

Targeted screening and quantitation of pesticide residues in green tea using a Quadrupole Time-of-Flight Mass Spectrometer

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1. Overview

The purpose of this study is to screen and identify pesticide chemicals in green tea. Compounds from green tea were extracted with QuEChERS method and analyzed with MS/MS measurement using a LC/Q-TOF-MS.

2. Introduction

With population growth and the development of agriculture and food industries, food safety is becoming a greater concern in recent years. The increasingly stricter regulations of residual food contaminants requires faster and more accurate analytic techniques. For this purpose, targeted screening by simultaneous MRM measurements using a triple quadrupole mass spectrometer is the most common strategy. Data acquisition using a high-resolution and accurate mass spectrometer (HRMS), such as a Time-of-Flight, is also carried out coupled with MRM-based analysis for the purpose of guaranteeing the robustness and reliability of screening. In addition, HRMS also provides a different advantage from MRM measurement because a compound which was not included as a target when acquiring data can be processed later without reacquiring data.

3. Methods

Compounds in green tea samples were extracted with QuEChERS extraction after grinding with cryogenic milling (Figure 2). The resulted acetonitrile layers were collected and purified by a fully automated solid phase extraction system (ST-400; Aisti Science, Wakayama, Japan). Pesticide mixtures or green tea extracts were analyzed using a Quadrupole Time-of-Flight Mass Spectrometer (LCMS-9030; Shimadzu Corporation, Kyoto, Japan) coupled with conventional flow liquid chromatography (Nexera X2; SHIMADZU). LC separation was performed using a Raptor Biphenyl (2.1 mm × 100 mm, RESTEK) with binary gradient of 2 mmol/L ammonium formate + 0.002% formic acid in water and 2 mmol/L ammonium formate + 0.002% formic acid in methanol. Assignments with MS/MS spectra and predicted fragments from a molecular structure are performed using a third party's software, ACD/MS Workbook Suite (ACD/Labs, Toronto, Canada).



Figure 1 LCMS-9030 quadrupole time-of-flight mass spectrometer

High-Resolution and Accurate Mass Spectrometer

Resolution power:
 > 30,000 FWHM at m/z 1,972 / 1,626
 Mass accuracy: 1 ppm
 Maximum acquisition rate: 100 Hz
 Minimum polarity switching rate: 1 second

