Application Note

Pesticide Analysis, Food Testing, Water Testing

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Rapid Separation of Paraquat and Diquat Using Hydrophilic Interaction Chromatography (HILIC) with LC/MS Detection

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Abstract

This Application Note describes the use of the next generation Agilent InfinityLab Poroshell 120 HILIC-Z phase chemistry to separate a particularly challenging paraquat and diquat pair using Hydrophilic Interaction Chromatography (HILIC). The two quaternary ammonium groups present on paraquat and diquat make these compounds highly polar; therefore, they are not easily retained or separated by reversed-phase chromatography. Previously, ion pairing agents were commonly used to induce retention of these compounds. However, this technique suffers from several limitations, such as system contamination, high background, and signal suppression, all of which are eliminated using HILIC columns.

Introduction

Reversed-phase chromatography uses a nonpolar stationary phase, which prevents the retention and separation of many polar pesticides.

Ion pairing is the more traditional technique used to retain and separate polar compounds. However, this technique comes with two major disadvantages:

- Ion pairing agents can contaminate LC/MS systems for an extended time after the agent has been used, resulting in high background and altered column selectivity.
- Operating in the mode that analyzes the ion pairing agent results in significant background and analyte signal suppression; therefore, MS analysis is limited to one ionization mode.

Conversely, HILIC is a technique that is capable of retaining and separating polar compounds without the issues caused by ion-pairing, while also using the same system and solvents as reversed-phase chromatography.

The strong positive charge on paraquat and diquat makes them a challenge for HILIC, because any interaction with the silanols on the surface will cause severe tailing and excessively long retention on many columns. The next-generation Agilent InfinityLab Poroshell 120 HILIC-Z overcomes this limitation using a novel zwitterion-based phase to achieve excellent peak shape.

Experimental

Reagents and Chemicals

All reagents were HPLC grade or higher. Ultra LC/MS grade acetonitrile was bought from J. T. Baker (Center Valley, PA, U.S.A.). 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, paraquat standards, and diquat standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Equipment and Materials

- Agilent InfinityLab fittings
  - **Column inlet:** Agilent Quick Connect (p/n 5067-5965)
  - **Column outlet:** Agilent Quick Turn (p/n 5067-5966)
- Agilent 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)
- Eppendorf pipettes and repeater
- Ultracentrifuge (VWR, Radnor, PA, USA)
- Vortexer and multitube vortexers (VWR, Radnor, PA, USA)
- 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)

Instrumentation

- Agilent 1290 Infinity II binary pump (G7120A)
- Agilent 1290 Infinity II Vialsampler (G7129B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent Ultralow dispersion kit for Agilent 1290 Infinity LC Series (p/n 5067-5189)
- Agilent MassHunter workstation software
- Agilent 6470 triple quadrupole LC/MS
- Agilent Jet Stream Electrospray ionization source

Sample Preparation

The paraquat and diquat standards were mixed to 0.25 mM in water, and analyzed with no further sample preparation.

Mobile Phase Preparation

A 200 mM ammonium formate stock solution was prepared in water and adjusted to pH 3 with formic acid. Mobile phase A (aqueous) was prepared by diluting the stock solution 9:1 in water, and mobile phase B (organic) was prepared by diluting the stock solution 9:1 in acetonitrile (final ionic strength of both mobile phases = 20 mM).

Instrument Conditions

HPLC Conditions	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z 2.1 × 100 mm, 2.7 µm (p/n 685775-924)
Mobile phase A	20 mM ammonium formate in water at pH = 3
Mobile phase B	20 mM ammonium formate at pH = 3 in 90 % acetonitrile
Flow rate	0.80 mL/min
Column temperature	30 °C
Injection volume	0.25 µL
Total run time	8 minutes
Gradient	Time (min) %B 0 80 1 80 5 73 6 80 8 80
MS Conditions	
Ionization mode	ESI Positive
Gas temperature	300 °C
Gas flow	7.0 L/min
Nebulizer	45 psi
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	3,500 V
Nozzle voltage	0 V

