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APPLICATION NOTE 21731

Sensitive UHPLC-UV determination of anthocyanins using innovative active eluent pre-heating technology

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Keywords

Environment and food safety, food and beverage analysis, UHPLC, DAD, UV, QA/QC, anthocyanins, antioxidants, nutraceuticals, delphinidin 3-glucoside, delphinidin 3,5-diglucoside, cyanadin 3,5-diglucoside, cyanidin 3-glucoside, pelargonidin 3-glucoside, pelargonidin 3,5-diglucoside, UltiMate 3000, Vanquish Flex, Acclaim 120 C18 RSLC 2.2 µm UHPLC column, columns pre-heating, SmartInject, reproducibility

Application benefits

- The Thermo Scientific™ Acclaim™ 120 C18, RSLC column proved capable of analyzing closely related anthocyanins.
- The method transfer between the Thermo Scientific™ UltiMate™ 3000 UHPLC system and the Thermo Scientific™ Vanquish™ Flex UHPLC system was successfully achieved with no changes to the chromatographic method avoiding re-validation time and costs.
- Active eluent pre-heating significantly improved the performance of the Vanquish Flex UHPLC system in terms of retention time and peak width reproducibility, giving greater method reproducibility while leaving retention times directly comparable.

Goal

To successfully demonstrate improved reproducibility of retention time, peak width at 50% height, and peak area during the method transfer of an anthocyanin separation from an UltiMate 3000 RS system to a Vanquish Flex system. To show the capability of the Acclaim 120 C18, RSLC, 2.2 µm column for the analysis of six anthocyanins commonly found in fruit products.

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Introduction

The inter- and intra-laboratory transfer of methods between different instruments is common over the lifetime of an HPLC method. The seamless transfer of methods is vital to ensure re-validation costs remain minimal. In this analysis, we demonstrate the transfer of a method from an UltiMate 3000 UHPLC system to a Vanquish Flex UHPLC system.

The Vanquish Flex UHPLC system provides the user the flexibility expected from a quaternary, low pressure mixing pump. However, the additional benefits of improved, proprietary autosampler and pump technology result in unrivalled retention time precision, providing the user with greater data confidence and more freedom in method development and application transfer.

Another important new feature of the Vanquish Flex UHPLC system is active pre-heating of the mobile phase, which ensures perfect temperature matching of the column and the entering solvent and thus avoids performance loss by temperature effects observed at UHPLC conditions. Utilization of the active eluent pre-heater technology greatly improved the relative standard deviation with respect to analyte retention times and peak width at 50% height. This resulted in greater method precision and confidence in results.

The Acclaim RSLC 120 C18 2.2 µm column is a high performance, reversed-phase column with strong hydrophobic retention. These columns have been designed with high surface area coverage and a low metal content, which results in high column efficiencies providing high resolution separations.

Anthocyanins are a sub-class of the flavonoids and are responsible for many of the brilliant red, orange, and blue colors of fruits and flowers. Due to their strong antioxidant properties, they are of considerable interest to the scientific community and the consumer market. This application demonstrates the separation of six of the most common anthocyanins by UHPLC with UV/Vis detection.

Experimental

Consumables and apparatus

- Acclaim 120 C18 RSLC, 2.2 µm UHPLC column, 150 mm × 2.1 mm (P/N 071399)
- MS grade 18 MΩ water from Thermo Scientific™ Barnstead™ Smart2Pure™ Water Purification system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Formic Acid, Optima™ LC/MS Grade, Fisher Chemical (P/N A117-50)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)

All standards were purchased from a reputable supplier.

Instrumentation

Analyses were performed using an UltiMate 3000 UHPLC system consisting of:

- LPG-3400RS Pump (P/N 5040.0036)
- WPS-3000TRS Autosampler (P/N 5840.0020)
- TCC-3000RS Column Oven (P/N 5730.0000)
- DAD-3000RS Diode Array Detector (P/N 5082.0020)
- Analytical Flow Cell for DAD-3000, 13 µL, 10 mm (P/N 6082.0100)

Analyses were also performed using a Thermo Scientific Vanquish Flex UHPLC system consisting of:

- Quaternary Pump F (P/N VF-P20-A)
- System Base Vanquish Flex (P/N VF-S01-A)
- Split Sampler FT (P/N VF-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active Pre-heater (P/N 6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- Thermo Scientific™ LightPipe™ Flow Cell, 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ vial identification system (P/N 60180-VT100)

Software

Thermo Scientific™ Chromeleon™ Chromatography data system 7.2 SR4

Sample preparation

Solutions of the six compounds shown in Table 1 were prepared by dissolving a known amount in mobile phase A to produce 1 mg/mL primary solutions. A mixed spiking solution was used to assess both systems and was prepared in mobile phase A at the concentrations identified in Table 1.

