A Multiresidue Method for Pesticide Analysis Using an Orbitrap Tribrid Mass Spectrometer and Automatic Background Exclusion

Paul Dewsbury, Anastasia Kalli, Seema Sharma, Charles Yang and Dipankar Ghosh, Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134, USA

ABSTRACT

Purpose: Develop a multiresidue method for pesticides quantitation and improved untargeted screening on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer

Methods: Chromatographic separation was performed on a Thermo Scientific™ Vanquish™ UHPLC system using a Thermo Scientific™ Accucore™ aQ C18 column

Mass spectrometric analysis was performed on an Orbitrap ID-X Tribrid mass spectrometer using AcquireX workflow, for automated generation of background exclusion list

Data processing was performed with the Thermo Scientific™ TraceFinder™ software utilizing the mzVault library for pesticide screening

Results: Excellent limits of quantitation, reproducibility, linearity and accuracies were obtained

Automated generation of background exclusion list, using the AcquireX workflow, resulted in a significant increase in the number of identified pesticides, through libraries matches, compared to DDA, at low ppb concentration levels

INTRODUCTION

Pesticides are routinely applied to crops for preventing, destroying or controlling pest activity. In order to protect the consumers and ensure they are not being exposed to pesticide levels harmful for their health, pesticides are regulated and several countries have established maximum residue levels (MRLs). Given the large number of pesticides used and the globalization of the food industry, multiresidue methods offer a great advantage allowing analysis of hundreds of pesticides in a single run. We have implemented a multiresidue method for the analysis of 250 pesticides on an Orbitrap ID-X Tribrid mass spectrometer utilizing AcquireX for automated generation of background exclusion list.

MATERIALS AND METHODS

Sample Preparation

Strawberry samples were obtained from a local retail store. Following homogenization, strawberry samples were extracted using a QuEChERS extraction kit. Briefly 10 g of sample was weighed into the QuEChERS extraction tube and 10ml of ACN was added. Samples were shaken, centrifuged and the supernatant was collected. Matrix-matched standards were prepared by spiking the pesticide standards (250 pesticides) into the extracted matrices at concentration levels ranging from 0.05 to 200 ppb.

Liquid Chromatography

Chromatographic separation was performed on a Vanquish UHPLC system using an Accucore aQ C18 column (100 × 2.1 mm, 2.6 μm). Mobile phase A consisted of 98% water, 2% methanol containing 5mM ammonium formate and 0.1% formic acid. Mobile phase B consisted of 98% methanol, 2% water containing 5mM ammonium formate and 0.1% formic acid. The flow rate was 300 μl/min and the column temperature was set at 25 ^{0}C. Analysis time was 15 min including 3 min equilibration time. Injection sample volume was 1 ul.

Mass Spectrometry

Mass spectrometric analysis was performed on an Orbitrap ID-X Tribrid mass spectrometer using AcquireX workflow, for automated generation of background exclusion list, or data dependent acquisition (DDA). The exclusion list was automatically generated from the matrix blank and automatically implemented in the acquisition method for all subsequent analyses to reduce background ion fragmentation. The AGC target value was set at 2e5 for the full MS and at 1e4 for the MS/MS scans. The resolution was set to 60,000 for the full MS scan and at 15,000 for the MS/MS scan events. HCD was performed with stepped collision energy (%) of 20, 40, and 70.

Data Processing

Data processing was performed with the TraceFinder software. Quantitation was performed on the precursor ion and mass tolerance was set to 5 ppm.

