Technical Overview

Feed Injection at Elevated Pressure Using the Agilent 1290 Infinity III Hybrid Multisampler

Optimization of a multipesticide method for the highest sensitivity through feed injection

Author

Edgar Naegele
Agilent Technologies, Inc.

Abstract

This technical overview demonstrates the capability of the Agilent 1290 Infinity III Hybrid Multisampler to operate at elevated pressure. Capable of operating up to 1300 bar and avoiding peak broadening at high injection volumes by means of the Feed Injection mode, this feature enables the use of longer columns with sub-2-micron material without gradient delay and improved peak shape, supporting automated integration and reliable quantification.

Introduction

Modern analytical methods for the determination of pesticide residues are based on multipesticide methods capable of determining hundreds of pesticides in a sample in one analytical LC/MS/MS run. For chromatographic separation of these pesticides, modern separation columns are used. Their dimensions are typically 2.1 × 150 mm, filled with 1.9 µm particle size material. This requires that the LC can work at high pressures up to 1300 bar. The Agilent 1290 Infinity III Hybrid Multisampler can operate within this pressure range. In addition, the Hybrid Multisampler can work in two different modes, the classic flow through mode and the Agilent Feed Injection mode. Feed Injection mode helps to prevent peak broadening and splitting effects as they can occur with the classic flow through mode for injection of samples in solvents with high elution strength and higher injection volumes.

Experimental

Instrumentation

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Hybrid Multisampler (G7137B)
- Agilent 1290 Infinity III MCT (G7116B)
- Agilent Ultivo Triple Quadrupole LC/MS with Agilent Jet Stream source

Software

- Agilent MassHunter Acquisition Software (v. 12.2)
- Agilent MassHunter Qualitative Analysis Software (v. 12.0)
- Agilent MassHunter Quantitative Analysis Software (v. 12.1)

Column

Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 × 150 mm, 1.9 µm (part number 699675-912)

Chemicals

- 5 M ammonium formate solution (G1946-85021)
- Formic acid for LC/MS (G2453-85060)

Standards

The standards used included the Canada Cannabis Pesticide Kit (2020), in 5 × 1 mL submixes (part number PST-CBS-CAN).

Method parameters

Table 1. UHPLC method parameters.

Parameter	Value
Flow Rate	0.6 mL/min
Solvents	A) 5 mM Ammonium formate + 0.1 % formic acid in water B) 5 mM Ammonium formate + 0.1 % formic acid in methanol
Gradient	Time (min) %B 0.00 5 8.00 95 9.00 100 Stop time: 9 min Post time: 2 min
Column Temperature	55 °C
Needle Wash	3 sec acetonitrile
Injection Volumes	2, 5, 10 µL
Feed Speed	60 µL/min
Overfeed Volume	4 µL

Table 2. MS method parameters.

Parameter	Value
Acquisition Mode	dMRM. All transitions with molecular weights, fragments, voltages, and collision energies are given in application note 5994-0429EN[1]
Polarity	Positive or Negative (compound-dependent)
Capillary Voltage	4,000 V in positive mode, 3,000 V in negative mode
Drying Gas Flow	10 L/min
Drying Gas Temperature	200 °C
Nebulizer Pressure	35 psi
Sheath Gas Temperature	200 °C
Sheath Gas Flow	10 L/min
Nozzle Voltage	300 V (either polarity)
Q1 and Q2: Resolution	Unit (0.7 amu), optimized by autotune
Delta EMV	0 V

Calibration

From a 1 ppm stock solution comprising all pesticides, the following concentrations were diluted for preparing calibration curves: 100, 20, 10, 2.0, 1.0, 0.2, 0.10, and 0.02 ppb. As dilution solvent, acetonitrile was used.

Solvents

- Agilent InfinityLab Acetonitrile for LC/MS (part number 5191-5101-001)
- Agilent InfinityLab Water for LC/MS (part number 5191-5121-001)

Results and discussion

Calibration curves for a multipesticide method were acquired to demonstrate the possible improvement of peak shape and the lower limits of quantification (LOQ) using Agilent Feed Injection mode in comparison to classical flow through injection mode. This method comprised approximately 120 pesticide compounds measured by dynamic MRM. The separation was performed on a 150 mm length column. With the chosen solvents and flow rate, a pressure range above 1000 bar was necessary. The calibration curves were acquired for concentrations down to 0.02 ppb (20 ppt), and each calibration point was measured three-fold. The concentrations of the calibration solutions were prepared by dilution using the strong-eluting solvent acetonitrile and injected directly by both injection modes with different injection volumes. An example chromatogram showing the elution pattern for the MRM transitions is displayed in Figure 1.