Table 1. Compound identification (in retention time order) and the concentration of each in the mixed standard.

Compound	Abbreviation	Concentration (µg/mL)
Delphinidin-3,5-diglucoside	Dp3, 5	10
Cyanadin-3,5-diglucoside	Cy3, 5	10
Delphinidin-3-glucoside	Dp3	10
Pelargonidin-3,5-diglucoside	Pg3, 5	10
Cyanidin-3-glucoside	Cy3	10
Pelargonidin-3-glucoside	Pg3	10

Vial labeling was supported by the Thermo Scientific Virtuoso Vial Identification System.

UHPLC conditions

HPLC column: Acclaim RSLC 120 C18, 2.2 µm UHPLC column, 150 mm × 2.1 mm

Mobile phase: A: Acetonitrile/formic acid/water 9:10:81 (v/v/v)
B: Acetonitrile/formic acid/water 36:10:54 (v/v/v)

Gradient conditions:

Time (min)	A %	B %
0.0	100.0	0.0
0.9	100.0	0.0
8.0	71.5	28.5
10.0	71.5	28.5
10.1	100.0	0.0

10 minutes post equilibration (100% A)

Flow rate: 0.475 mL/min

Column temperature: 30°C (still air mode for Vanquish Flex UHPLC system; forced air mode for UltiMate 3000 UHPLC system)

Injection details: 0.5 µL

UV detection: 540 nm

Backpressure: Approximately 700 bar maximum for both systems

Gradient mixer: 350 µL static + 50 µL capillary

Results and discussion

Full resolution of six anthocyanins was achieved within four minutes on both the UltiMate 3000 system and the Vanquish Flex system using the Acclaim 120 C18 RSLC, 2.2 µm UHPLC column (Figure 1).

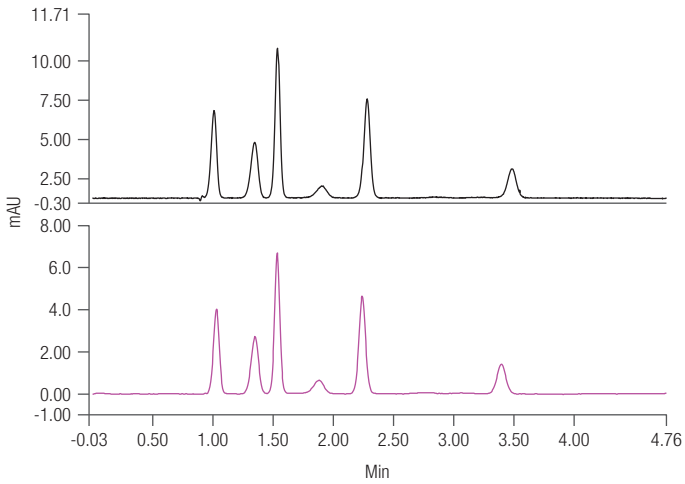

Figure 1. Chromatogram showing the separation of six anthocyanins on the Vanquish Flex system (top trace) and the UltiMate 3000 system (bottom trace).

In the current configuration both systems are equipped with quaternary, low pressure mixing pumps with very similar dwell volumes. The average peak retention time of 25 injections per system was utilized to assess system reproducibility. With respect to retention time, both systems were comparable and therefore no adjustment to system dwell volume was required (Figure 2).