## RESULTS

Figure 1. Representative calibration curves
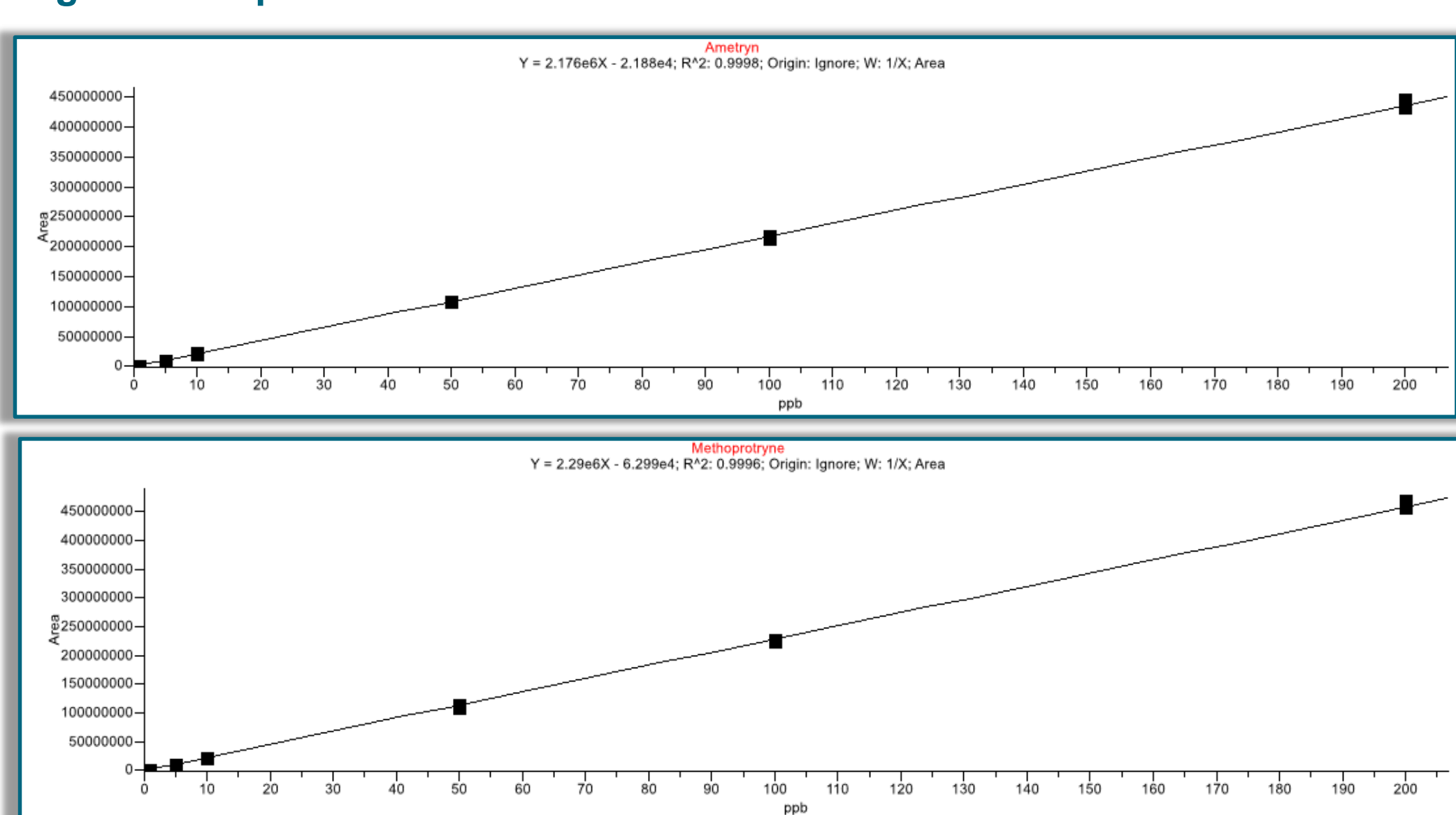
Calibration curves for ametryn and methoprotryne. For methoprotryne calibration range was from 0.1 to 200 ppb. For ametryn calibration range was from 0.5 to 200 ppb.

Table 1. Obtained % Diff, % RSD and %CV at LOQ levels for representative pesticide residues

