



Rapid and sensitive UHPLC screening for water soluble vitamins in sports beverages

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Keywords

Vanquish Flex, Acclaim PolarAdvantage II, rapid analysis, water soluble vitamins, beverage analysis, UHPLC, Pyridoxine HCl, Ascorbic acid, Nicotinic acid, Nicotinamide, D-Pantothenic acid, Cyanocobalamin, Folic acid

Goal

- To demonstrate the capability of the Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2) and Thermo Scientific™ Vanquish™ Flex Binary UHPLC system combination for the rapid separation of water soluble vitamins in sports beverages with excellent linearity, reproducibility, resolution, and recoveries.
- To show the capability of the Vanquish Flex Binary UHPLC system to provide superior retention time precision through its novel pump features.

Introduction

Vitamins are a group of compounds that are essential for human health. They cannot be readily produced by the body and so must be consumed in the diet. To ensure sufficient vitamin intake many people use vitamin supplements, often in the form of vitamin-enhanced beverages. Sports drinks are a particularly popular type of beverage due to their enhanced electrolyte, carbohydrate, and in many cases vitamins contents. Since the lack, or excess, of vitamins in the diet can cause health problems, it is important to be able to accurately measure the vitamin content of these products.

Due to its wide range of selectivity, reversed-phase chromatography is an excellent technique for the analysis of vitamins. Water-soluble vitamins are

readily soluble in reversed-phase eluents and have strong visible and UV absorbance properties. This method demonstrates the separation of seven water soluble vitamins that can be found in sports drinks by UHPLC with UV detection.

Water soluble vitamins are structurally diverse and include some highly polar compounds that do not retain well on traditional C18 columns. The logP value for seven vitamins are presented in Table 1. The Acclaim PolarAdvantage II (PA2) columns have a polar-embedded stationary phase that operates over a wider range of chromatographic conditions than possible with conventional reversed-phase stationary phases. Its unique chemistry provides enhanced hydrolytic stability from pH 1.5 to 10 with 100% aqueous mobile phases. The Vanquish Flex Binary UHPLC system allows the user the method speed expected from a binary high pressure mixing pump.

Table 1. Theoretical logP values for seven water-soluble vitamins.

Vitamin	logP value
Pyridoxine HCl	-0.95
Ascorbic acid	-1.90
Nicotinic acid	-0.17
Nicotinamide	-0.39
D-Pantothenic acid	-1.36
Cyanocobalamin	-7.0
Folic acid	0.68

Experimental

Consumables

- Acclaim PolarAdvantage II (PA2) 100 mm x 3 mm, 2.2 µm (P/N 071607)
- 18 MΩ•cm water from Thermo Scientific™ Smart2Pure™ system (P/N 50129845)
- Fisher Scientific™ HPLC grade acetonitrile (A/0626/17) (P/N 10407440)
- Fisher Scientific HPLC grade methanol (M/4056/17) (P/N 10675112)
- Fisher Scientific™ Optima™ grade formic acid (P/N A117-50)
- Potassium hydroxide (P/5640/65) (P/N 10142010)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (P/N 60180-VT400)

- Thermo Scientific™ 30 mm Target 2™ 0.45 µm nylon syringe filter (P/N F2500-1)

All standards were purchased from a reputable supplier.

Instrumentation

Analyses were performed using a Vanquish Flex Binary UHPLC system consisting of:

- Binary Pump F (P/N VF-P10-A-01)
- 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, 2 µL 10 mm (P/N 6083.0100)

Thermo Scientific™ Virtuoso™ vial identification system (P/N 60180-VT-100)

Software

Thermo Scientific™ Chromeleon™ 7.2 SR4 MUb (8525)

Sample preparation

Solutions of the seven vitamins shown in Table 2 were prepared by dissolving the solid compound in water to produce 1 mg/mL primary solutions, with the exception of folic acid. Due to the limited solubility of folic acid in water, the free acid was converted to potassium folate by addition of a few drops of dilute potassium hydroxide. A mixed working standard solution containing all compounds was then prepared in mobile phase A and diluted as appropriate.

Linearity preparation

Mixed calibration standards were prepared in mobile phase A covering the concentration detailed in Table 4.

