Application Note

Food & Beverage Testing

Improved Peak Shape and Lower LOQs in Pesticide Analysis

Using the Agilent 1260 Infinity II Hybrid Multisampler in the LC/MS analysis of polar pesticides

Suitable for Agilent
1260 Infinity III LC

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Abstract

This application note demonstrates the use of an Agilent 1260 Infinity II Hybrid Multisampler in Agilent Feed Injection mode for the multiresidue LC/MS analysis of polar pesticides dissolved in strong eluting organic solvent. The 1260 Infinity II Hybrid Multisampler is used to infuse the sample at various feed speeds, mitigating solvent effects and trapping and enriching the polar compounds on the column. This technique eliminates breakthrough and provides better peak shapes, which improves detection, quantification, and fully automated peak integration. A typical multiresidue method where this technique can be applied is the analysis of pesticides extracted from fruits and vegetables using pure acetonitrile in QuEChERS sample preparation.

Introduction

A general problem in LC/MS analysis of polar compounds is that their chromatographic separation suffers from the elution strength of the sample solvent. With increasing sample solvent elution strength in reversed-phase chromatography and increasing injection volume, the peak performance of early-eluting polar compounds is destroyed, even resulting in complete breakthrough with the solvent front.

The mode of sample injection into an HPLC instrument can also have a large influence on the separation of the compounds in the sample. Modern HPLC instruments are highly optimized to minimize their dispersion volume for best peak performance. Unfortunately, this optimization makes it more likely that polar compounds will be very concentrated in the sample solvent when it reaches the column head. To prevent split or breakthrough peaks, the transition of polar compounds into the starting solvent is normally facilitated by, for example, sandwich injection or additional mixers. The 1260 Infinity II Hybrid Multisampler can deliver a sample by Feed Injection, which mixes the sample with the starting solvent without changing the flow path and compromising peak performance. This injection mode does not require any manual interaction with the LC system. The 1260 Infinity II Hybrid Multisampler can also operate in a classic flow-through mode: all that is required is a single mouse click in the method setup, enabling method compatibility with earlier flow-through methods.

The previously described analytical challenges often occur in the multiresidue analysis of polar pesticides from vegetables and fruits, because in the QuEChERS sample preparation procedure, pesticides are ultimately dissolved in acetonitrile.

This application note demonstrates the application of a newly developed injection principle, Feed Injection. This injection mode enables solvent modulation by sample infusion for multiresidue LC/MS analysis of polar pesticides dissolved in solvents such as acetonitrile, as is typical after QuEChERS sample preparation. The positive effect of solvent modulation by sample infusion on peak performance and maximum sensitivity by increasing injection volumes will be shown and discussed.

Experimental

Instrumentation

- Agilent 1260 Infinity II Hybrid Multisampler (G7167C)
- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent Ultivo triple quadrupole LC/MS (G6465B)

Column

Agilent ZORBAX StableBond C18, 2.1 × 100, 1.8 µm (part number 858700-902)

LC method

Parameter	Value
Solvent	A) Water + 5 mM ammonium formate (AmFormate) + 0.1% formic acid (FA), B) Methanol (MeOH) + 5 mM AmFormate + 0.1% FA
Flow Rate	0.5 mL/min
Gradient	0 min – 5% B 5 min – 30% B
Stop Time	5 min Post time: 2 min
Column Temperature	40 °C
Flow-Through Injection	Injection volume: 0.5, 1.0, 1.5, and 2.0 µL
Feed Injection	Injection volume: 0.5, 1.0, 1.5, and 2.0 µL Feed speed: 35 µL/min Overfeed volume: 6 µL Feed solvent (S2): water + 5 mM AmFormate + 0.1% FA Inner wash: 150 µL S2 Reconditioning: S2
Needle Wash (S1)	3 s, water/acetonitrile (ACN) (50/50) + 0.1% FA

