

Increased Productivity in Pesticide Residue Analysis – Quantifying 440 Pesticides Following China GB 2763-2014: The Pesticide Explorer Collection – Standard Quantitation

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Key Words

Pesticides analysis, food safety, TSQ Endura, TraceFinder, MRL, tSRM, residue analysis

Goal

Developing a robust, sensitive, high-throughput method for quantitation of 440 pesticide residues in bell pepper in a regulated environment.

Introduction

In recent years, growing concerns over food safety and the expanding world agricultural trade have led to the promulgation and enforcement of stricter pesticide regulations. In 2014, China's Ministry of Agriculture and Ministry of Health jointly issued a revised national food safety standard, GB 2763-2014 - Maximum Residue Limits for Pesticides in Food.¹ This new standard expanded the number of categories of pesticide residues and the total number of maximum residue limits (MRLs). Together with the Japanese Positive List System² and EU/EC Directive No. 752/2014,³ these standards constitute some of the strictest food safety regulations globally and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues. While it is critical to address the challenge of developing sensitive, robust analytical methods for pesticide residues, most existing solutions lack the ability to quantify multiple pesticide residues in one single experiment.

Here, a method utilizing the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer and Thermo Scientific™ TraceFinder™ software is described for the simultaneous, high-throughput, quantitative analysis of 440 pesticide residues in bell pepper.

Experimental

Sample Preparation

Pesticide standards were obtained from ULTRA Scientific (North Kingstown, RI). The stock solution was prepared in acetonitrile at a concentration of 2.5 µg/mL.

Calibration solutions were prepared by serial dilution of the pesticide stock solution in acetonitrile/water (40/60 v:v).

Bell pepper samples, provided by the California Department of Food and Agriculture (CDFA), were extracted using a QuEChERS method in which 5 g of homogenized bell pepper and 15 mL of acetonitrile were used. The final QuEChERS extracts were diluted with 1.5 times their volume of ultrapure water. Finally, the extracts were spiked with the pesticides standard, mixed, and vortexed thoroughly to produce a set of solutions with concentrations of 0.001 to 200 pg/µL (ppb).

Liquid Chromatography Method

Chromatographic separation was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 ultra-high-performance liquid chromatography system, equipped with an UltiMate HPG3400-RS Rapid Separation Binary High-Pressure Gradient Pump, WPS-3000TRS Rapid Separation Well Plate Autosampler, and TCC-3000RS Rapid Separation Thermostatted Column Compartment.

The chromatographic conditions were as follows:

Column	Thermo Scientific™ Accucore™ aQ (100 x 2.1 mm, 2.6 µm), P/N 17326-102130
Mobile phases	Aqueous phase: Water + 5 mM ammonium formate + 0.1% formic acid Organic phase: Methanol + 5 mM ammonium formate + 0.1% formic acid
Flow rate	300 µL/min
Column temperature	30 °C

Gradient

Time (min)	% Aqueous	% Organic
0.0	98	2
0.5	98	2
2.0	60	40
20.0	5	95
22.0	5	95
22.1	98	2
25.0	98	2

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Mass Spectrometry Method

Compounds were detected using the TSQ Endura MS equipped with a Thermo Scientific™ Easy-Max NG™ HESI III heated electrospray ionization source. Timed selected-reaction monitoring (tSRM) scan mode employing fast polarity switching was used. The tSRM times were based on the peak width differences of the pesticide residues; they were set to 1 min for the majority of compounds and up to 5 min for tridemorph, propiconazole and a small number of other substances.

The MS conditions were as follows:

Vaporizer temperature	450 °C
Ion transfer tube temperature	200 °C
Spray voltage	3500 V (ESI+); 2500 V (ESI-)
Sheath gas	60 arb
Auxiliary gas	5 arb
Sweep gas	1 arb
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7
Collision cell pressure	1.5 mTorr
tSRM scan cycle time	1.2 s

Data Processing

Method development, data acquisition, and data processing were performed with TraceFinder software. TraceFinder software uses a compound database (CDB) that includes retention times and CAS numbers, plus other relevant information needed for confirmation of pesticides (Figure 1). Using the CDB, standard samples are no longer necessary for method optimization and development. Instead, relevant conditions can be imported from the database to directly conduct sample analysis.

The various tSRM conditions, including retention time, SRM fragmentations, RF lens voltage, and collision energy, were imported directly from the CDB within TraceFinder software (Figure 2). The drag-and-drop method editor accelerated method development and supported the flexible customization of various method templates.