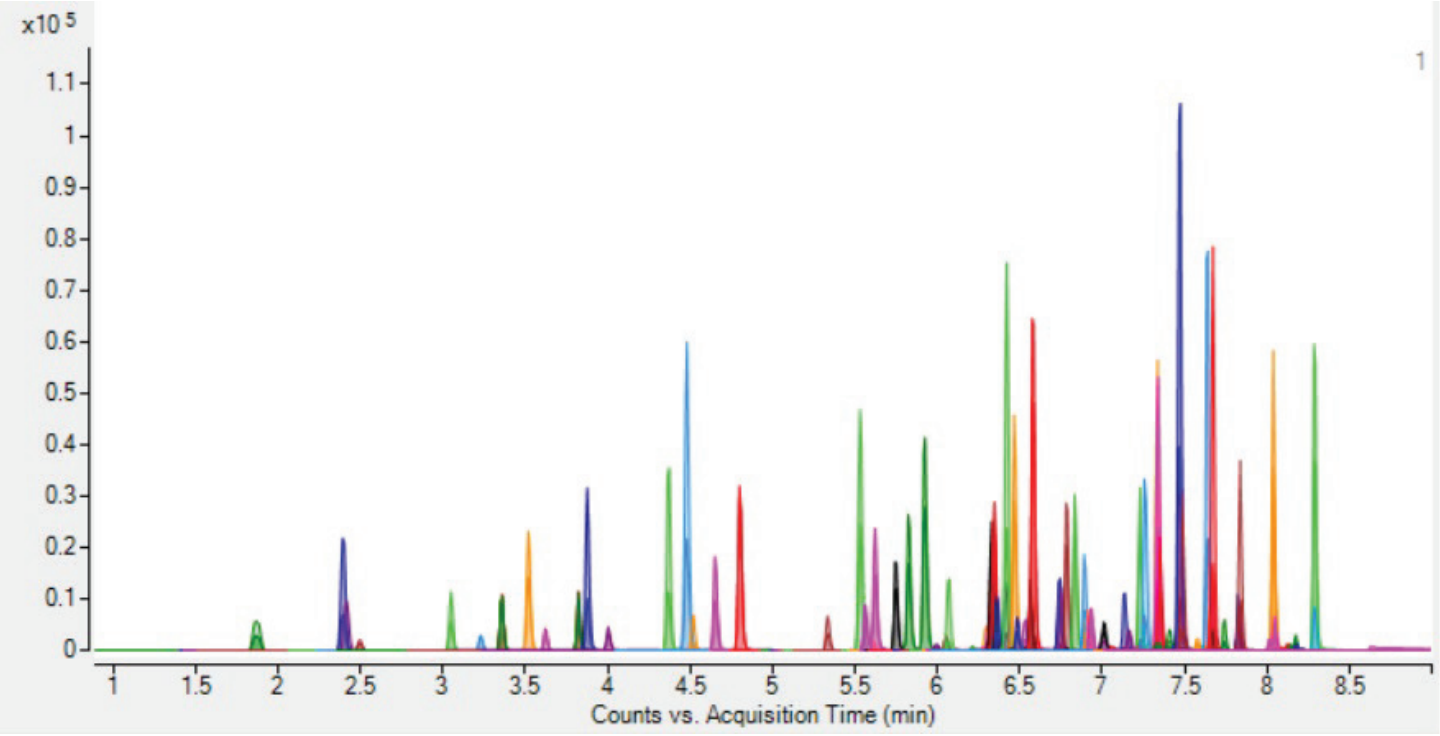

Figure 1. MRM of the multipesticide method acquired by Feed Injection mode with a 2 µL injection at a concentration of 10 ppb.

To demonstrate the effect of the injection modes at different injection volumes, five peaks were selected with a distance in elution time of approximately 0.5 to 1 minute apart. The first eluting compound was Dinotefuran, while the last selected one was Propoxur. In this retention time range (up to about 4.5 minutes), 25 compounds were eluting and represent half of the total run time. The selected peaks injected with both injection modes at a volume of 2 µL are shown at a glance in Figure 2. For this injection volume, peak shapes achieved using both injection modes are comparable, except for the first eluting compound that showed a slight shoulder in flow through mode.