MS method

Parameter	Value
Source	Agilent Jet Stream
Gas Temperature	120 °C
Gas Flow	12 L/min
Sheath Gas Temperature	325 °C
Sheath Gas Flow	12 L/min
Nebulizer Pressure	45 psi
Capillary Voltage	+3,500 V
Nozzle Voltage	+300 V
Time Filter	0.02 min
MRM Conditions	See Table 1

Software

- Agilent MassHunter acquisition software for Ultivo LC/TQ V1.1
- Agilent MassHunter Qualitative Analysis software V10.0
- Agilent MassHunter Quantitative Analysis software V10.0

Pesticides

Methamidophos, acephate, aminocarb, omethoate, propamocarb, pymetrozine, dinotefuran, and oxamyl.

Calibration

Stock solutions of the eight individual pesticides were mixed to achieve a concentration of 1,000 ppb. This solution was diluted to a calibration series with concentrations of 100, 20, 10, 2, 1, and 0.2 ppb in acetonitrile.

Samples

QuEChERS extracts from strawberries in acetonitrile, spiked to a concentration of 10 ppb for all pesticides, and unspiked.

Sample preparation

1. Weigh 10.00 ±0.01 g homogenized strawberry sample into a 50 mL centrifuge tube.
2. Spike as required and add 10 mL acetonitrile, cap, and vortex for 1.5 minutes.
3. Add Agilent QuEChERS extraction kit, original method (part number 5982-5550) extraction salt (4 g) and vortex for 1.5 minutes.
4. Centrifuge at 4,000 rpm for 4 minutes.
5. Transfer 6 mL supernatant to a 15 mL Agilent QuEChERS dispersive universal SPE tube (part number 5982-5056).
6. Vortex for 1.5 minutes and centrifuge at 4,000 rpm for 4 minutes.
7. Transfer supernatant to a clean test tube.

Table 1. MRM conditions for pesticide selection.

	Precursor (*m/z*)	Fragmentor (V)	Quantifier Fragment (*m/z*)	CV (V)	Qualifier Fragment (*m/z*)	CV (V)
Methamidophos	142.0	88	125.9	12	93.9	12
Acephate	184.0	60	143.0	4	49.1	20
Omethoate	214.0	88	155.0	12	125.0	20
Propamocarb	189.2	106	102.0	16	74.0	28
Aminocarb	209.1	101	137.0	24	122.0	48
Pymetrozine	218.1	106	105.1	24	78.0	48
Dinotefuran	203.1	78	157.0	4	129.1	8
Oxamyl	237.1	70	90.0	4	72.0	16

8. Filter with an Agilent Captiva premium syringe filter (part number 5190-5083) into an autosampler vial.

Solvent and chemicals

- All solvents were purchased from Merck, Germany.
- Chemicals were purchased from Merck, Germany.
- Fresh ultrapure water was obtained from a Milli-Q integral system equipped with LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and discussion

To demonstrate the influence of the injection mode (i.e., either Feed Injection or flow-through injection mode), a mixture of eight polar, early-eluting pesticides was dissolved in acetonitrile at a final concentration of 10 ppb for each compound. This sample was injected at different injection volumes of 0.5, 1.0, 1.5, and 2.0 µL to examine the peak performance achieved with both injection modes. Figure 1 highlights the MRM quantifier peak shape of the first eluting pesticide, methamidophos, in both injection modes. At an injection volume of 0.5 µL, Feed Injection provided a well-shaped peak (Figure 1: A1). The peak obtained from the flow-through injection mode also showed a good peak shape, but with slight splitting at the top (Figure 1: A2). With increasing injection volume, the peak height increased in Feed Injection mode, while excellent peak shape was maintained (Figure 1: B1 to D1). By contrast, in flow-through injection mode, the peak started to split at an injection volume of 1.0 µL (Figure 1: B2). At larger injection volumes of 1.5 and 2.0 µL, the peak of the pesticide methamidophos started to break through with the solvent front (Figure 1: C2 to D2).