As shown in Figure 3, TraceFinder software streamlines the laborious process of analyzing hundreds of pesticide residues simultaneously.

The screenshot displays the Thermo TraceFinder LC software interface. The main window is titled 'Thermo TraceFinder LC' and shows a 'Compound Database - ChinaEFS_DB@Endura' view. The interface is divided into several sections:

- Method Development:** Includes sections for Method View, Acquisition, Quantitation, Processing, Compounds, Limits, Groups, Intel Seq, and Reports.
- Compound Database:** A table listing various pesticides with their names in Chinese and English, and their corresponding SRM and Formula values. The table is searchable and filterable.
- Compound Detail:** Provides detailed information for a selected compound, 'aspon 丙硫特普'. It includes the experiment type (SRM), category (insecticides), CAS number (3244-90-4), formula (C12H28O5P2S2), ionization method (ESI), response threshold (5000), and neutral mass (378.08534).
- Target Peaks:** Shows the precursor mass (379.115) and product mass (210.890) for the selected compound, along with confirming peaks and their collision energies.

Figure 1. Pesticide detection method from TraceFinder CDB database.

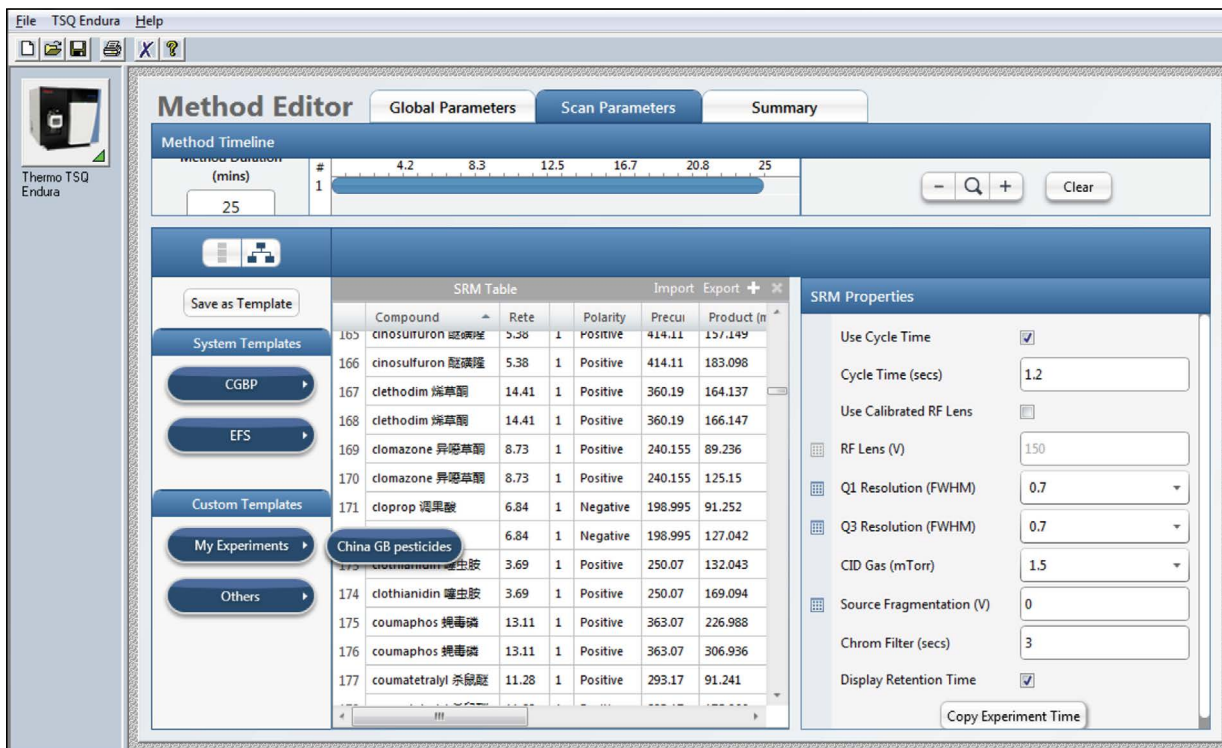


Figure 2. TraceFinder Method Editor, showing experimental conditions.

