

# Analysis of a Sports Beverage for Electrolytes and Sugars Using Multi-Mode Chromatography with Charged Aerosol Detection

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## Key Words

Acclaim Trinity P2, Corona Veo charged aerosol detector, electrolytes, sugars, food, beverage

## Abstract

Sports beverages are advertised to replenish electrolytes and water lost during vigorous exercise. The product labels indicate these beverages contain sugars, electrolytes, flavors, vitamins, coloring, and preservatives. Analysis for the purpose of quality control or label guarantee presents some interesting challenges, which can be overcome with the correct HPLC column and detector.

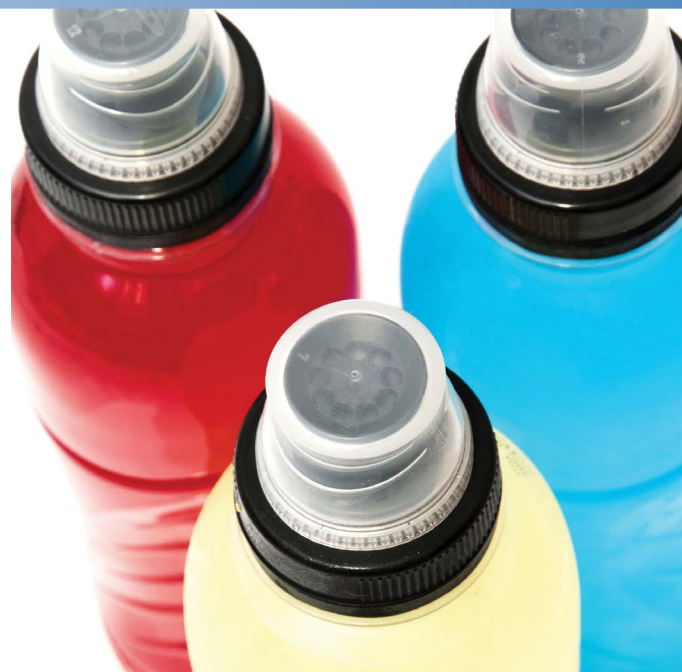
The Thermo Scientific™ Acclaim™ Trinity™ P2 column is a newly developed multi-mode column based on Nanopolymer Silica Hybrid technology. It separates both anions and cations within one analysis under simple salt-gradient conditions, and separates sugars under isocratic HILIC-mode conditions.

The Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector (CAD) provides sensitive detection of nonvolatile and semi-volatile analytes; it is especially useful for analytes lacking a strong UV chromophore. In this work, one such beverage is analyzed to demonstrate the power of this combination of new technologies.

## Introduction

The analysis of sports beverages provides an interesting analytical challenge in that the carbohydrates, anions, and cations are all functional ingredients that require determination. Typical solutions often involve separate analytical techniques and devices for each group of analytes. Advances in multi-mode chromatography permit all three groups to be resolved using a single HPLC column and a single HPLC detector.

The Acclaim Trinity P2 column is based on Nanopolymer Silica Hybrid (NSH™) technology, which consists of high-purity, porous, spherical silica particles coated with charged nanopolymer beads. The inner-pore area of the silica particles is modified with a covalently bonded hydrophilic layer that provides cation exchange retention; the outer surface is modified with anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion-exchange and cation-exchange regions. In addition, its hydrophilic surface makes it a good HILIC column useful for retaining highly polar molecules, such as sugars.



The Corona Veo Charged Aerosol Detector is the latest generation of this technology. It converts the column effluent into a dry aerosol and applies an electrical charge to the particles; the amount of charge measured by the electrometer is proportional to the mass of analyte. The Corona Veo CAD is the detector of choice for this application. UV absorbance simply cannot detect most of the analytes of interest, and refractive index detectors are incompatible with gradient elution and lack sensitivity. The detection limits for evaporative light scattering detection are also inferior to the charged aerosol detector. Sports drinks often contain some ingredients that would foul HPLC columns, in particular vegetable gum and food coloring. In this work, Thermo Scientific™ Dionex™ OnGuard™ II P cartridges, packed with cross-linked polyvinylpyrrolidone, were used to remove color and particulate matter from aqueous samples.