| Pesticide Residue	LOQ (ppb)	% Diff Injection 1	% Diff Injection 2	% Diff Injection 3	% RSD	% CV
Ametryn	0.5	-0.07	-6.27	-8.01	4.38	4.48
Carbaryl	1.0	4.14	-6.79	3.43	6.10	5.10
Chloridazon	1.0	-0.13	-3.33	8.17	5.84	5.86
Clomazone	0.5	0.54	4.46	-9.50	7.31	7.24
Cyanazine	0.5	-4.15	-1.42	-1.71	1.54	1.53
Cyazofamid	0.5	-4.48	-4.40	-7.92	2.13	2.01
Dicrotophos	0.5	3.66	2.87	9.13	3.24	3.62
Dinotefuran	1.0	2.14	8.90	1.67	3.88	4.88
Fensulfothion	0.5	1.08	-4.20	5.61	4.87	4.74
Fuberidazole	0.5	-1.24	7.81	1.97	4.46	4.47
Hexazinone	0.5	-5.17	-0.35	-2.01	2.51	2.55
Heptenophos	0.5	-0.37	3.72	-0.37	2.34	2.40
Methabenzthiazuron	0.5	3.38	6.83	2.54	2.18	2.13
Metosulam	1.0	-1.21	-1.81	-2.98	0.92	0.72
Ofurace	0.5	6.41	-1.51	-0.11	4.16	4.14
Tebufebpyrad	1.0	1.39	-5.94	6.06	6.02	5.60
Thiabendazole	0.5	1.49	-2.71	-5.99	3.84	3.81
Tricyclazole	0.5	-1.04	-4.31	-2.49	1.68	1.73

The % Diff represents the difference between the calculated amount and the expected amount, divided by the expected amount. % RSD is based on the calculated amount and % CV is based on the peak areas.

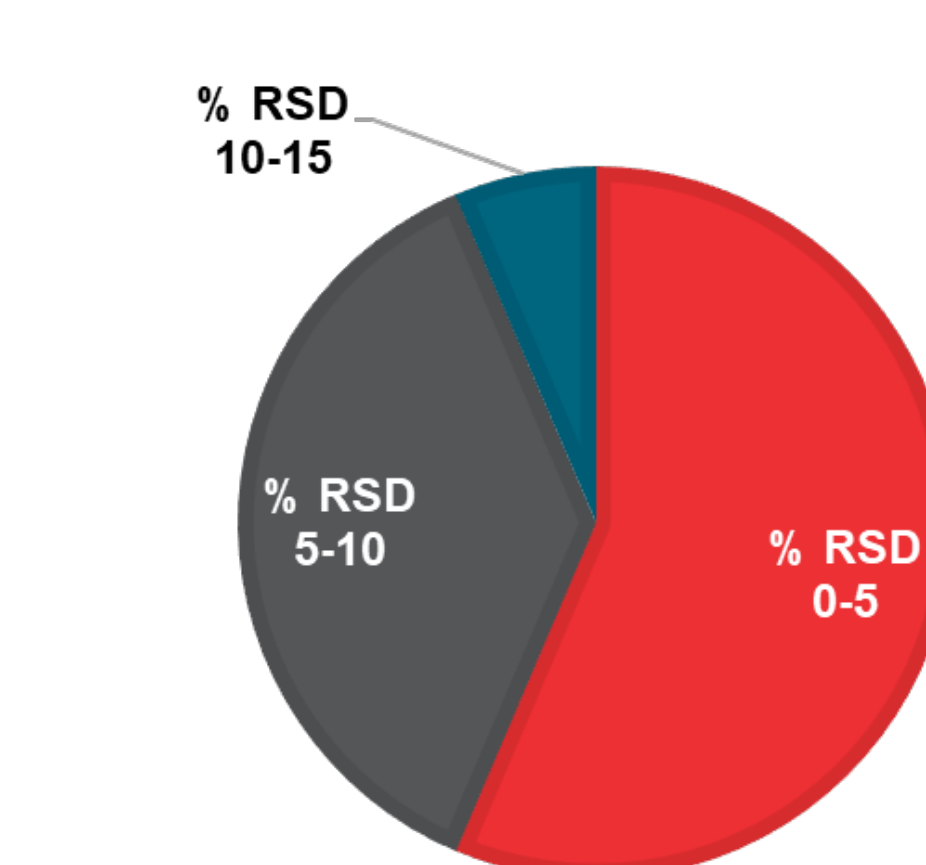### Figure 2. Obtained LOQ levels per number of analytes
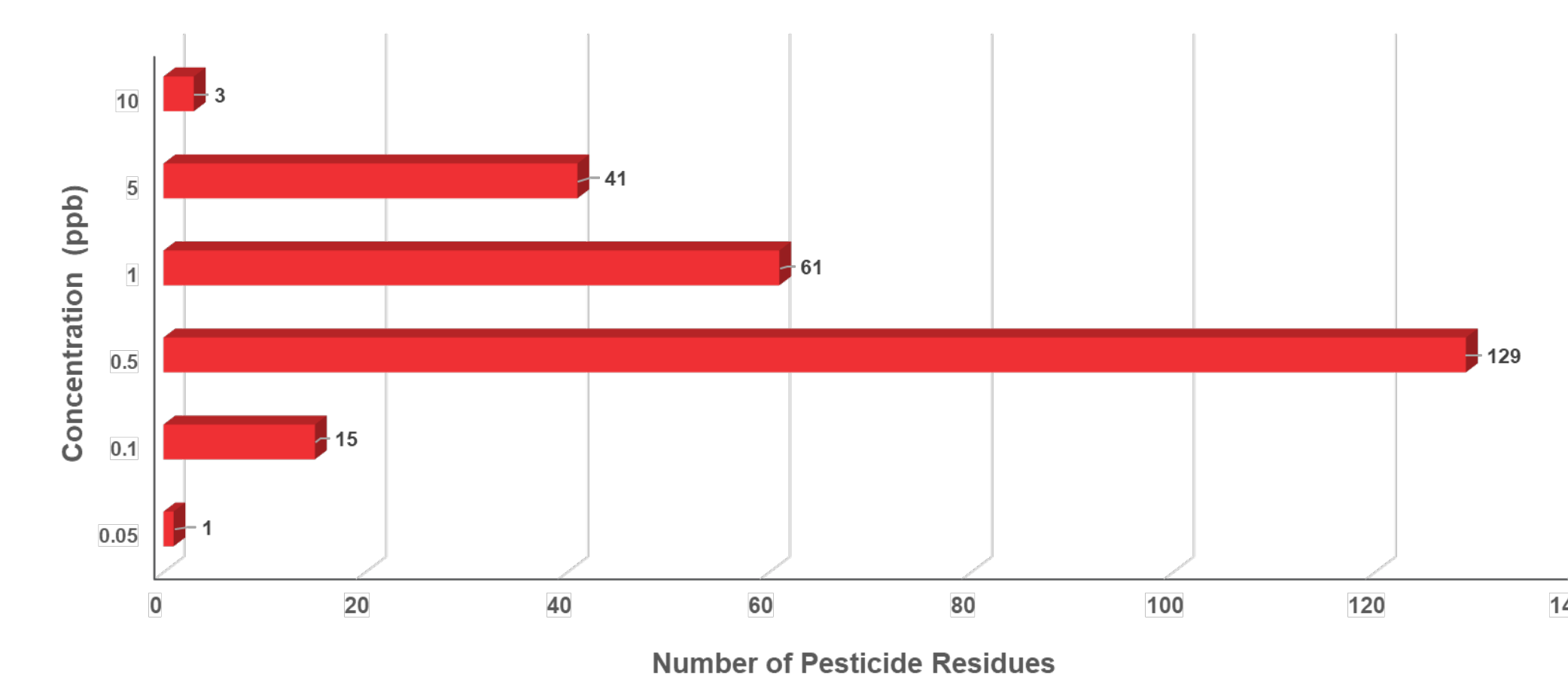
LOQ levels were at or below 1 ppb for 82% of the pesticide residues tested.

