

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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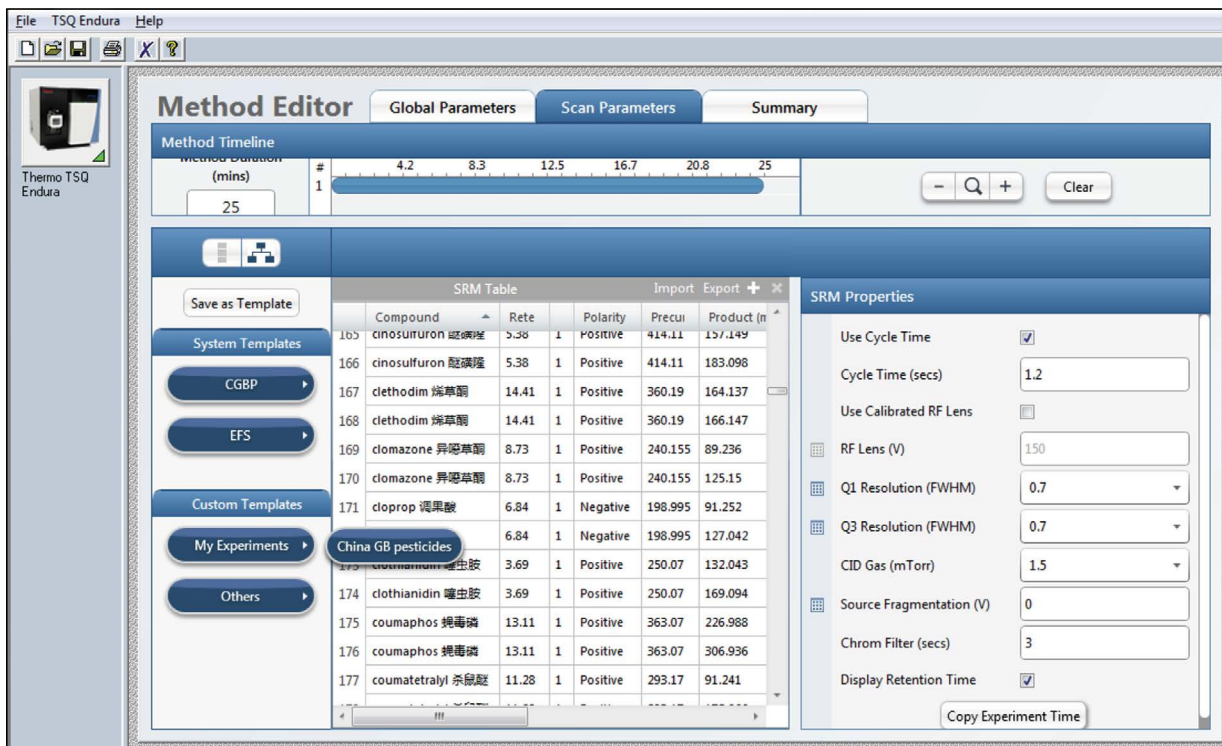


Figure 2. TraceFinder Method Editor, showing experimental conditions.

