



# Determination of common food and beverage additives by UHPLC-UV using a mixed mode WAX column

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## Keywords

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citrate, acesulfame, saccharin,  
UltiMate 3000, Vanquish Flex  
UHPLC, Acclaim Mixed Mode  
WAX-1

## Application benefits

- The method transfer between the Thermo Scientific™ UltiMate™ 3000 UHPLC system and a Thermo Scientific™ Vanquish™ Flex UHPLC system is seamless and, in this case, for an isocratic separation requires no method redevelopment.
- The Thermo Scientific™ Acclaim™ Mixed Mode WAX-1 column proved capable of the analysis of neutral and ionic compounds that would be otherwise poorly retained on solely reversed-phase columns.
- The %RSD of peak area was found to be at least 50% lower for the majority of compounds analysed using the Thermo Scientific Vanquish Flex systems proprietary autosampler technology.

## Goal

To demonstrate the seamless method transfer from an UltiMate 3000 RS system to a Vanquish Flex system using an Acclaim Mixed Mode WAX-1, 5  $\mu\text{m}$  column, for the analysis of seven additives found in carbonated beverages to show improved reproducibility.

## 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. The additional benefits of improved, proprietary pump and autosampler 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 Mixed Mode WAX-1 column is a silica-based column that contains both hydrophobic and weak anion exchange characteristics. The dual functionality allows for the analysis of complex mixtures, which contain both neutral and ionic compounds, from samples across many markets, including food and beverage.

Additives are widely used in the beverage industry for reasons such as flavor enhancement, preservation, and stimulus. Although the individual recipes are proprietary it is necessary for quality, health and safety reasons to determine the presence and concentration of a number of such additives during or after production. The current method demonstrates the separation of seven commonly used beverage additives.

## Experimental

### Consumables and apparatus

- Acclaim Mixed Mode WAX-1, 5  $\mu\text{m}$  HPLC column, 150 mm  $\times$  4.6 mm (P/N 064984)
- LC/MS grade 18 M $\Omega$  water from Thermo Scientific™ Barnstead™ Smart2Pure™ Water Purification system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (P/N A/0626/17)
- Fisher Scientific™ methanesulfonic acid (P/N 10346910)
- Fisher Scientific™ potassium dihydrogen phosphate (P/N 12900504)
- 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  $\mu\text{L}$ , 10 mm (P/N 6082.010)

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)
- 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™ 7.2 SR4

### Sample preparation

Solutions of the seven compounds shown in Table 1 were prepared from their salts by dissolving a known amount in methanol or water 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	Concentration ( $\mu\text{g/mL}$ )
Caffeine	4
Aspartame	12
Sorbate	20
Benzoate	20
Citrate	120
Acesulfame	40
Saccharin	12

Vial labeling was supported by the Virtuoso vial identification system.

### UHPLC conditions

HPLC column: Acclaim Mixed Mode WAX-1, 5  $\mu\text{m}$  HPLC column, 150 mm  $\times$  4.6 mm

Mobile phase: 120 mM potassium dihydrogen phosphate pH 3.0 / acetonitrile (45:55 v/v)

