

Screening of Pesticides in Lipid-rich Food Matrices by Using High Resolution GC/Q-TOF and Accurate Mass Pesticide Library

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Introduction

The screening of a broad scope of pesticides in various food commodities is considered as one of the most demanding GC/MS applications in modern pesticide residue analysis laboratories. This application requires untargeted acquisition of full scan mass spectra of all GC amenable pesticides present in a sample, which offers the benefits of a more comprehensive data analysis, particularly in cases when unexpected or new contaminants emerge. The ability to identify pesticides of low concentration in complex matrices is also imperative to meet strict regulatory requirements on maximum residue levels (MRL).

High resolution accurate mass GC/Q-TOF serves as a fit-for-purpose tool in pesticide screening as it improves compound identification and reduces screening detection limits. In this study, we demonstrate a novel GC/Q-TOF based workflow to screen pesticides in lipid-rich food matrices with added confidence.

Experimental

Sample Preparation

Organic peanut oil, avocado and salmon were extracted using QuEChERS (EN) and followed by a cleanup with EMR-Lipid dSPE and polish with dry steps. A mixture of 120 pesticide standards were then spiked at 5 and 10 ng/mL in the extracts. Avocado extract was also spiked with 5-200 ng/mL of the standards for matrix matched calibration.



Figure 1. 7200 Series GC/Q-TOF System

Experimental

Instrumental Analysis

The samples were analyzed in EI full spectrum acquisition mode by an Agilent 7200 Series High Resolution Accurate Mass GC/Q-TOF (Figure 1). The system is configured with a mid-column backflushing setup (Figure 2). A 20 minute constant flow retention time locked (RTL) method has been utilized for chromatographic separation. Parameters of GC and MS are listed in Table 1.

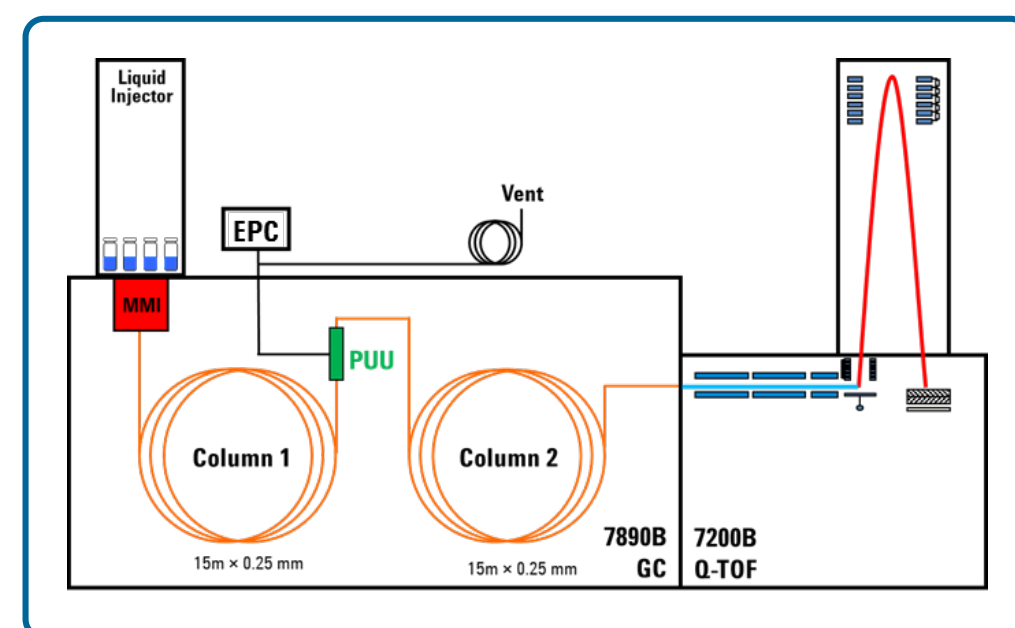


Figure 2. Configuration using Mid-column Backflushing.

