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Improved Performance and Cycle Time for the Analysis of Lipid Soluble Antioxidants Using CORTECS T3 Columns on the ACQUITY Arc System

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APPLICATION BENEFITS

- Demonstrate the transfer of a gradient method from HPLC to UHPLC using an ACQUITY® Arc™ System
- Improved resolution, reduced sample run time, and solvent consumption using CORTECS® T3 Columns

WATERS SOLUTIONS

CORTECS T3 Columns

Atlantis® T3 Columns

Alliance® HPLC System

ACQUITY Arc System

Empower® 3 CDS

KEYWORDS

CORTECS T3, Alliance HPLC, ACQUITY Arc, Atlantis T3, Empower 3, antioxidants

INTRODUCTION

Processed foods can have a long shelf life. In part, this is due to antioxidants added to slow the degradation of fatty acids from oxidative free radical chain reactions. The analysis for such food additives is important to determine if the additives used are those approved by food regulatory agencies.

One example for the analysis of lipid soluble antioxidants was previously performed using a 5 µm Atlantis T3 HPLC Column on an Alliance HPLC[1] System. While the separation was adequate, it can be improved using new column technology and LC instrumentation. This application note transfers the original HPLC method to a UHPLC method using an ACQUITY Arc System and a 2.7 µm CORTECS T3 Column. The result is better resolution with reduced sample run time and solvent consumption.

EXPERIMENTAL

LC method conditions

LC systems:	Alliance HPLC ACQUITY Arc
Columns:	Atlantis T3, 5 µm, 4.6 x 150 mm (p/n 186003747) CORTECS T3, 2.7 µm, 3.0 x 75 mm (p/n 186008488)
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	Methanol
Mobile phase D:	2% Formic acid in water (blended to 0.1% formic acid)
Gradient (Atlantis T3):	Starting mobile phase conditions 60:17.5:17.5:5 A:B:C:D, changing linearly to 40:27.5:27.5:5 over four minutes. Change linearly to 14:40:41:5 in two minutes, hold for nine minutes, and return to starting conditions. Hold at starting conditions for 4 minutes. Total run time 20 minutes.
Gradient (CORTECS T3):	Starting mobile phase conditions 60:17.5:17.5:5 A:B:C:D, changing linearly to 40:27.5:27.5:5 over two minutes. Change linearly to 14:40:41:5 in one minute, hold for four and half minutes, and return to starting conditions. Hold at starting conditions for 2 minutes. Total run time 10 minutes.
Gradient SmartStart:	Gradient starts 133 µL before injection.
Flow rate:	2.0 mL/min (Atlantis T3) 0.85 mL/min (CORTECS T3)
Column temp.:	30 °C
Detection (UV):	280 nm
Injection volume:	10.0 µL (Atlantis T3) 2.1 µL (CORTECS T3)
Data management:	Empower 3 CDS
Sample Preparation:	A sample mix containing the following compounds at the described concentrations was created using 65:17.5:17.5 water:methanol:acetonitrile. Compound structures are shown in Figure 1.

1. Propyl Gallate (0.05 mg/mL)
2. 2,4,5-Trihydroxybutyrophenone (0.05 mg/mL)
3. Tert-butylhydroquinone (0.1 mg/mL
4. Butylated hydroxyanisole (0.1 mg/mL)
5. 2,6-Di-tert-butyl-4-hydroxymethylphenol (0.1 mg/mL
6. Octyl Gallate (0.05 mg/mL)