Sample preparation

The sports drinks were placed in an ultrasonic bath for 5 minutes to degas, diluted 1:1 in mobile phase A, then filtered through a 0.45 µm nylon syringe filter. Aliquots (900 µL) of each diluted sample were spiked with 100 µL of the mixed working standard solution. Samples were prepared as above but these were spiked with the same amount of mobile phase A instead of the mixed working standard solution. Calibration standards were prepared

with equivalent dilution factors applied. Vial labelling was supported by the Virtuoso vial identification system.

All samples were sourced from a local supermarket. Three different flavors were chosen for evaluation purposes (Table 1).

Table 2. Sample identification.

Sample	Drink	Flavor
Sports drinks	A	Orange
	B	Tropical
	C	Mixed Fruits

UHPLC conditions

HPLC column: Acclaim PolarAdvantage II (PA2)
100 mm × 3 mm, 2.2 μm

Mobile phase A: 0.015% Formic acid in water

Mobile phase B: Methanol/acetonitrile (20:80 v/v)

Gradient conditions:	Time (min)	%A	%B
	-3.0	100.0	0.0
	0.0	100.0	0.0
	2.0	100.0	0.0
	6.0	55.0	45.0
	6.0	0.0	100.0
	7.0	0.0	100.0
	7.0	100.0	0.0

Flow rate: 0.50 mL/min

Column temperature: 50 °C (still air mode)

Pre-heater temperature: 50 °C

Injection volume: 5 μL

UV detection: 210 nm (solvent blank background subtracted)

Backpressure: Approximately 240 bar maximum

Gradient mixer: 50 μL capillary + 150 μL static

Results and discussion

Full resolution of all seven vitamins (>1.5 EP resolution) was achieved within approximately six minutes on the Vanquish Flex Binary system using an Acclaim PolarAdvantage II (PA2) column (Figure 1).

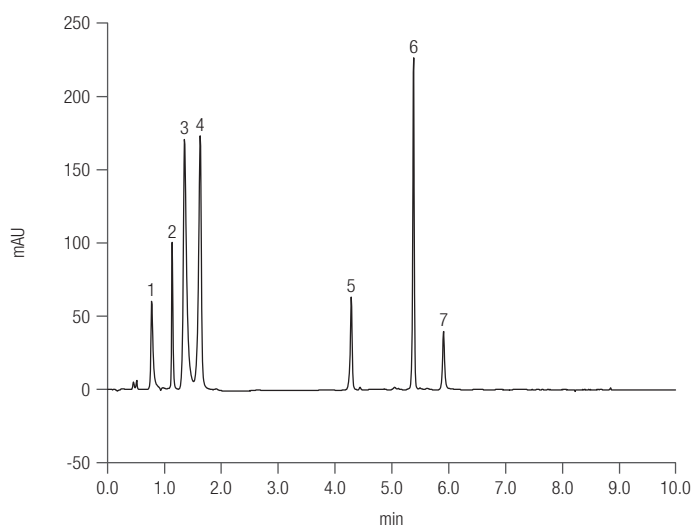


Figure 1. Chromatogram showing the separation of seven vitamins in a mixed standard at mid calibration point for each compound on the Vanquish Flex Binary system.

Method reproducibility

Excellent method reproducibility for retention time and peak area was achieved for all vitamins (Table 3).

Table 3. Comparison of % RSD of retention time (RT) and peak area for seven vitamins in a mixed standard at mid calibration point for each compound.

Compound	Peak Number	% RSD	
		RT	Area
Pyridoxine HCl	1	0.073%	0.061%
Ascorbic acid	2	0.088%	0.711%
Nicotinic acid	3	0.086%	0.087%
Nicotinamide	4	0.091%	0.055%
D-Pantothenic acid	5	0.270%	0.890%
Cyanocobalamin	6	0.038%	0.045%
Folic acid	7	0.019%	1.822%

Method linearity

All calibration curves' R^2 values were found to be between 0.9953 and 0.9983 (Table 4).

Table 4. Calibration curve details.