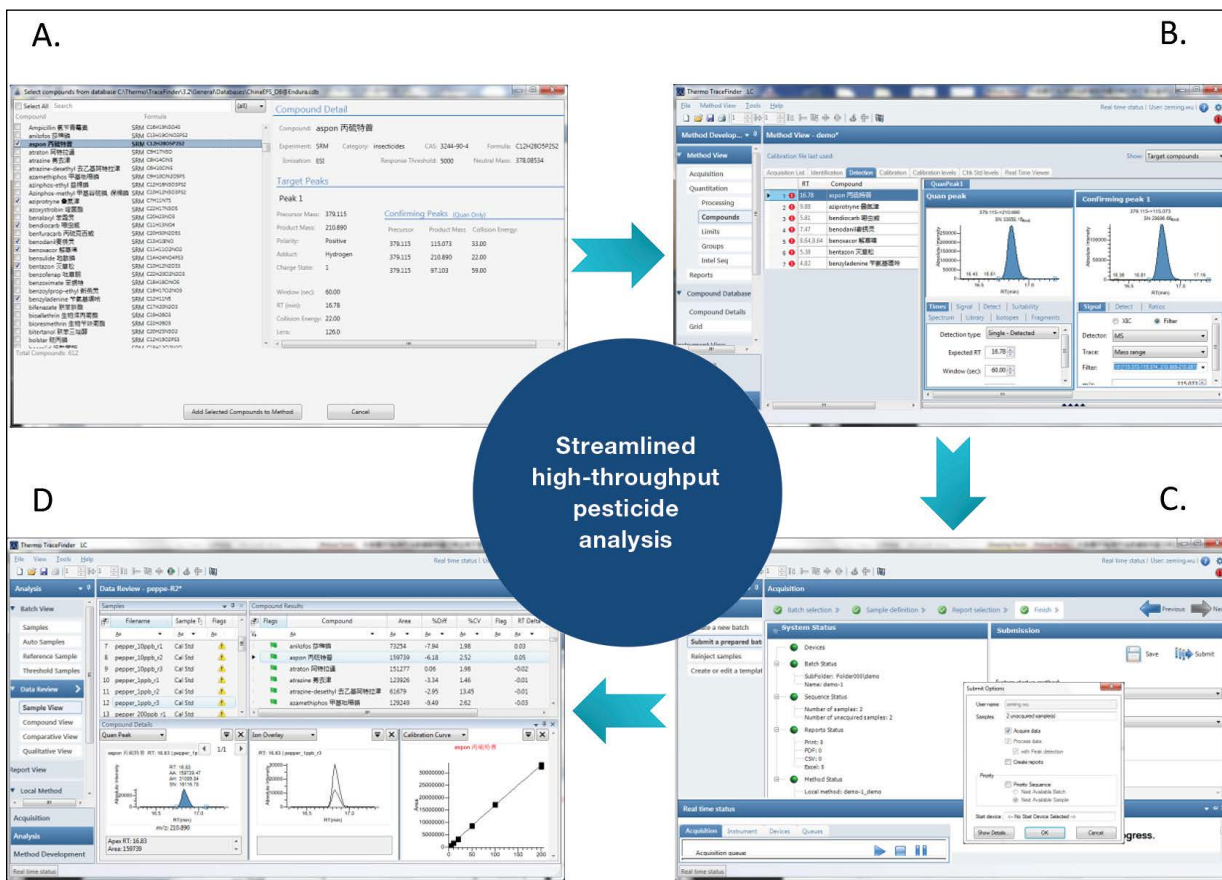


Figure 3. TraceFinder software streamlines pesticide residues analysis. (A) Choose pesticide residues needed for analysis from CDB. (B) Create instrument method and data processing method. (C) Compile analysis, operation, and data collection sequence. (D) Analyze data, browse results, and create reports.

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.

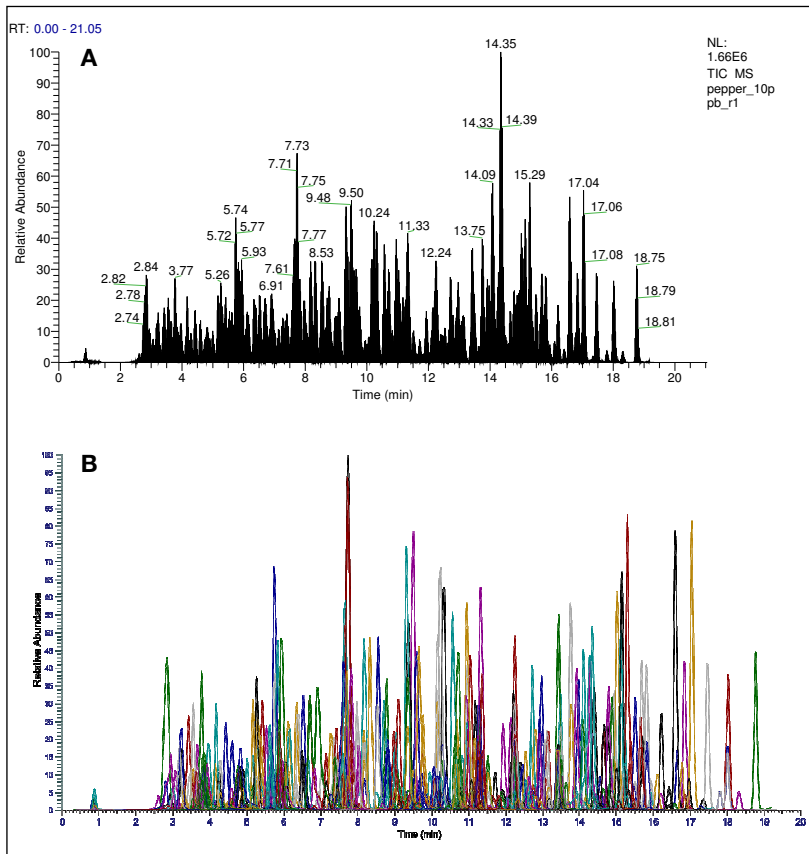


Figure 4. (A) Total ion chromatogram of 440 pesticide residues simultaneously detected in bell pepper; (B) Extracted ion chromatogram of 440 pesticide residues (10 pg/ μ L).