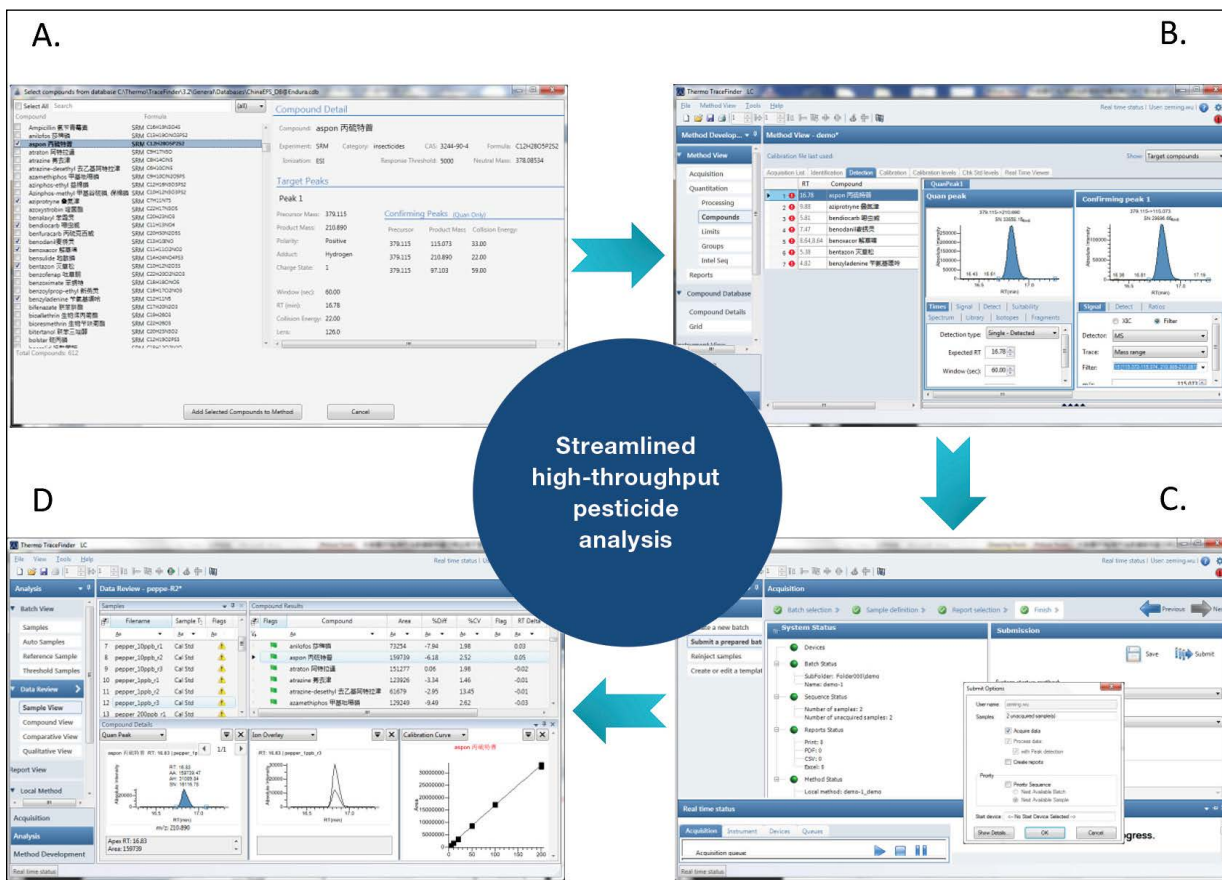


Figure 3. TraceFinder software streamlines pesticide residues analysis. (A) Choose pesticide residues needed for analysis from CDB. (B) Create instrument method and data processing method. (C) Compile analysis, operation, and data collection sequence. (D) Analyze data, browse results, and create reports.

Results and Discussion

The TSQ Endura MS, which uses simple tSRM scan functions, can quickly calculate the correct dwell time needed to run hundreds of pesticides simultaneously within a rapid gradient to achieve sensitive detection (Figure 4). Figure 5 displays the optimized chromatographic conditions needed to detect the 440 pesticide residues.

