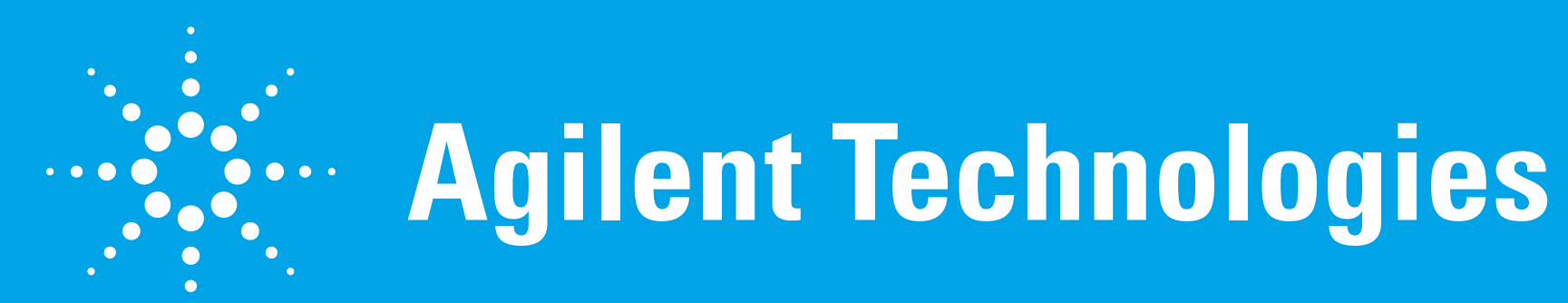


Analysis of Multiple Pesticide Residues in Fruits and Vegetables using GC/Q-TOF and EI Accurate Mass Pesticide Library

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Kai Chen,¹ Philip L. Wylie,² Joan Stevens,²

¹Agilent Technologies, Inc., 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA; kai_chen@agilent.com; ²Agilent Technologies, Inc., Wilmington, DE, USA

Introduction

Given that more than 900 different pesticides are in use around the globe, there is a strong demand to screen for a wide scope of pesticides to ensure a safe food supply. Various governmental authorities have set laws to regulate which pesticides can be legally used on a given crop, and the maximum residue limits (MRLs) of those pesticides after harvest. To achieve such a challenging ambition, it requires a technique with untargeted data acquisition and good screening sensitivity. It is also desired in the pesticide testing community to screen and quantitate a large number of pesticides within one run for improved efficiency.

Accurate mass GC/Q-TOF serves as a fit-for-purpose tool for this demanding GC/MS application in pesticide analysis laboratories, as it offers untargeted acquisition of full scan EI mass spectra of all GC amenable pesticides at low concentrations. The rich accurate mass spectral information allows enhanced confidence in pesticide identification and easy expansion of surveillance scope when unexpected new pesticides emerge. In this study, we demonstrated a novel GC/Q-TOF and accurate mass pesticide library based workflow for screening and calibrating more than 100 pesticides in peach and avocado samples.

Experimental

Sample Preparation

Organic peach (15 g) was extracted using QuEChERS (AOAC) extraction protocol and followed by a cleanup with Agilent Bond Elut QuEChERS Disperse Kit for general fruits and vegetables (AOAC). Organic avocado (10 g) was extracted by QuEChERS (EN) extraction protocol and followed by a cleanup with Agilent QuEChERS Enhanced Matrix Removal (EMR)-Lipid dSPE and polish with dry steps. A mixture of 120 pesticide standards were then spiked at 5, 10, 20, 50, 100 and 200 ng/mL in the extracts to evaluate screening capability at low concentrations and matrix matched calibrations.