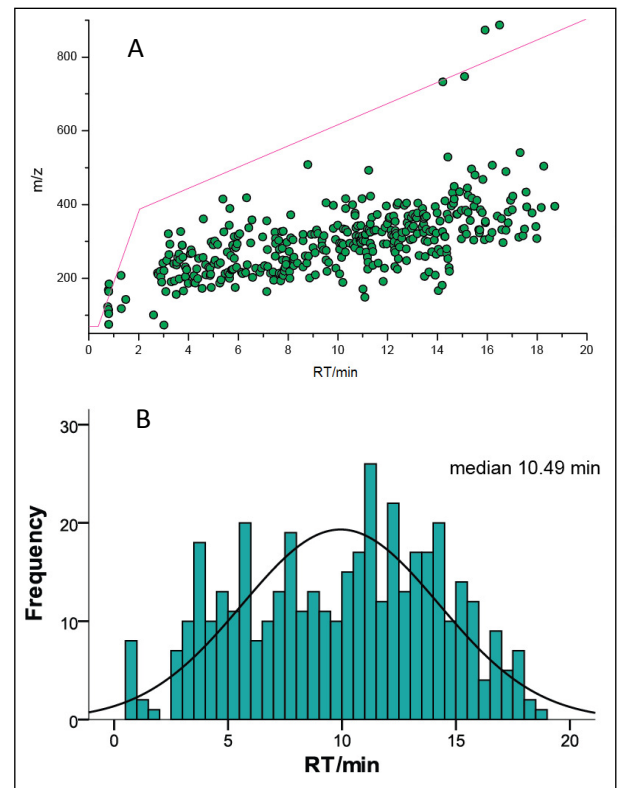


Figure 5. (A) Scatter plot of pesticide residue m/z vs. retention time; (B) Frequency distribution of retention times.

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

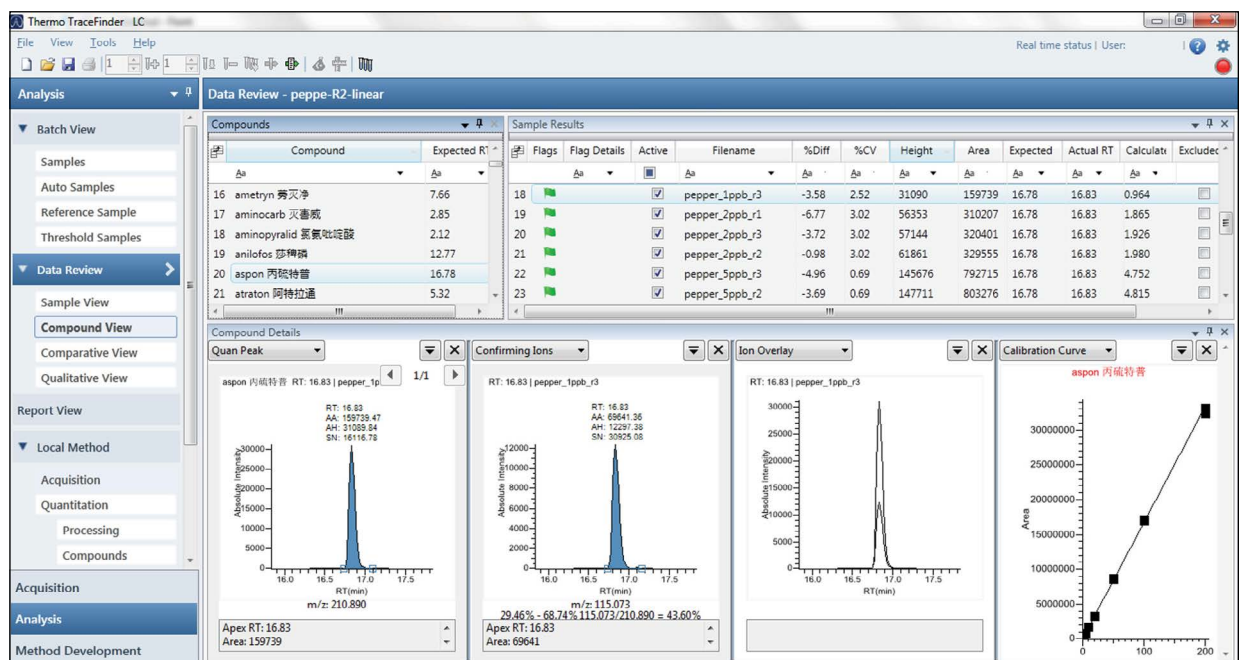


Figure 6. TraceFinder software Data Review page, showing the Compound View with the results for specific compounds.

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.

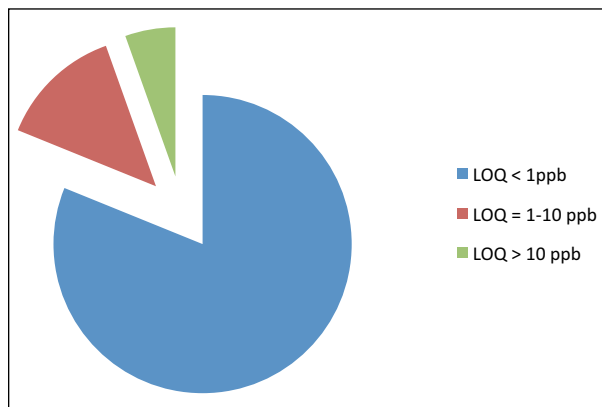


Figure 7. Representation of the LOQs detected in bell pepper matrix.

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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References

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