Application Note

Food Testing and Agriculture Pesticides

Agilent

Trusted Answers

Enhanced Sensitivity in Multiresidue Pesticide Analysis

Using LC/MS/MS with high-pressure Feed Injection

Authors

Peter Kornas and
Edgar Naegele
Agilent Technologies, Inc.

Abstract

High-organic QuEChERS extracts often cause solvent-strength-related distortions such as peak broadening and splitting, particularly for early-eluting compounds. These challenges are compounded when using long, sub-2 µm particle columns operated at elevated pressure to achieve the required separation efficiency. Feed Injection introduces the sample directly into the mobilephase stream rather than into a fixed loop, creating dilution that mitigates solvent-mismatch effects. This study evaluates how Feed Injection improves peak shape, response linearity, and sensitivity in a multiresidue pesticide workflow monitoring > 1,500 transitions. Improved peak shapes enable reliable automated integration for quantification at lower sensitivity with less manual interaction.

Introduction

The determination of pesticides in food matrices such as fruits and vegetables is commonly performed using the QuEChERS sample preparation method. As a result, target analytes are typically extracted into acetonitrile, which introduces challenges during subsequent LC/MS/MS analysis. Owing to the high elution strength of acetonitrile, pronounced solvent effects may occur in reversed-phase chromatography, particularly for early-eluting compounds, leading to peak broadening or peak splitting. Modern pesticide residue analysis relies on comprehensive multiresidue methods capable of detecting hundreds of analytes within a single LC/MS/MS run, placing high demands on chromatographic performance.[1] To achieve sufficient separation efficiency, columns with an inner diameter of 2.1 mm, lengths up to 150 mm, and sub-2 µm particle sizes are typically employed. The use of such high-performance columns requires liquid chromatography systems that can reliably operate at pressures exceeding 1000 bar.

This application note demonstrates the use of the Agilent 1290 Infinity III Hybrid Multisampler, which can operate up to 1,300 bar, to overcome unwanted solvent effects by offering two injection modes: classical flow-through injection and Feed Injection. In Feed Injection mode, the sample is introduced directly into the mobile phase stream via a dedicated valve, enabling controlled dilution and analyte focusing prior to chromatographic separation. This effectively minimizes solvent mismatch effects and prevents peak broadening or splitting, resulting in improved peak shape, enhanced sensitivity, and reliable automated peak integration.

Experimental

LC/MS/MS system

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Hybrid Multisampler (G7137B)
- Agilent 1290 Infinity III Multicolumn Thermostat (G7116B)
- Agilent 6495D triple quadrupole LC/MS (G6495D)

Dynamic MRM transition

The development of dynamic MRM transitions for 764 pesticide compounds was based on the Agilent Pesticide tMRM Database for LC/TQ systems (G1733CA).[2]

Column

Agilent ZORBAX Rapid Resolution High Definition Eclipse Plus C18 column, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)

Software

Agilent MassHunter Acquisition Software for LC/MS systems (version 12) and Agilent MassHunter Quantitative Analysis (version 12)

Standards

- Agilent LC/MS Pesticide Comprehensive test mix (p/n 5190-0551)
- Agilent Custom Pesticide test mix (p/n CUS-00000635 to CUS-00000643, CUS-000004663)
- AccuStandard Custom Pesticide standards (p/n S-96086-01 to S96086-10)

LC/MS/MS method

Parameter	Value
Mobile Phase A	Water + 5 mM ammonium formate + 0.1 % formic acid
Mobile Phase B	Methanol + 5 mM ammonium formate + 0.1 % formic acid
Flow Rate	0.4 mL/min
Gradient	0 min: 5% B; 0–3 min: 30% B; 3–17 min: 100% B; 17–20 min: 100% B
Post Time	3 min
Feed Injection	Feed speed: 10 µL/min (fixed), automatic overfeed volume (4.4 µL), Flush Out Solvent: Mobile Phase A (S2)
Needle Wash	1. Methanol (S1), 3 sec 2. Acetonitrile (S3), 3 sec Injection path cleaning inner wash mode: Off
Reconditioning	Mobile phase A (S2)
Column Temperature	40 °C

