Optimized reproducibility using a state of the art UHPLC system for the analysis of catechins found in tea

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Keywords

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Application benefits

- The Thermo Scientific™ Acclaim™ RSLC 120 C18 column proved capable for the analysis of catechins.

- The method transfer between a Thermo Scientific™ UltiMate™ 3000 RS system and a Thermo Scientific™ Vanquish™ Flex UHPLC system was successfully achieved with no changes to the chromatographic method.

- The performance of the Vanquish Flex UHPLC system in terms of reproducibility (%RSD) was significantly superior to the UltiMate 3000 system by at least 50% for most samples, providing the user with reliability and confidence in chromatographic separations.

Goal

To successfully demonstrate improved reproducibility for retention time, peak width at 50% height, and peak area during the method transfer of a catechins-based separation from an UltiMate 3000 RS system to a Vanquish Flex system. To show the capability of the Acclaim RSLC 120 C18 column for the analysis of catechins commonly found in food and beverages.

Introduction

The inter- and intra-laboratory transfer of methods between different instruments is common over the lifetime of an HPLC method.

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The seamless transfer of methods is vital to ensure re-validation costs remain minimal. In this analysis, the transfer of a method from a Thermo Scientific UltiMate 3000 UHPLC system to a Thermo Scientific Vanquish Flex UHPLC system is demonstrated.

The Vanquish Flex UHPLC system provides the user the flexibility expected from a quaternary, low pressure mixing pump. However, the additional benefits of improved 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.

The Acclaim 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.

Catechins are antioxidants found in several food and beverages including tea and are thought to provide health benefits to the consumer. Dependent on the extent of processing the tea undergoes, the catechin content can vary significantly. The exposure to heat and oxidative atmospheres can significantly reduce the amount of these labile compounds. This method demonstrates the separation of seven commonly found catechins by UHPLC with UV detection.

Experimental

Consumables and apparatus
- Acclaim 120 C18 RSLC, 2.2 μm HPLC column, 150 mm x 2.1 mm (P/N 071399)

- LC/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)

- Fisher Scientific™ Trifluoroacetic acid (TFA) (P/N A116-50)

- 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-3000RS Autosampler (P/N 5840.0010)

- 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 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)

- LightPipe™ Flow Cell, 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT-100)

Software
Thermo Scientific™ Chromeleon™ Chromatography Data System 7.2 SR4

Sample preparation
Solutions of the seven 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.

Vial labelling was supported by the Virtuoso Vial Identification System.

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

Compound	Concentration (µg/mL)
Gallocatechin	15
Epigallocatechin	15
Catechin	15
Epicatechin	15
Epigallocatechin gallate	15
Gallocatechin gallate	15
Epicatechin gallate	15

UHPLC conditions

HPLC column:	Acclaim RSLC 120 C18, 2.2 µm, 150 mm × 2.1 mm
Mobile phase:	A: 0.1 % TFA, 5 % acetonitrile in water (v/v)
	B: 0.1 % TFA in acetonitrile (v/v)

Gradient conditions:

Time (min)	A %	B %
0.0	100.0	0.0
1.2	100.0	0.0
15.5	71.5	28.5
17.0	71.5	28.5
17.0	100.0	0.0
25.0	100.0	0.0

Flow rate:	0.45 mL/min
Column temperature:	25 °C (still air mode)
Injection details:	1 µL
UV detection:	280 nm
Backpressure:	Approximately 600 bar maximum for both systems
Gradient mixer:	350 µL static + 50 µL capillary

Results and discussion

Full resolution of all seven catechins was achieved within thirteen minutes on both the UltiMate 3000 system and the Vanquish Flex system, using an Acclaim 120 C18 column and the same chromatographic method (Figure 1).
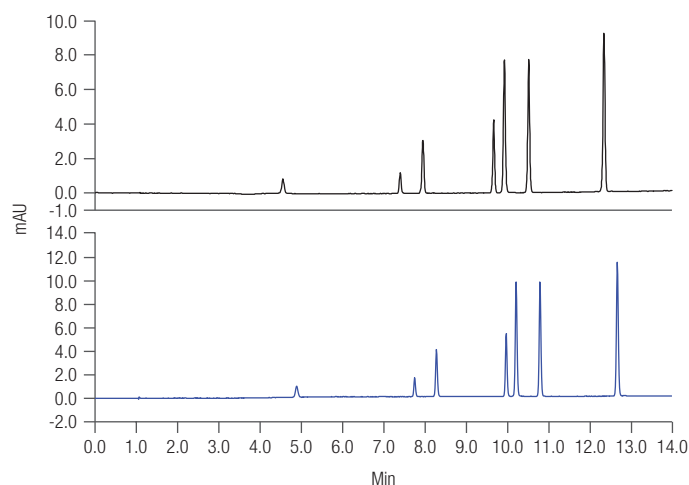

Figure 1. Chromatograms showing the separation of seven catechins on both the UltiMate 3000 system (top trace) and the Vanquish Flex system (bottom trace).