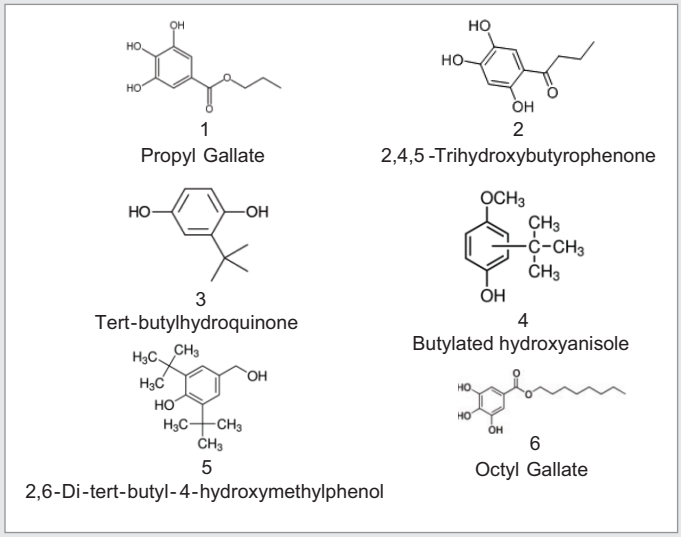

Figure 1. Compound structures of lipid soluble antioxidants.

RESULTS AND DISCUSSION

The transfer of a gradient method has two steps. First, the gradient profile must be scaled to account for changes in column geometry and particle size between the original method and the transferred method. This is most easily done with the Waters Column Calculator.[2] Second, the difference in the dwell volume (gradient delay volume) between LC systems must be accounted for. Dwell volume, the volume between the pump and the column, is easily measured.[3] With Empower, the analyst can then use the Gradient SmartStart feature to adjust for the difference in dwell volume.[4]

The ACQUITY Arc System has multi-flow path technology, allowing it to operate similarly to both an HPLC and a UHPLC system with only a minor change to an instrument method. The ACQUITY Arc solvent manager has two fluidic flow paths, which mimic the flow paths of HPLC (path 1) and UHPLC (path 2) instruments. By selecting the appropriate flow path in the instrument method, the ACQUITY Arc System can operate similarly to either type of instrument. Table 1 shows the dwell volume of the Alliance and the ACQUITY Arc flow path 1, as measured by the authors. These dwell volumes were determined by performing a gradient separation using a UV absorbing compound in mobile phase B, i.e. acetone, with a union in place of the column. After the dwell volumes were measured, the method used on the ACQUITY Arc System had to be altered to account for the difference. A Gradient SmartStart setting of "Gradient starts 133 uL before injection" was therefore used to effectively reduce the affect of system dwell on the separation.

System	Dwell volume (mL)
Alliance HPLC	1.080
ACQUITY Arc (Path 1)	1.213

Table 1. Dwell volume measurements of the systems used.

Figure 2 shows the original separation of lipid soluble antioxidants using an Atlantis T3, 4.6 x 150 mm, 5 µm Column (p/n 186003747) on an Alliance HPLC System. A good separation was achieved for the six major components, with three impurities (two of which co-elute, see inset) between peaks 3 and 4. The overall run time for this separation, including column re-equilibration, is 20 minutes, during which time 40 mL of mobile phase is consumed.