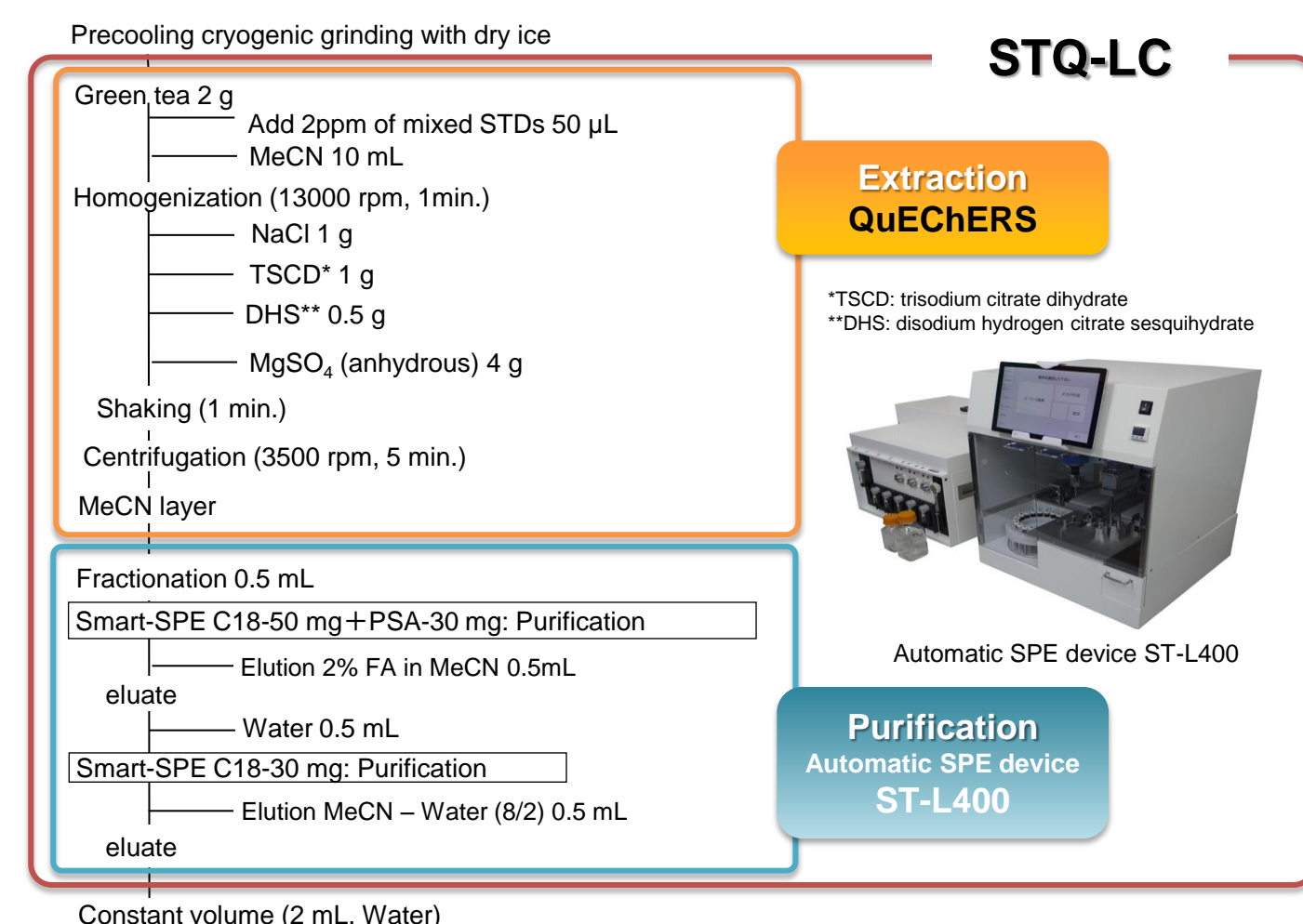


Figure 2. Sample preparation of pesticides in green tea

4. Results

4-1. Analysis conditions for pesticides

We prepared a mixed standard sample of 157 pesticide compounds frequently used in local tea agriculture. The theoretical m/z of each chemical was calculated and listed up. Mass chromatogram was confirmed by a mass error tolerance of ± 10ppm. The retention time of each chemical was recorded by injection of standard sample by using the following instrument parameters.

UHPLC (Nexera X2)	
Analytical Column:	Restek Raptor™ Biphenyl (2.1 mmI.D. x 100 mmL., 2.7 µm)
Mobile Phase A:	2 mmol/L Ammonium formate + 0.002% Formic acid – Water
Mobile Phase B:	2 mmol/L Ammonium formate + 0.002% Formic acid – Methanol
Gradient Program:	3%B (0 min) – 10%B (1 min) – 55%B (3 min) – 100%B (10.5 – 12 min) – 3%B (12.01 – 15 min)
Flow Rate:	0.4 mL/min
Column Temperature:	35 °C
Injection Volume:	10 µL
MS (LCMS-9030)	
Ionization:	ESI positive
Acquisition mode:	MS
Nebulizing Gas Flow:	3 L/min
Drying Gas Flow:	10 L/min
Heating Gas Flow:	10 L/min
Interface Temperature:	350 °C
DL Temperature:	150 °C
HB Temperature:	300 °C
TOF range:	m/z 120 - 1000
Event Time:	0.4 s (Pulser inj.: 794)
ID:	off

Figure 3 shows MRM chromatograms of 157 pesticide chemicals. It took 12 minutes per LC-MS/MS analysis. Excellent separation and high sensitivity detection were obtained.

