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Enhanced Sensitivity in Multi-Residue Pesticide Analysis by LC-MS/MS Using High-Pressure Feed Injection

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Introduction

Challenges in Pesticide Analysis

The determination of pesticides in food matrices such as fruits and vegetables is commonly performed using the QuEChERS sample preparation method. As a result of this approach, the target analytes are typically extracted into acetonitrile, which can introduce 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 multi-residue methods capable of detecting hundreds of analytes within a single LC-MS/MS run, placing high demands on chromatographic performance. To achieve sufficient separation efficiency, columns with an internal diameter of 2.1 mm, lengths of 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.

Overcoming Solvent Effects

The Agilent 1290 Infinity III Hybrid Multisampler, which can operate up to 1300 bar, helps overcome unwanted solvent effects by offering two injection modes: classical flow injection and feed injection.
In feed injection mode, the sample is introduced directly into the mobile phase stream via a dedicated valve, enabling controlled on-line dilution 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.

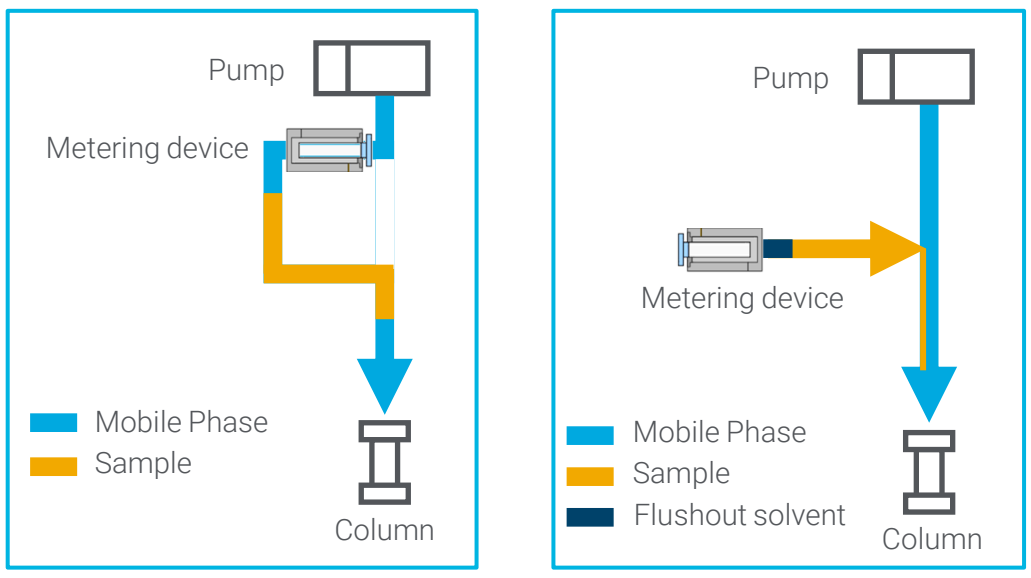


Figure 1. Flow injection (left) vs. feed injection (right)

Conclusions

- The Agilent 1290 Infinity III Hybrid Multisampler is designed for ultra-high-pressure operation and can be used at pressures of up to 1300 bar.
- It offers two injection modes: classical flow injection and feed injection, providing flexibility for different analytical requirements.
- When samples are injected using solvents with high elution strength, classical flow injection can lead to solvent effects such as peak broadening or peak splitting.
- In flow injection mode, peak height no longer increases proportionally with increasing injection volume beyond a certain limit, indicating saturation effects.
- In contrast, feed injection dilutes the sample with the mobile phase during injection, 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. Naegele, E. et al., Feed Injection at Elevated Pressure Using the Agilent 1290 Infinity III Hybrid Multisampler, *Agilent Technologies Application Note,* Publication Number 5994-8478EN, 2025
2. Naegele, E- at al., Improved Peak Shape and Lower LOQs in Pesticide Analysis, *Agilent Technologies Application Note,* Publication Number 5994-6125EN, 2024
3. Kornas, P. et al., Analysis of 764 Pesticides in Tomato Using an Agilent 6495D Triple Quadrupole LC/MS System, *Agilent Technologies Application Note,* Publication Number 5994-8004EN, 2025
4. Quantitative Analysis of Multiresidue Pesticides in Food Matrices Using Agilent 6470 Triple Quadrupole LC/MS system – Method Protocol, 2022

Experimental

LC-MS/MS System

1290 Infinity III High Speed Pump (G7120A)
1290 Infinity III Hybrid Multisampler (G7137B)
1290 Infinity III Multicolumn Thermostat (G7116B)
6495D Tripple Quadrupole LC/MS (G6495D)