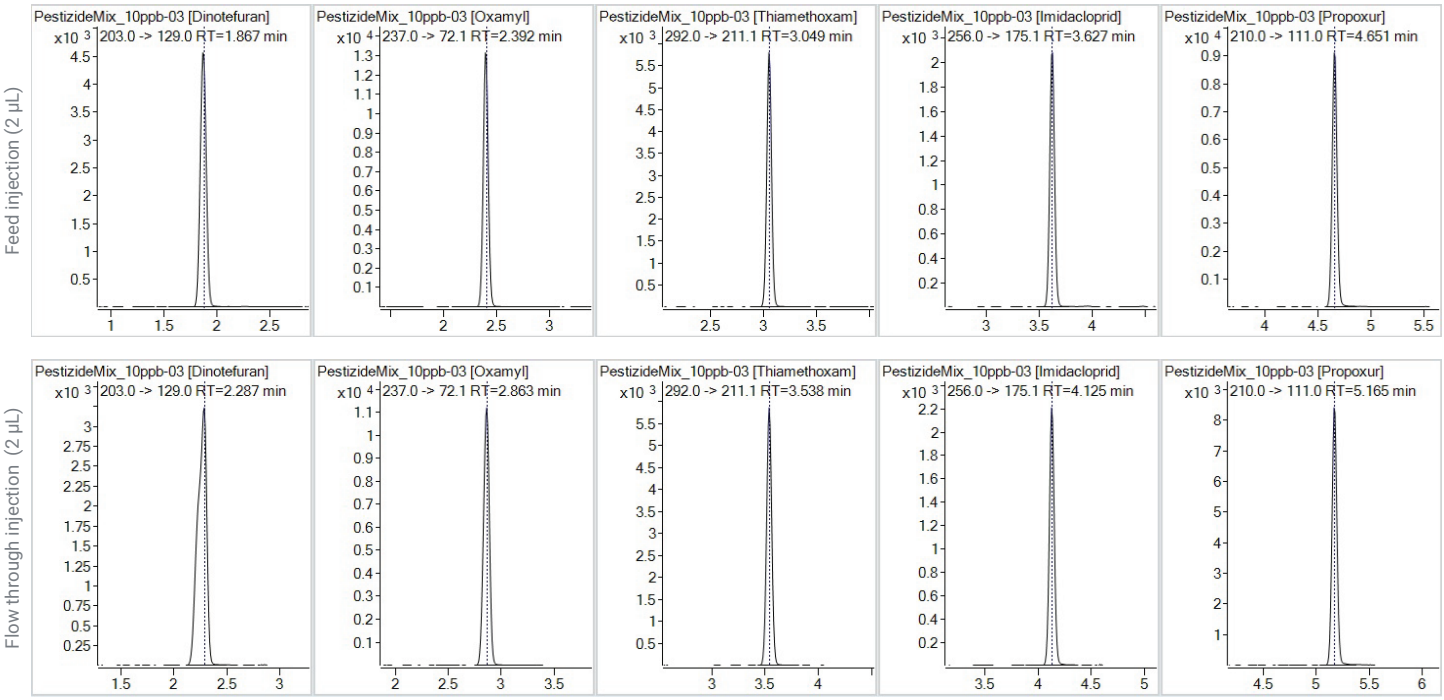

Figure 2. Calibration point at 10 ppb injected in Feed Injection mode (top row) and classical flow through mode (bottom row) at an injection volume of 2 µL. The shift in retention time is due to a larger delay volume in flow through mode.

With an increase of the injection volume to 5 µL for both injection methods, the influence of the larger volume of high elution strength solvent can be seen for the peak shapes obtained in flow through mode (Figure 3, bottom row). The first peak of the compound Dinotefuran showed a clear split, and Oxamyl, the second compound, showed the beginning of a split. The third peak for the compound Thiamethoxam showed a fronting shoulder, and the later eluting compounds were not affected by the solvent effect. For Feed Injection mode, there was no visible influence on peak shape under these conditions (Figure 3, top row). Generally, the peaks obtained under Feed Injection mode showed larger peak heights and a higher signal-to-noise ratio.