The results obtained for all eight pesticides, acquired in both injection modes with injection volumes of 1.0 and 1.5 µL, are shown for MRM quantifier and qualifier peaks in Appendix Figures A and B. The compounds eluting later than methamidophos showed good peak shapes without splitting at an injection volume of 0.5 µL in both injection modes. However, in flow-through mode, some peaks started to split at 1.0 µL injection volume (Appendix: Figure A). At an injection volume of 1.5 µL, all peaks started to split or break through with the solvent front (Appendix: Figure B). Even the last pesticide, eluting at approximately 3.7 minutes, began to split at a 2.0 µL injection volume.

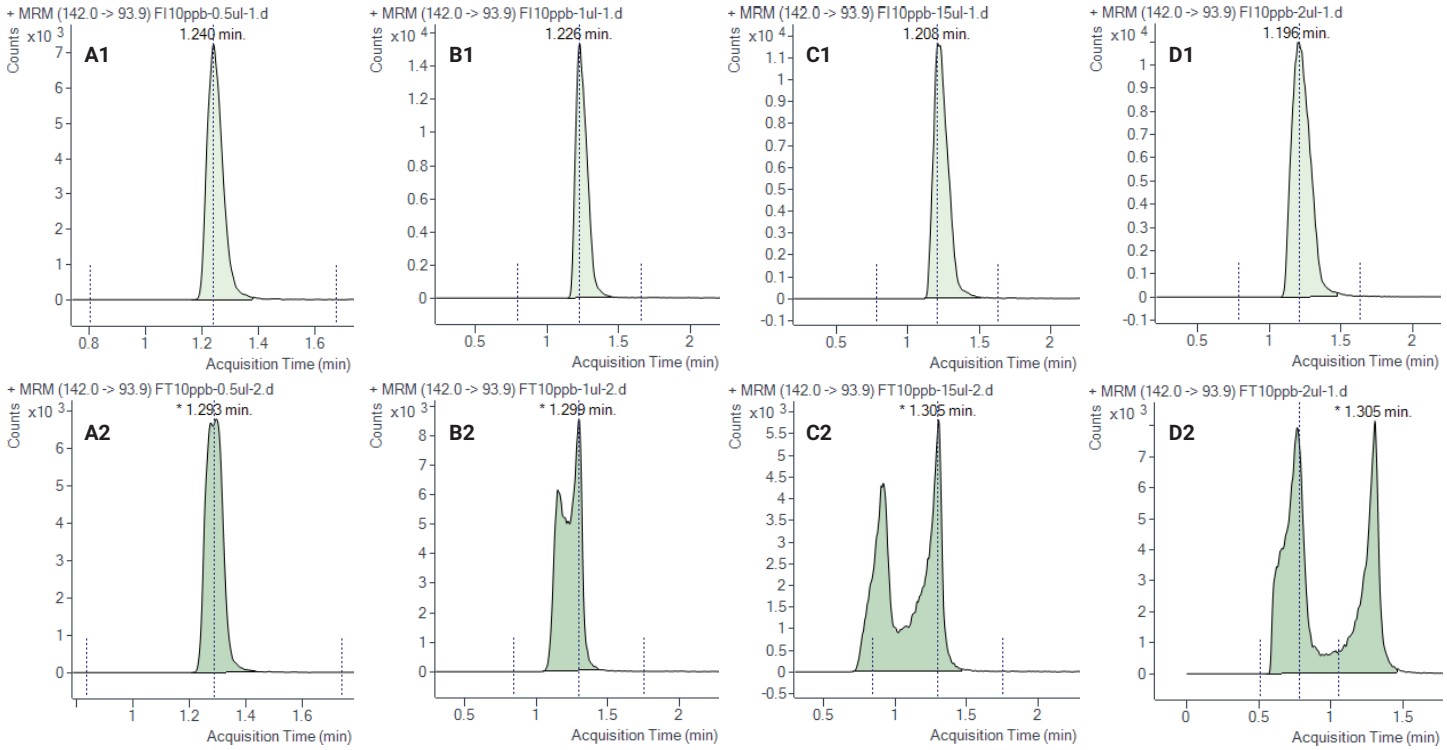


Figure 1. Comparison of the polar, early-eluting pesticide methamidophos (10 ppb in acetonitrile) in Agilent Feed Injection mode (series 1, upper row) and flow-through injection mode (series 2, bottom row) at different injection volumes (A: 0.5 µL; B: 1.0 µL; C: 1.5 µL; D: 2.0 µL).

For a more quantitative look at the results, calibration curves from 0.2 to 100 ppb were acquired for all compounds in both injection modes and all mentioned injection volumes (n = 6 per calibration level, in acetonitrile).