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
Oven program	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)
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	EL 70 eV
Source temperature	300°C
Quadrupole temperature	180°C
Spectral Acquisition	45 to 550 m/z, 5 spectra/sec, 4 GHz high res mode

Data Analysis

Compound identification used a curated GC/Q-TOF accurate mass pesticide library and Find by Fragment workflow in pre-released MassHunter Qualitative Analysis (B.08) with enhancements to facilitate review of screening results. The identified compound information can also be easily transferred to pre-released MassHunter Quantitative Analysis software (B.08) for calibration over a wide linear dynamic range using an innovate profile-based algorithm.

Results and Discussion

Matrix	Peanut Oil		Avocado		Salmon	
Matrix Complexity (relative)	Low		Medium		High	
Spike Level (ng/mL)	5	10	5	10	5	10
Found by Auto-DA ^a	119	120	112	116	110	116
Found by Manual Extraction only ^b	1	n.a.	2	3	3	1
Total	120	120	114	119	113	117

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 ions found);
b. EIC manually extracted (mass extraction window of 25 ppm), at least 1 EIC with S/N >=3;

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

Pesticide Detectability

The 120 spiked pesticides represent a large variety of pesticide categories, including carbamate, nitroaniline, triazole, organochlorine, organophosphorus, and pyrethroid. An accurate mass pesticide library containing retention time and curated mass spectrum of each compound was used to perform the screening analysis. Over 110 spiked pesticides at concentrations of 5 ng/mL and 115 spiked pesticides at 10 ng/mL were identified by automated data analysis (Find by Fragment) in all three investigated matrices. The detailed detectability and data analysis parameters are tabulated in Table 2.

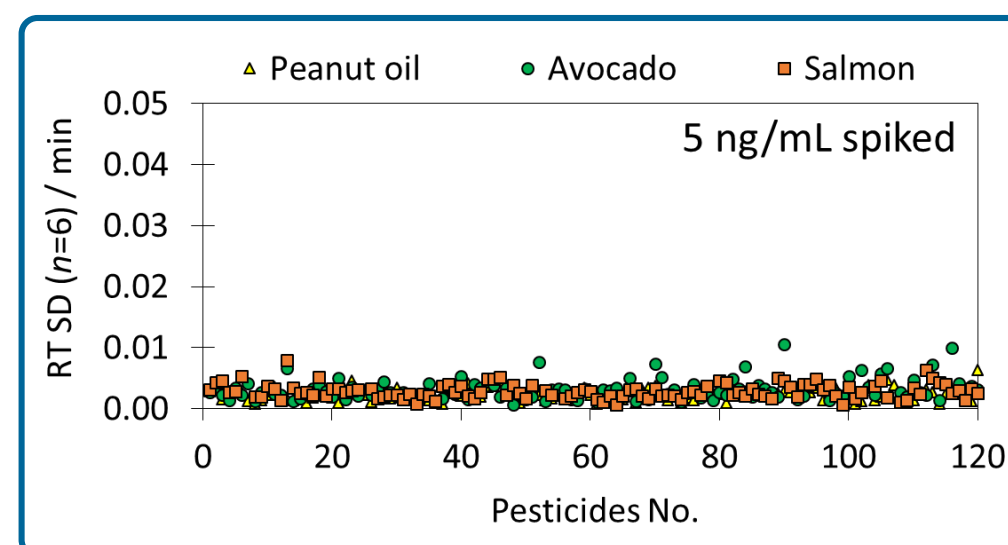


Figure 3. Standard Deviation of Retention Time.

Retention Time and Response Repeatability

The RTL backflushing method ensured retention time stability, with standard deviation (SD) ≤ 0.01 min observed for all identified pesticides even at 5 ng/mL in all three matrices (Figure 3). The instrument precision is illustrated by RSD distribution of identified pesticides at both 5 and 10 ng/mL (Figure 4). Most pesticides showed single digit %RSD, suggesting that the majority of screened pesticides can be detected at even lower concentrations.