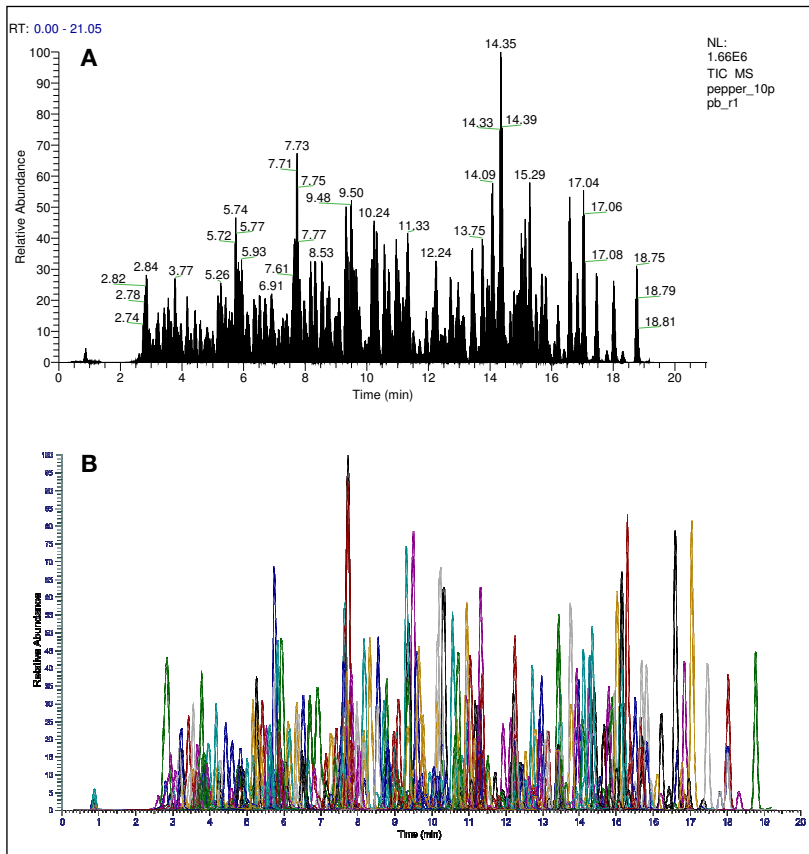


Figure 4. (A) Total ion chromatogram of 440 pesticide residues simultaneously detected in bell pepper; (B) Extracted ion chromatogram of 440 pesticide residues (10 pg/ μ L).

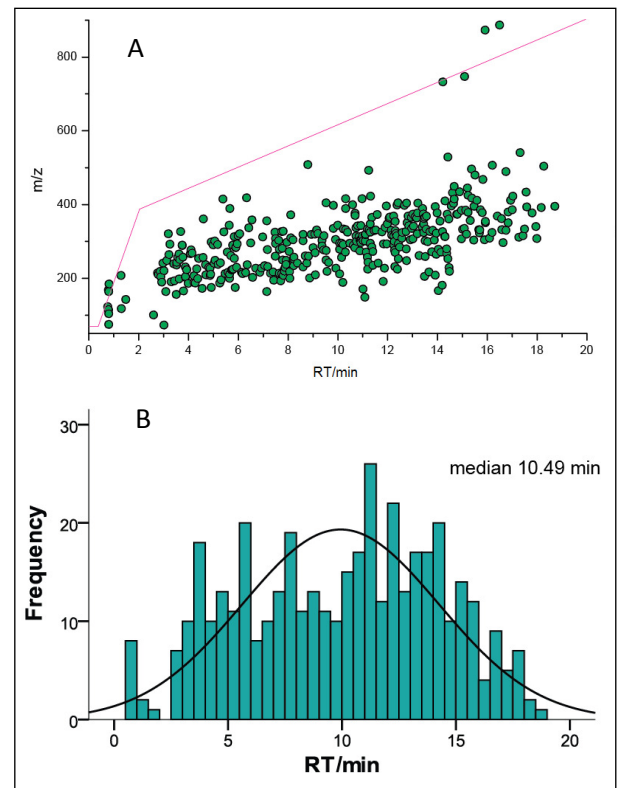


Figure 5. (A) Scatter plot of pesticide residue m/z vs. retention time; (B) Frequency distribution of retention times.