MS parameters

Parameter	Value
Source	AJS
Ionization Mode	Positive/negative
Drying Gas	200 °C, Flow: 12 L/min
Sheath Gas	400 °C, Flow: 12 L/min
Nebulizer	35 psi
Nozzle Voltage	0 V (positive/negative)
Capillary Voltage	+2500 V/–3000 V
Scan Type	Dynamic MRM (dMRM)
Total MRMs	1,590

Sample preparation

- 10 ± 0.1 g of homogenized organic spinach was weighed into a 50 mL centrifuge tube. A QuEChERS extraction was performed by adding 10 mL acetonitrile and shaking for 2 minutes with a mechanical shaker.
- Then, QuEChERS extraction salts (p/n 5982-5650CH) were added, followed by another shaking and centrifugation.
- A pesticide standard mix was added to the supernatant to achieve a final concentration of 1 ng/mL (equivalent to 10 µg/kg).The postspiked extracts were directly used for LC/MS/MS analysis.

Chemicals and reagents

Agilent InfinityLab Acetonitrile for LC/MS (p/n 5191-5101*), Agilent InfinityLab Methanol for LC/MS (p/n 5191-5111*), Agilent InfinityLab Water for LC/MS (p/n 5191-5121), and 5M ammonium formate solution (p/n G1946-85021) were used for the study. LC/MS-grade formic acid was purchased from VWR. All other solvents used were HPLC grade and were procured from Sigma-Aldrich.

* Only available in select countries

Results and discussion

A spinach QuEChERS extract fortified with a multiresidue pesticide standard mixture was analyzed using injection volumes ranging from 0.2 to 2 µL. The resulting separation of 764 pesticides with more than 1,500 transitions separated in a 20-minute run is shown in Figure 1. Due to the fact that this analysis was performed using classical flow-through injection, some early-eluting compound peaks show peak broadening and peak splitting.

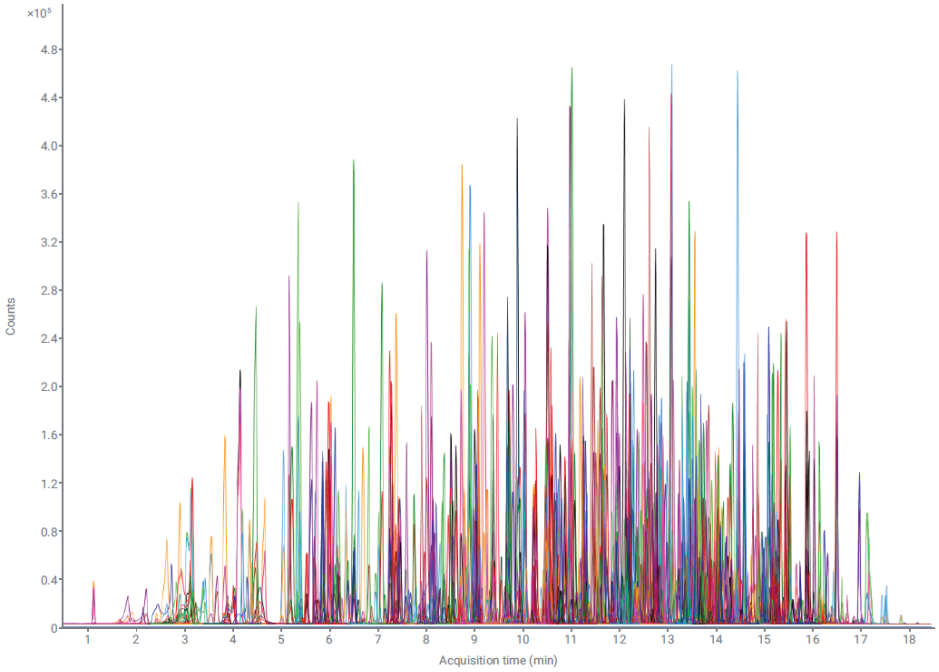

Figure 1. Overlay of MRM chromatograms of 764 pesticides injected by means of classical flow-through injection (10 µg/L, injection volume: 2 µL).

Classical flow-through injection

Within the injection volume range of 0.2 to 1 µL, no significant peak broadening was observed except for pymetrozine, which showed early signs of peak distortion. When the injection volume was increased to 2 µL, noticeable peak broadening and, in some cases, peak splitting were observed for early-eluting compounds, caused by solvent effects at higher injection volumes (Figure 2).

