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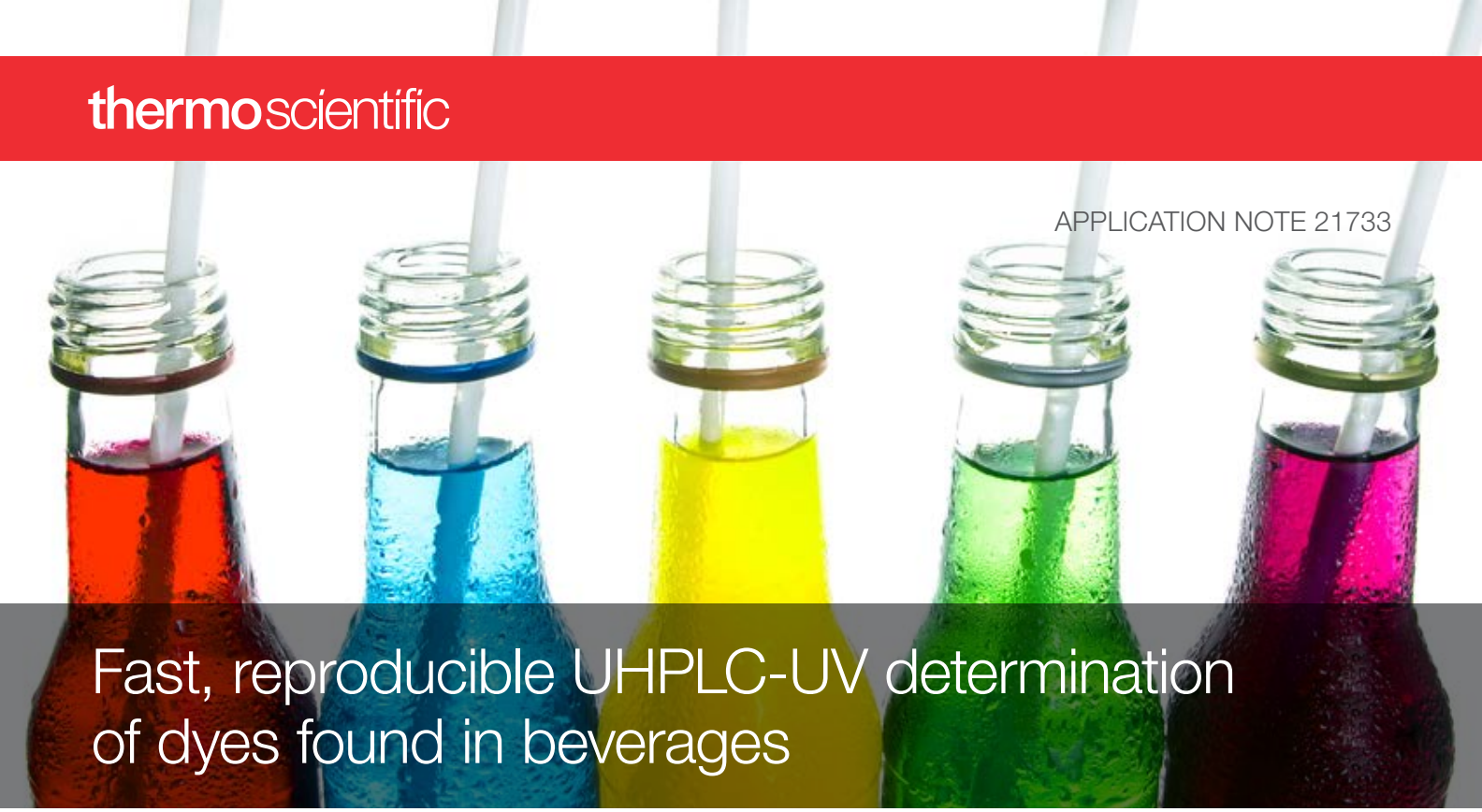
APPLICATION NOTE 21733