Flow rate: 1.5 mL/min

Column temperature: 30  $^{\circ}\text{C}$

Injection details: 5  $\mu\text{L}$

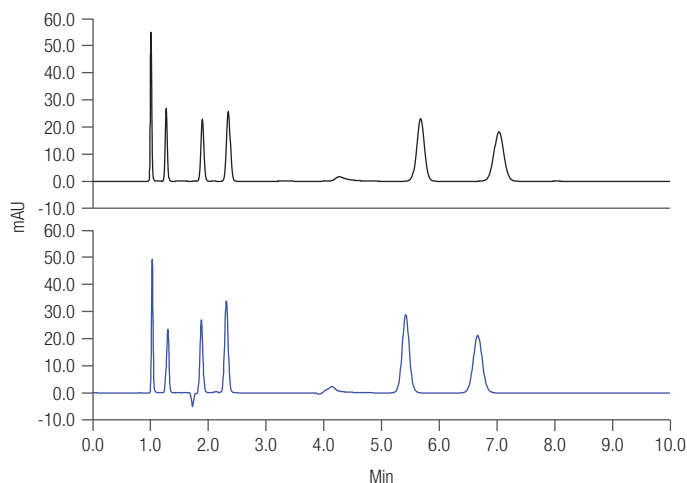
UV detection: 210 nm

Backpressure: Approximately 120 bar maximum for both systems

Gradient mixer: 350  $\mu\text{L}$  static + 50  $\mu\text{L}$  capillary

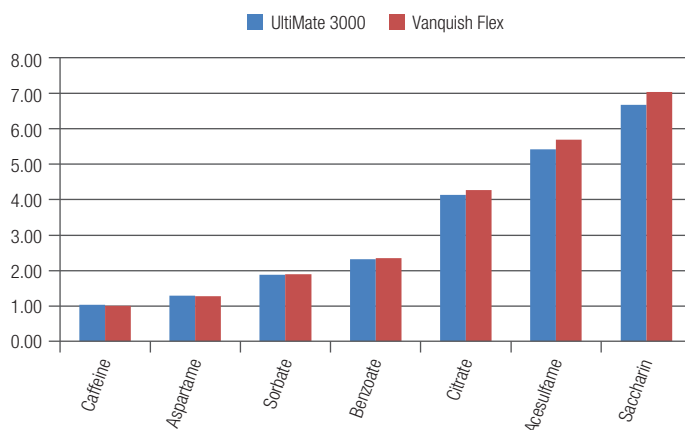
## Results and discussion

Full resolution of all seven additives was achieved within eight minutes on both the UltiMate 3000 system and the Vanquish Flex system using the Acclaim Mixed Mode WAX-1 column and the same isocratic chromatographic method (Figure 1).



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

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  $n=9$  injections for the two systems were found to be comparable (Figure 2).



**Figure 2. Average retention time ( $n=9$ ) for seven additives on the UltiMate 3000 system and the Vanquish Flex system.**

The relative standard deviation (%RSD) of peak area was utilized to assess system reproducibility by evaluating nine injections for each system. The results for reproducibility show that the Vanquish Flex system has significantly improved the performance previously achieved by at least 50% compared to the UltiMate 3000 system (Figure 3). The %RSD of the citrate peak was quite high on both systems with the variability in peak area due to poor peak shape affecting the consistency of automatic integration on both systems.

The Vanquish Flex autosampler utilizes the proprietary Thermo Scientific SmartInject technology to reduce flow inconsistencies during injection and pressure shocks to the HPLC column. This and other improvements applied to the pump and autosampler technology increase method reproducibility of the Vanquish Flex UHPLC system.

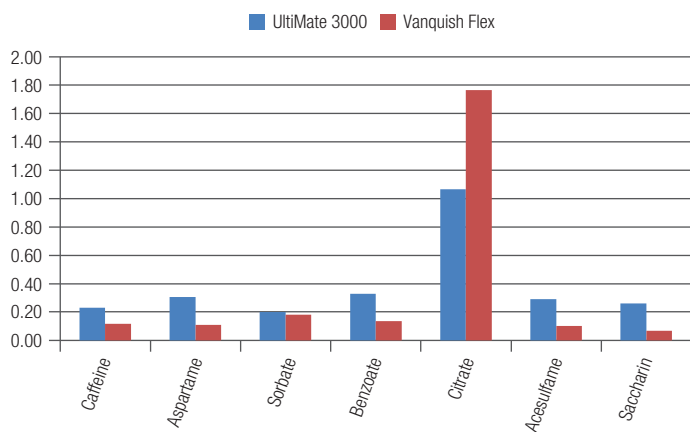


Figure 3. %RSD of peak area (n=9) for seven additives on the UltiMate 3000 system and Vanquish Flex system.

## Conclusions

This application note demonstrates the following:

- The method transfer between the UltiMate 3000 UHPLC system and Vanquish Flex UHPLC system is seamless and in this case, for an isocratic separation requires no method redevelopment.
- The Acclaim Mixed Mode WAX-1 column proved capable of the analysis of neutral and ionic compounds that would be otherwise poorly retained on solely reversed-phase columns.
- %RSD of peak area was found to be at least 50% lower for the majority of compounds analysed using the Vanquish Flex systems proprietary autosampler technology.

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