

Analysis of Inorganic Anions Using HILIC Chromatography and the Agilent InfinityLab LC/MSD iQ Mass Selective Detector

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Abstract

This application note describes the analysis of inorganic anions using hydrophilic interaction chromatography (HILIC) with mass detection using the Agilent InfinityLab LC/MSD iQ. This method provides an alternative solution for the separation of polar and ionic compounds besides ion exchange (IEX) or ion chromatography (IC). The method employs a smart and easy-to-use system incorporating the main benefits of the InfinityLab LC/MSD iQ and OpenLab CDS software.

Introduction

Analysis of inorganic anions has always been a challenge in reversed-phase chromatography due to the nature of polar anions in a relatively nonpolar environment. IEX and IC are typically used for small anion analysis, even if they imply longer equilibration and analysis times, require more specialized chemicals and instrument configurations, and are rather challenging to make MS-compatible.

HILIC is a robust and reliable analysis, which can be easily implemented in a laboratory where reversed-phase chromatography is already routinely used.¹ With its recent advancements, HILIC offers an alternative analysis method for polar compounds. Using HILIC, the main advantages over IEX and IC are the simple mobile phase system (typically water-acetonitrile) without any strong acidic or basic additives; simple method setup where no after-treatment such as regeneration or system clean up is needed; and the possibility for the simultaneous analysis of different analytes (cations, anions, and neutral species in a single analytical run).

This work is based on a previously published application note where inorganic anions and cations were simultaneously analyzed with an Agilent 1260 Infinity LC using an Agilent ELSD for detection.² An Agilent InfinityLab Poroshell 120 HILIC-Z column with its zwitterionic chemistry offered the best stationary phase, providing excellent peak shape and minimal secondary interactions for the inorganic anions.

Mass detection was used with the InfinityLab LC/MSD iQ mass selective detector due to the enhanced selectivity and sensitivity over ELSD. As the mobile phase and its additives strongly

influence the detector response, the mobile phase composition has been investigated in detail. In HILIC, increasing the buffer concentration decreases the retention time and provides better peak shape.¹ However, the response of the mass spectrometer detector, and thus the assay sensitivity is also affected by buffer concentration, requiring an optimum balance to be determined for the overall analysis.

Experimental

Standards and chemicals

All reagents and solvents were HPLC or LC/MS grade. Water and acetonitrile were purchased from Agilent Technologies (Santa Clara, CA, USA). Ammonium formate, ammonium hydroxide, and the inorganic ion standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation

The analytical system consisted of the following modules:

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II vialsampler (G7129B) with integrated column compartment with 3 μ L heat exchanger, 40 μ L analytical head, and 20 μ L loop (default setup) and sample thermostat (option #101)
- Agilent InfinityLab LC/MSD iQ (G6160A)

Sample preparation

Inorganic ions were purchased as salts and dissolved in water to generate stock solutions of 1 g/L. The samples for analysis were diluted to a final concentration of 5 μ g/mL and injected individually with no further sample preparation.

Table 1. Information on the investigated inorganic ions.

Inorganic Ions	Retention Time (min)	Target Mass (<i>m/z</i>)	Fragmentor (V)
Chloride	3.262	35	120
Bromide	1.169	79	120
Nitrite	0.782	46	120
Nitrate	0.783	62	100
Iodide	0.574	127	120
Hexafluorophosphate	0.439	145	100

Table 2. Agilent 1290 Infinity II LC method parameters.

Parameter	HPLC Method Value	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 \times 100 mm, 2.7 μ m at 30 $^{\circ}$ C (p/n 685775-924)	
Mobile Phase A	5 mM Ammonium formate in water	
Mobile Phase B	Acetonitrile	
Gradient	Time (min)	%B
	0.00	95
	2.00	95
	11.00	60
	11.01	95
14.00	95	
Stop Time	14 min	
Post-Time	3 min	
Flow Rate	0.4 mL/min	
Injection Volume	1 μ L	

OpenLab CDS

Agilent OpenLab CDS software was used for data acquisition, processing, and reporting. OpenLab CDS provides compliance features that support data integrity in accordance with US FDA 21 CFR Part 11, EU Annex 11, and other similar regulations. The 1290 Infinity II LC and InfinityLab LC/MSD iQ are designed to ensure reliable and robust LC/MS for routine applications in GxP laboratories.