The first parameter to highlight is the RSD of the peak area under the influence of the injection mode, in relation to the compound and the injection volume of 1.5 µL (Figure 2). The RSD values were typically below 2% for all compounds at the lower injection volumes of 0.5 and 1.0 µL in both injection modes. However, the peak area RSD increased dramatically with an increase of the injection volume to 1.5 µL for flow-through injections. For the three compounds methamidophos, acephate, and omethoate, the RSD increased up to 14%. The later-eluting compounds propamocarb and aminocarb were strongly impacted by a 2.0 µL injection volume, with RSD values up to 30%.

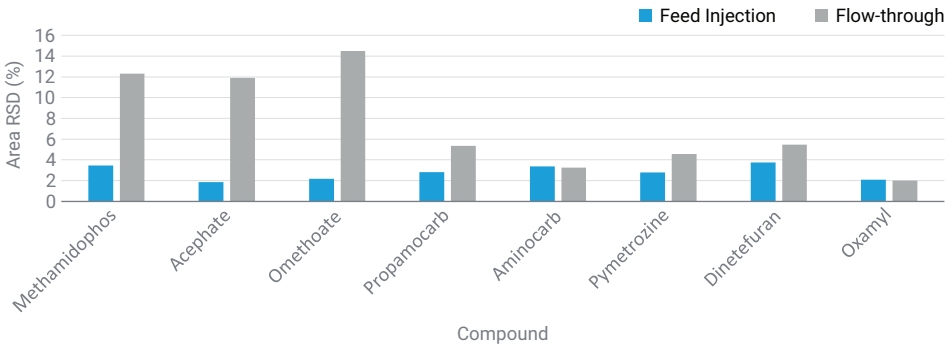


Figure 2. The influence of the injection mode on the peak area RSD depended on the compound (10 ppm each) at 1.5 µL injection volume.

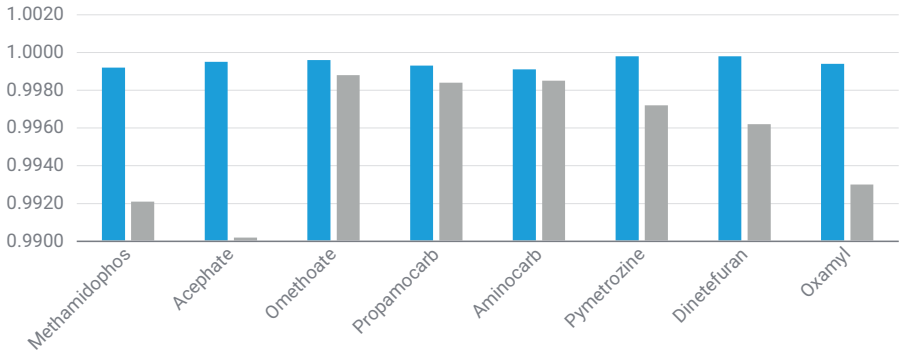
Later-eluting compounds showed similar behavior, but on a lower level. With Feed Injection, all compounds showed a peak area RSD with typical values below 3%. Even for the highest injection volume, peak area RSDs typically did not exceed 5%.