## Experimental Details

Consumables	Part Number
SPE cartridges, Dionex OnGuard II P	057087
Polypropylene vials, 1.5 mL	079812
3 mL all-plastic syringes	14-817-27
Acetonitrile, Fisher Optima™ LC/MS grade	A955
Formic acid, >98%	
Ammonium formate	
Deionized water	

### Sample Preparation Supplies

The sample of sports beverage was purchased from a local food market.

Conditioning stage:	Using a syringe, apply 1 mL of water to the Dionex OnGuard II P cartridge and allow it to rehydrate for 1 min.
Application stage:	Using a syringe, apply 2 mL of sample at 2 mL/min. Discard the first 1 mL of liquid; retain the second 1 mL for analysis.
	For electrolyte analysis, the sample was not treated further. For sugar analysis, the sample was diluted 40x with mobile phase (80:20 acetonitrile / buffer).

Sample Preparation	Part Number	
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 system	
Column:	Acclaim Trinity P2, 3 μm, 50 × 3 mm	085433
Mobile phase A:	Acetonitrile	
Mobile phase B:	Water	
Mobile phase C:	100 mM ammonium formate, pH 3.65 (6.35 g/L NH <sub>4</sub> HCO <sub>2</sub> + 4.5 g/L HCO <sub>2</sub> H)	
Flow rate:	0.60 mL/min	
Detection:	Corona Veo charged aerosol detector, evaporator temperature 55 °C, gas pressure 60 psi, data rate 5 Hz, filter 2 s, power function 1.50	

### Experiment 1 – Separation of electrolytes

LC gradient conditions:	See Table 1
Column temperature:	30 °C
Injection volume:	2 μL

Time (min)	A	B	C
-8	0	90	10
0	0	90	10
1	0	90	10
11	0	0	100
15	0	0	100

Table 1: LC gradient conditions for electrolyte analysis

### Experiment 2 – Separation of sugars

Isocratic conditions:	80% A, 0% B, and 20% C (v/v)
Column temperature:	60 °C
Injection volume:	5 μL

## Data Processing

Software:

Thermo Scientific™ Chromeleon™ 6.80 SR13

## Results

Acclaim Trinity P2 columns provide the required selectivity for the separation of mono- and multi-valent anions and cations using a standard salt-gradient elution program. Baseline resolution of a total of twelve ions including phosphate, sodium, potassium, chloride, malate, bromide, nitrate, citrate, fumarate, sulfate, magnesium, and calcium is shown in Figure 1. This desirable selectivity is provided by the unique phase design in which the cation- and anion-exchange capacity are carefully balanced for optimal resolution. This separation cannot be realized on any other separation media.

The selectivity of anions versus cations can be adjusted in the range of pH 3–4.5. However, the standard starting point for method development is pH 3.65 and in this case satisfactory results were obtained at this pH, as shown in Figure 2. All the identified peaks matched declared ingredients in the product, except for peak 8. This matched the retention time of sulfate, which was not a listed ingredient.

Under the chromatographic conditions used for electrolyte analysis the sugars eluted in the void volume, and therefore a second analysis was required. Under HILIC conditions, using a mobile phase consisting of 80% acetonitrile and 20% buffer, the same Trinity P2 column successfully separated sugars in this sports drink (Figure 3).

This particular beverage contained high-fructose corn syrup, so the major carbohydrates present are fructose, glucose, and a small amount of residual maltose. In solution, these sugars exist as an equilibrium mixture of anomeric forms. In acidic and neutral conditions, the equilibrium is slow enough to cause undesirable partial separation of anomers, resulting in peak broadening and crowded chromatograms. Thus, an elevated temperature of 60 °C was employed to overcome this challenge.