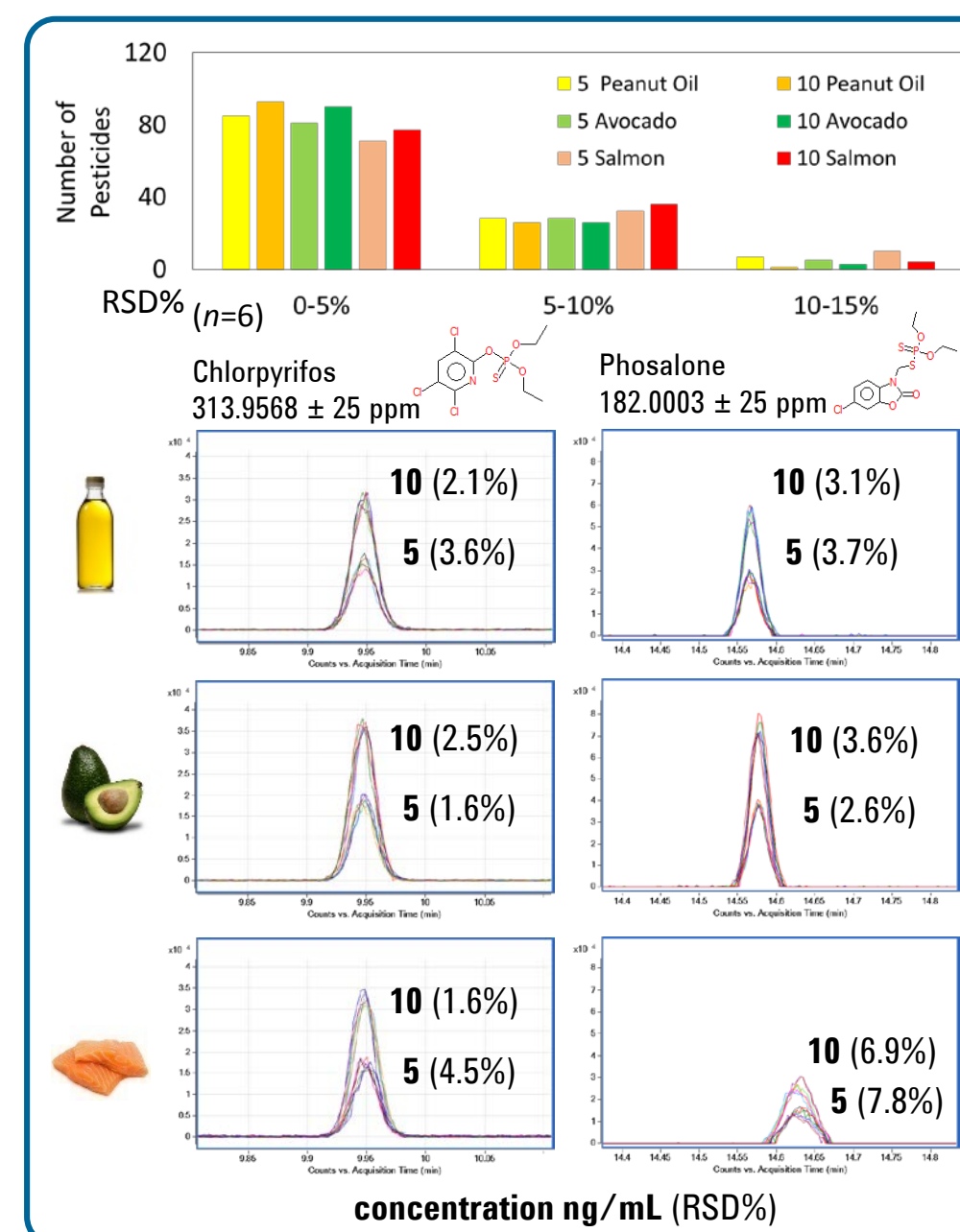


Figure 4. Response RSD% of Pesticides in Food Matrices.

Long Term Stability

The long term system stability has also been evaluated by a sequence of alternate injecting 5 and 10 ng/mL pesticides in avocado, with 36 injections in total. Figure 5 shows the long term response stability of four example pesticides.