The retention time RSD values, which were also calculated from the measured data, were typically below 0.1% for Feed Injection and flow-through injection. Retention times obtained in flow-through mode were slightly higher due to the additional sampling loop delay volume.

From all measured calibration curves, the obtained linearity was compared in terms of the R^2 value. From the calibration curves created using Feed Injection, the obtained R^2 values for all compounds were typically >0.999

at all applied injection volumes. In flow-through injection mode, at injection volumes of 1.5 µL and upward, a decline of the R^2 value could be seen for all compounds (Figure 3). For the calibration curves created from the results obtained by flow-through injections at 0.5 and 1.0 µL injection volume, the R^2 values for methamidophos, acephate, and omethoate were typically >0.99. The later-eluting pesticides typically showed R^2 values of >0.999. For an injection volume of 2.0 µL, the obtained R^2 values were strongly compromised and were typically between 0.93 and 0.99.

The lower area precision and linearity for flow-through injections at larger injection volumes also affects the limit of quantification (LOQ). The LOQs (S/N = 10) were calculated from the lowest accessible calibration point. The achieved values were typically between 30 and 40 ppt with Feed Injection over the complete injection volume range. The LOQ was between 70 and 120 ppt with flow-through injection at 1.5 µL injection volume (Figure 4). Flow-through injection showed a steadily increasing LOQ with increasing injection volume for methamidophos, acephate, and aminocarb. For all pesticides, the LOQ obtained from a 2.0 µL injection was very high, at around 200 ppt. For the later-eluting pesticides, dinetefuran and oxamyl, this effect was weaker due to their better retention.



Feed Injection	0.9992	0.9995	0.9996	0.9993	0.9991	0.9998	0.9998	0.9994
Flow-through	0.9921	0.9902	0.9988	0.9984	0.9985	0.9972	0.9962	0.9930

Figure 3. Linearities obtained from calibration curves for all measured pesticides using Agilent Feed Injection and flow-through injection at 1.5 µL injection volume.

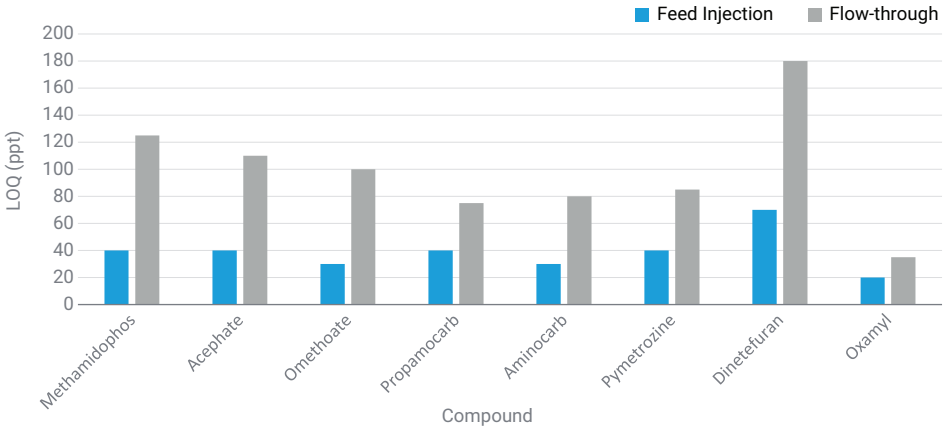


Figure 4. LOQs obtained from the calibration curves created by Agilent Feed Injection mode with a 1.5 µL injection volume.