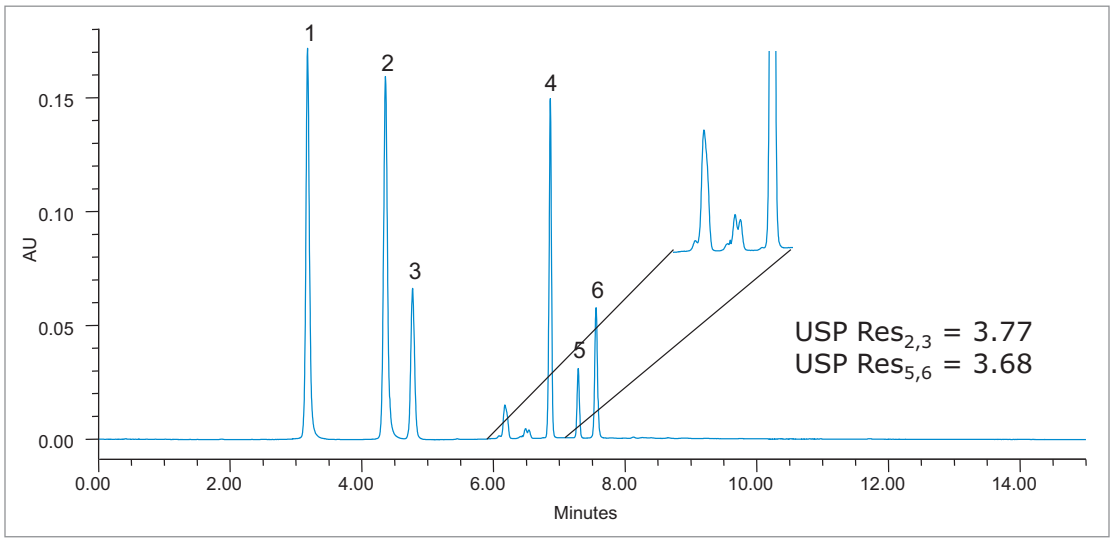

Figure 1. Separation of six lipid soluble antioxidants using an Atlantis T3, 4.6 x 150 mm, 5 µm Column on an Alliance HPLC System.

The transferred method was then run on the ACQUITY Arc System with the CORTECS T3, 3.0 x 75 mm, 2.7 µm Column (p/n 186008488). Figure 3 shows the chromatogram of the antioxidants. The resolutions of the main components are improved as are the separations for the impurities (where two peaks no longer co-elute, see inset). The sample run time is reduced by 50%, from 20 minutes to 10 minutes, with a nearly 5X reduction of mobile phase consumption.

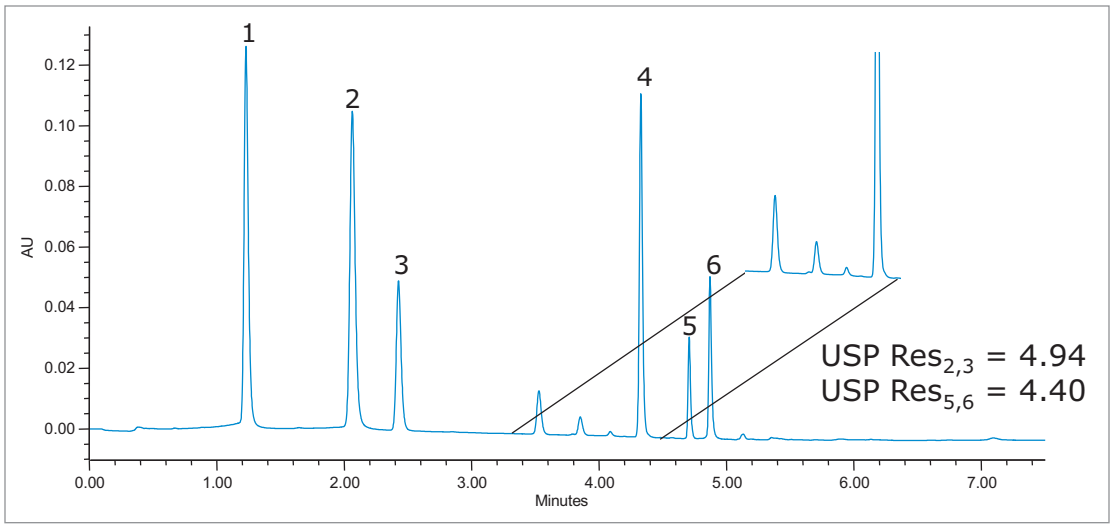

Figure 2. Separation of six lipid soluble antioxidants using a CORTECS T3, 3.0 x 75 mm, 2.7 µm Column on an ACQUITY Arc System.

CONCLUSIONS

Traditional HPLC methods often achieve acceptable chromatography, but at the cost of lengthy run times and high solvent consumption. By taking these older methods and transferring them to newer columns and LC instrumentation, the separation can often be improved while using less analysis time and solvent. This translates into a higher quality result with savings of both time and money. A separation of lipid soluble antioxidants was transferred from an HPLC system using a 5 µm Atlantis T3 Column to a UHPLC system using a 2.7 µm CORTECS T3 Column, resulting in improved peak resolutions, a 50% reduction in sample run time, and nearly a 5X reduction in solvent consumption.

References

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