Results and discussion

Analysis of inorganic anions using mass detection

The optimum MS parameters needed to be found to achieve the highest sensitivity with the InfinityLab LC/MSD iQ system for the analysis. Based on empirical observation, the gas temperature did not have a significant effect on the signal intensity, and neither did the capillary voltage – only a slight influence could be observed. The fragmentor voltage, however, showed a rather significant impact on the results. In general, higher fragmentor voltage (approximately 120 V) provided better overall intensity. Therefore, an optimal fragmentor voltage needed to be found for each anion in selected ion monitoring (SIM) mode (Table 1).

The influence of the fragmentor voltage is illustrated in Figure 1. Fragmentor voltages of 70, 100, and 120 V are compared for the nitrite ion showing the 120 V signal to be the most abundant.

Example data can be seen in Figure 2, showing the SIM extracted ion chromatograms (EICs) of the targeted anions at a concentration of 5 µg/mL. Chloride (m/z 35), bromide (m/z 79), nitrite (m/z 46), nitrate (m/z 62), iodide (m/z 127), and hexafluorophosphate (m/z 145) could be detected separately this way.

Table 3. Agilent InfinityLab LC/MSD iQ parameters.

Parameter	InfinityLab LC/MSD iQ Method Value
Ion Source	ESI
Polarity And Scan Type	Negative scan and SIM (Dwell time: 43 ms)
Acquisition Mode	Advanced Acquire Mode
Points/S	2
Drying Gas Temperature	350 °C
Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Capillary Voltage	3.0 kV
Fragmentor Voltage	70 to 140 V
Scan Range	m/z 10 to 150

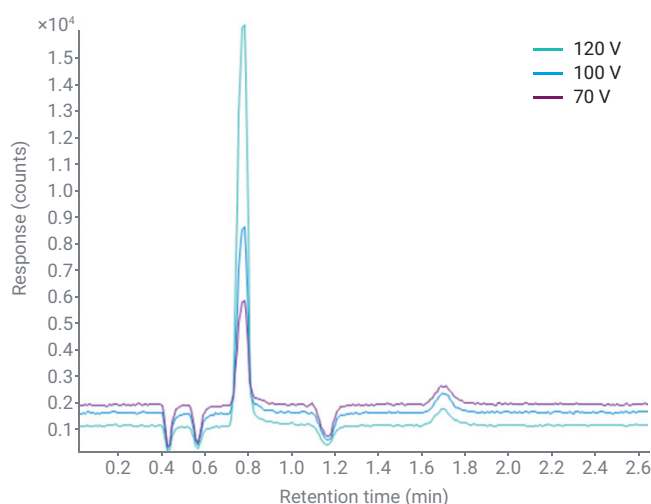


Figure 1. Fragmentor voltages of 70, 100, and 120 V are compared for the nitrite ion (SIM at m/z 46) signal.

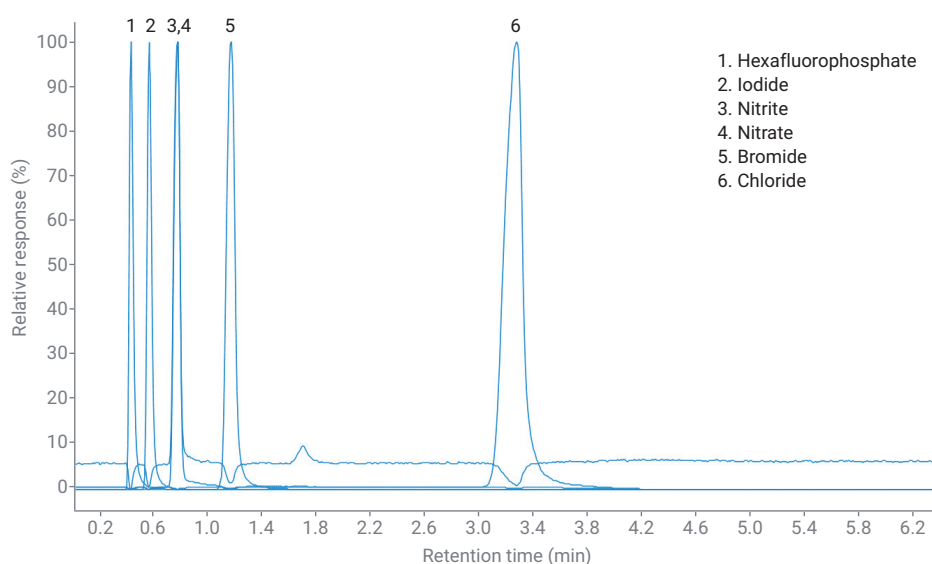


Figure 2. Overlaid SIM EIC chromatograms of targeted anions at 5 µg/g: chloride (m/z 35), bromide (m/z 79), nitrite (m/z 46), nitrate (m/z 62), iodide (m/z 127), and hexafluorophosphate (m/z 145).