In the current configuration, both systems are equipped with quaternary, low pressure mixing pumps with very similar dwell volumes. The retention times for both systems are comparable and therefore no adjustment to system dwell volume was required.

The relative standard deviation (%RSD) was utilized to assess system reproducibility by evaluating 15 injections for each system with respect to peak width at half height, retention time, and peak area. The results for reproducibility show that the Vanquish Flex system has significantly improved the performance by at least 50% for most samples compared the UltiMate 3000 system (Figures 2-4).

The Vanquish Flex autosampler utilizes the proprietary Thermo Scientific™ SmartInject technology to reduce flow inconsistencies during injection and pressure shocks to the HPLC column, ultimately extending column lifetime. The optimized autosampler and pump technology of the Vanquish Flex UHPLC system compared to the UltiMate 3000 system translates into excellent %RSD in retention time, peak width, and peak area.
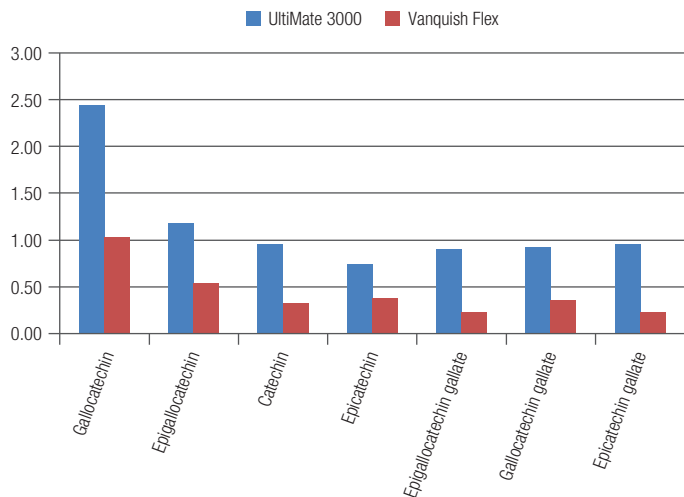
Figure 2. %RSD of the peak widths at half height (n=15) for seven catechins on the UltiMate 3000 system and Vanquish Flex system.
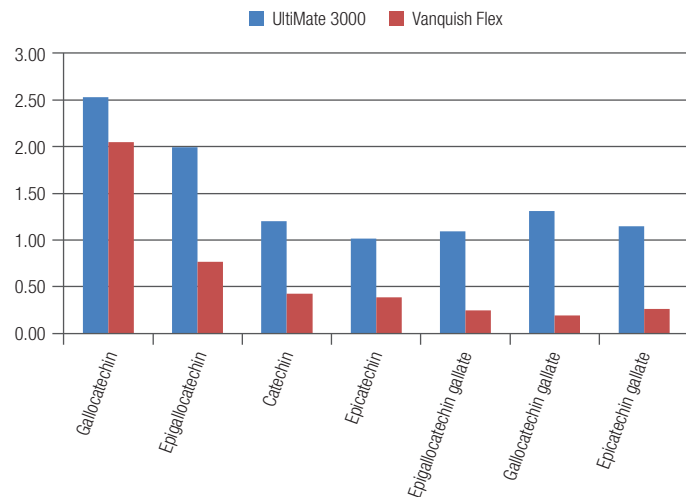

Figure 4. %RSD of the peak area (n=15) for seven catechins on the UltiMate 3000 system and Vanquish Flex system.
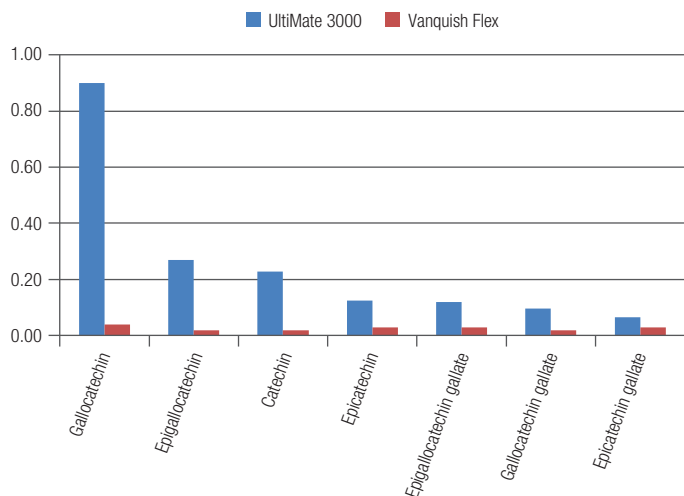

Figure 3. %RSD of the retention times (n=15) for seven catechins on the UltiMate 3000 system and Vanquish Flex system.

Conclusions

This application note demonstrates the following:

- The Acclaim RSLC 120 C18 column proved capable for the analysis of catechins.

- The method transfer between the UltiMate 3000 system and Vanquish Flex UHPLC system was successfully achieved with no changes to the chromatographic method.

- The performance of the Vanquish Flex UHPLC system with regards to reproducibility (%RSD) was significantly superior to the UltiMate 3000 system by at least 50% for most samples, providing the user with reliability and confidence in chromatographic separations.

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