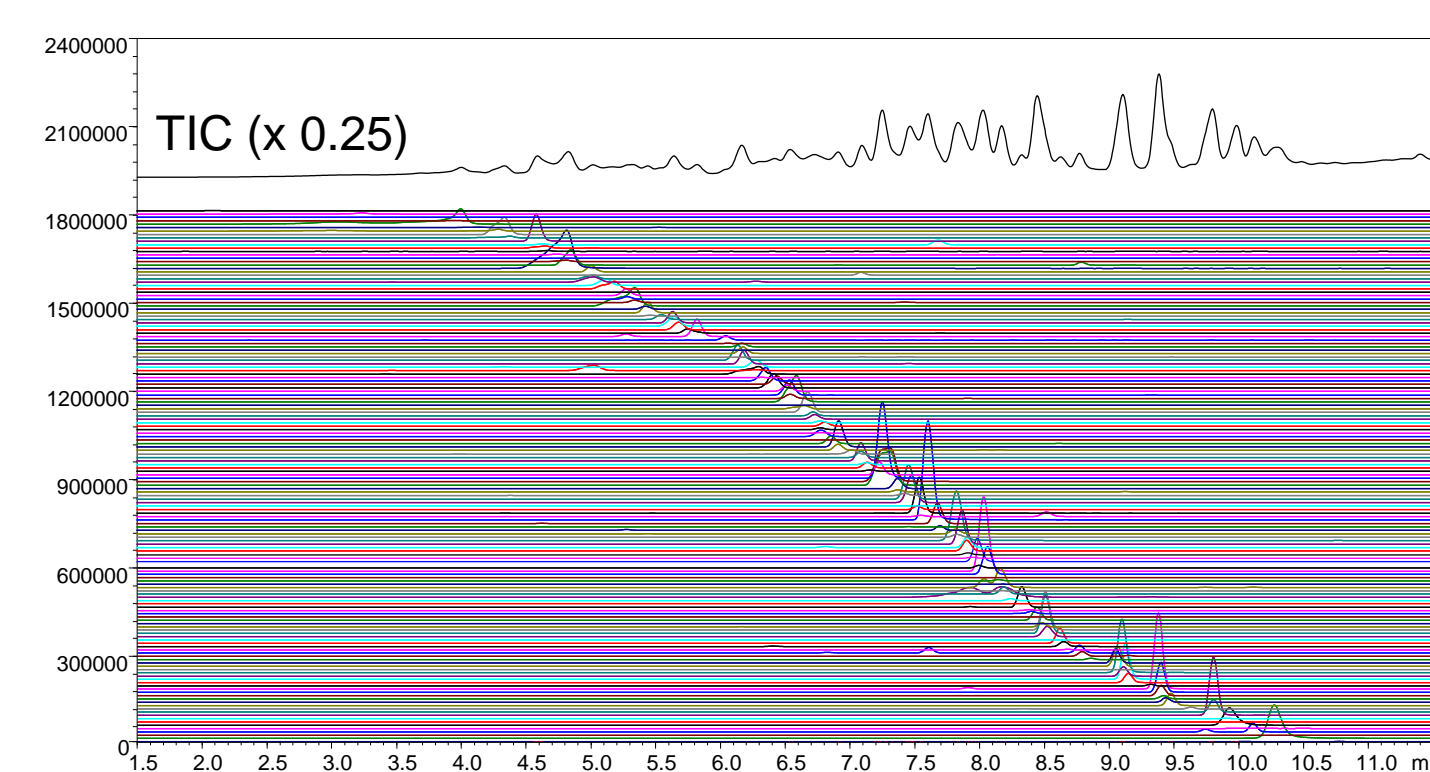


Figure 3 Trace amounts of 157 pesticides (10 ppb) were quantified in one run of LCMS analysis with their fragmental MS/MS information. All peaks were extracted by theoretical m/z (± 10 ppm) from one full scan.