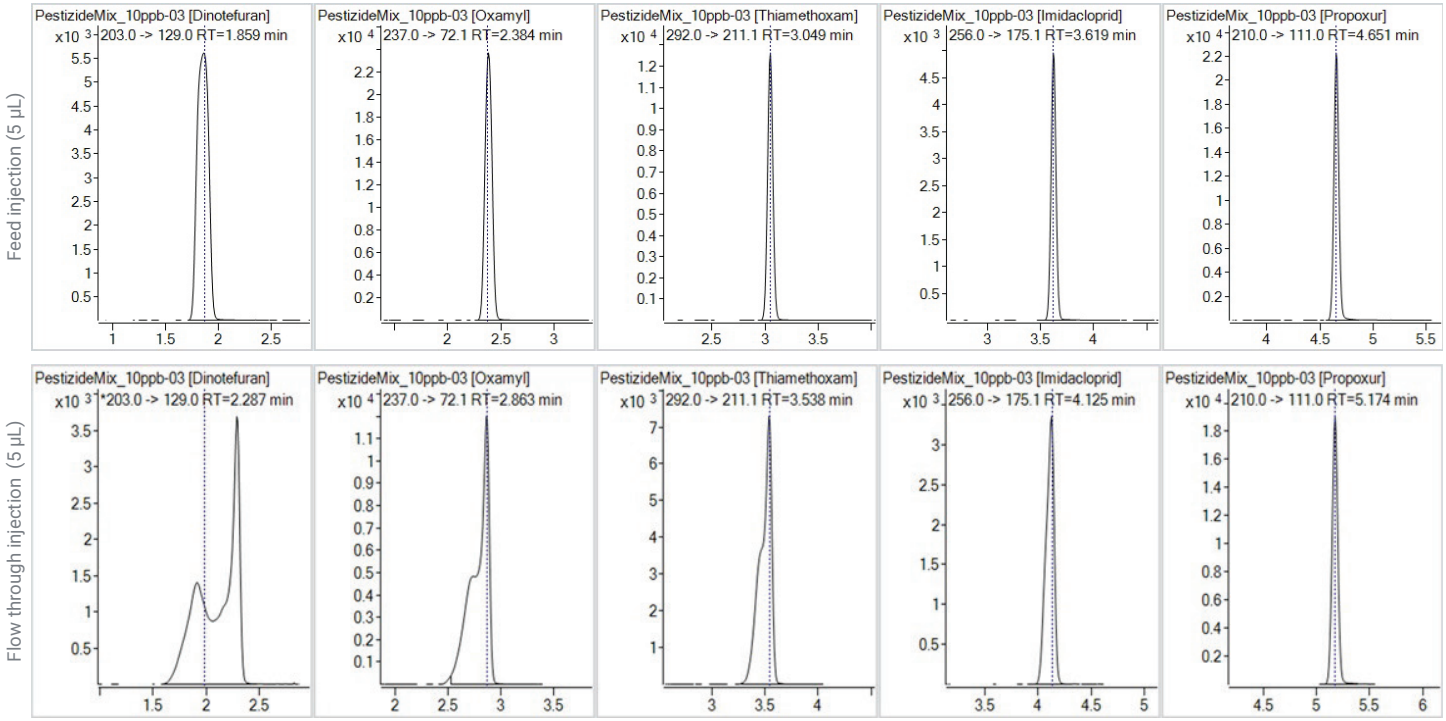

Figure 3. Calibration point at 10 ppb injected in Feed Injection mode (top row) and classical flow through mode (bottom row) at an injection volume of 5 µL.

Enlarging the injection volume up to 10 µL, the influence on peak shape for classical flow through injection can be seen for all compounds (Figure 4, bottom row). The peak of the first eluting compound was even breaking through the retention, partially leaving the MRM window with a loss for quantification. The second peak had broad split fronting, and later eluting peaks suffered from large fronting. These effects resulted in lower sensitivity and caused problems for automated integration without manual intervention. In contrast, for Feed Injection mode, there was only an influence of peak broadening on the first two eluting compounds. No other peaks showed a split or shoulder (Figure 4, top).