When evaluating peak area and peak height as a function of injection volume, a clear linear relationship between injection volume and peak area was observed across the investigated range (Figure 3). In contrast, peak height does not increase proportionally at higher injection volumes; instead, a saturation effect becomes evident in the range between 1 and 2 µL (Figure 4).

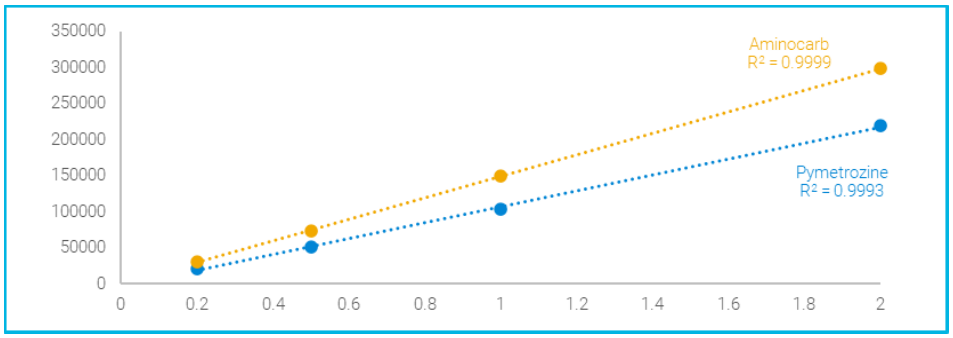

Figure 3. Flow-through injection peak area versus injection volume (µL) for aminocarb and pymetrozine.

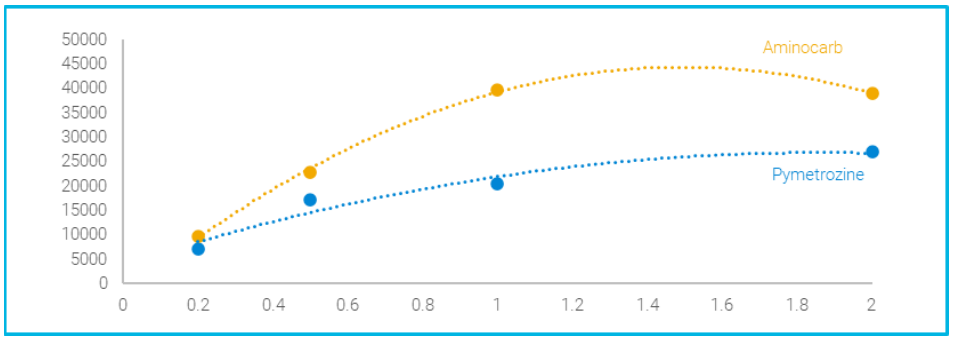

Figure 4. Flow-through injection peak height versus injection volume (µL) for aminocarb and pymetrozine.