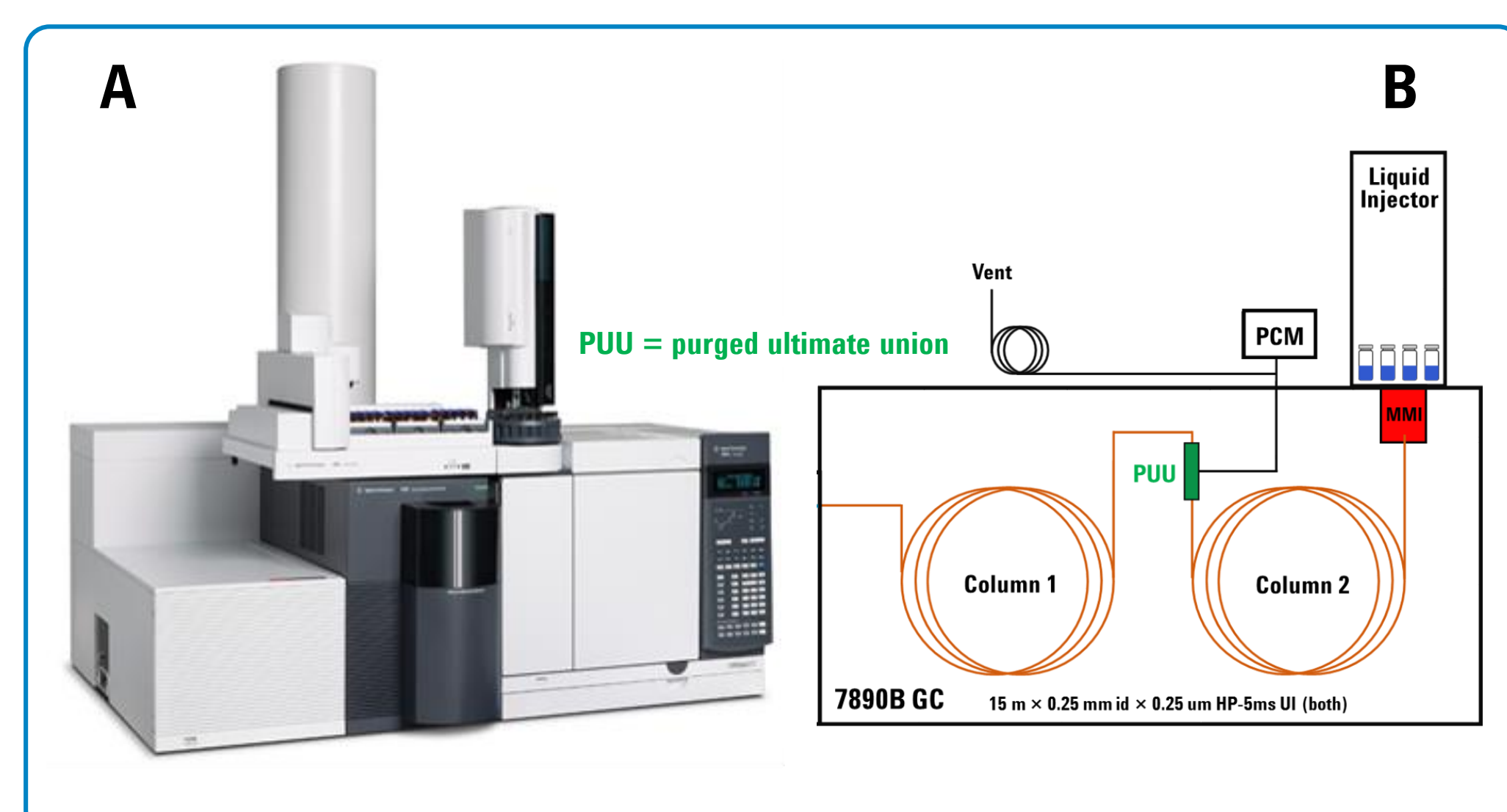


Figure 1. 7200 Series GC/Q-TOF (A) and Configuration using Mid-column Backflushing (B).

Instrumental Analysis

The samples were analyzed in EI full-spectrum acquisition mode by Agilent 7200 Series high resolution accurate mass GC/Q-TOF. The system is configured with a mid-column backflushing setup (Figure 1). A 20 minute constant flow retention time locked (RTL) method has been utilized for chromatographic separation, with operational GC and MS parameters of listed in Table 1.

Table 1. GC/Q-TOF Operational Conditions.