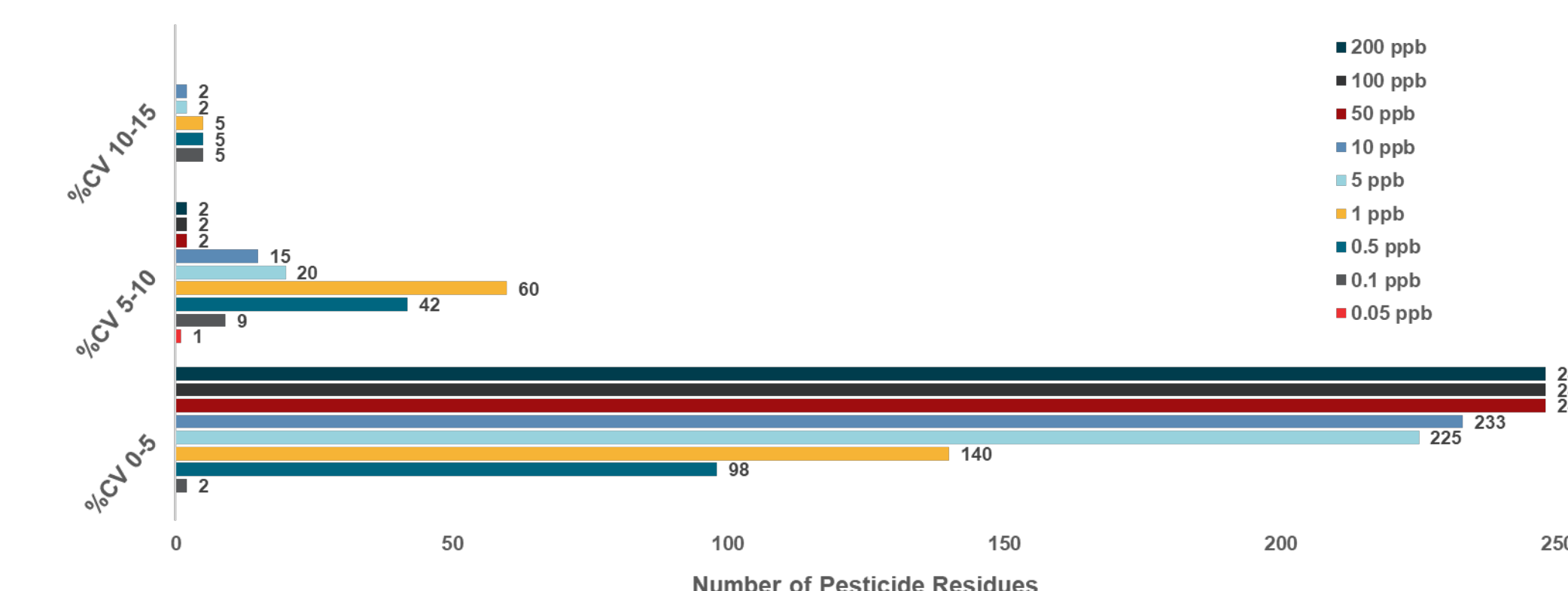### Figure 3. Obtained % CV at LOQ levels
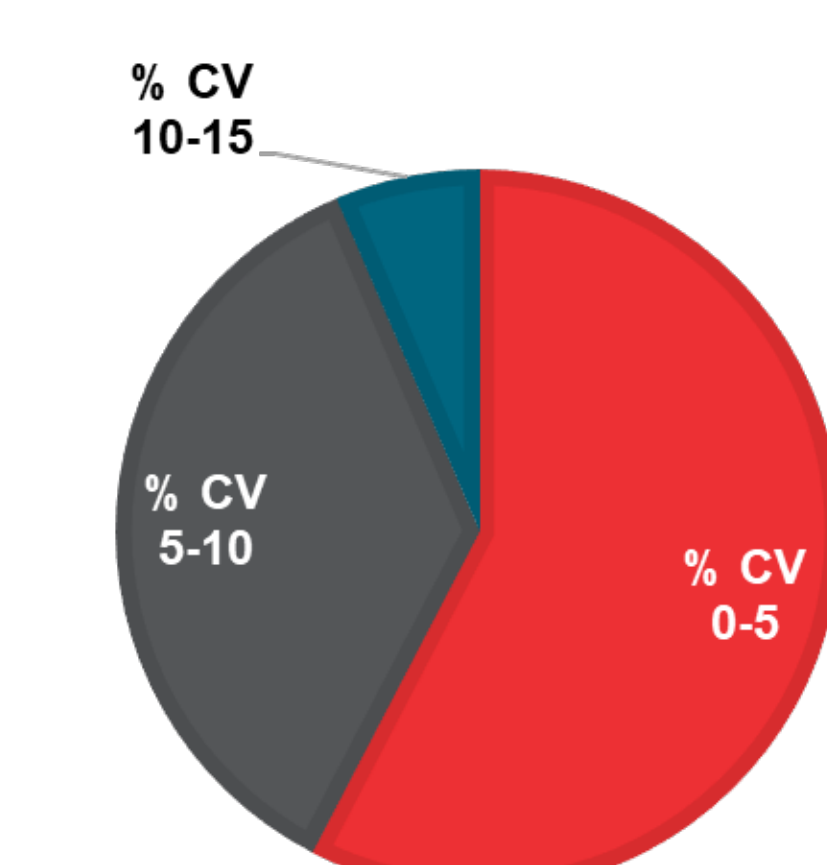
Obtained % CV values at LOQ levels were within 10% for 94% of all pesticides tested.

