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APPLICATION NOTE 21734

Sensitive HILIC UHPLC-UV determination of steviol glycoside natural sweeteners

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Keywords

Environment, food safety, food and beverage analysis, UHPLC, HILIC, DAD Flow Cell LightPipe, UV, QA/QC, natural sweeteners, steviol glycosides, dulcoside A, stevioside, rebaudioside C, steviobioside, rebaudioside A, rebaudioside B, UltiMate 3000, Vanquish Flex Quaternary, Acclaim Trinity P1

Application benefits

- Sensitivity of steviol glycoside determination by HILIC UHPLC was found to increase by up to 40% by utilizing the newly developed Thermo Scientific™ LightPipe™ Diode Array Detector (DAD) Flow Cell technology.
- Compared to the Thermo Scientific™ UltiMate™ 3000 system, the %RSD of peak width at half height was found to be up to 80% lower in the case of dulcoside A using the Thermo Scientific™ Vanquish™ Flex system's improved proprietary pump technology.
- The unique chemistry of the Thermo Scientific™ Acclaim™ Trinity P1 columns allows for the HILIC separation of highly polar and structurally similar steviol glycosides, which are difficult to separate on traditional reversed-phase columns.

Goal

To demonstrate the seamless method transfer from an UltiMate 3000 RS system to a Vanquish Flex system using an Acclaim Trinity P1, 3 µm column with unique chemistry and functionality for the HILIC analysis of steviol glycosides. To demonstrate the increased reproducibility and sensitivity of the Vanquish Flex system obtained by improved pump features and newly developed DAD Flow Cell LightPipe technology.

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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 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 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 Trinity P1 column is a novel, high-efficiency, silica-based column designed for maximum flexibility in method development through unique chemistry and functionality. The inner-pore surface is modified with an organic layer that provides both reversed-phase and anion-exchange properties, while the outer-pore surface is modified with cation-exchange functionality. This method uses the column in hydrophilic interaction liquid chromatography (HILIC) mode, not only for the separation of six steviol glycosides, but with the additional advantage of volatile mobile phases that can be used for charged aerosol or mass spectrometric detection (CAD or MS) giving further flexibility to the method.

Steviol glycosides are natural sweeteners widely used for beverages, which are significantly sweeter than sugar and do not have a caloric value. The chromatographic separation, however, can be challenging as they are structurally highly related (Figure 1). Due to their strong polarity, analysis by reversed-phase HPLC is particularly challenging. This method demonstrates the full resolution of six steviol glycosides using a HILIC based method.

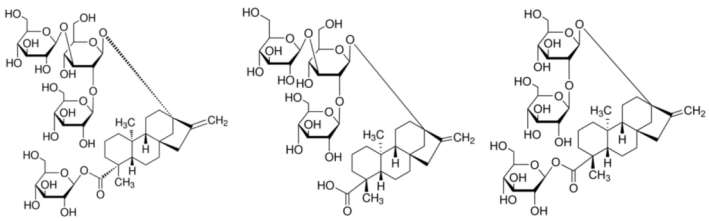

Figure 1. Chemical structure of rebaudioside A, rebaudioside B and stevioside.

Experimental

Consumables and apparatus

- Acclaim Trinity P1, 3 µm HPLC column, 100 mm × 2.1 mm (P/N 071389)
- Acclaim Trinity P1, guard, 3 µm, 10 mm × 2.1 mm (P/N 071391)
- Acclaim SST Guard cartridge holder (P/N 069580)
- 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™ Formic acid (P/N 10559570)
- Fisher Scientific™ Ammonium formate (P/N 10131850)
- 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, 2 µL 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 six compounds were prepared by dissolving a known amount in mobile phase to produce 2 mg/mL or 1 mg/mL (rebaudioside C) primary solutions. A mixed spiking solution was used to assess both systems and was prepared in mobile phase at a concentration of 0.19 mg/mL per compound.

Vial labelling was supported by the Virtuoso Vial Identification System.

UHPLC conditions

HPLC columns:	Acclaim Trinity P1, 3 µm HPLC column, 100 mm × 2.1 mm
	Acclaim Trinity P1, guard, 3 µm, 10 mm × 2.1 mm
Mobile phase:	10 mM ammonium formate pH 3.0 / acetonitrile (19:81 v/v)
Flow rate:	0.30 mL/min
Column temperature:	40 °C
Pre-heater temperature:	40 °C
Thermostatting mode:	ForcedAir
Injection volume	5 µL
UV detection:	210 nm
Mixer:	350 µL static + 50 µL capillary

Results and discussion

Relative separation of all six sweeteners was achieved within 10 minutes on both the UltiMate 3000 system and the Vanquish Flex system using an Acclaim Trinity P1 column and the same chromatographic method (Figure 2).