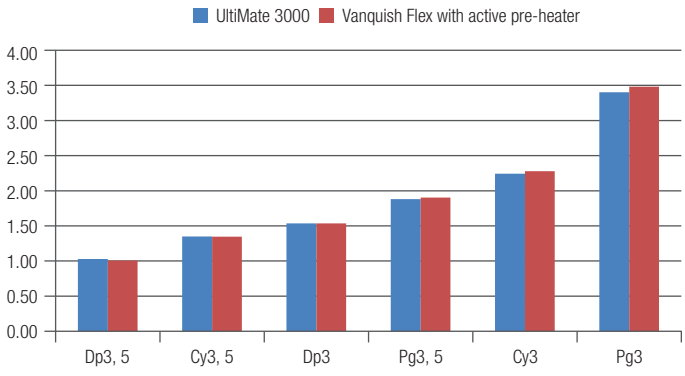

Figure 2. Comparison of the peak retention times (n=25) for six anthocyanins on the UltiMate 3000 system and the Vanquish Flex system with active pre-heater.

An additional feature of the Vanquish Flex UHPLC system compared to the UltiMate 3000 UHPLC system is the active eluent pre-heater. This study was used to assess the effectiveness of the active pre-heater on method reproducibility for the separation of the anthocyanins. When the active pre-heater was not in use, the performance of the system was assessed under otherwise identical conditions.

The effects were explored by running the anthocyanin method with and without the eluent pre-heater enabled in the instrument method. The relative standard deviation (%RSD) was utilized to assess system reproducibility by evaluating 25 injections with respect to retention time and peak width at 50% height (Figures 3 and 4).

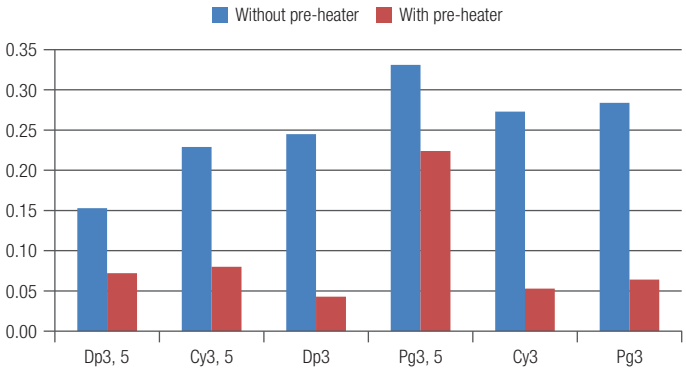

Figure 3. %RSD of the retention times (n=25) for six anthocyanins on the Vanquish Flex system, with and without eluent pre-heater enabled.

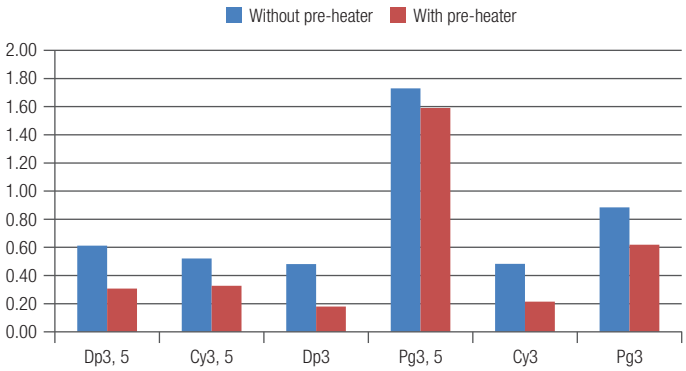

Figure 4. %RSD of the peak width at 50% height (n=25) for six anthocyanins on the Vanquish Flex system, with and without eluent pre-heater enabled.

In this application note, it was found that the use of active eluent pre-heating significantly improved the %RSD values for both retention time and peak width at 50% height. UHPLC analysis utilizing the active pre-heating feature of the Vanquish Flex system therefore improved reproducibility and reduced variations in analyte retention time. By using the active pre-heating technology with still air column thermostatting mode, minimal thermal mismatch by viscous heating under UHPLC conditions was achieved, translating into the optimal UHPLC efficiency and resolution. The retention times for all components with and without active heating were also found to be comparable.

A comparison of the %RSD values for retention time, peak area, and peak width at 50% height was made for the Vanquish Flex system with active pre-heating initialized and the UltiMate 3000 system (Figures 5-7). The results show that use of the active pre-heater on the Vanquish Flex system improved retention time and peak area precision by an overall reduction in %RSD values for this application. The %RSD for the Pg3, 5 retention time was higher with the Vanquish Flex system; however, this was due to variability in peak apex identification due to poor peak shape, which affected the consistency of automatic integration for this peak.