Figure 4. Obtained % RSD at LOQ levels

Obtained % RSD values at LOQ levels were within 10% for 94% of all pesticides tested.

Figure 5. Obtained % CV at all calibration levels for all pesticides residues tested
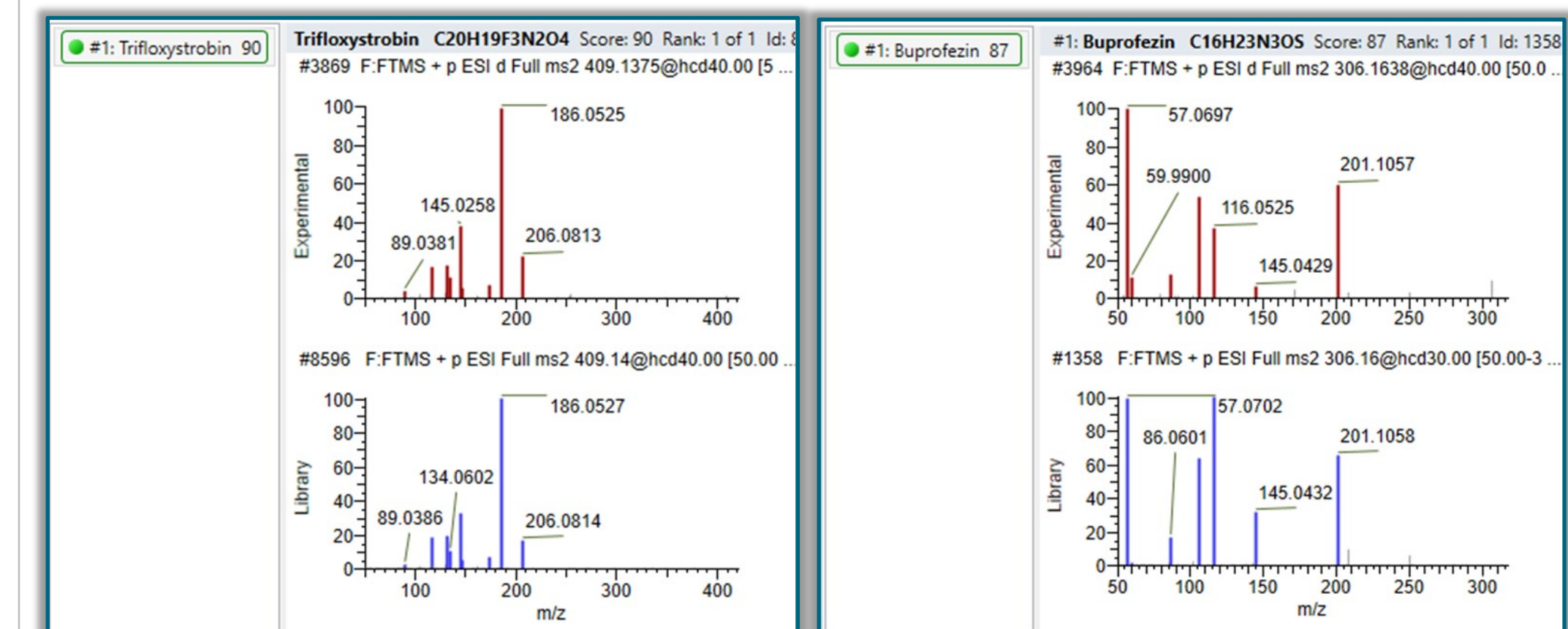

Obtained % CV were within 5% for the vast majority of the pesticides residues tested.