GC and MS Conditions:	Value
Columns (2 ea.)	HP-5 MS UI, 15 meter, 0.25 mm ID, 0.25 µm film
Inlet	MMI, 4-mm UI liner single taper w wool
Injection	2 µL, cold splitless
Carrier gas	Helium
Inlet flow (column 1)	~1 mL/min
PUU flow (column 2)	column 1 flow + 0.2 mL/min 60 °C for 1 min 40 °C/min to 170 °C, 0 min 10 °C/min to 310 °C, 3min (Run time 20.75 min)
Oven program	
Backflushing conditions	5 min (Post-run), 310 °C (Oven) 50 psi (Aux EPC pressure), 2 psi (Inlet pressure)
Transfer line temperature	280 °C
Ion source	EI, 70 eV
Source temperature	300 °C
Quadrupole temperature	180 °C
Spectral Acquisition	45 to 550 m/z, 5 spectra/sec, 4 GHz mode

Experimental

Data Analysis

Compound identification used a curated GC/Q-TOF accurate mass pesticide library (containing 800+ compounds with RTs and spectra) and *Find by Fragment* workflow in pre-released MassHunter Qualitative Analysis (B.08) with enhancements to facilitate review of screening results. The qualified ions of identified compounds can also be easily transferred to pre-released MassHunter Quantitative Analysis software (B.08) to set up a calibration method for quantitative screening.

Results and Discussion

Pesticide Detectability

The 120 spiked pesticides represent a large variety of categories, including carbamate, triazole, organochlorine, organophosphorus, pyrethroid, etc. Detectability and data analysis parameters are reported in Table 2.

Table 2. Number of Detected Pesticides (out of 120)

Matrix	Peach		Avocado	
Spike Level (ng/mL)	5	10	5	10
Found by Auto-DA ^a	117	118	112	116
Found Manually ^b	1	2	2	3
Total	118	120	114	119

a. Use *Find by Fragment* (mass extraction window of 25 ppm; coelution score >=70; S/N >= 3; RT diff <=0.15 min; at least 2 out of 6 ions qualified);
b. Only found by manually extracting EIC (mass extraction window of 25 ppm), at least 1 EIC with S/N >=3;

Retention Time and Response Repeatability

The RTL backflushing method ensured RT stability, with SD <=0.01 min for almost all pesticides (except α -Endosulfan in peach). The instrument precision is shown by %RSD of identified pesticides (Figure 2) at trace levels, with most pesticides showing single digital %RSD.