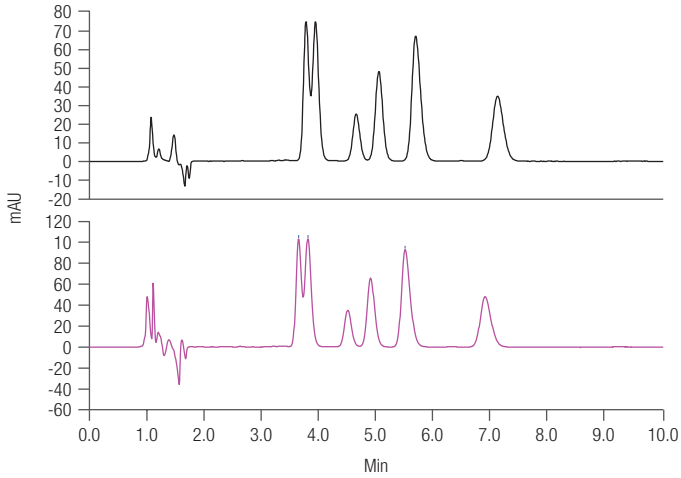

Figure 2. Chromatograms showing the separation of six sweeteners on both the UltiMate 3000 system (top trace) and the Vanquish Flex system (bottom trace).

In the current configuration, both systems are equipped with quaternary, low pressure mixing pumps with very similar dwell volumes. The average component retention time of n=6 injections for the two systems were found to be comparable and therefore no adjustment to system dwell volume was required (Figures 2 and 3).

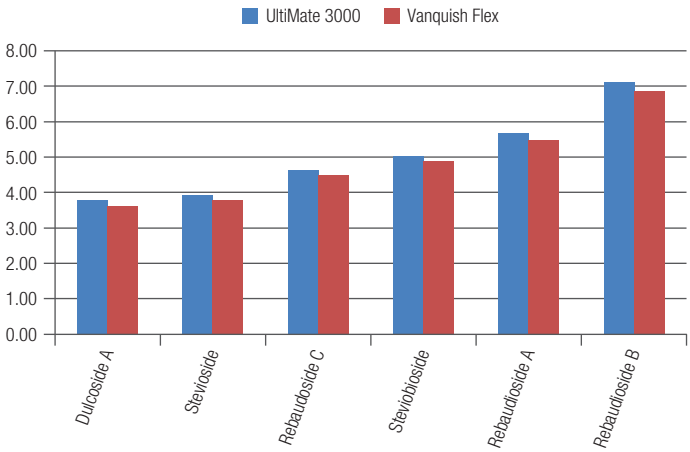

Figure 3. Average retention times (n=6) for six sweeteners on the UltiMate 3000 system and Vanquish Flex system.

The UltiMate 3000 system and Vanquish Flex system both exhibit comparable peak widths at 50% height, demonstrating similar chromatographic efficiencies of the two. In both cases the majority of peak widths at 50% height are less than 0.2 minutes (Figure 4).

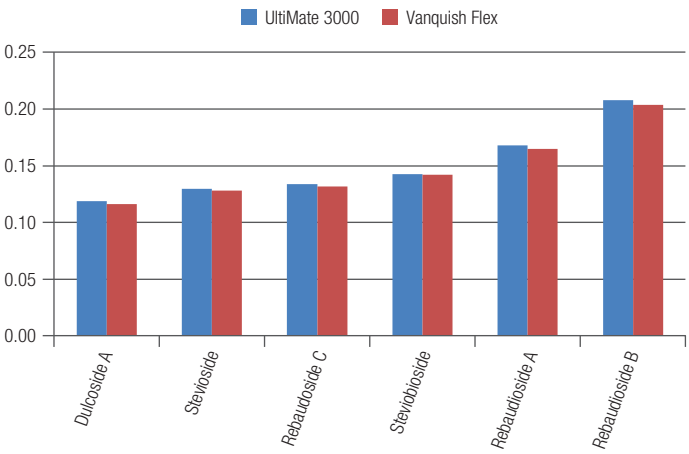

Figure 4. Average peak widths (min) at half height (n=6) for six sweeteners on the UltiMate 3000 system and Vanquish Flex system.

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 adopted to the Vanquish Flex pump technology differentiate it in terms of precision. When comparing the %RSD of peak widths at 50% height for six injections it is apparent that the Vanquish Flex system is superior to the UltiMate 3000 system, leading to greater confidence in analytical results (Figure 5).