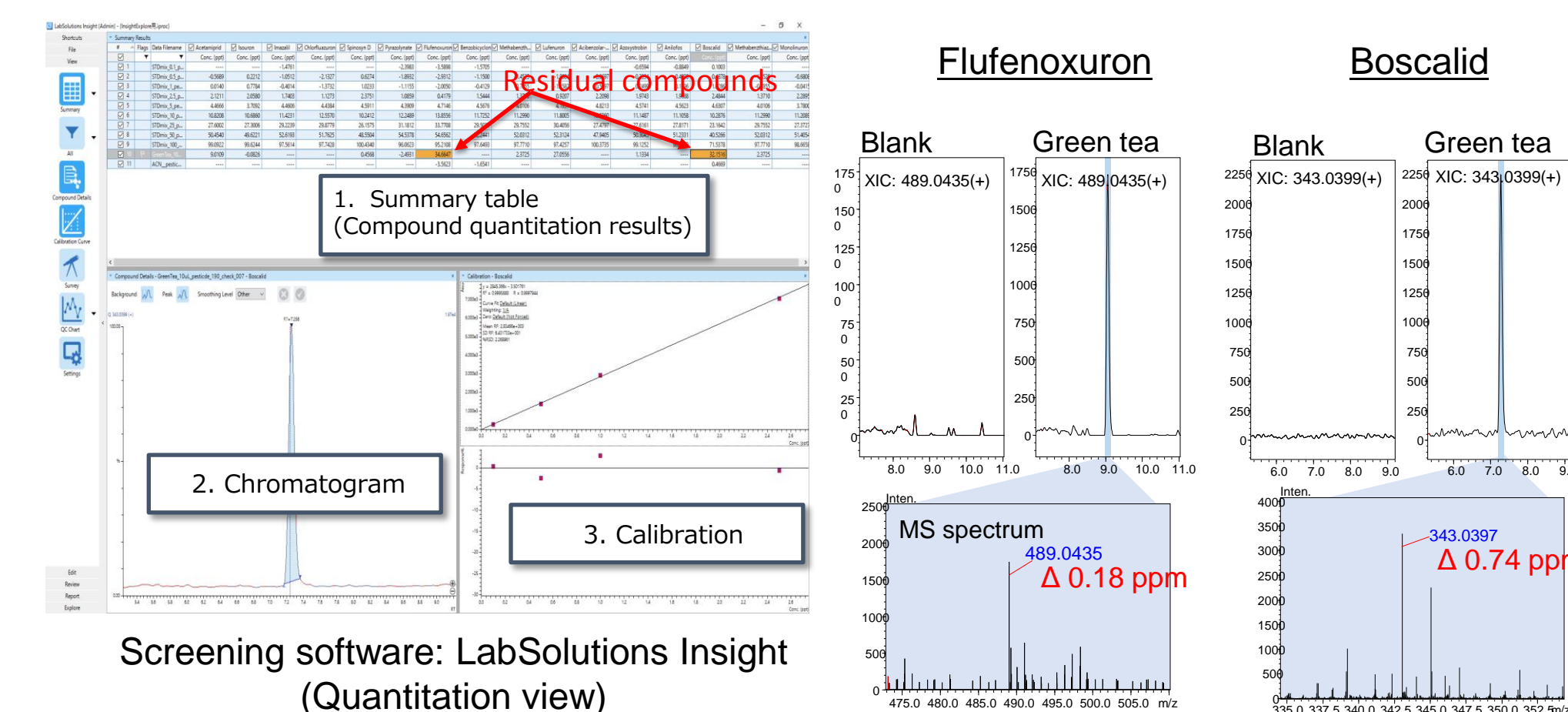


Figure 4 Screening result of pesticide residues in green tea

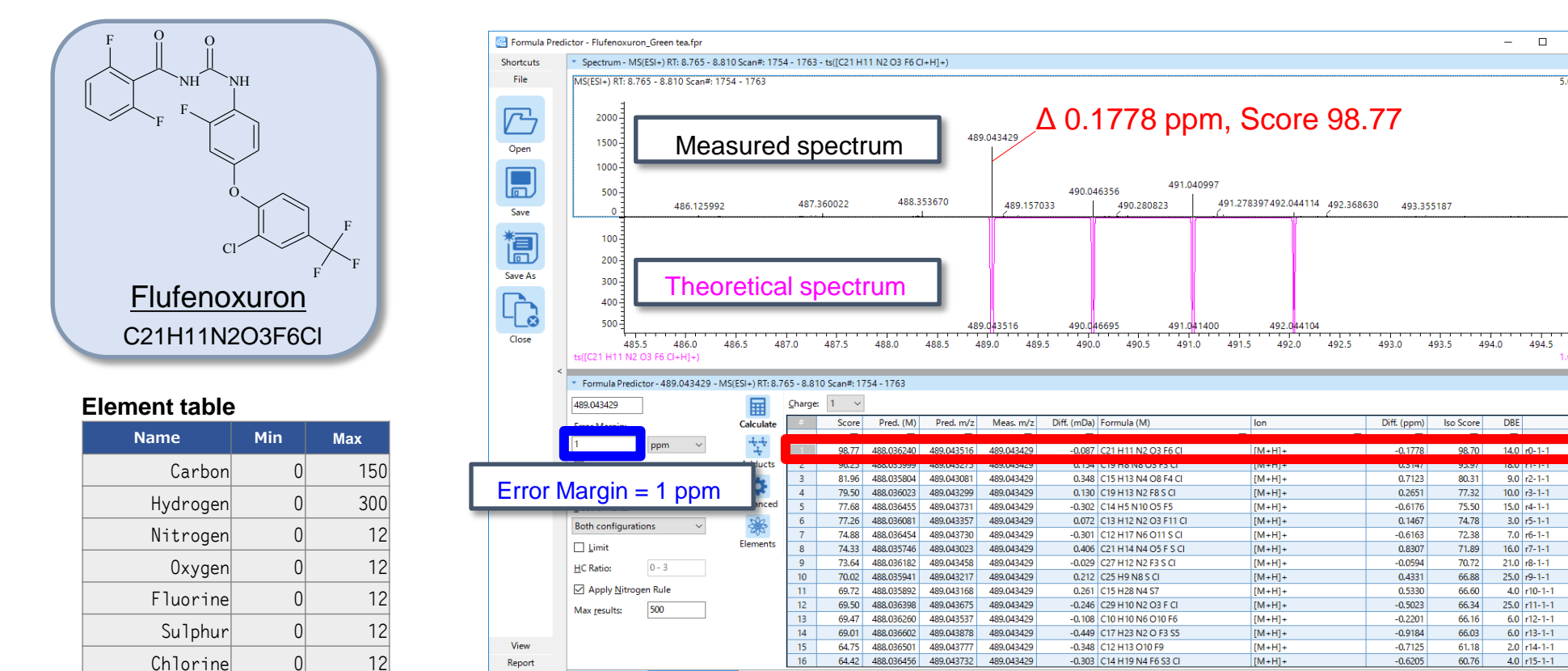


Figure 5 Example of formula prediction (LabSolutions Insight Explore)

4-2. Calibration curve for quantitative analysis

Calibration curves for quantitative analysis are generated using the green tea extracts spiked with pesticide mixture. Among 157 pesticides, flufenoxuron and boscalid, were detected from the green tea extracts, estimated concentrations as 0.142 ppb and 0.231 ppb, respectively. As a result of formula prediction, the composition