Results and Discussion

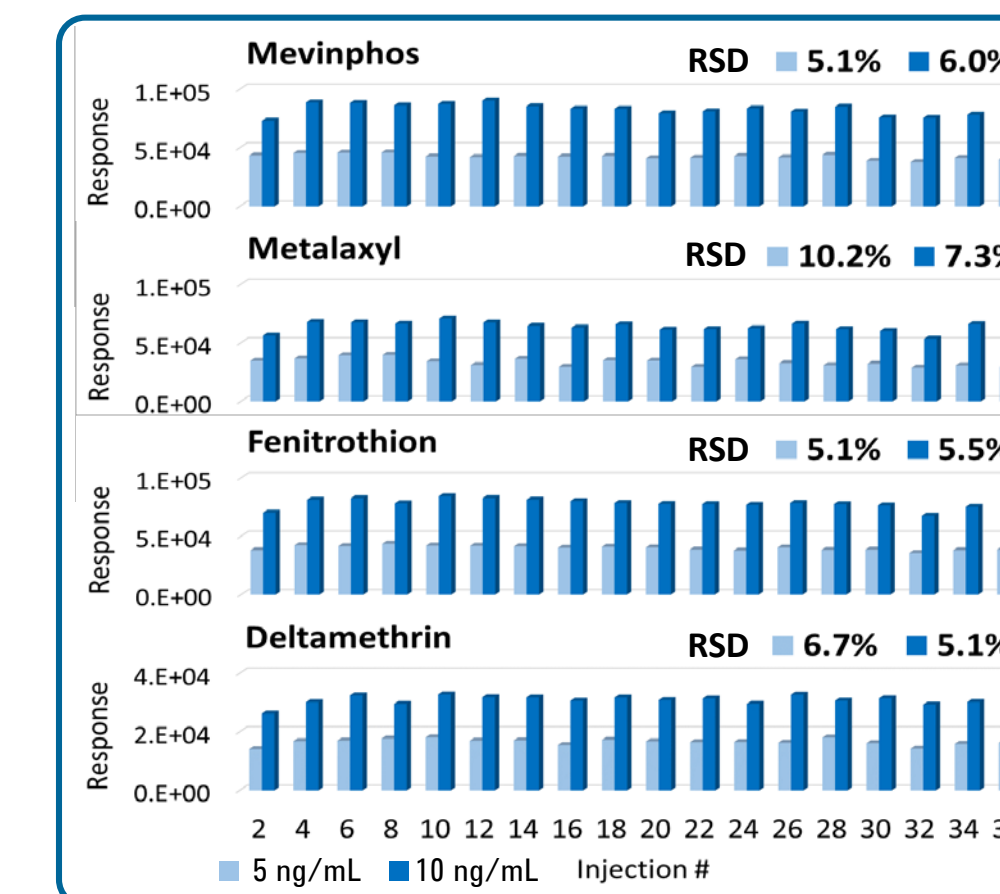


Figure 5. Long Term Stability in Avocado

Ion Ratio (IR)

Over 90% of the identified pesticides yielded at least one pair of qualified ions with relative IR within 30% variance to that in the corresponding library spectrum. The IR of almost all identified pesticides is < 30% when compared to the measured spectrum using pesticide standards. The stability of IR is illustrated by two examples in Figure 6.