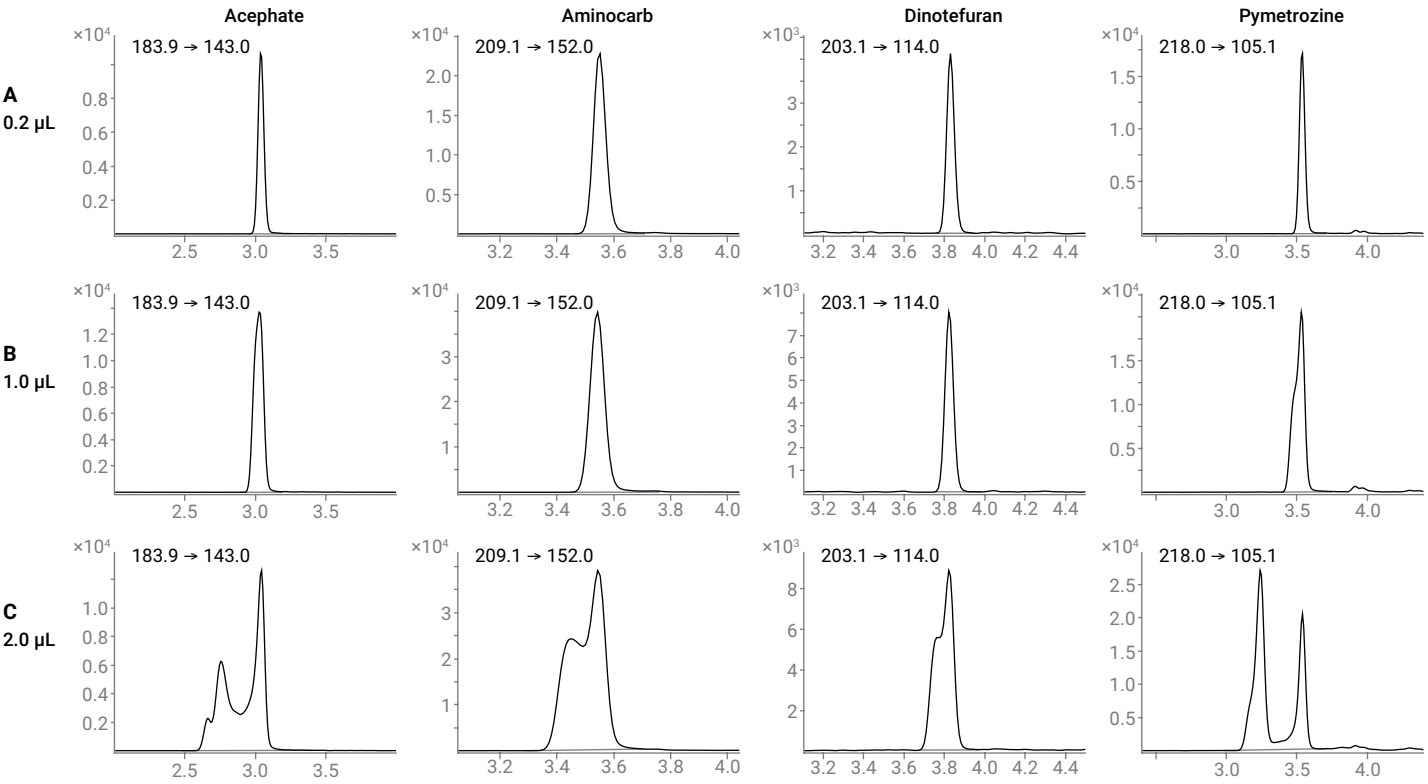

Figure 2. Chromatograms of flow-through injections with different injection volumes (A) 0.2 µL, (B) 1.0 µL, and (C) 2.0 µL of the highly polar, early-eluting pesticides acephate, aminocarb, dinotefuran, and pymetrozine.

While increasing the injection volume continues to result in a linear increase in peak area, the chromatographic peaks do not become more intense, but rather exhibit increased peak broadening, indicating a limitation in peak capacity at higher injected amounts.

Feed Injection

The same spinach QuEChERS extract and the same range of injection volumes were applied for the measurements using the 1290 Infinity III hybrid multisampler. A feed speed of 10 µL/min was applied, corresponding to an effective 1:40 dilution of the sample with the mobile phase before entering the column. Under these conditions, no solvent-related effects were observed in the chromatograms, even when higher injection volumes were used, indicating efficient sample dilution with mobile phases and stable chromatographic performance (Figure 5).