In a test using real QuEChERS acetonitrile extraction conditions, the recovery was determined from a strawberry matrix spiked with 10 ppb of the eight pesticides. For all pesticides, a recovery between 85% and 105% could be measured in Feed Injection mode, which is within the required tolerance of 80% to 120%.[2] The recoveries of the early-eluting pesticides tended to be between 85% and 95%. The two pesticides that eluted last showed a recovery between 100% and 105% (Figure 5). The percentage recoveries achieved in flow-through injection mode were within the same range, with an identical tendency to increase with the later-eluting pesticides. The pesticide pymetrozine could only be determined with a very low recovery in both cases due to a high matrix suppression for this pesticide at the obtained retention time.

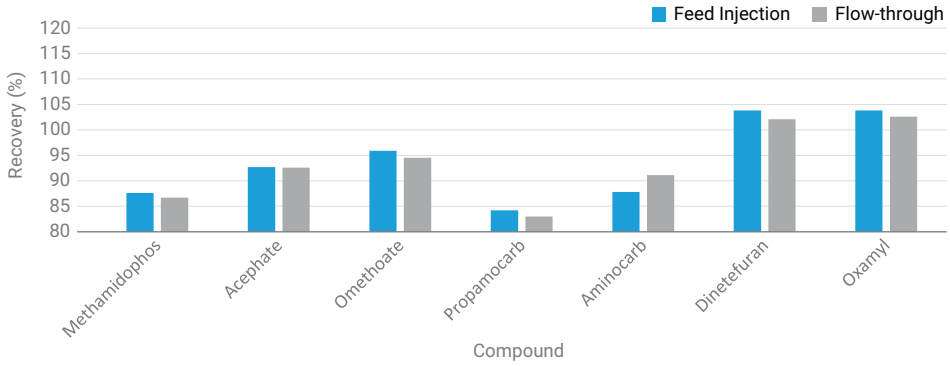


Figure 5. Recoveries of eight early-eluting pesticides from a strawberry matrix spiked with 10 ppb of each pesticide, determined in Agilent Feed Injection mode and flow-through injection mode at 1.5 µL injection volume.

Conclusion

This application note demonstrates the performance of the Agilent 1260 Infinity II Hybrid Multisampler for the analysis of polar pesticides in a multiresidue method using Agilent Feed Injection mode. As the 1260 Infinity II Hybrid Multisampler can also work in flow-through injection mode, comparative measurements are also shown. It has been demonstrated that Feed Injection typically provides superior peak shapes for the early-eluting polar pesticides from solution in acetonitrile. This excellent performance also has a positive effect on the linearity of calibration curves, and LOQs are typically lower when Feed Injection is applied. The recoveries obtained from QuEChERS extracts in acetonitrile meet the requirement to be between 80% and 120%.

Feed Injection enables a UHPLC instrument to be used in its dispersion-optimized configuration without any additional mixing volume modifications. Due to the optimum peak shape achieved through using Feed Injection, time-consuming data review can also be reduced.

References

1. Herschbach, H.; Naegele, E. Performance Characteristics of the Agilent 1260 Infinity II Hybrid Multisampler. *Agilent Technologies technical overview*, publication number 5994-5952EN, **2023**.
2. European Commission: Health & Consumer Protection Directorate—Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, SANTE/12682/2019.

