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 the 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 as it improves compound identification and reduces screening detection limits. In this study, we demonstrates 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

Instrumental Analysis

The samples were analyzed in EI full spectrum acquisition mode by 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 min constant flow retention time locked (RTL) method has been utilized for chromatographic separation. Parameters of GC and MS are listed in Table 1.

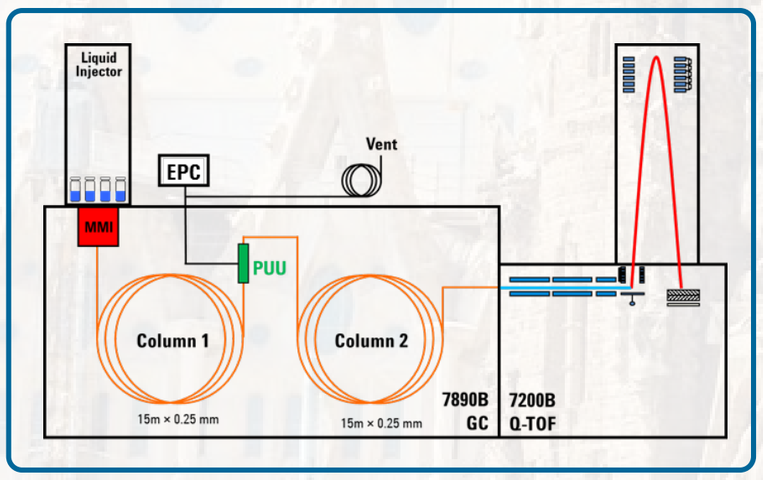

Figure 2. Configuration using Mid-column Backflushing.

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	2uL, 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	EI, 70eV
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 MassHunter Qualitative Analysis software (B.08) with enhancements to facilitate review of screening results. The identified compound information can also be easily transferred to MassHunter Quantitative Analysis software (B.08) for calibrated quantitation over a wide dynamic range using an profile-based innovative algorithm.

Results and Discussion

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

Matrix	Peanut Oil		Avocado		Salmon	
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 qualified);
b. EIC manually extracted (mass extraction window of 25 ppm), at least 1 EIC with S/N >=3;

Pesticide Detectability

The 120 spiked pesticides represents a large variety of categories, which includes carbamate, nitroaniline, triazole, organochlorine, organophosphorus, pyrenthoid. 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 in 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.

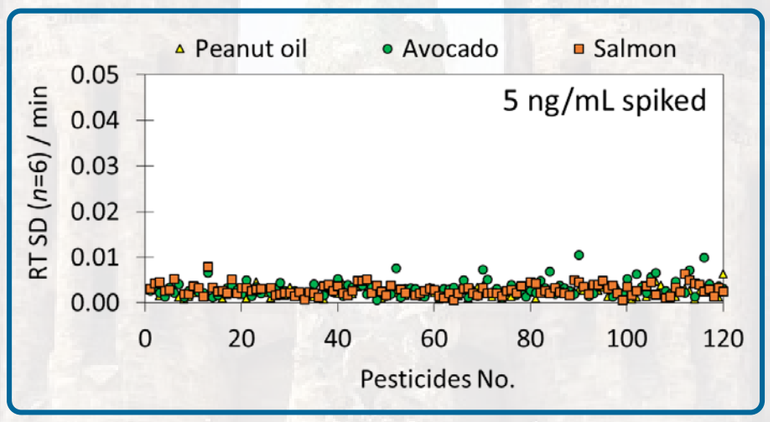

Figure 3. Standard Deviation of Retention Time.

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 at even 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), and most of pesticides showed single digital %RSD. This also suggests that the majority of pesticides can be detected at even lower concentrations.

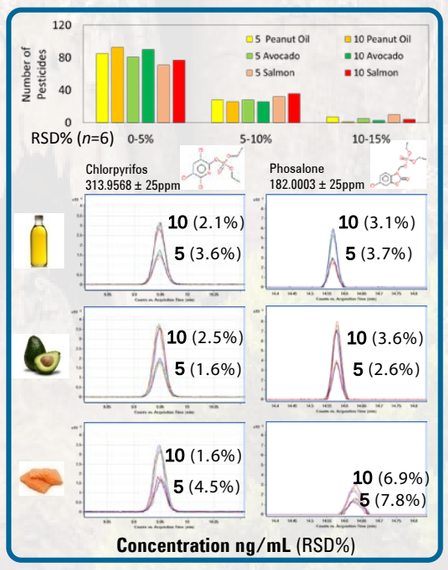

Figure 4. Response RSD% of Pesticides in Food Matrices.

