Application Note

Inorganic Ions, Water Testing, Minerals, Metals, Basic Chemicals

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Analysis of Metals, Halides, and Inorganic Ions Using Hydrophilic Interaction Chromatography

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Abstract

This Application Note describes how Hydrophilic Interaction Chromatography (HILIC) is used to separate a range of inorganic ions. Analysis of these highly polar compounds was previously restricted to specialized methods such as ion exchange (IEX) and ion chromatography (IC). With the development of advanced HILIC phase chemistries, such as Agilent InfinityLab Poroshell 120 HILIC-Z and InfinityLab Poroshell 120 HILIC-OH5, it is now possible to retain and analyze ionic species on a standard HPLC with a water-acetonitrile gradient.

Introduction

Analysis of inorganic ions is a core function of many labs, but cannot easily be accomplished by reversed-phase chromatography. This is due to the poor retention of highly polar ions on the relatively nonpolar phase chemistries. Ion exchange (IEX) and ion chromatography (IC) have been the only options for many years, despite the longer analysis times and more specialized equipment requirements.

With the development of more advanced Hydrophilic Interaction Chromatography (HILIC) phase chemistries, it is possible to analyze a wide range of highly polar compounds, including inorganic ions. HILIC also provides several unique advantages over IEX and IC methods:

- Cations, anions, and polar neutral compounds can be analyzed simultaneously in a single injection.
- Standard water-acetonitrile eluents are used, without any strongly acidic or alkaline additives.
- Systems do not require regeneration, suppressors, or eluent generators.
- HILIC can use higher efficiency silica-based media instead of polymeric resins.

In addition to these advantages, the Agilent InfinityLab Poroshell 120 HILIC-Z and Agilent InfinityLab Poroshell 120 HILIC-OH5 use 2.7 µm superficially porous particles to achieve UHPLC levels of efficiency and resolution, without the high backpressure.

Experimental

Reagents and Chemicals

All reagents were ACS grade or higher. HPLC-grade acetonitrile was bought from Honeywell (Muskegon, MI, USA). Water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany). Reagent-grade formic acid (FA) (p/n G2453-85060) was from Agilent Technologies. Ammonium formate and inorganic ion standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Equipment and Materials

- Eppendorf pipettes and repeater
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, 3 ports, 1 vent valve (p/n 5043-1219)
- Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk. (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190-7024)
- Agilent vial insert, 250 µL, deactivated glass with polymer feet (p/n 5181-8872)
- Agilent InfinityLab Quick Connect and Quick Turn fittings (p/n 5067-5957 and p/n 5067-5966)
- Low dispersion kit (p/n 5067-5963)

Instrumentation

An Agilent 1260 binary LC system with an Agilent G4218A ELSD was used for this work. To minimize system dispersion, all connecting capillaries were short, with a 0.12 mm internal diameter. Agilent OpenLab software was used to control the system and process the data.

Sample Preparation

Inorganic ions were analyzed as salts, were dissolved in water, and injected individually with no further sample preparation.

Mobile Phase Preparation

Ammonium formate was weighed to achieve the desired concentration, and mixed with water. Eluents were then adjusted to pH 3 with formic acid. HPLC grade acetonitrile was used with no further preparation.

Note: Ammonium formate buffers can degrade over time, and it is recommended that eluents be mixed in 1 L quantities or less, and replaced frequently.

Salt Standards

Salt	Concentration
Calcium chloride	2.4 mmol/L (0.4 mg/mL)
Lithium bromide	5.0 mmol/L (0.4 mg/mL)
Magnesium chloride	3.0 mmol/L (0.3 mg/mL)
Potassium bromate	2.1 mmol/L (0.3 mg/mL)
Potassium iodide	2.7 mmol/L (0.5 mg/mL)
Potassium phosphate	2.5 mmol/L (0.3 mg/mL)
Sodium bromide	3.4 mmol/L (0.3 mg/mL)
Sodium chlorate	3.4 mmol/L (0.4 mg/mL)

Note: Salt mixtures must take solubility into consideration to prevent formation of insoluble salts (for example, calcium phosphate).