Results

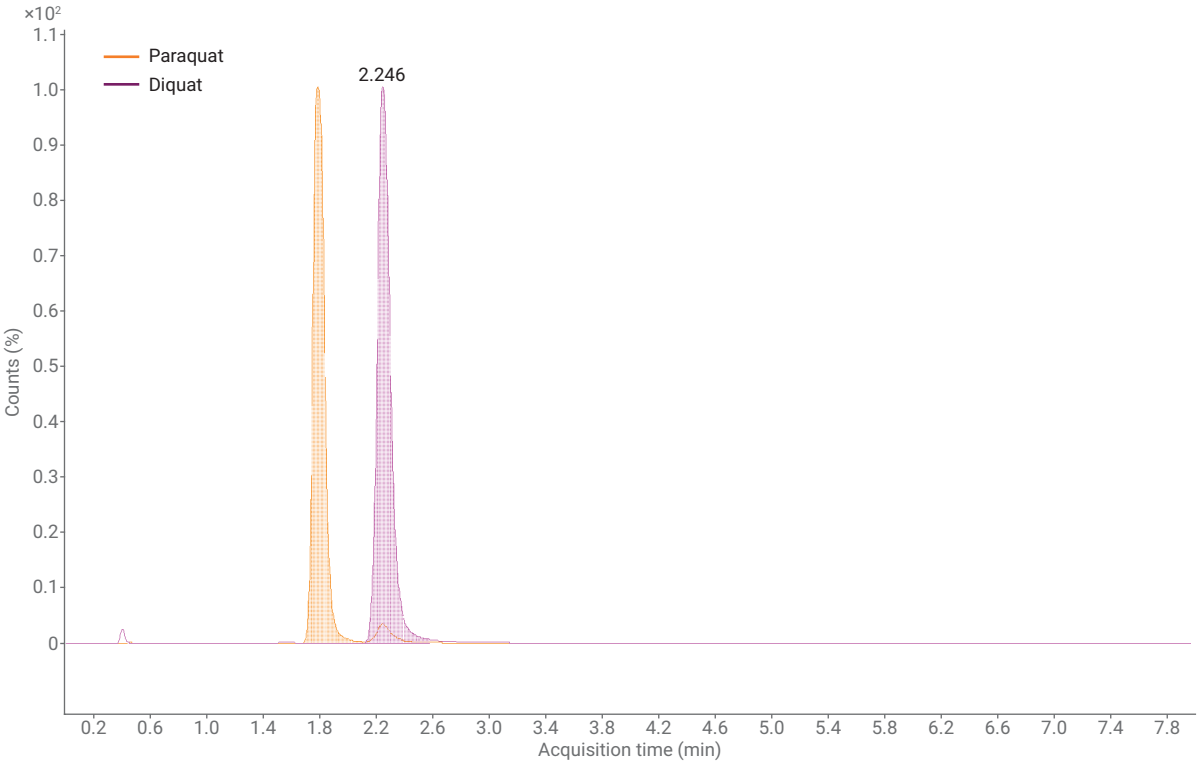

Figure 1. Separation of paraquat and diquat on an Agilent 6470 triple quadrupole LC/MS with an Agilent InfinityLab Poroshell 120 HILIC-Z column.

Conclusions

The Agilent InfinityLab Poroshell 120 HILIC-Z was successfully used to resolve the challenging paraquat-diquat pair with conditions and solvents that did not cause ion suppression or contamination, providing an MS-friendly workflow.

Peaks were baseline-resolved with minimal tailing, demonstrating the importance of a charge-neutral surface, as well as the separating capabilities of InfinityLab Poroshell HILIC-Z columns.

Compared to traditional ion pairing, the HILIC workflow with the InfinityLab Poroshell HILIC-Z gives high-quality chromatography for polar compounds without system contamination or limitations on MS usability.

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Printed in the USA, December 18, 2017
5991-8830EN

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