of corresponding peak on the MS spectrum completely agree with that of flufenoxuron and boscalid with the top score.

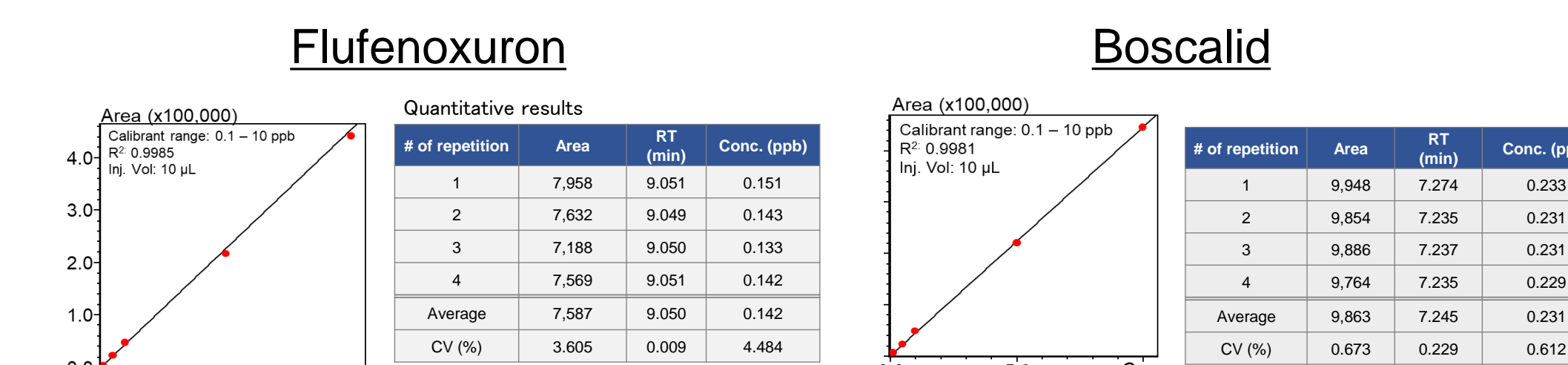


Figure 6 Quantitative result of pesticide residues in green tea

4-3. Identification of pesticides by structural information from MS/MS fragments.

In order to confirm the validity of the identification results, we further performed structural analysis by MS/MS acquisition of the corresponding peak. All of the major MS/MS fragments were assigned to structural fragments of flufenoxuron and boscalid with accuracies of 1 mDa or less.