Instrument Conditions

Parameter	Value
HPLC Conditions	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924) Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm (p/n 685775-601) Agilent InfinityLab Poroshell 120 HILIC, 2.1 × 100 mm, 2.7 µm (p/n 695775-901T)
Flow rate	0.40 mL/min
Column temperature	30 °C
Injection volume	1 µL
Mobile phase A	100 mM ammonium formate in water at pH = 3
Mobile phase B	Acetonitrile
ELSD Conditions	
Temperature	40 °C
Pressure	3.5 psi
Data rate	30 Hz

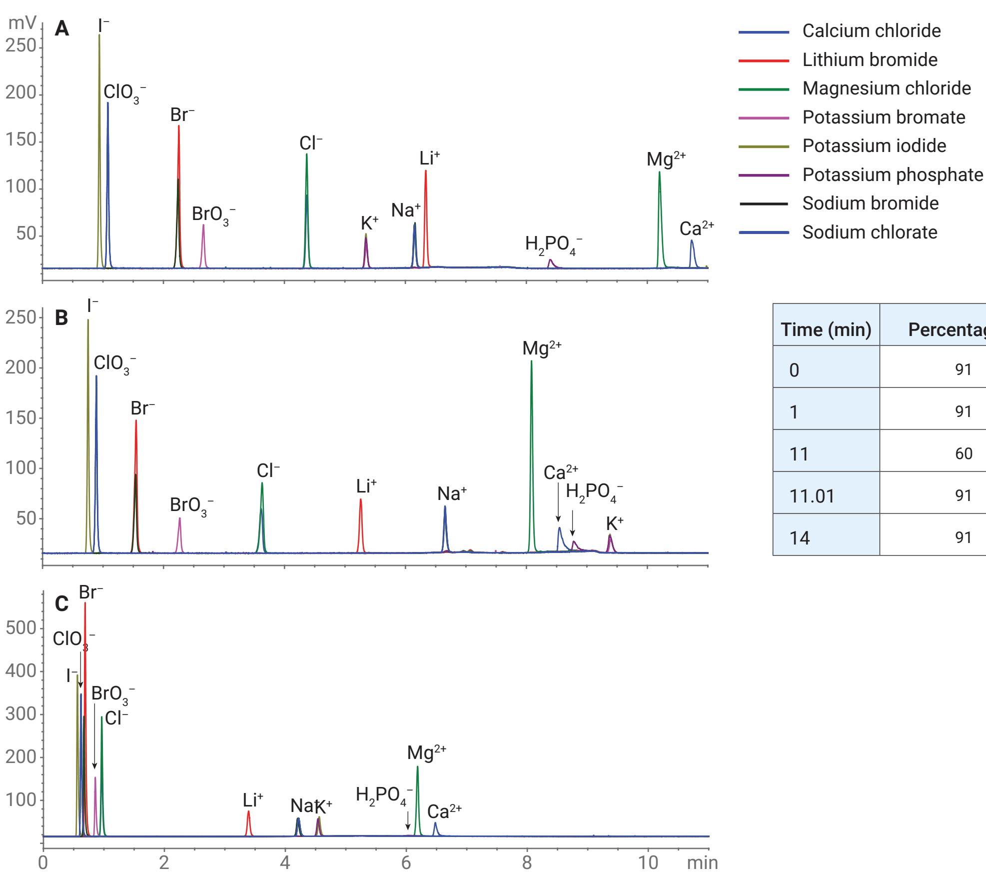
Selectivity Comparison

Each of the three InfinityLab Poroshell 120 HILIC phases give very different selectivities for inorganic ions.

Table 1. Inorganic ion concentration and retention between Agilent InfinityLab Poroshell 120 HILIC phases.

Analyte*	Concentration	Agilent InfinityLab Poroshell 120 HILIC-Z retention time	Agilent InfinityLab Poroshell 120 HILIC-OH5 retention time	Agilent InfinityLab Poroshell 120 HILIC retention time
Chloride (Cl^-)	6.0 mmol/L (0.21 mg/mL)	4.37	3.62	0.97
Chlorate (ClO_3^-)	3.4 mmol/L (0.28 mg/mL)	1.08	0.89	0.62
Bromide (Br^-)	5.0 mmol/L (0.40 mg/mL)	2.26	1.55	0.69
Bromate (BrO_3^-)	2.1 mmol/L (0.27 mg/mL)	2.66	2.27	0.86
Iodide (I^-)	2.7 mmol/L (0.34 mg/mL)	0.94	0.75	0.56
Phosphate ($H_2PO_4^-$)	2.5 mmol/L (0.24 mg/mL)	8.40	8.79	6.05
Lithium (Li^+)	5.0 mmol/L (0.034 mg/mL)	6.34	5.26	3.39
Sodium (Na^+)	3.4 mmol/L (0.078 mg/mL)	6.15	6.65	4.21
Potassium (K^+)	2.7 mmol/L (0.11 mg/mL)	5.35	9.40	4.55
Magnesium (Mg^{2+})	3.0 mmol/L (0.072 mg/mL)	10.20	8.09	6.19
Calcium (Ca^{2+})	2.4 mmol/L (0.096 mg/mL)	10.73	8.55	6.48

*Note: The highest concentration was used for salts with the same cations or anions.