TraceFinder software provides a comprehensive system for high-throughput pesticide residue analysis that incorporates built-in methods for commonly found pesticides, processing methods, library searching capabilities, data review, and reporting with built-in, customizable templates. Figure 6 shows the results displayed graphically. Sample and reference mass spectra can be inspected, peak integration evaluated, different curve fits reviewed, and ion ratio values observed easily and fully interactively

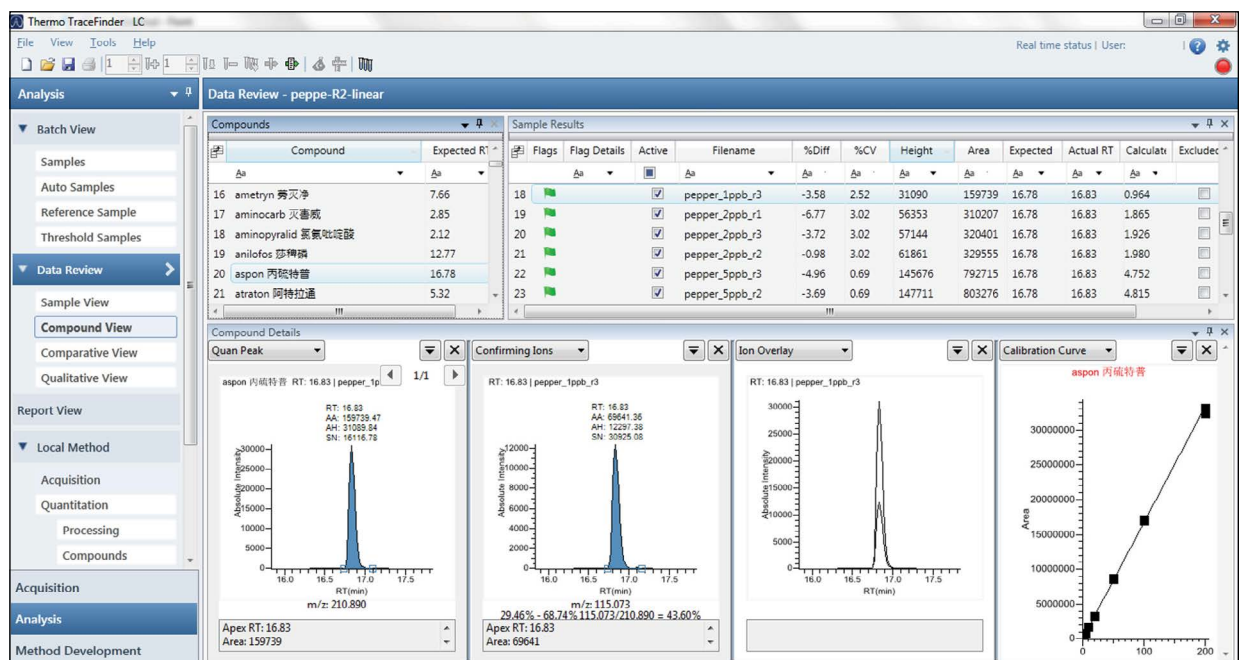


Figure 6. TraceFinder software Data Review page, showing the Compound View with the results for specific compounds.

Three factors—the coefficient of variation (CV) of the peak area, the peak shape, and the signal-to-noise ratio—were analyzed to determine the LOD and LOQ of the 440 pesticide residues in bell pepper. The CV for the reproducibility and stability of the three sample injections was less than 30% at the LOD concentrations and less than 20% at the LOQ concentrations. LOQs are represented in Figure 7.

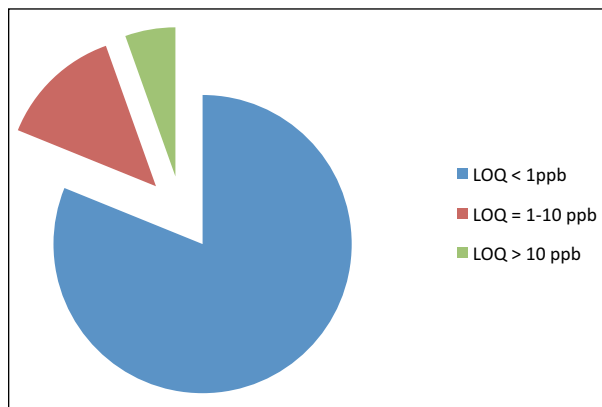


Figure 7. Representation of the LOQs detected in bell pepper matrix.

Conclusion

Addressing a critical challenge of developing a sensitive, robust, reproducible quantitative assay to quantify pesticide residues, a multi-residue method was developed for the screening and determination of 440 pesticides in a single run on the TSQ Endura triple quadrupole mass spectrometer. Data analysis was streamlined by using TraceFinder software, which is ideally suited for quantitation of large amounts of data. For this multi-pesticide residue study, a timed SRM experiment provided accurate and sensitive results for the analysis of each compound per experiment. The majority of the pesticides were detected in the spiked matrices at concentrations lower than the MRLs established by China, Japan, and the EU.

Acknowledgement

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