Separation of Calcium, Magnesium and Counterions in a Dietary Supplement Using Multi-mode Liquid Chromatography with Charged Aerosol Detection

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Key Words
Acclaim Trinity P2, calcium, magnesium, dietary supplement, Corona Veo charged aerosol detector, counterion

Abstract
This work relates to the simultaneous determination of calcium, magnesium and their counterion (citrate) in a dietary supplement under gradient elution conditions using a Thermo Scientific™ Acclaim™ Trinity™ P2 column and Thermo Scientific™ Dionex™ Corona™ Veo™ Charged Aerosol Detector (CAD).

Introduction

Calcium and magnesium are essential nutrients and are commonly formulated into dietary supplements. The form of the mineral can affect the rate of absorption, so various counterions are included in the formula. The analysis of mineral supplements provides an interesting analytical challenge in that both the anions and cations are functional ingredients that require determination. In the past, separate assays for anions and cations would be used. Advances in multi-mode chromatography permit both anions and cations to be resolved in one run using a single HPLC column and a universal 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 particles. The inner-pore structure of the silica particles is modified with a covalently bonded hydrophilic layer that provides cation exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion exchange and cation exchange regions.

The Corona Veo charged aerosol detector represents the latest advancement of this technology. Compared with other aerosol based detectors (e.g., ELSD), the Corona Veo features superior limit of detection, better reproducibility (RSD), and greater ease of use. Because Calcium and Magnesium have no UV chromophore, the Corona Veo is the detector of choice for this application.

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Experimental Details

Consumables	Part Number
Acetonitrile, Fisher Optima™ LC/MS grade	A955
Formic acid, >98%	
Ammonium formate, 99.995%	
Deionized water	
3 mL all-plastic syringes	14-817-27
0.2 µm nylon membrane filter, 17 mm, Hyperclean	60183-212
Polypropylene vials, 1.5 mL	079812

Sample Preparation

The sample was obtained from a local market, in a powder form contained in gelatin capsules. The content of one capsule was dissolved in 3 mL of formic acid, diluted to 500 mL with water, then a portion filtered with a 0.2 µm membrane filter.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 system	
Column:	Acclaim Trinity P2, 3 µm, 50 × 3 mm	085433
Mobile phase A:	Water	
Mobile phase B:	100 mM ammonium formate, pH 3.65 (6.35 g/L NH_4HCO_2 + 4.5 g/L HCO_2H)	
Flow rate:	0.60 mL/min	
Column temperature:	30 °C	
Injection volume:	2 µL	
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)	

Time (min)	A	B
-8.0	90	10
0.0	90	10
1.0	90	10
11.0	0	100
20.0	0	100

Table 1: LC gradient conditions

Data Processing

Software:	Thermo Scientific™ Dionex™ 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 buffer-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 capacities are carefully balanced for optimal resolution. This separation cannot be realized on any other HPLC column.

The mineral supplement product used in this example prominently featured calcium and magnesium with citrate and aspartate on the label. Since Acclaim Trinity P2 columns provide both cation and anion-exchange retention mechanisms at the same time, they can adequately retain both anions and cations. The unique chemistry of the Acclaim Trinity P2 column, in which cation-exchange and anion-exchange regions are spatially separated, allows for great flexibility in method optimization by adjusting mobile phase buffer concentration, pH, and/or organic solvent content. The mineral supplement's declared ingredients were consistent with the chromatographic separation of the standards.

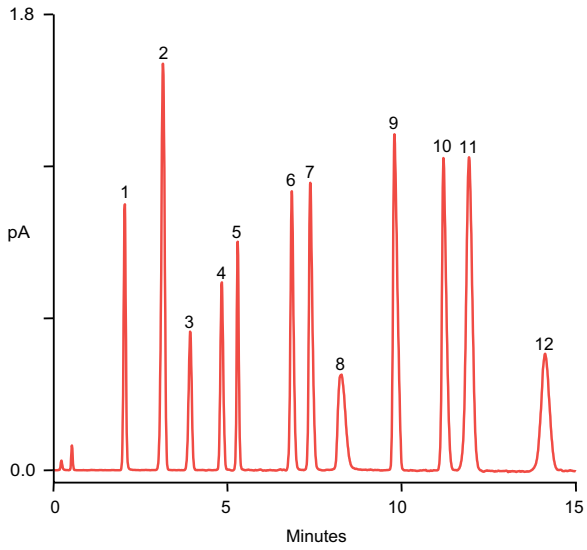

Figure 1: Separation of common anions and cations under standard salt-gradient conditions
Peaks 1: phosphate, 2: sodium, 3: potassium, 4: chloride, 5: malate, 6: bromide, 7: nitrate, 8: citrate, 9: fumarate, 10: sulfate, 11: magnesium, 12: calcium.

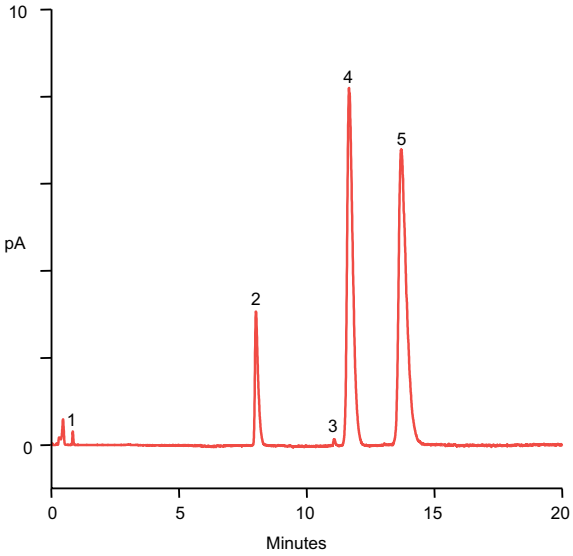

Figure 2: Analysis of a mineral supplement
Peaks 1: aspartate, 2: citrate, 3: unidentified, 4: magnesium, 5: calcium.

Conclusion

- The Acclaim Trinity P2 column offers ideal selectivity for simultaneous separation of monovalent and divalent anions and cations using a simple acetonitrile and ammonium formate mobile phase system
- The Corona Veo detector provides the best solution for detecting non-volatile analytes with no or weak chromophore

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