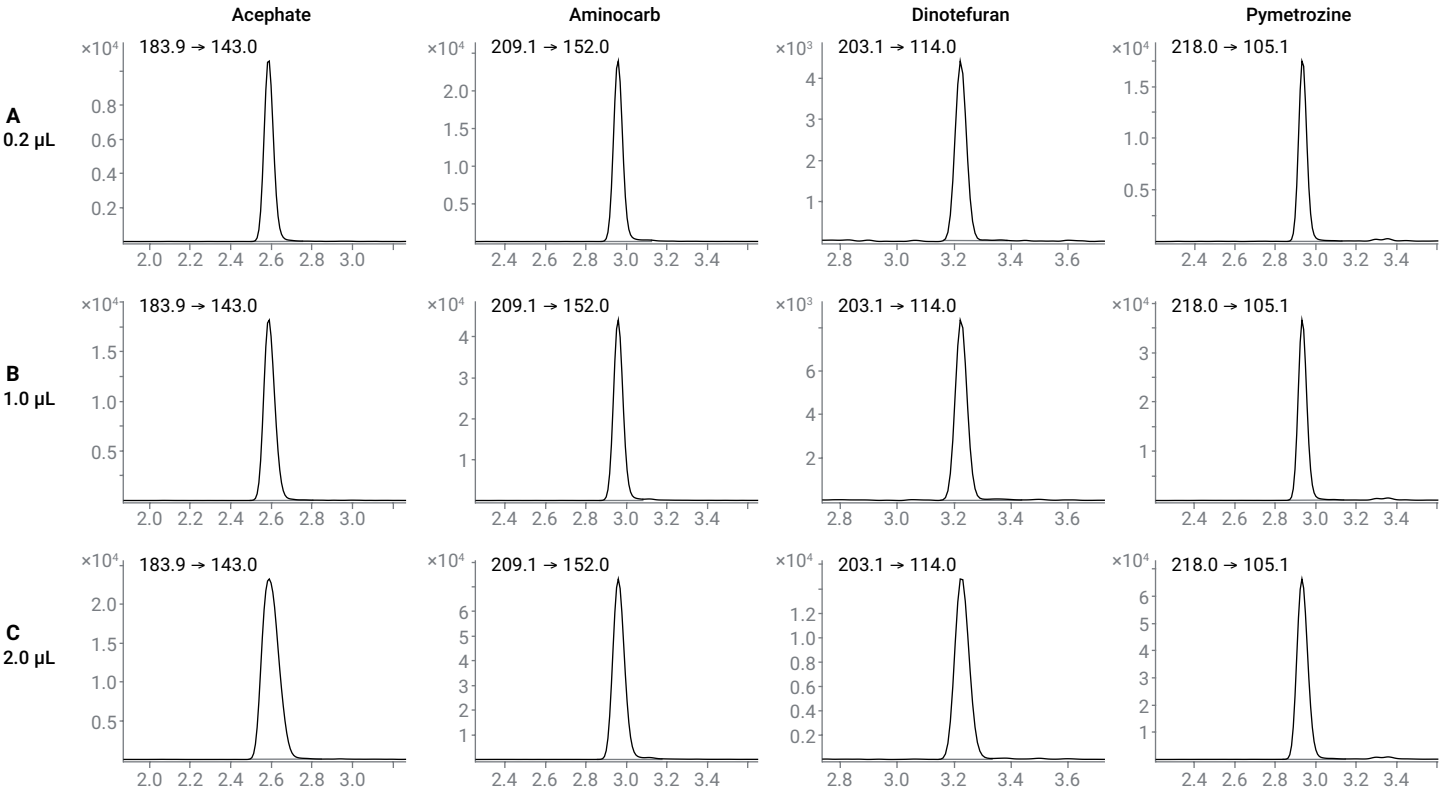

Figure 5. Chromatograms for Feed Injection with different injection volumes (A) 0.2 µL, (B) 1.0 µL, and (C) 2.0 µL of the highly polar, early-eluting pesticides qcephate, aminocarb, dinotefuran, and pymetrozine.

This behavior is also reflected in the linear response of both peak area and peak height as a function of injection volume (Figures 6 and 7). Increasing the injection volume from 1 µL to 2 µL results in a proportional doubling of the peak height, demonstrating that no saturation occurs within this range (Figure 7).

The capability to inject larger sample volumes without compromising peak shape results in a substantial enhancement of analytical sensitivity. This produces well-defined, symmetrical peaks, which reduces the need for manual reintegration and facilitates faster, more straightforward data review. This increased sensitivity is particularly beneficial for the reliable detection and quantification of trace-level compounds present at very low concentrations. By enabling lower limits of detection without the need to alter chromatographic conditions or implement additional sample concentration steps, this approach maintains method simplicity and robustness while extending the method's applicability to challenging analytical tasks. Both aspects, the increased sensitivity due to better peak shape for early-eluting pesticides and the injection of increased volumes for later-eluting peaks under UHPLC conditions are detailed in separate application notes.[3,4]