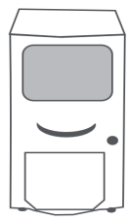
Column

Agilent ZORBAX RRHD Eclipse Plus C18,
2.1 x 150 mm, 1.8 µm (PN 959759-902)

Standards

Agilent LC/MS Pesticide Comprehensive Test Mix (PN 5190-0551), Agilent Custom Pesticide Test Mix (PN CUS-00000635 – CUS-00000643, CUS-000004663), AccuStandard Custom Pesticide Standards (PN S-96086-01 – S96086-10)

Agilent QuEChERS
EN Extraction Kit
(PN 5982-5650CH)



Shaker

Centrifuge

Sample Preparation

10 ± 0.1 g of spinach sample 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 were added, followed by another shaking and centrifugation.

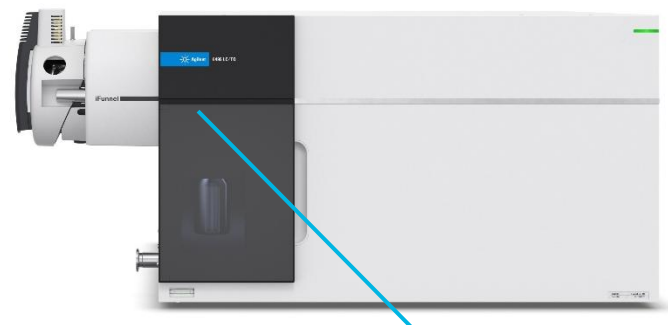
The acetonitrile extract was used directly for LC-MS/MS analysis.

LC-MS/MS Method

Mobile Phase A:	Water + 5 mM Ammonium Formate + 0,1 % Formic Acid
Mobile Phase B:	Methanol + 5 mM Ammonium Formate + 0,1 % Formic Acid

Temperature: 40 °C

Feed Injection:
Feed Speed: 10 µL/min
Automatic Overfeed Volume (4,4 µL)
Flushout Solvent: Mobile Phase A
Outer wash: 1. Methanol 3 sec
2. Acetonitrile 3 sec
Reconditioning: Mobile Phase A



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

MS Parameter:
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
Capillary Voltage: +2500 V / -3000 V
Scan Type: Dynamic MRM (dMRM)
Total MRMs: 1590

Results and Discussion

Classical Flow Injection:

A spinach QuEChERS extract fortified with a multi-residue pesticide standard mixture was analyzed using injection volumes ranging from 0.2 to 2 µL. 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.

Feed Injection:

The same spinach QuEChERS extract and the same range of injection volumes were employed for the measurements using the 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 prior to injection. 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.

Classical Flow Injection:

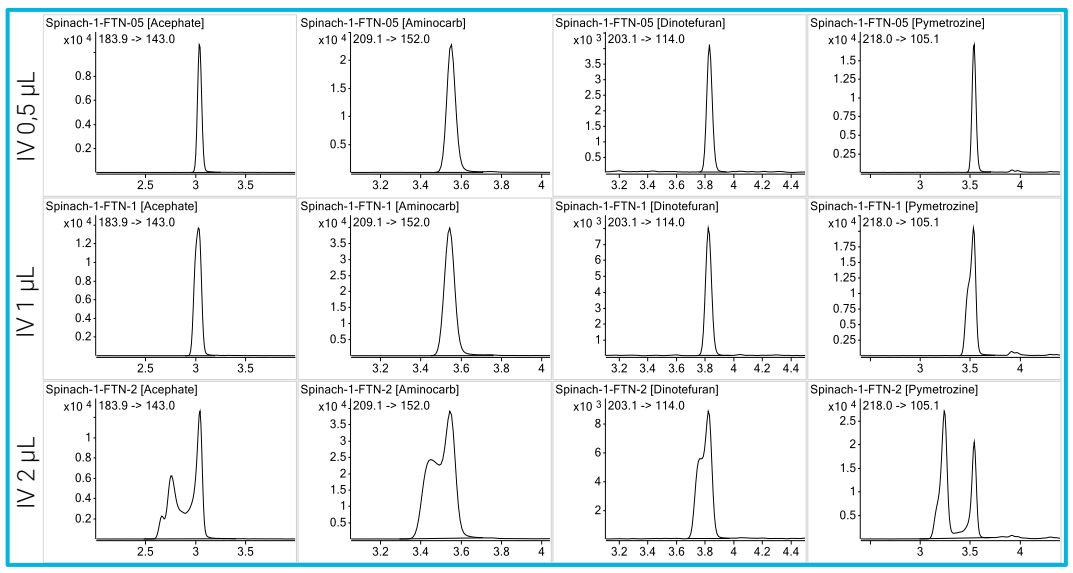


Figure 3. Chromatograms for flow injection with different injection volumes

When evaluating peak area and peak height as a function of injection volume, a clear linear relationship between injection volume and peak area is observed across the investigated range as can be seen in Figure 4 for Aminocarb and Pymetrozine. 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 (Figure 5). While increasing the injection volume continues to result in a linear increase in peak area, the chromatographic peaks do not become taller but rather exhibit increased peak broadening, indicating a limitation in peak capacity at higher injected amounts.