Appendix

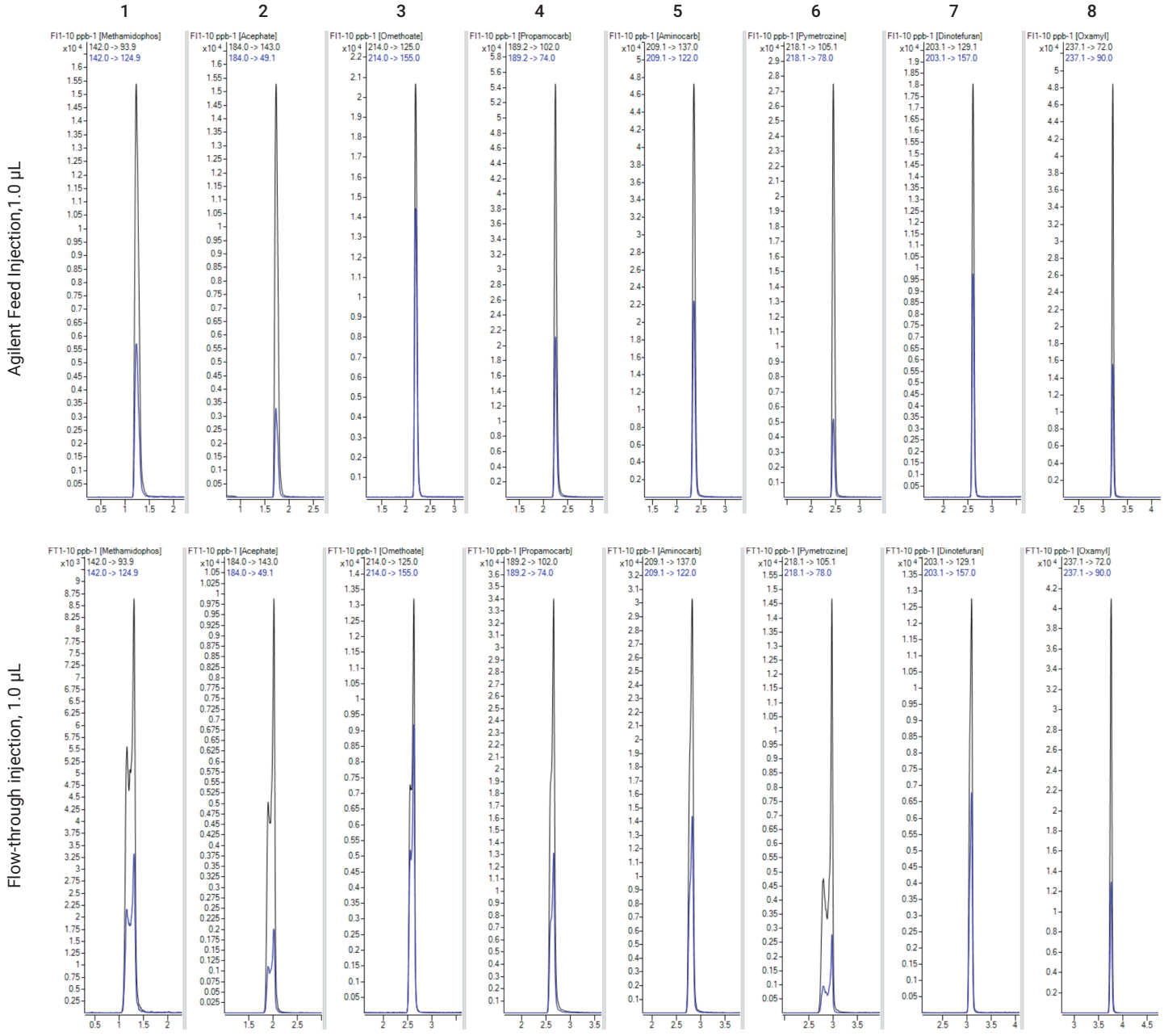


Figure A. Injection of 1.0 µL of a mixture of eight polar pesticides by Agilent Feed Injection and by flow-through injection (quantifier = gray, qualifier = blue). 1) Methamidophos; 2) acephate; 3) omethoate; 4) propamocarb; 5) aminocarb; 6) pymetrozine; 7) dinotefuran; 8) oxamyl.