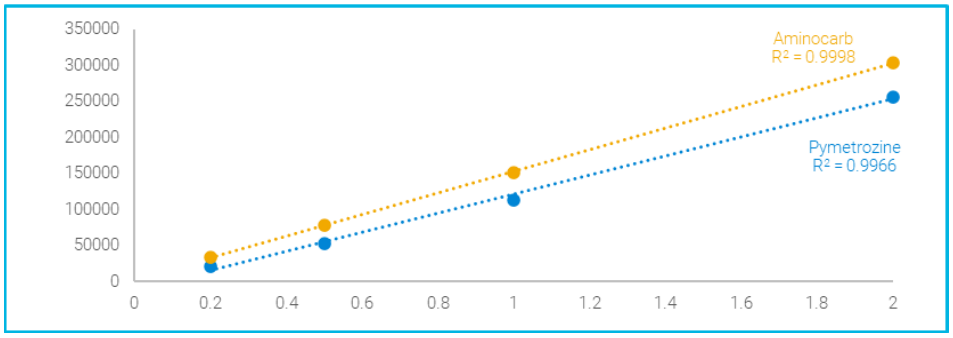

Figure 6. Feed Injection peak area versus injection volume (µL) for aminocarb and pymetrozine.

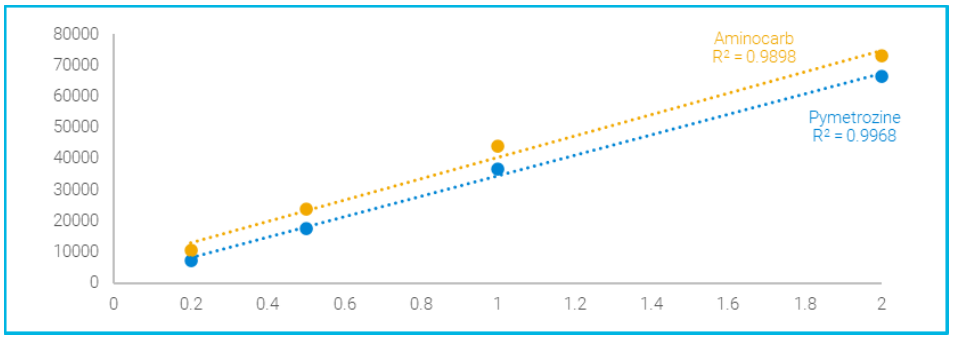

Figure 7. Feed Injection peak height versus injection volume (µL) for aminocarb and pymetrozine.

Conclusion

The Agilent 1290 Infinity III Hybrid Multisampler is designed for ultrahigh-pressure operation and can be used at pressures of up to 1,300 bar. It offers two injection modes: classical flow-through injection and Feed Injection, providing flexibility for different analytical requirements.

When samples are injected using solvents with high elution strength, classical flow-through injection can lead to solvent effects such as peak broadening or peak splitting. In flow-through injection mode, peak height no longer increases proportionally with increasing injection volume beyond a certain limit, indicating peak broadening and break through effects. In contrast, Feed Injection dilutes the sample with the mobile phase during injection and helps to trap analytes in front of the column, effectively minimizing solvent mismatch effects.

As a result, Feed Injection prevents peak broadening and splitting and allows higher injection volumes to be used without compromising peak shape. Feed Injection can therefore significantly increase the sensitivity of the measurement method. In addition, Feed Injection produces well-defined and symmetrical peaks, enabling reliable automated integration and simplifying data review.

References

1. Kornas, P.; Wu, L.; Zhao, H.; Analysis of 764 Pesticides in Tomato Using an Agilent 6495D Triple Quadrupole LC/MS System; *Agilent Technologies application note*, publication number 5994-8004EN, **2025**.
2. Quantitative Analysis of Multiresidue Pesticides in Food Matrices Using Agilent 6470 Triple Quadrupole LC/MS System—Method Protocol; *Agilent Technologies*, **2022**.
3. Naegele, E.; Improved Peak Shape and Lower LOQs in Pesticide Analysis; *Agilent Technologies application note*, publication number 5994-6125EN, **2024**.
4. Naegele, E.; Feed Injection at Elevated Pressure Using the Agilent 1290 Infinity III Hybrid Multisampler; *Agilent Technologies application note*, publication number 5994-8478EN, **2025**.

www.agilent.com

DE-016423

Printed in the USA, August 26, 2026
5994-9396EN

Agilent
Trusted Answers