Figure 6. AcquireX Workflow for Background Exclusion

Matrix Blank Injection → Exclusion List Generation → Instrument Method Update with Exclusion list → Sample Analysis with Updated Method to Exclude Background Ions Fragmentation
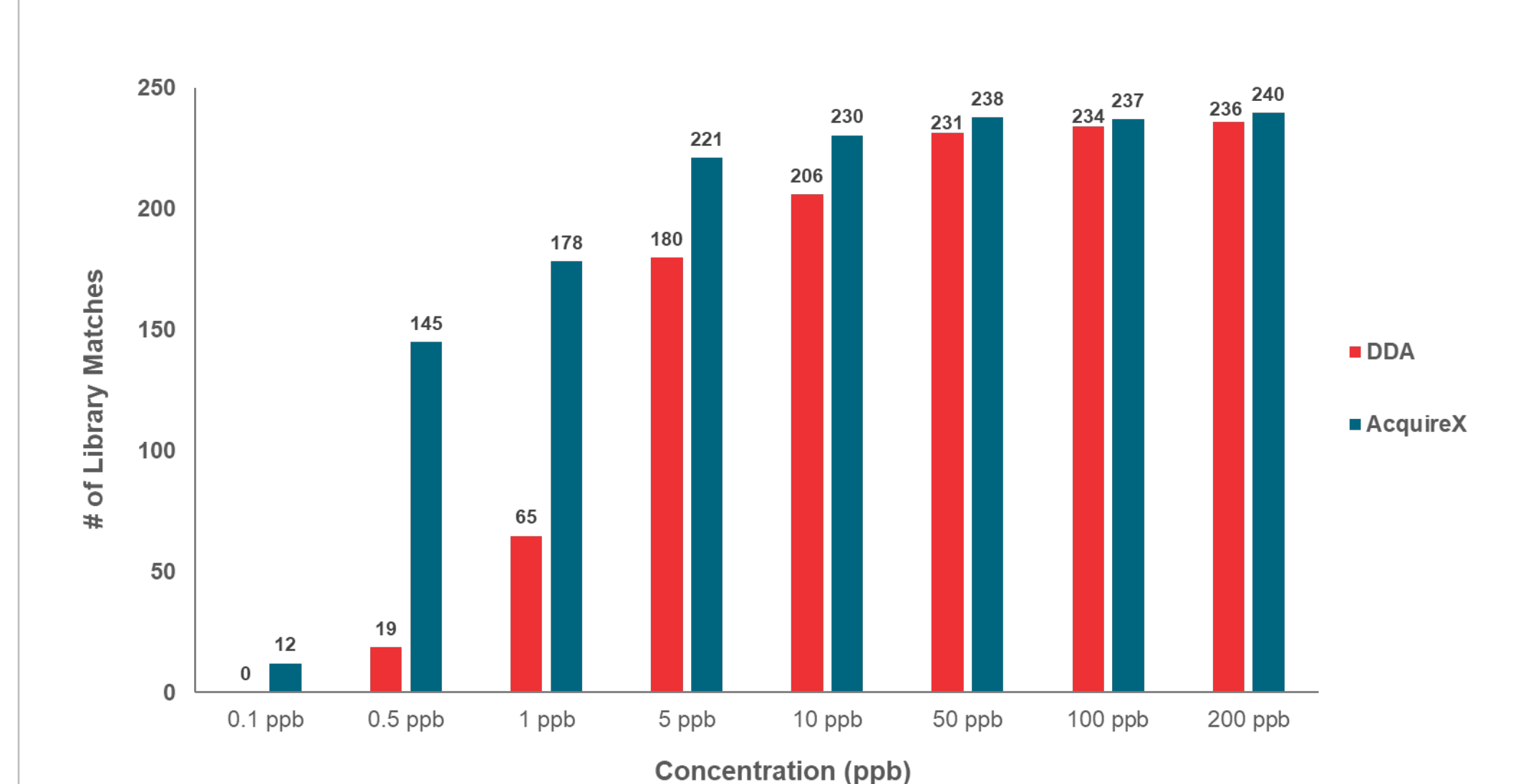
### Figure 7. Representative Examples of mzCloud Spectral Library Matches

Spectra library matches for trifloxystrobin at a spiked concentration of 0.5 ppb and buprofezin at spiked concentration of 1 ppb.

Figure 8. Number of Identified Pesticides with Background Exclusion using Acquire X and Data Dependent Acquisition (DDA)

Automated generation of background exclusion list with AcquireX workflow significantly increases the number of identified pesticides, compared to DDA, at low ppb concentration levels.