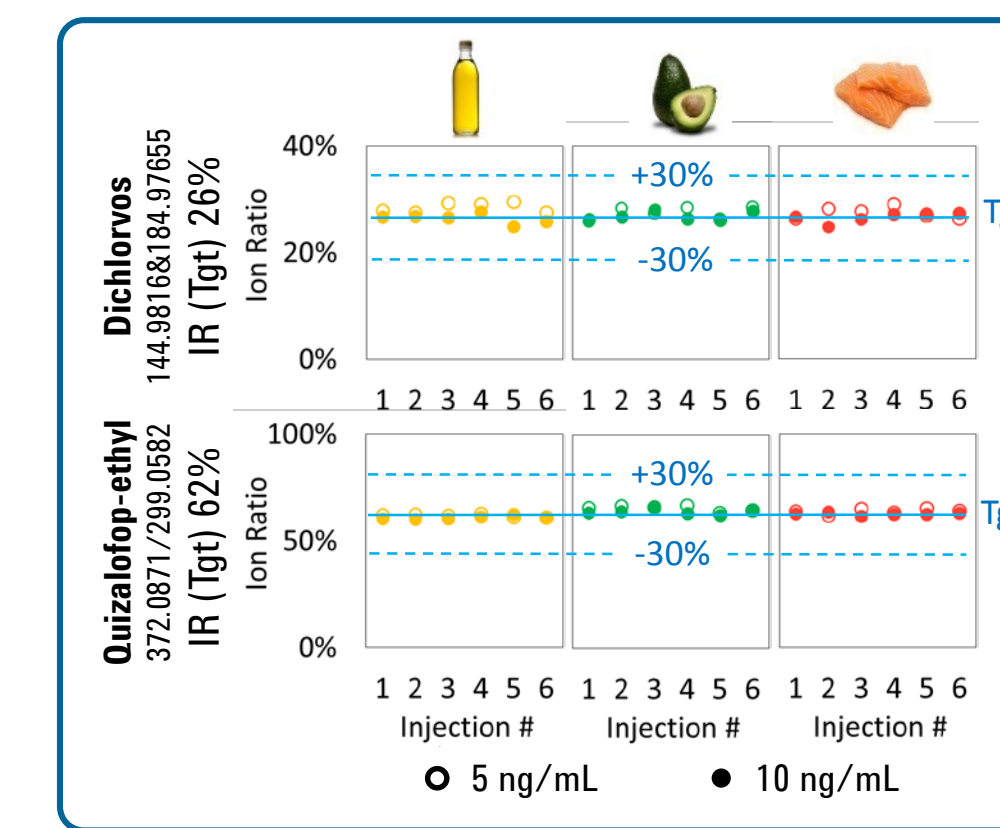


Figure 6. Ion Ratio (IR) Stability in Food Matrices.

Mass Accuracy (MA)

The analysis of these pesticides by GC/Q-TOF provided excellent mass accuracy for matrices of medium to higher complexity (Figure 5). The mass accuracy of each pesticide was calculated using the average spectrum extracted over the entire compound peak. 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)
Peanut Oil	120
Avocado	108
Salmon	107

Increased Dynamic Range

An innovative algorithm was used to calibrate the identified pesticides over a wide dynamic range in avocado. The calibration of 5-200 ng/mL (triplicates) yielded good linearity (R²>0.99) for 105 pesticides in this complex matrix, with results of two example compounds shown in Figure 7.

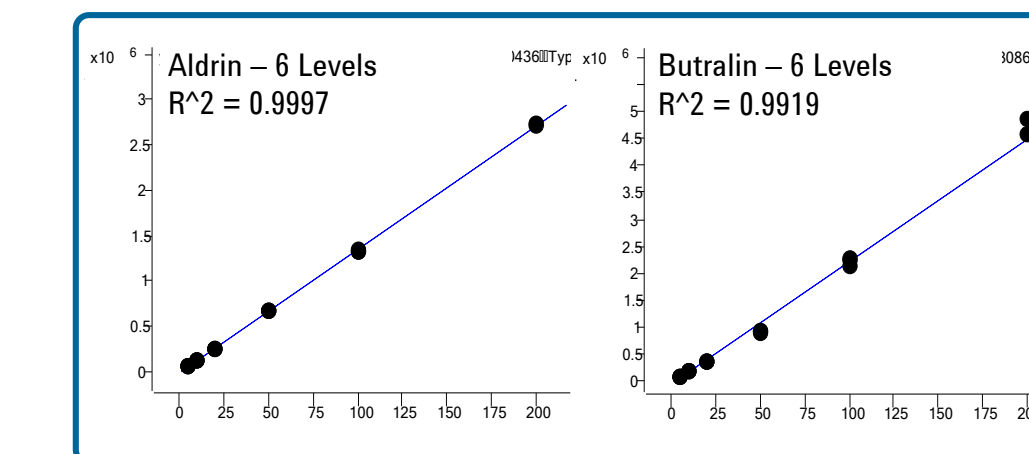


Figure 7. Calibration Result Examples in Avocado

Conclusions

- High resolution accurate mass GC/Q-TOF and an accurate mass library has been combined to successfully screen pesticides at low concentrations in lipid-rich complex food matrices.
- The confidence in identification is enhanced by stable RT, repeatable response and good mass accuracy.
- A wide dynamic range calibration of 5-200 ng/mL can be achieved using innovative algorithmic techniques.