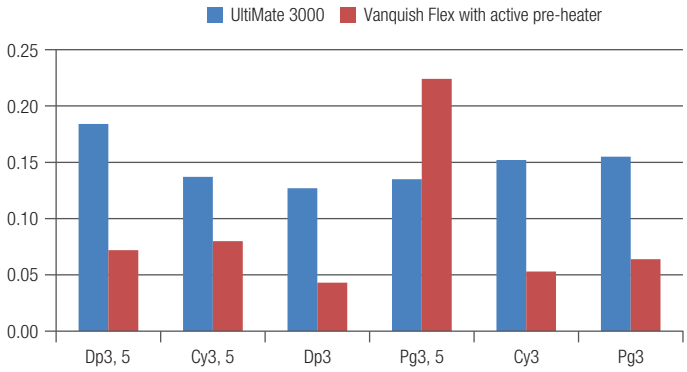

Figure 5. %RSD of retention time for n=25 injections containing six anthocyanins on the Vanquish Flex system with active eluent pre-heating versus the UltiMate 3000 system.

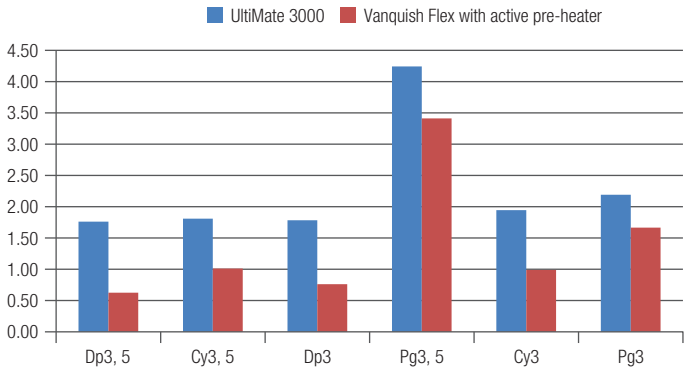

Figure 6. %RSD of peak area for n=25 injections containing six anthocyanins on the Vanquish Flex system with active eluent pre-heating versus the UltiMate 3000 system.

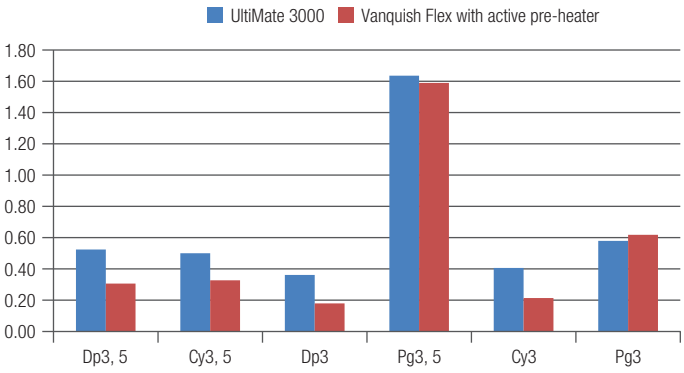

Figure 7. %RSD of peak width at 50% height for n=25 injections containing six anthocyanins on the Vanquish Flex system with active eluent pre-heating versus the UltiMate 3000 system.

The Vanquish Flex autosampler utilizes the proprietary Thermo Scientific SmartInject technology to reduce flow inconsistencies during injection and pressure shocks to the HPLC column. The improvements made to the pump and autosampler technology of the Vanquish Flex UHPLC system differentiate it in terms of reduced %RSD in retention time, peak width, and peak area.

Conclusions

This application demonstrates the following:

- The Acclaim 120 C18 RSLC column proved capable for the analysis of anthocyanins.
- The method transfer between the UltiMate 3000 UHPLC system and the Vanquish Flex UHPLC system was successfully achieved with no changes to the chromatographic method, avoiding re-validation time and costs.
- Active eluent pre-heating significantly improved the performance of the Vanquish Flex UHPLC system in terms of retention time and peak width reproducibility, giving greater method reproducibility while leaving retention times directly comparable.

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