Compound	Calibration Line with a 1/x Weighting		
	Peak Number	R^2 Values	Linear Range ($\mu\text{g/mL}$)
Pyridoxine HCl	1	0.9960	0.25–20
Ascorbic acid	2	0.9967	37.5–300
Nicotinic acid	3	0.9982	1.25–100
Nicotinamide	4	0.9980	2.5–100
D-Pantothenic acid	5	0.9953	1.25–100
Cyanocobalamin	6	0.9974	0.25–20
Folic acid	7	0.9983	0.078–5

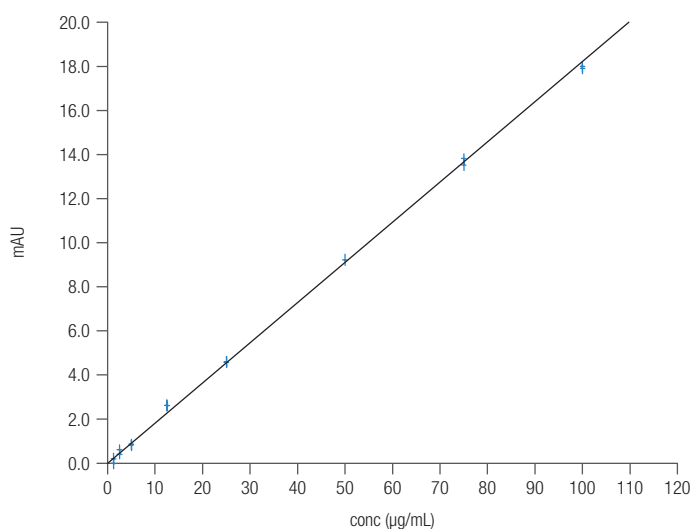


Figure 2. Calibration line of D-pantothenic acid with a correlation coefficient (R^2) of 0.99528.

Many vitamin-enhanced drinks contain other additives such as flavorings, colorings, gums, and preservatives to emulsify and stabilize the drink. These add complexity to the chromatography as peaks of interest must be resolved from matrix peaks. Figure 3 is an example of the chromatography obtained from drink A with all seven vitamins spiked in to show good resolution from matrix peaks.

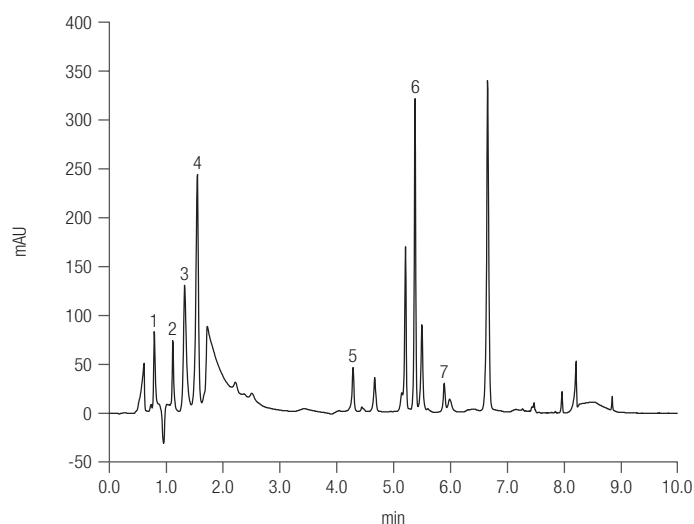


Figure 3. Sample A spiked with all seven vitamins.

Excellent spiked recoveries were observed in each sample for all seven vitamins. All recoveries were $\geq 80\%$. This shows the method has minimal matrix interference (Figure 4).