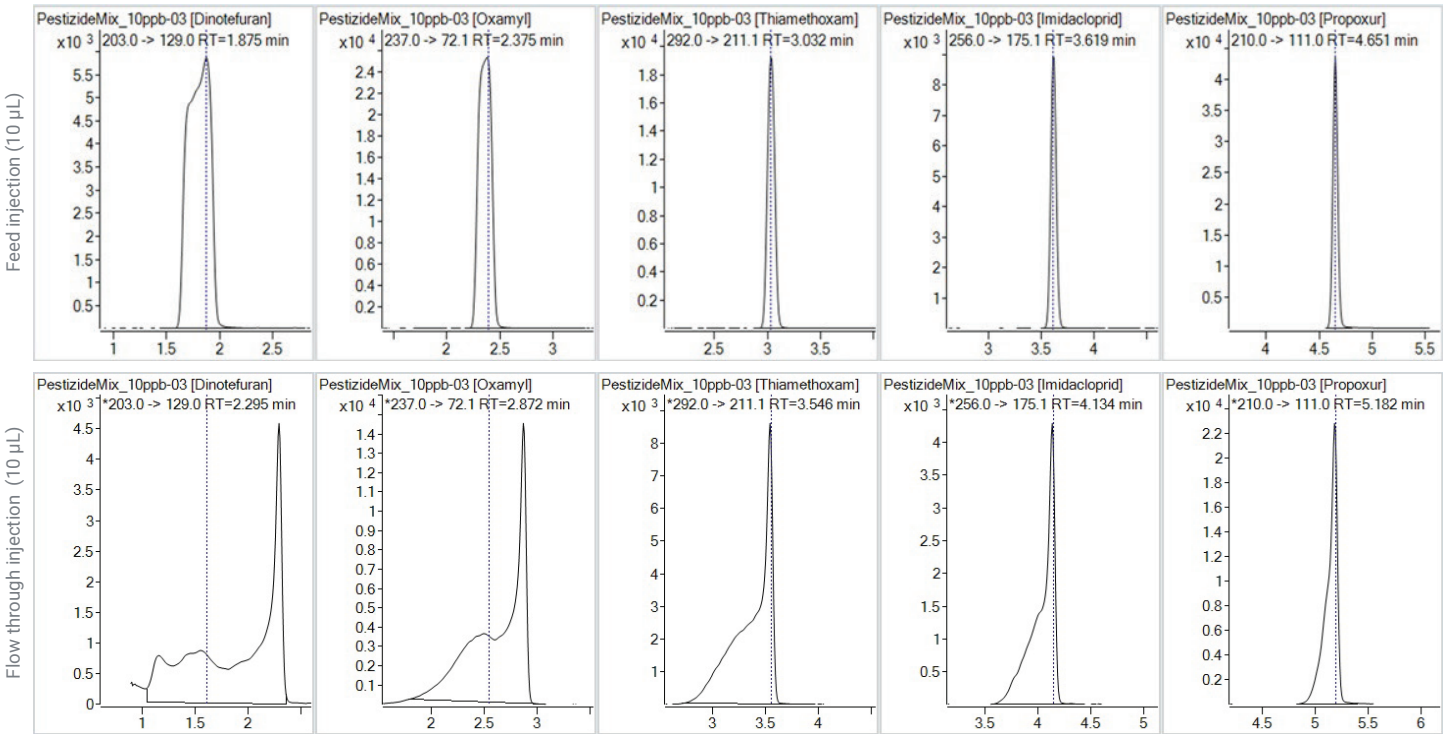

Figure 4. Calibration point at 10 ppb injected in Feed Injection mode (top row) and classical flow through mode (bottom row) at an injection volume of 10 µL.

For a more quantitative view, the LOQs were calculated from the calibrant point closest to a signal-to-noise ratio of 10 (Figure 5). At an injection volume of 2 µL, the LOQs obtained for Feed Injection mode and flow through injection mode were in the same order. For the injection of 5 µL, Feed Injection mode showed an improvement in obtained LOQ by about a factor of 2 for the first compound and up to a factor of 5 for later eluting compounds. For the 10 µL injection, Feed Injection mode showed an improvement in sensitivity by a factor of 3 for Dinotefuran and up to a factor of 25 for the later eluting compounds. Compounds like Propoxur or later eluting ones showed a similar sensitivity for the applied injection volume.