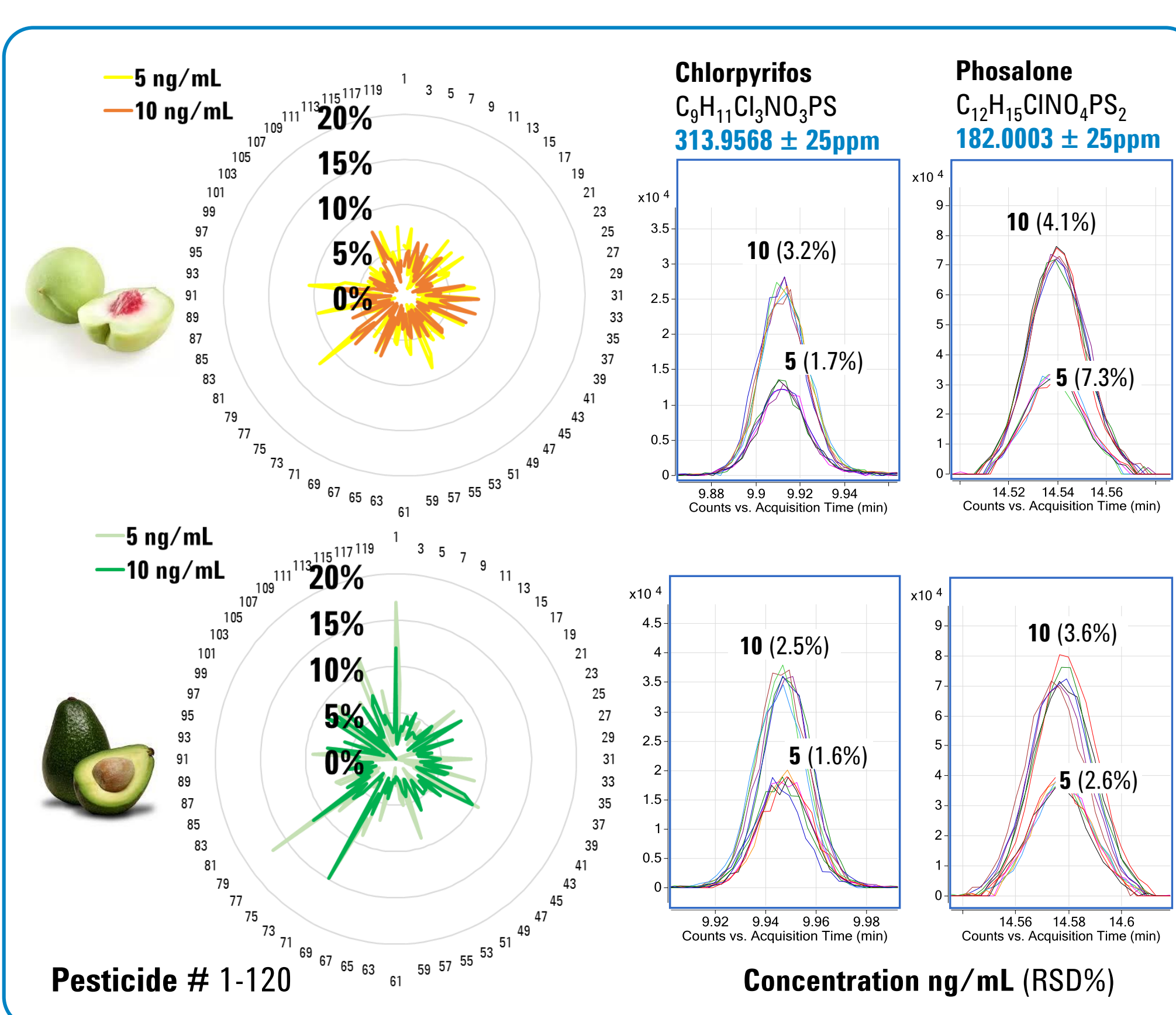


Figure 2. Response RSD% (n=6) of Pesticides in Food Extracts

The long term response stability was evaluated by a sequence of alternate injecting 5 and 10 ng/mL pesticides in avocado, with 36 injections in total. Figure 3 shows the results of two example pesticides.