Figure 9. Results Reproducibility with AcquireX Workflow
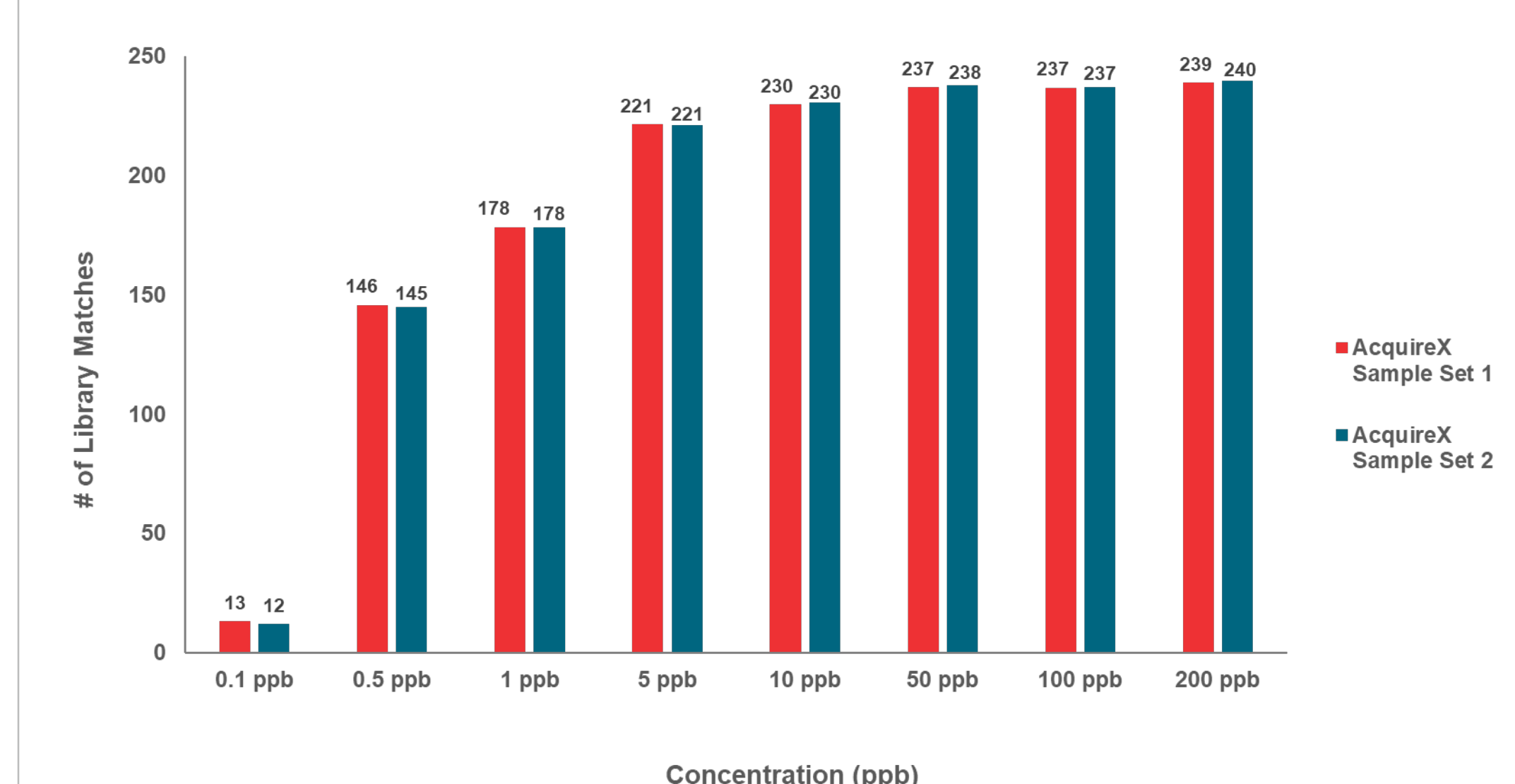
Number of identified pesticides obtained with automated generation of background exclusion list with AxquireX workflow are highly reproducible. In the data presented above matrix-matched standards were prepared and analyzed on two different days

CONCLUSIONS

The Orbitrap ID-X Tribrid mass spectrometer delivers confident, accurate and reproducible quantitation

LOQ levels for 82% of the pesticide residues tested were at or below 1 ppb

AcquireX workflow enables the generation of background exclusion list in an automated fashion to increase productivity, efficiency and quality of results

Automated generation of background exclusion list with AcquireX workflow significantly increases the number of identified pesticide residues at low ppb levels compared to DDA

TRADEMARKS/LICENSING

Thermo Fisher SCIENTIFIC