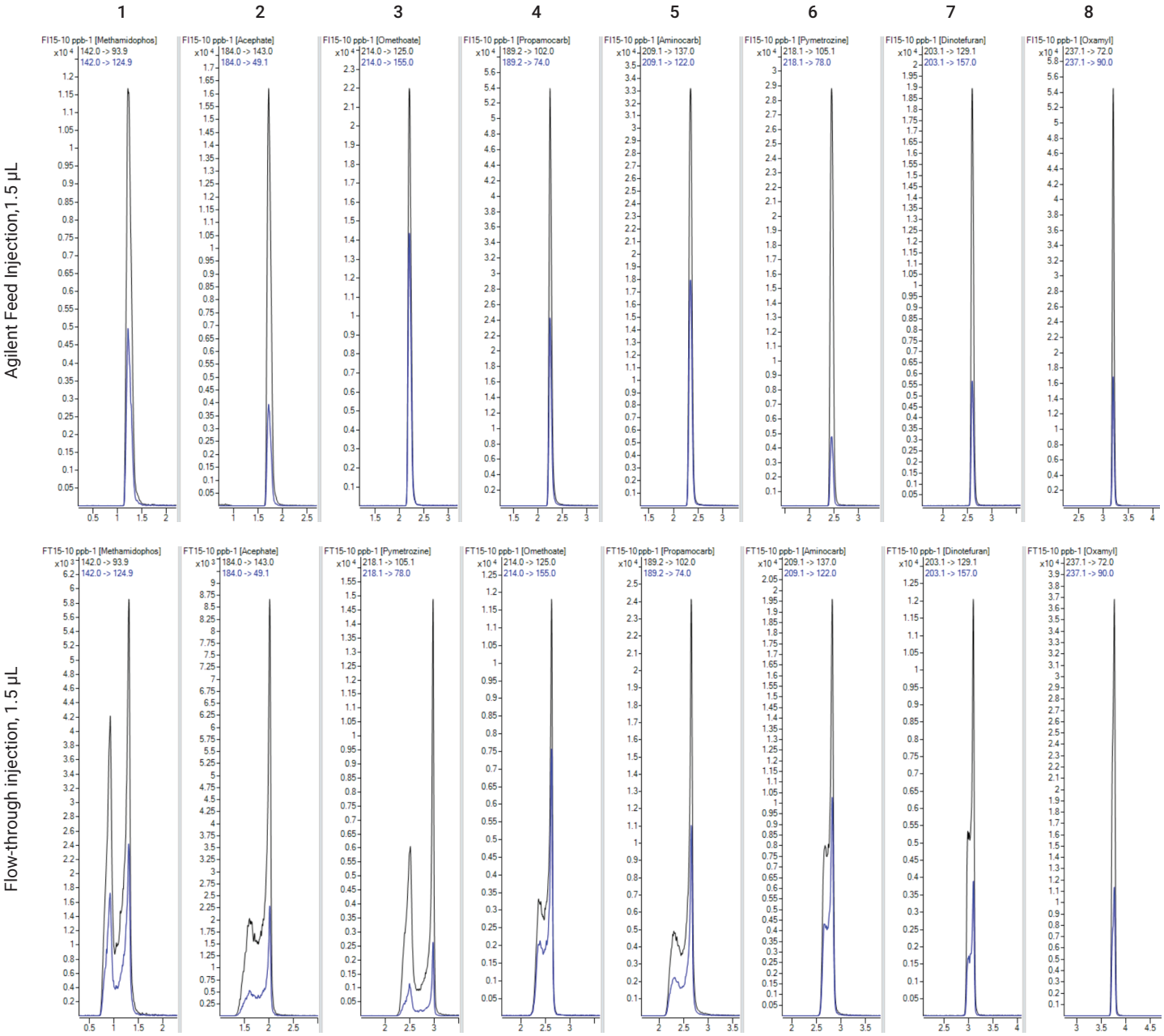


Figure B. Injection of 1.5 µL of a mixture of eight polar pesticides by Agilent Feed Injection and by flow-through injection (quantifier = gray, qualifier = blue). 1) Methamidophos; 2) acephate; 3) omethoate; 4) propamocarb; 5) aminocarb; 6) pymetrozine; 7) dinotefuran; 8) oxamyl.

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