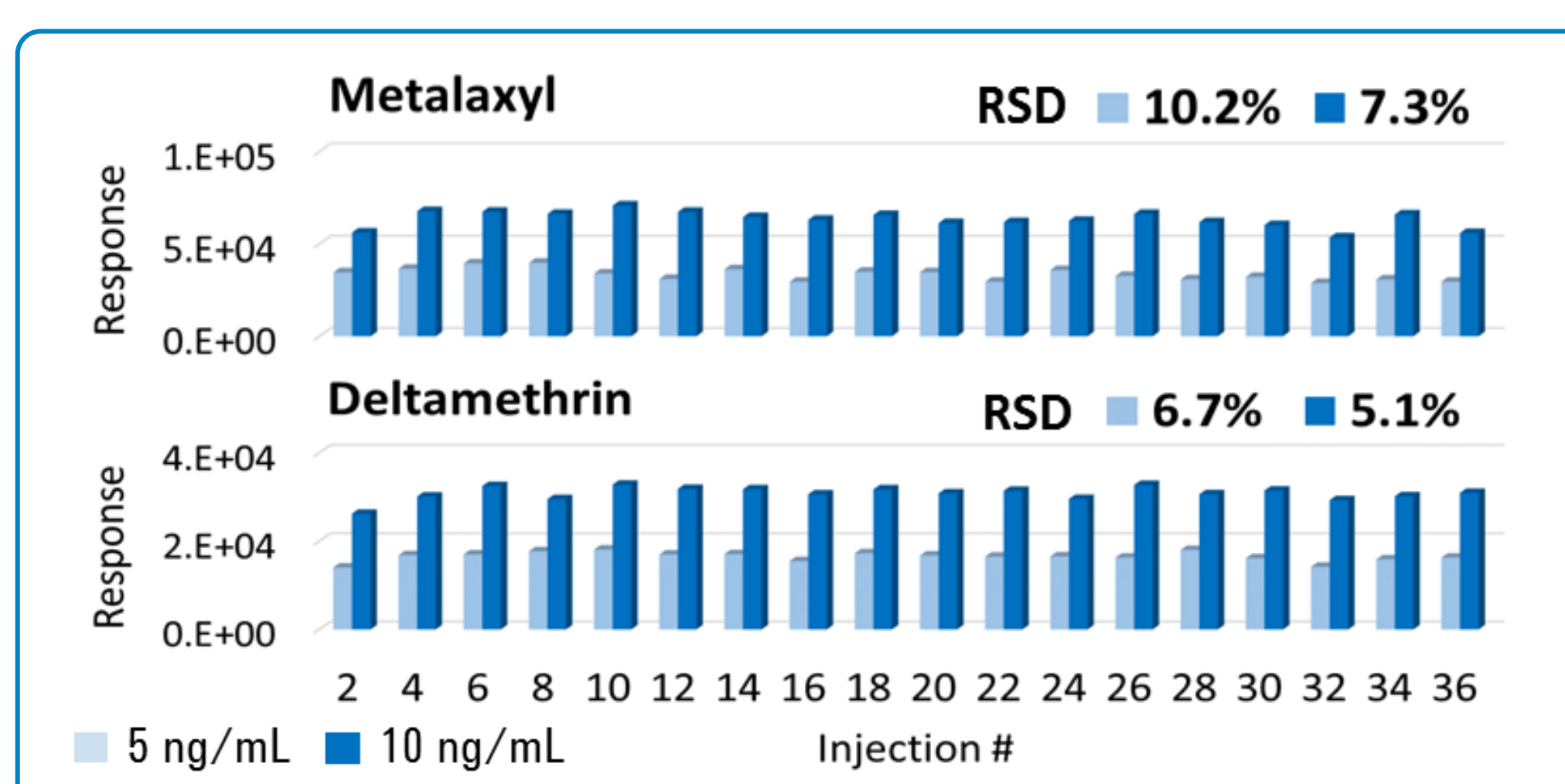


Figure 3. Long Term Stability in Avocado

Results and Discussion

Ion Ratio (IR) and Mass Accuracy (MA)

Over 90% of identified pesticides yielded at least one pair of ions with relative IR within 30% variance to that in the corresponding library spectrum. The IR of almost all identified pesticides is < 30% when comparing to the measured spectra with reference pesticide standards. The stability of IR is illustrated by two example pesticides in Figure 4.

