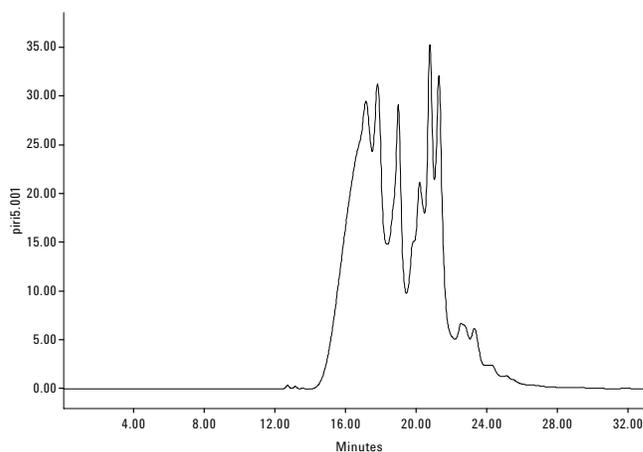


Figure 2. Oligopin analyzed on 3 Agilent OligoPore columns.

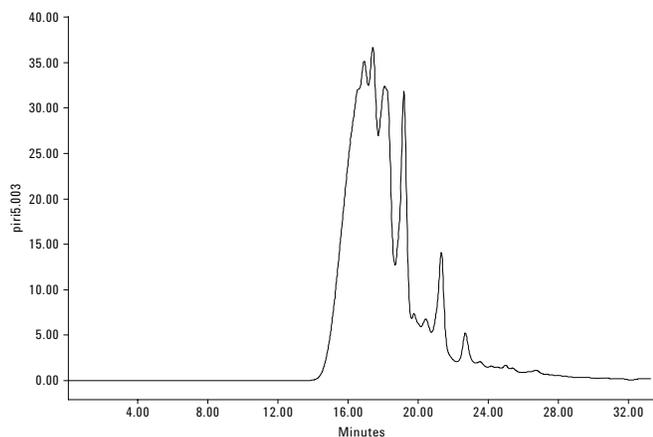


Figure 3. Vitaflavan analyzed on 3 Agilent OligoPore columns.

Conclusions

Clearly, the OligoPore column set resolves the oligomers of the Oligopin and Vitaflavan to a greater degree than the individual pore size column set. This is because the OligoPore set focuses in on the low molecular weight region (resolving up to 4,000 g/mol polystyrene in tetrahydrofuran) and also because the structure of the media in the Agilent PlusPore columns is designed to give increased pore volume over traditional GPC/SEC columns, resulting in higher resolution. As this application note clearly demonstrates, OligoPore columns are the columns of choice for low molecular weight oligomer separations.

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