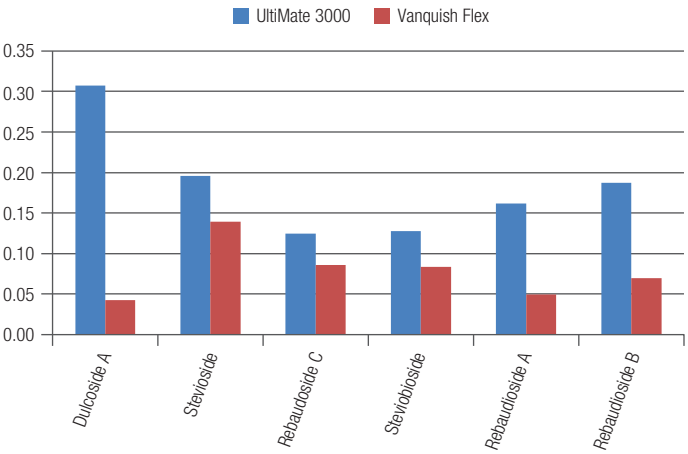

Figure 5. %RSD of peak widths at half height (n=6) for six sweeteners on the UltiMate 3000 system and Vanquish Flex system.

The Vanquish Flex system also exhibits the new LightPipe flow cell technology designed for the Diode Array Detector, which provides the user with increased sensitivity for analytes due to the very long light path and minimum peak dispersion due to small internal volume. These features coupled with total internal reflection maximize the incident UV light intensity, thus increasing the sensitivity for low level analytes. This can be observed when comparing peak heights and peak areas for the analysis of steviol glycosides. Here the Vanquish Flex system was found to increase peak heights and areas by up to 40% compared to the UltiMate 3000 system when analyzing the same standard at the same time (Figures 6 and 7).

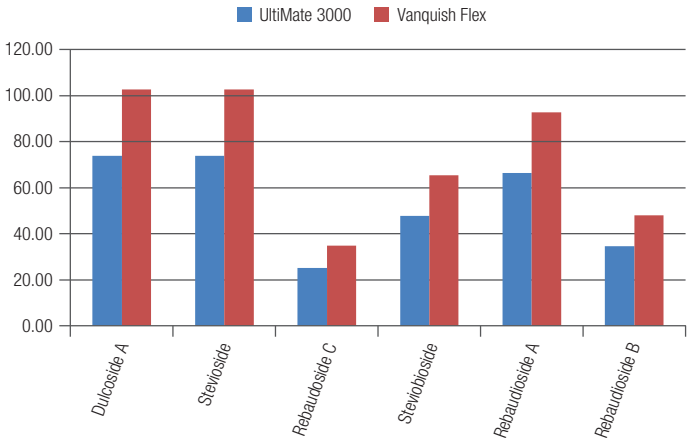

Figure 6. Average peak height (n=6) for six sweeteners on the UltiMate 3000 system and Vanquish Flex system.

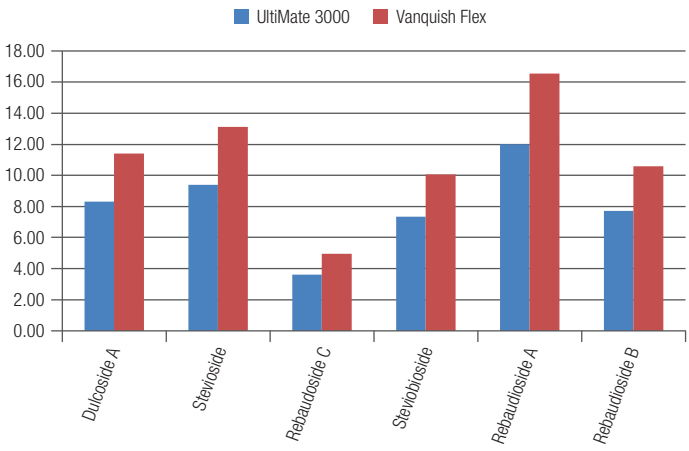

Figure 7. Average peak area (n=6) for six sweeteners on the UltiMate 3000 system and Vanquish Flex system.

Conclusions

- Sensitivity of steviol glycoside determination by HILIC UHPLC was found to increase by up to 40% by utilizing the newly developed DAD Flow Cell LightPipe technology.

- Compared to the UltiMate 3000 system, the %RSD of peak width at half height was found to be up to 80% lower in the case of dulcoside A using the Vanquish Flex system's improved pump and autosampler technology.

- The unique chemistry of the Acclaim Trinity P1, 3 µm columns allows for the HILIC separation of highly polar and structurally similar steviol glycosides, which are difficult to separate on traditional reversed-phase columns.

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