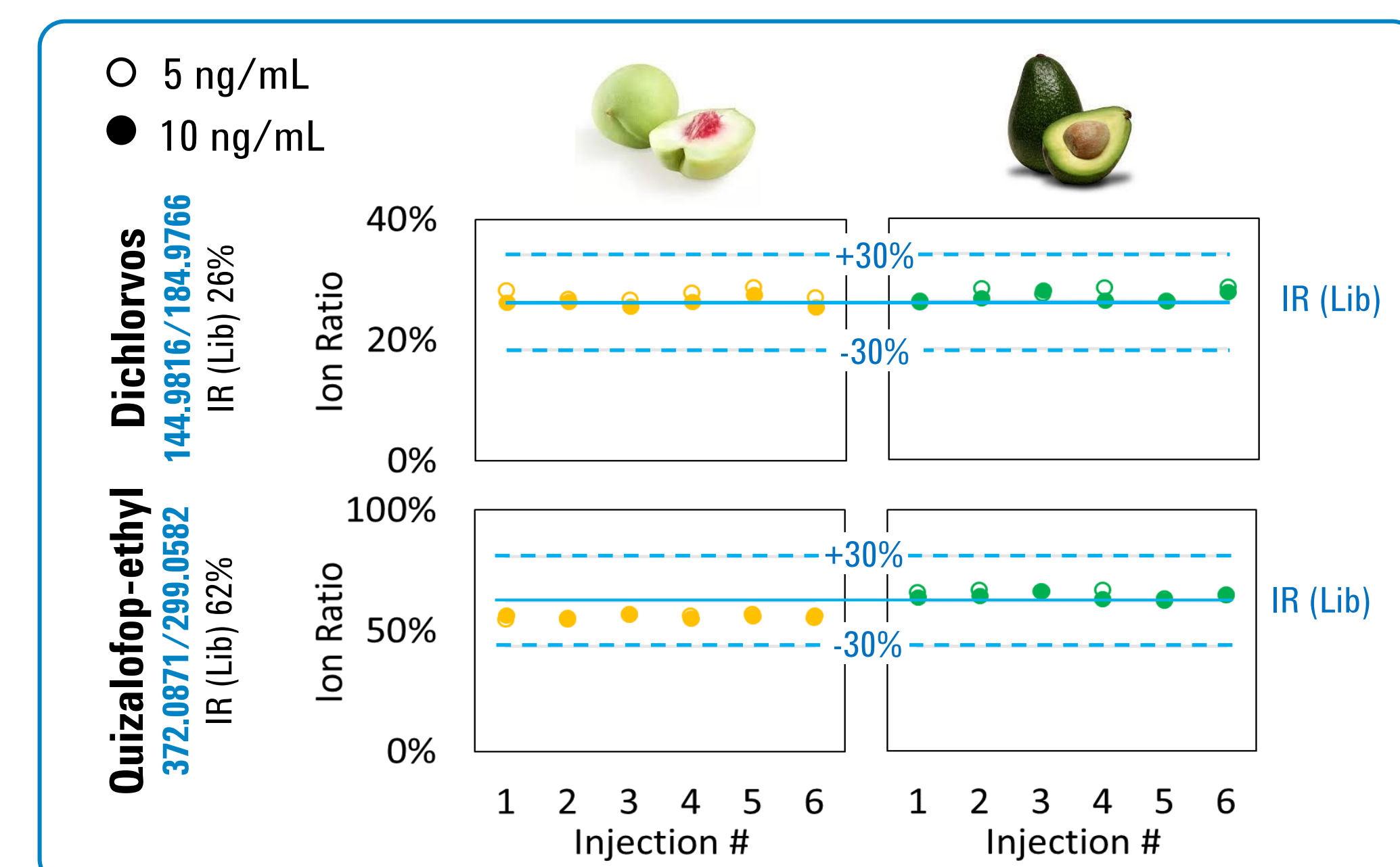


Figure 4. Ion Ratio Stability in Food Extracts

The analysis of these pesticides by GC/Q-TOF provided excellent mass accuracy for most pesticides in the investigated complex matrices (Table 3). Compound peak average spectrum was used to assess the mass accuracy. For those pesticides with MA > 5 ppm, the majority had at least 3 ions identified with S/N >=3 for the corresponding EICs and had relative IR variance < 30% compared to their reference spectra, thus meeting identification criteria in major guidelines.

Table 3. Mass Accuracy at 10 ng/mL in Food Matrices

Matrix	Number of Pesticides (MA < 5 ppm)
Peach	117
Avocado	108

Increased Dynamic Range

The *SureMass* feature, an innovative profile based algorithm, was used to calibrate the identified pesticides over a wide dynamic range. The matrix matched calibrating standards of 5-200 ng/mL (triplicates) yielded good linearity (R²>0.99) for 105 pesticides in the complex matrices, with calibration curves of two example compounds shown in Figure 5.