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.

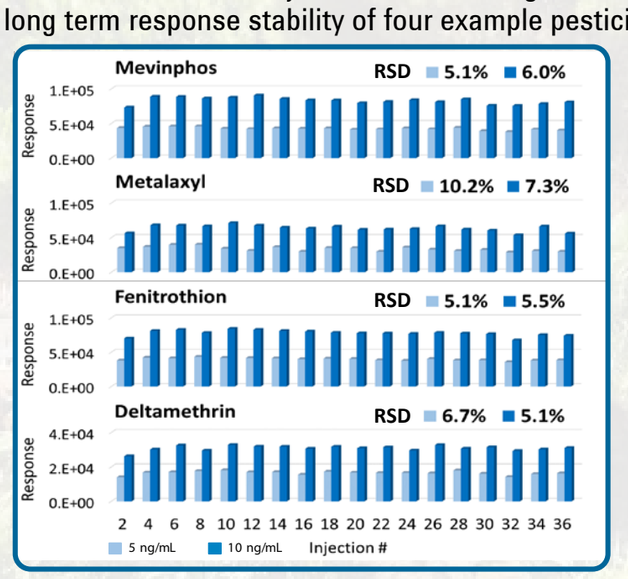

Figure 5. Long Term Stability in Avocado.

Ion Ratio (IR)

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

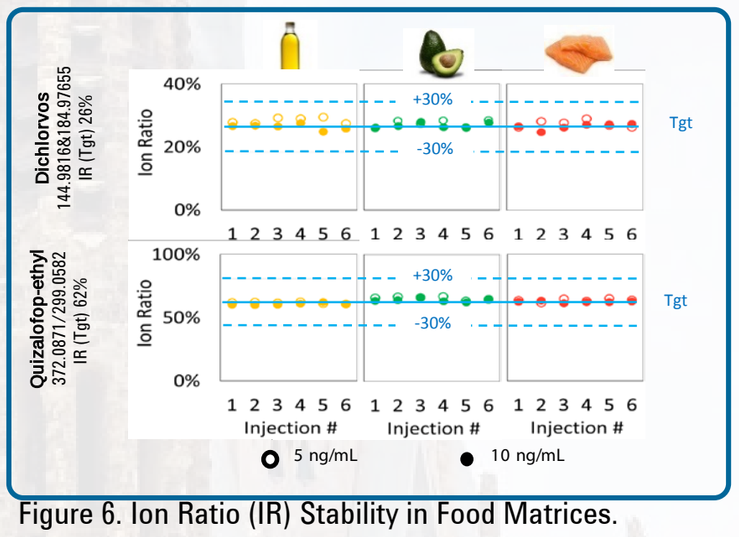

Figure 6. Ion Ratio (IR) Stability in Food Matrices.

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

Extended 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 (R^2>0.99) for 105 pesticides in this complex matrix, with results of two example compounds shown in Figure 7.

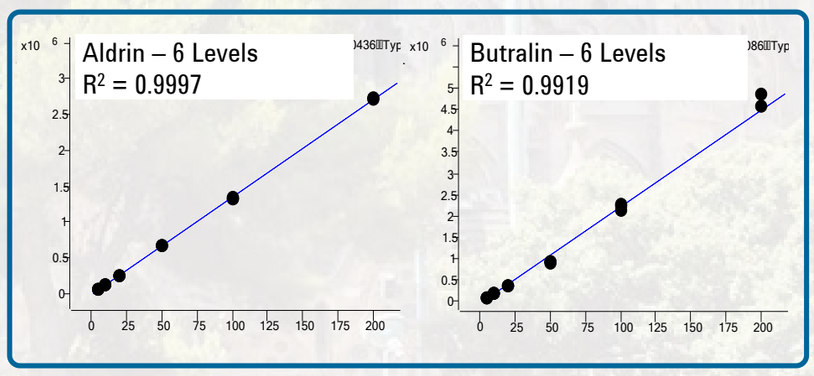

Figure 7. Calibration Result Examples in Avocado.

Conclusions

- High resolution GC/Q-TOF and an accurate mass library has been combined to successfully screen pesticides of 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 the innovative algorithm.