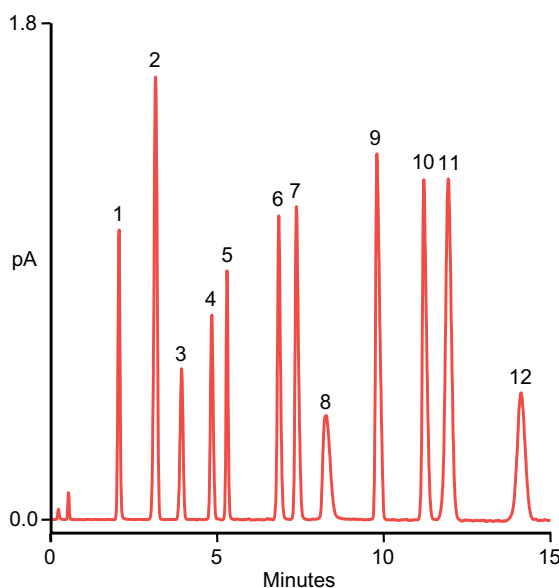


Figure 1: Separation of common anions and cations under standard salt-gradient conditions. Peaks 1: phosphate, 2: sodium, 3: potassium, 4: chloride, 5: malate, 6: bromide, 7: nitrate, 8: citrate, 9: fumarate, 10: sulfate, 11: magnesium, 12: calcium.

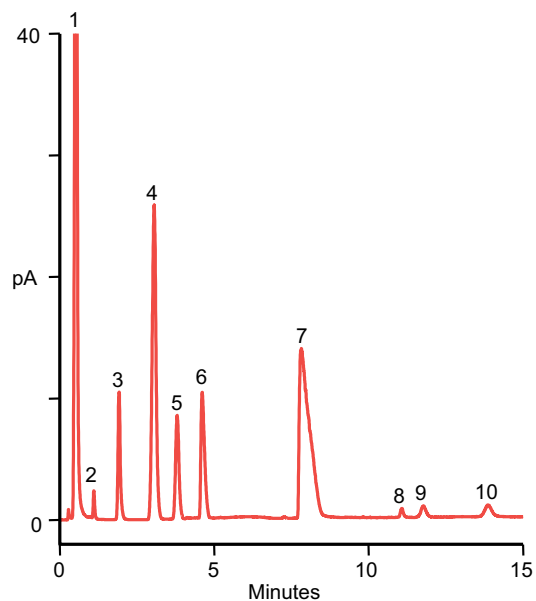


Figure 2: Electrolytes in a sports beverage using standard salt-gradient elution conditions. Peaks 1: sugars, 2: ascorbate, 3: phosphate, 4: chloride, 5: sodium, 6: potassium, 7: citrate, 8: unidentified, 9: magnesium, 10: calcium.

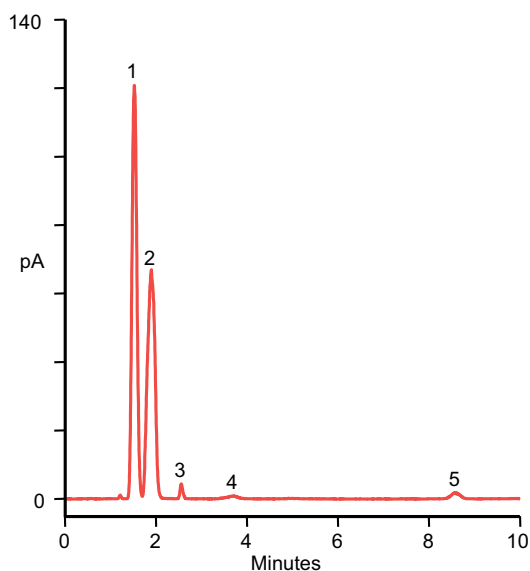


Figure 3: Sugars in a sports beverage using isocratic HILIC-mode elution conditions. Peaks 1: fructose, 2: glucose, 3: chloride, 4: maltose, 5: sodium.

## Conclusion

- The Acclaim Trinity P2 column offers ideal selectivity for simultaneous separation of monovalent and divalent anions and cations using ion exchange conditions.
- The Acclaim Trinity P2 column can operate under HILIC conditions for separation of sugars.
- Both separations are carried out using a simple mobile phase system of acetonitrile and ammonium formate buffer.
- The Corona Veo detector can be used for both analyses and replaces the multiple detectors traditionally used.
- The OnGuard II P cartridge was used for simple sample preparation.

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