When using SIM mode, chromatographically coeluting anions can be detected separately by mass. This way, nitrite and nitrate ions could be differentiated and separately analyzed even though they coelute. Figure 3 illustrates the individual SIM signals of the two anions: nitrite at m/z 46 and nitrate at m/z 62, both eluting at ~ 0.8 minutes.

Investigation of mobile phase composition

In HILIC, the mobile phase additives – therefore the pH value – as well as the buffer concentration have a significant influence on the detector response. The ionization efficiency of the mass spectrometer strongly depends on the mobile phase composition. Different mobile phase systems and buffer concentrations have been investigated to compare the detection sensitivity: 100, 10, and 5 mM ammonium formate as well as 0.3% ammonium hydroxide as the water-based component and acetonitrile with or without 0.3% ammonium hydroxide as the organic-based component.

The comparison can be seen in Figure 4, selecting chloride ion (SIM at m/z 35) as an example. Using 5 mM ammonium formate provided the overall best signal intensity. Using 0.3% ammonium hydroxide did not provide sufficient retention, while 100 mM ammonium formate showed significant ion suppression, therefore weak signal intensity.

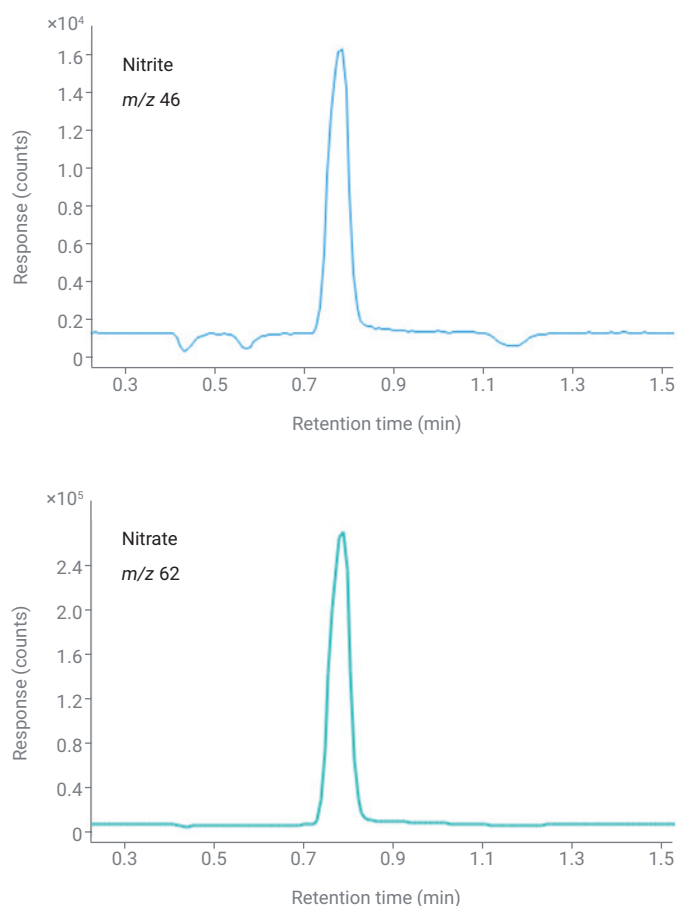


Figure 3. SIM EICs of coeluting anions at ~ 0.8 minutes: nitrite at m/z 46 and nitrate at m/z 62, respectively, at the concentration level of 5 $\mu\text{g/mL}$.