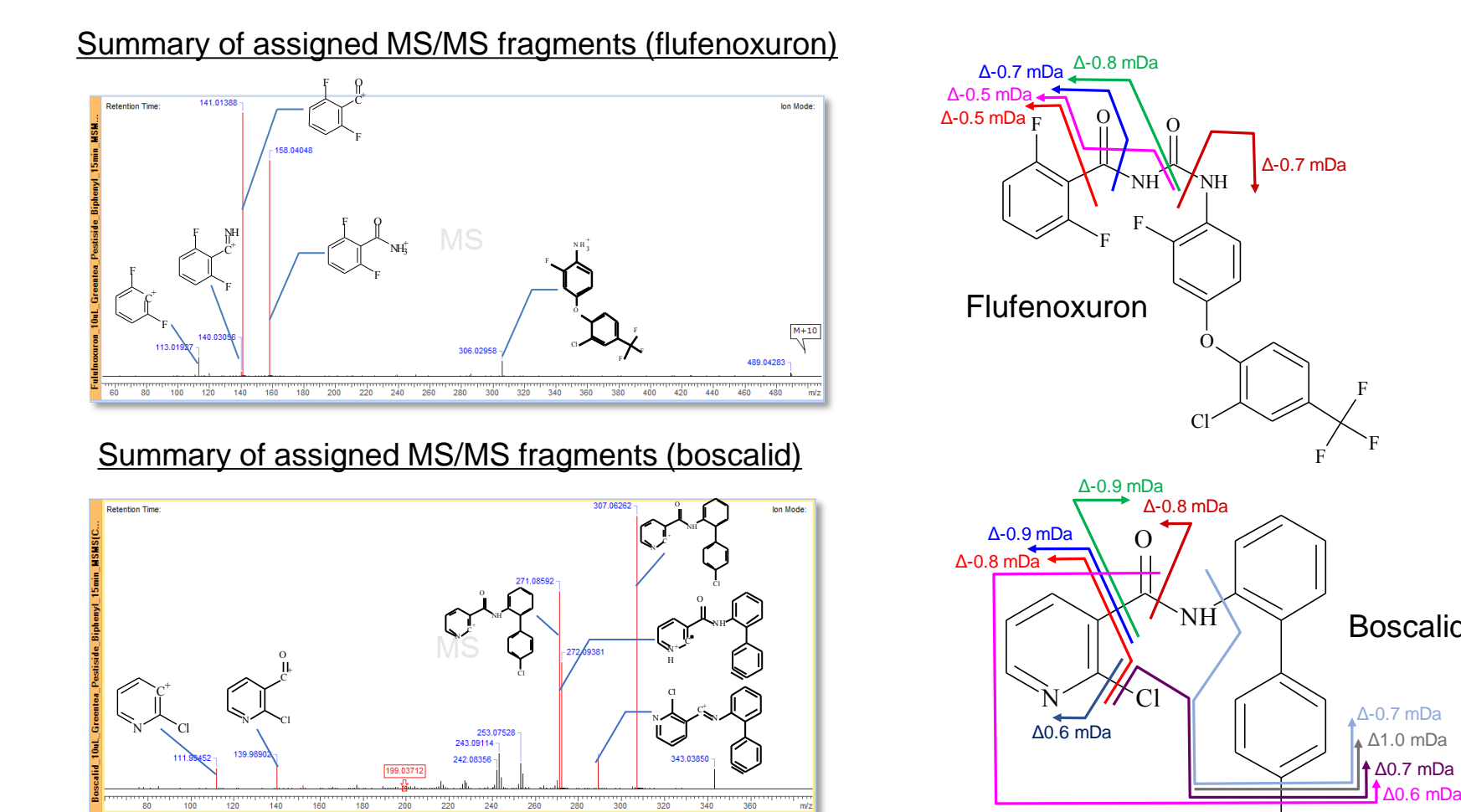


Figure 7 Accurate MS/MS fragments help identify flufenoxuron and boscalid; MS/MS spectra (left), fragmentation patterns and mass error (right)

5. Conclusions

According to the official guideline of CODEX, and Japanese Ministry of Health, residual concentrations quantified here is far below the criteria defined for them. Thus, our results indicate that LCMS-9030 system successfully achieves robust and reliable residual screening in foods by coexistence with both high-sensitivity quantitation and qualification with high mass accuracy.