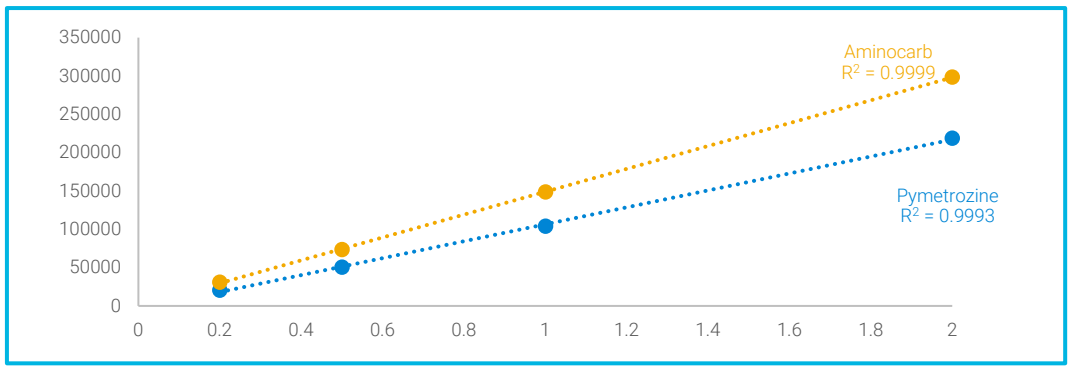


Figure 4. Flow injection peak area vs. volume (µL)

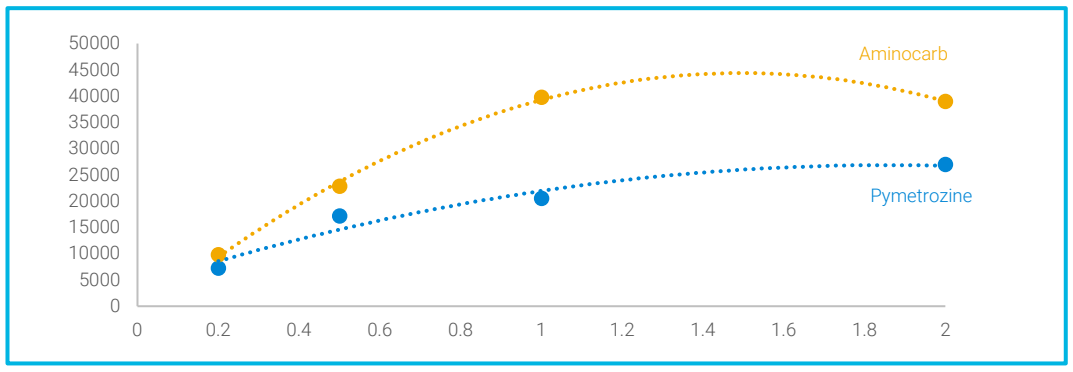


Figure 5. Flow injection peak height vs. volume (µL)

Feed Injection:

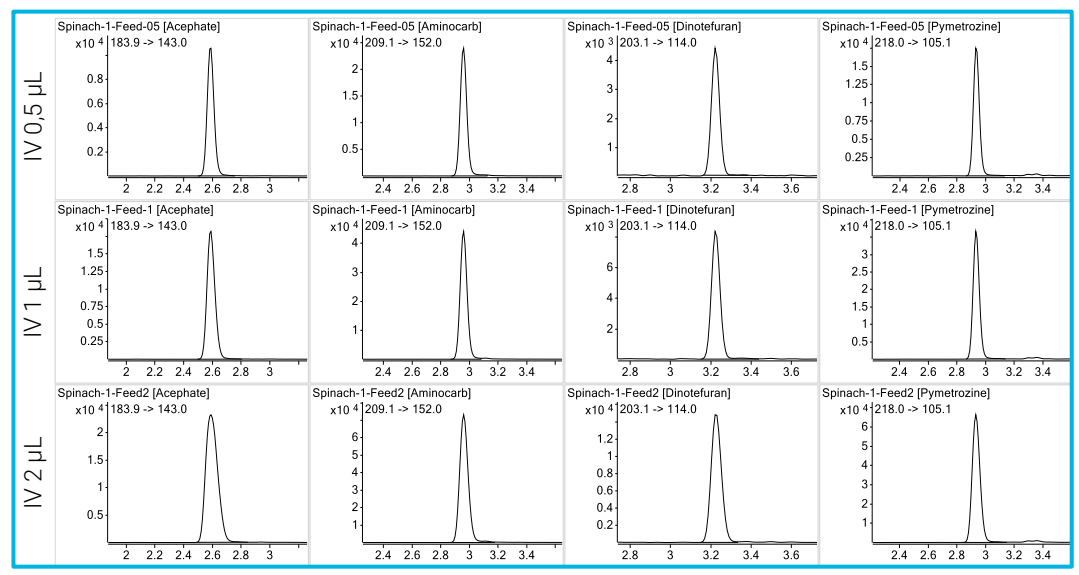


Figure 6. Chromatograms for feed injection with different injection volumes

This behavior is also reflected in the linear response of both peak area and peak height as a function of injection volume as shown for the two example substances aminocarb and pymetrizine in Figures 7 and 8. 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. This improvement leads to a significant enhancement of the method's sensitivity and, moreover, yields well-defined peak shapes. As a result, manual reintegration is no longer required, contributing to a more robust and efficient data evaluation process.

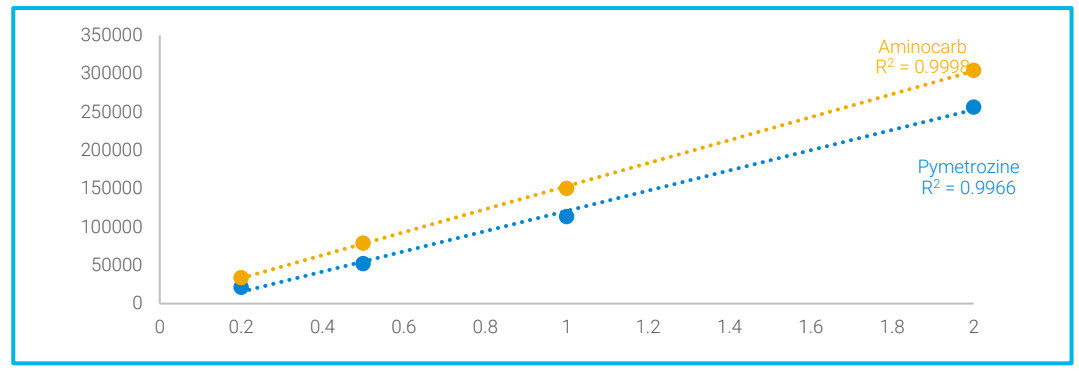


Figure 7. Feed injection peak area vs. volume (µL)

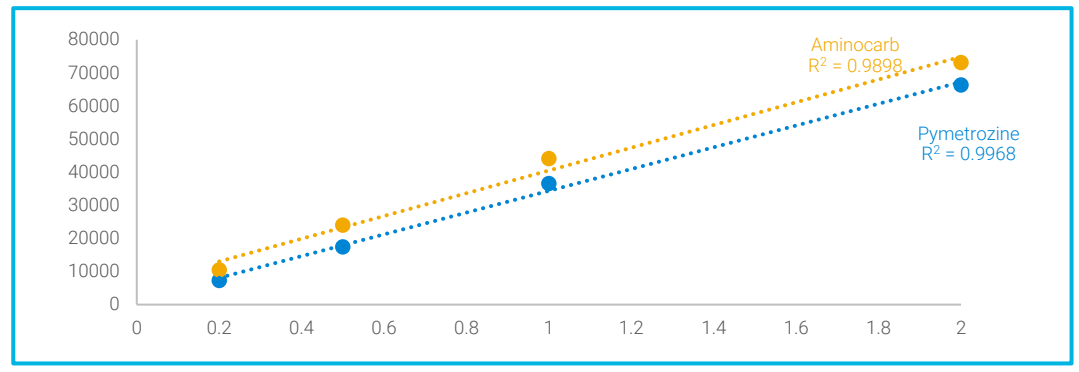


Figure 8. Feed injection peak height vs. volume (µL)

The capability to inject larger sample volumes without compromising peak shape results in a substantial enhancement of analytical sensitivity and produces well-defined, symmetrical peaks, which reduces the need for manual reintegration and facilitates faster, more straightforward data review (Figure 3 and 6). 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 to be achieved 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.

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