Time (min)	Percentage B
0	91
1	91
11	60
11.01	91
14	91

Figure 1. Selectivity comparison between the Agilent InfinityLab Poroshell 120 HILIC-Z (A), HILIC-OH5 (B), and HILIC (C) columns.

Gradient optimization

The method can be further optimized using a two-part gradient to further improve the speed, peak shape, and separation of critical pairs.

Optimal gradient: Agilent InfinityLab Poroshell 120 HILIC-Z	
Time (min)	Percentage B
0	91
1	91
6	80
11	20
11.01	91
14	91

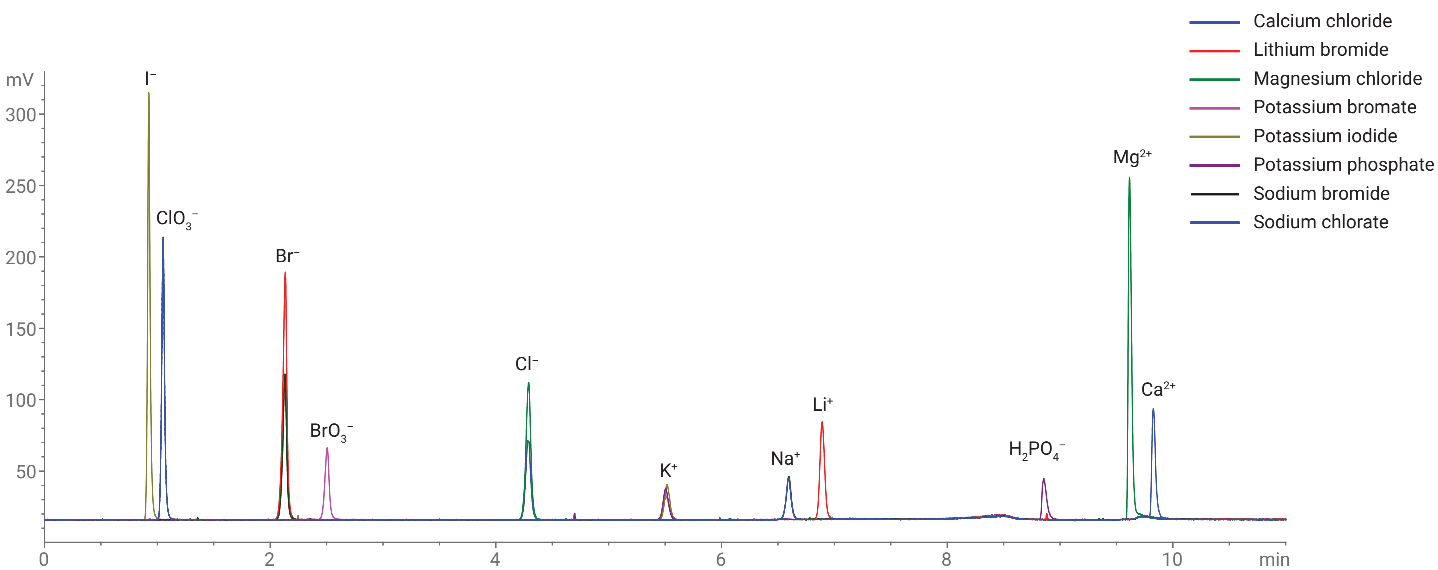


Figure 2. Optimized separation of inorganic ions on the Agilent InfinityLab Poroshell 120 HILIC-Z phase.

Conclusions

An HILIC method for the separation of inorganic ions was successfully developed and used to quickly resolve various cations and anions of interest. The fructan-bonded Agilent InfinityLab Poroshell 120 HILIC-OH5 and zwitterion-bonded Agilent InfinityLab Poroshell 120 HILIC-Z show orthogonal selectivity to each other.

Both the InfinityLab Poroshell 120 HILIC-Z and InfinityLab Poroshell 120 HILIC-OH5 showed significant improvement for retention and separation of most ions, especially anions, over the silica-based HILIC phase.

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