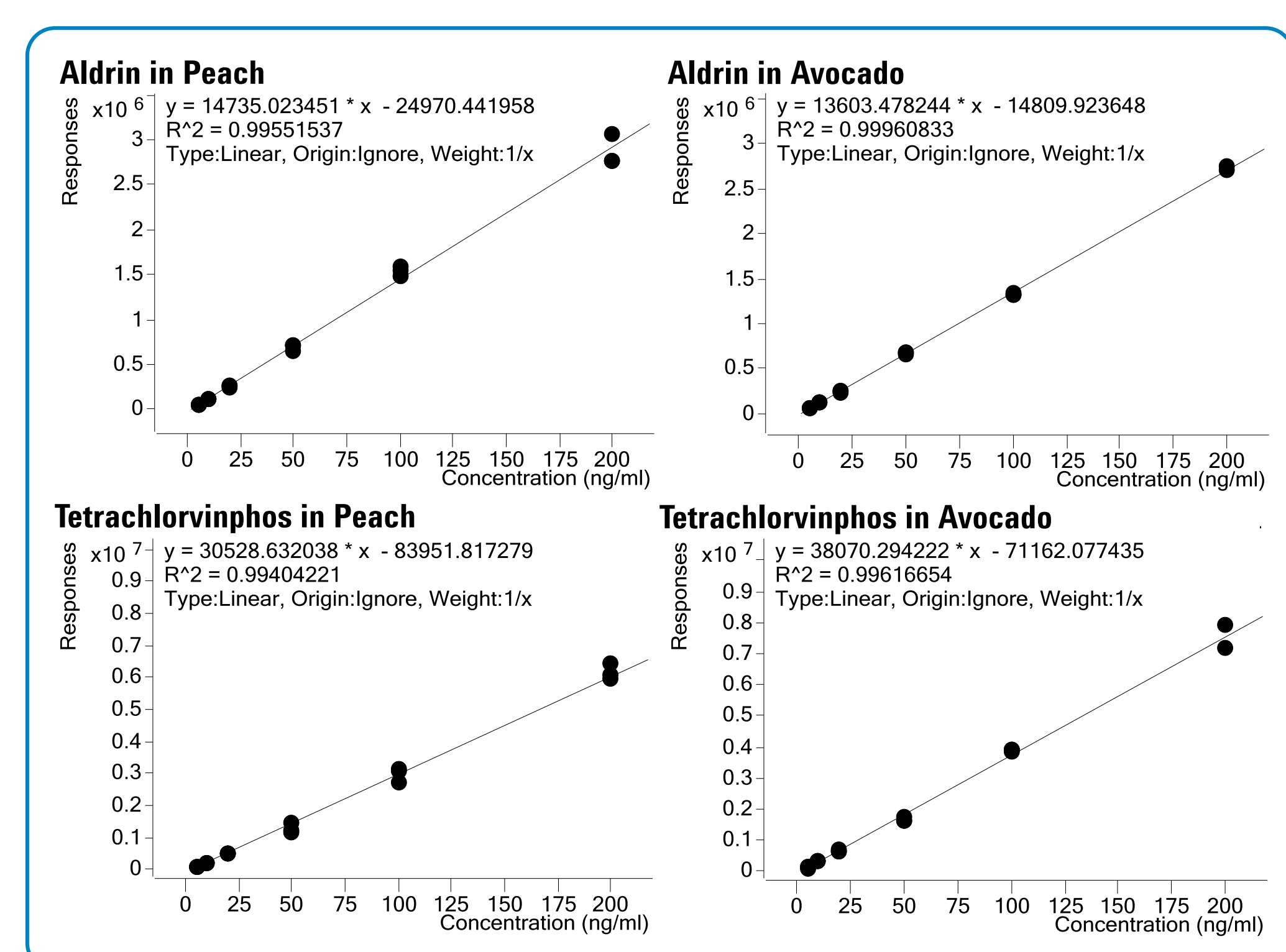


Figure 5. Matrix Matched Calibration Examples 5-200 ng/mL

Conclusions

- High resolution GC/Q-TOF and an accurate mass library has been combined to successfully screen pesticides in complex food matrices with enhanced confidence.
- An increased dynamic range calibration for data acquired at 4GHz mode can be achieved by the novel algorithm.
- Cleanup of the avocado extract using EMR-Lipid helps give stable RTs and responses because it efficiently removes lipids.