Fast, reproducible UHPLC-UV determination of dyes found in beverages

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Keywords

Environment, food safety, food and beverage analysis, UHPLC, DAD, UV, QA/QC, food dyes, sports drinks, tartrazine, indigo carmine, new coccine, sunset yellow FCF, fast green FCF, eosin Y, erythrosine, phloxine B, bengal rose B, UltiMate 3000, Vanquish Flex Quaternary, Acclaim PA2

Application benefits

- The Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2) column and Thermo Scientific™ Vanquish™ Flex system proved capable for the rapid UHPLC analysis of nine food dyes in less than five minutes.
- The method transfer between the Thermo Scientific™ UltiMate™ 3000 RS system and Vanquish Flex UHPLC system was seamless, requiring no adjustment to gradient delay volume.
- Up to an 80% decrease in retention time %RSD was observed in the case of indigo carmine compared to previous instrument generation due to proprietary improvements applied to instrument autosampler and pump technology.

Goal

To demonstrate the seamless method transfer from an UltiMate 3000 RS system to a Vanquish Flex system using an Acclaim PA2 3 µm column for the analysis of nine dyes found in carbonated beverages, showing excellent retention time precision.

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, it is shown that the transfer of a method from an UltiMate 3000 RS system to a Vanquish Flex UHPLC system is easy and straightforward.

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 excellent retention time precision, providing the user with greater data confidence and more freedom in method development and application transfer.

The Acclaim PA2 3 µm column is a high performance, reversed-phase column with enhanced hydrolytic stability. These columns feature surface chemistry that incorporates an amide-embedded polar group and multi-point attachments between ligands and the silica surface. This unique chemistry provides enhanced hydrolytic stability from pH 1.5 to 10 with 100% aqueous mobile phases and exhibits high reversed-phase capacity with selectivity complementary to conventional C18 columns.

Reversed-phase chromatography is an excellent technique for the analysis of dyes. Many dyes are hydrophilic and therefore readily soluble in reversed-phase eluents, and all have strong visible and UV absorbance properties. This method demonstrates the separation of nine dyes, both approved and not approved, which can be found in soft drinks by using UHPLC with UV detection.

Experimental

Consumables and apparatus

- Acclaim PA2, 3.0 µm HPLC column, 75 mm × 3.0 mm (P/N 066277)
- 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™ Ammonium phosphate dibasic (P/N 10509263)
- Fisher Scientific™ Potassium hydroxide pellets (P/N 10575355)
- 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 RS 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 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 nine 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.

Peak	Compound	Concentration (µg/mL)
1	Tartrazine	10
2	Indigo carmine	10
3	New coccine	10
4	Sunset yellow FCF	10
5	Fast green FCF	10
6	Eosin Y	10
7	Erythrosine	10
8	Phloxine B	10
9	Bengal rose B	10

UHPLC conditions

HPLC column: Acclaim PA2, 3.0 µm, 75 mm × 3.0 mm

Mobile phase: A: 20 mM $(NH_4)_2HPO_4$, pH 8.8 in water
B: 20 mM $(NH_4)_2HPO_4$, pH 8.8 / acetonitrile (50:50 v/v)

Gradient conditions:

Time (min)	A %	B %
-5.0	88.0	12.0
0.0	88.0	12.0
3.5	0.0	100.0
4.5	0.0	100.0
4.6	88.0	12.0

Flow rate: 0.71 mL/min

Column temperature: 30 °C (still air mode)

Injection details: 3 µL

UV detection: 254 nm

Backpressure: Approximately 180 bar maximum for both systems

Gradient mixer: 350 µL static + 50 µL capillary mixer

Results and discussion

Full resolution of all nine dyes was achieved within approximately four minutes on both the UltiMate 3000 system and the Vanquish Flex system using an Acclaim PA2 column and the same chromatographic method (Figure 1).