In case of the presence of pesticides more polar than Dinotefuran, lower injection volumes must be applied. This has already been demonstrated in application note 5994-0429EN.[2]

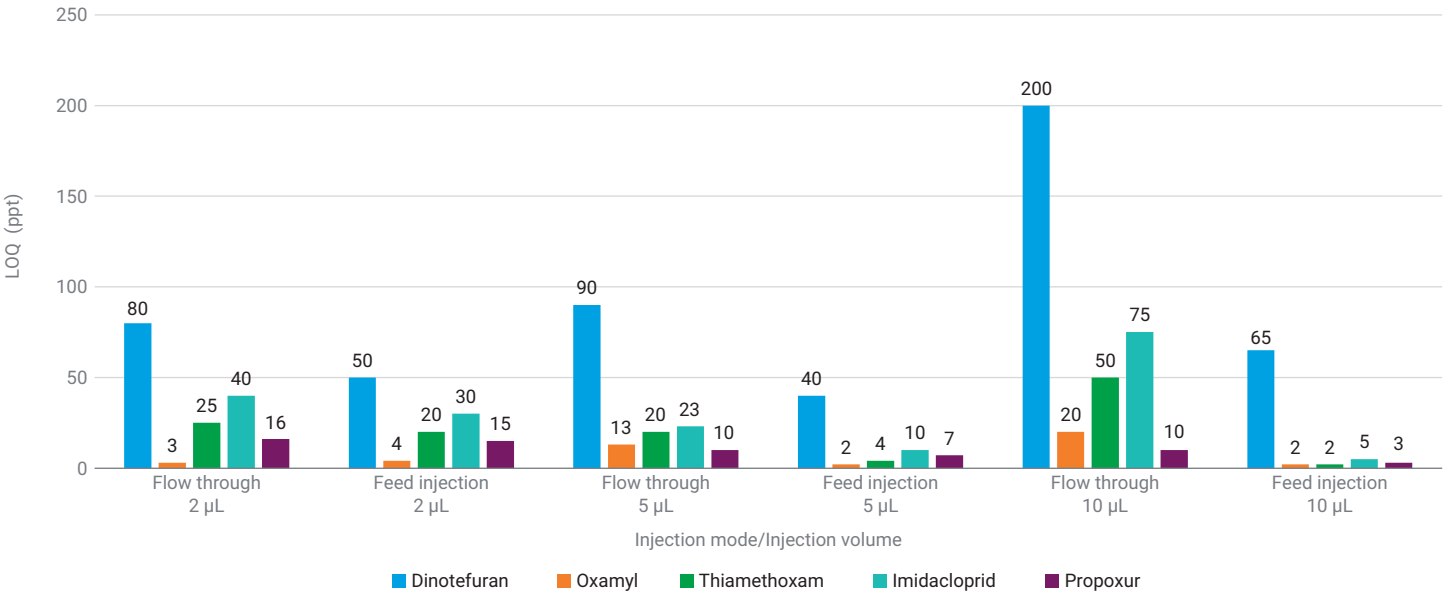

Figure 5. Limits of quantification (ppt) in comparison to injection mode and injection volume.

Conclusion

This technical overview demonstrates the use of the Agilent Feed Injection mode available with the Agilent 1290 Infinity III Hybrid Multisampler in comparison to the classical flow through injection mode for the analysis of pesticides by a multipesticide method. We demonstrated that the use of Agilent Feed Injection mode improves the obtained peak shape for injection of samples dissolved in high elution strength solvent compared to classical flow through mode. With the improved peak shape obtained by Feed Injection mode, signal to noise ratios were improved, resulting in higher sensitivity. Due to retained peak shape, no manual interaction for peak integration was necessary when using feed injection.

References

1. Roy, J-F.; Deckers, C.; Honnold, R.; Stone, P.; Hitchcock, J.; Macherone, A.; Geiling, B; Young, S. A Sensitive and Robust Workflow to Measure Residual Pesticides and Mycotoxins from the Canadian Target List in Dry Cannabis Flower. *Agilent Technologies application note*, publication number 5994-0429EN, **2020**.
2. Naegele; E. Improved Peak Shape and Lower LOQs in Pesticide Analysis. *Agilent Technologies application note*, publication number 5994-6125EN, **2024**.

www.agilent.com

DE-007903

Printed in the USA, July 1, 2025
5994-8478EN

Agilent
Trusted Answers