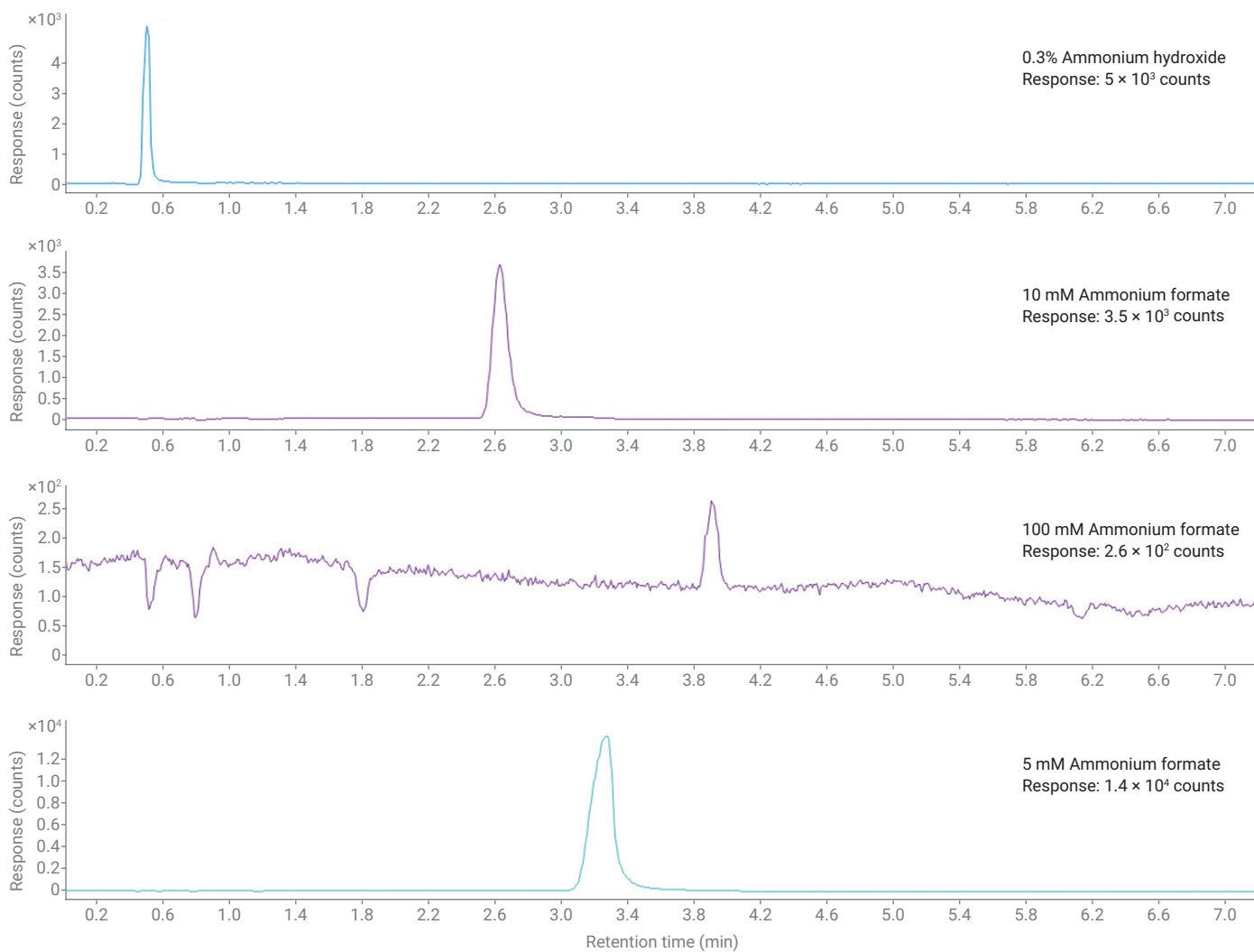


Figure 4. Comparison of the different mobile phases used for the detection of inorganic anions. The results for the chloride ion (SIM at m/z 35) as an example are demonstrated here.

Conclusion

This study demonstrates the successful analysis of several small inorganic anions using a HILIC method on an Agilent 1290 Infinity II LC coupled to an Agilent LC/MSD iQ mass selective detector for selective and sensitive detection. The Agilent InfinityLab Poroshell 120 HILIC-Z column can retain polar ions with typical reverse phase systems and all the ions were baseline separated except for the nitrite and nitrate ions. Nevertheless, the nitrite and nitrate can be easily separated when detecting them separately by mass using the SIM mode of the mass detector. Different mobile phases were investigated, as well as the fragmentor voltages have been optimized to achieve the best signal intensity of the system.

The Agilent InfinityLab LC/MSD iQ mass selective detector is well suited for single- and polyatomic ion analysis where the low mass of analytes can be a struggle to detect.

References

1. Hydrophilic Interaction Chromatography Method Development and Troubleshooting. *Agilent Technologies technical overview*, publication number 5991-9271EN, **2018**.
2. Mack, A.; Bivens, B. Analysis of Metals, Halides, and Inorganic Ions Using Hydrophilic Interaction Chromatography. *Agilent Technologies application note*, publication number 5991-8602EN, **2017**.

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