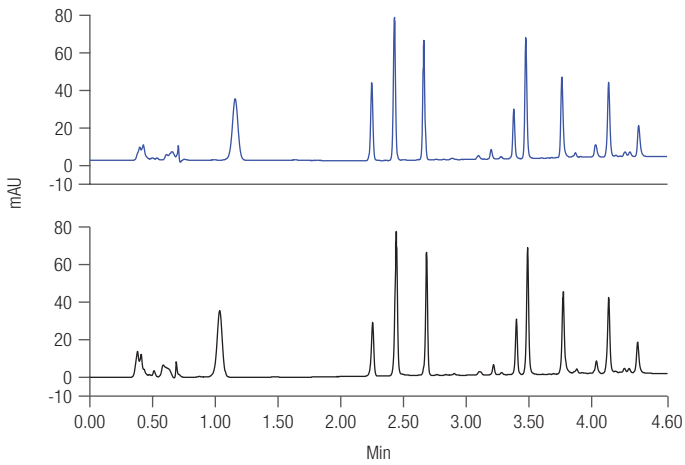

Figure 1. Chromatograms showing the separation of nine dyes on both the UltiMate 3000 system (top trace) and the Vanquish Flex system (bottom trace).

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 (Figures 1 and 2).

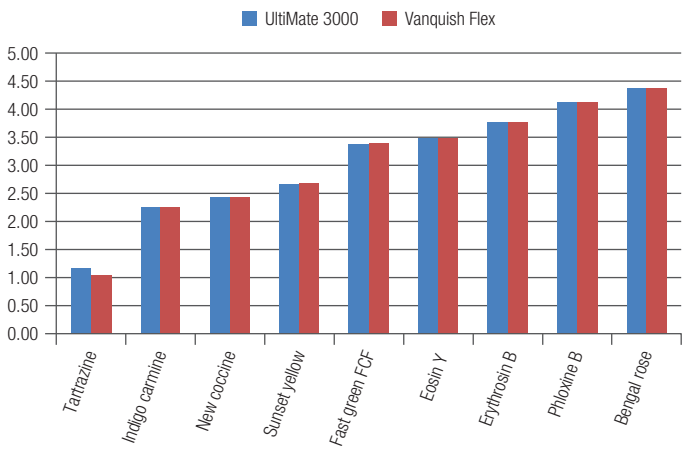

Figure 2. Average retention time (n=6) for nine dyes on the UltiMate 3000 system and Vanquish Flex system.

The relative standard deviation (%RSD) was utilized to assess system reproducibility by evaluating six injections for the two systems with respect to retention time. Altogether the results are significantly better with the Vanquish Flex UHPLC system (Figure 3).

The Vanquish Flex autosampler utilizes the proprietary SmartInject technology to reduce flow inconsistencies during injection and pressure shocks to the HPLC column, ultimately extending column lifetime and injection reproducibility.

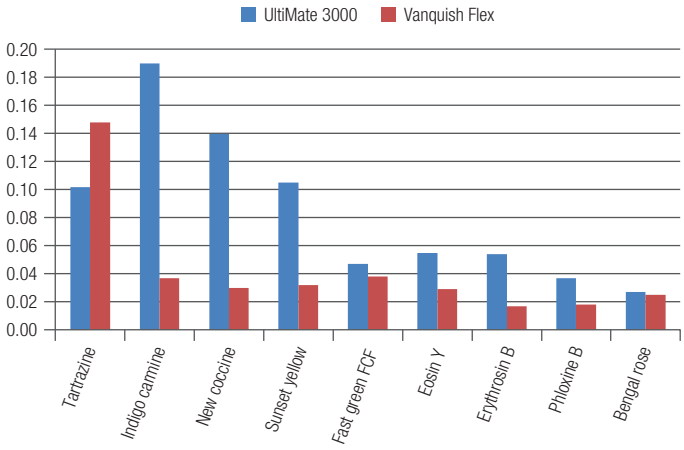

Figure 3. %RSD of retention time (n=9) for nine dyes on the UltiMate 3000 system and Vanquish Flex system.

Conclusions

This application note demonstrates the following:

- The Acclaim PA2 column and Vanquish Flex system proved capable for the rapid UHPLC analysis of nine food dyes in less than five minutes.
- The method transfer between the UltiMate 3000 RS system and Vanquish Flex UHPLC system was seamless, requiring no adjustment to gradient delay volume.
- Up to an 80% decrease in retention time %RSD was observed in the case of indigo carmine compared to previous instrument generation due to proprietary improvements applied to instrument autosampler and pump technology.

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