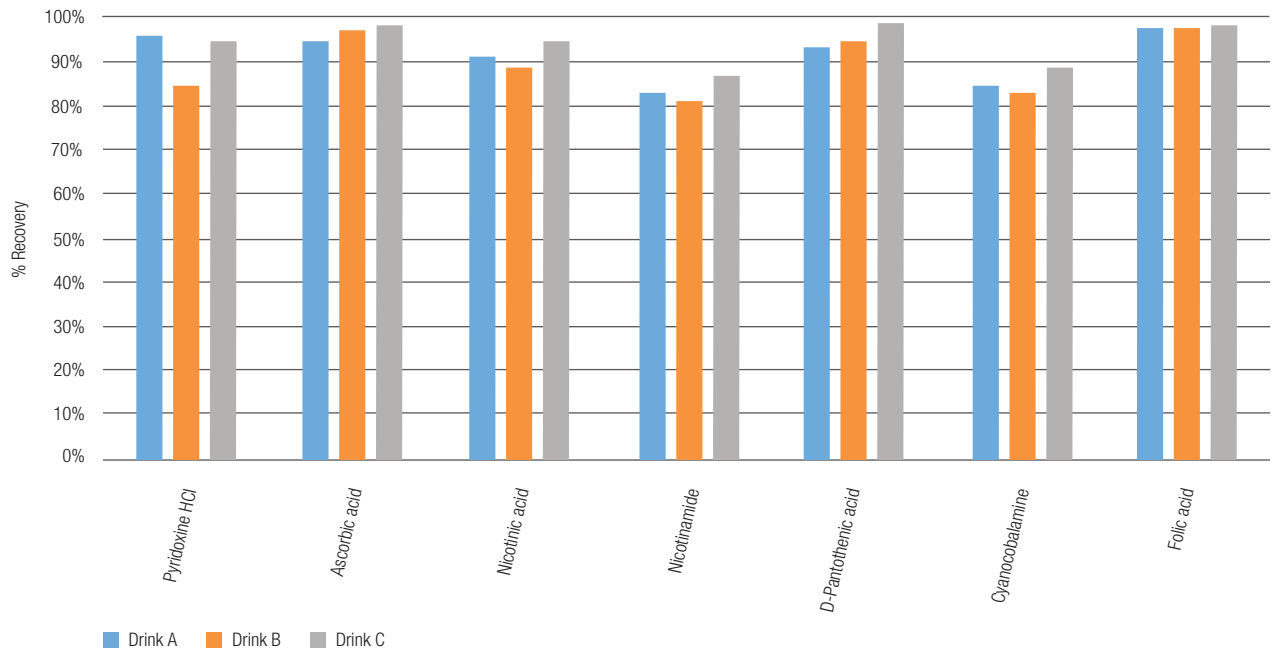


Figure 4. Percent recoveries of seven vitamins from three samples.

Calculated concentrations generated with this assay were in close agreement with the stated values on the product information label on each of the three flavors of sports drink as presented in Table 5.

Table 5. Comparison of concentration of vitamins on the product label compared to measured concentrations.

Drink	Vitamin	Concentration (µg/mL)	
		Label	Calculated
A	Niacin (as Nicotinic acid)	5.4*	0.44
	Niacin (as Nicotinamide)		4.7
	Vitamin B6 (as Pyridoxine HCl)	0.5	0.61
	Vitamin B12 (Cyanocobalamin)	0.0009	Below LLOQ
	Vitamin B5 (Pantothenic acid)	2.0	2.9
B	Niacin (as Nicotinic acid)	5.4*	0.43
	Niacin (as Nicotinamide)		4.8
	Vitamin B6 (as Pyridoxine HCl)	0.5	0.71
	Vitamin B12 (Cyanocobalamin)	0.0009	Below LLOQ
	Vitamin B5 (Pantothenic acid)	2.0	2.9
C	Niacin (as Nicotinic acid)	5.4*	0.46
	Niacin (as Nicotinamide)		4.8
	Vitamin B6 (as Pyridoxine HCl)	0.5	0.66
	Vitamin B12 (Cyanocobalamin)	0.0009	Below LLOQ
	Vitamin B5 (Pantothenic acid)	2.0	2.9

* Label on each drink stated a concentration for niacin, which includes both nicotinic acid and nicotinamide.

Conclusions

This application demonstrates the following:

- The capability of the Acclaim PolarAdvantage II (PA2) column and Vanquish Flex Binary system combination for the rapid separation of vitamins in sports beverages with excellent linearity, reproducibility, resolution, and recoveries.
- The capability of the Vanquish Flex Binary system to provide superior retention time precision through advanced features giving consistent flow control and injection reproducibility.

Find out more at thermofisher.com/LC-columns

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