Application of QuEChERS Technique With UHPLC-Q-TOF Tandem Mass Spectrometry for High-Throughput Screening Multiple Pesticides in Food Matrixes

Application Note

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Abstract

With the implementation of strict regulations on the maximum allowable residue levels (MRLs) of pesticides in food matrixes worldwide, sensitive, reliable, and high-throughput analytical methodologies are urgently required for rapid screening and monitoring of the level of pesticide residues in foodstuffs. In this application note, we have detailed a method on QuEChERS extraction and cleanup protocol combined with ultra high performance liquid chromatography (UHPLC) quadrupole time-of-flight tandem mass spectrometry (Q-TOF-MS/MS) for multiple pesticides screening based on the work we published previously [1]. With optimized QuEChERS protocol, UHPLC-Q-TOF-MS/MS separation and detection conditions, we validated the analytical performance of the method by examining 281 pesticide compounds spiked into three representative matrixes of apple, tomato, and cabbage. The compounds were initially identified based on an accurate mass database with fixed retention time under the selected LC condition, and were further confirmed by matching their accurate Q-TOF MS/MS spectra against the established accurate MS/MS spectra library. The most abundant mono-isotopic ion species under TOF MS scanning mode was selected for quantitation. Using this method, all 281 pesticides spiked in the matrixes at primarily 5.0, 10.0, and 20.0 µg/kg were exclusively identified and quantified. A great majority (> 98%) of average recoveries (n = 5) were within the range of 70%~120% at the three spiked levels for all three matrixes. The relative standard deviations (RSDs) were lower than 20%. Using the matrix-matched calibration concentration primarily from 2.5 to 100.0 µg/kg, most compounds showed excellent linear responses, with correlation coefficients (R^2) of 0.99 or above. Limits of detection (LOD) of these compounds in the three matrixes ranged from 0.010–4.5 µg/kg, which met the most strict requirement of EU regulations, with the MRLs of 10 µg/kg for most pesticide residues. The developed method has been successfully applied to screen the target pesticide residues in real samples; therefore it can be used as a routine monitoring method for these pesticide residues in food matrixes.

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Introduction

Hundreds of pesticides have been used globally to enhance crop yield and improve product quality. However, use of pesticides without proper supervision has led to agricultural products with elevated pesticide residues, presenting a potential threat to public health. Many countries and international organizations have enacted strict regulations on the maximum allowable residue level (MRL), covering hundreds of pesticides in a variety of food matrixes [2]. China recently issued a new regulation on maximum allowable pesticides residue levels (GB-2763-2014) that specifies the 387 frequently used pesticides in China and covers MRLs in 3,650 food matrixes. Facing such a variety of pesticides and food matrixes, a rapid, easy, efficient, high-throughput method is urgently required for routine screening of pesticides in food commodities. Currently, pesticide detection involves two major steps, sample treatment and instrumental measurement. QuEChERS has been demonstrated to be a rapid, easy, safe, and efficient extraction and cleanup method [3], and is widely used in pesticide analysis by coupling with GC or LC and mass spectrometric detection [4,5]. Ultra-high performance LC with Q-TOF accurate mass spectrometry can provide high efficiency separation, accurate mass on both MS and MS/MS levels, as well as satisfactory dynamic range for quantitation. By combining with QuEChERS extraction and cleanup, it has become an attractive technique for the high-throughput screening of pesticide residues in foodstuffs [6]. In China, the current reference methods for pesticide measurement (GB method) are mostly based on solid phase extraction (SPE), which is very time-consuming compared to QuEChERS. This application note describes the combination of QuEChERS with LC-QTOF MS/MS to simultaneously screen and quantitate several hundreds of pesticides in vegetables and fruits.

Experimental

Materials and reagents

281 pesticide standard compounds (purity ≥ 95%) were obtained from Dr. Ehrenstorfer GmbH (Germany). Acetonitrile, formic acid, and ammonium acetate were all of HPLC grade. Other chemicals were of analytical grade. Most standard compounds were prepared using pure methanol at a concentration of 10.0 mg/mL, except a few that could not dissolve completely in methanol, which were prepared using acetonitrile.

The matrix-matched calibration standard solutions were prepared by spiking a certain amount of pesticides into the blank matrix extract which was obtained following the sample preparation procedure in the next section. The calibration concentrations ranged from 1.3 µg/kg to 556.1 µg/kg, with primarily from 2.5 µg/kg to 100.0 µg/kg. For the spiking test, the pesticide standards were spiked into the blank matrix at three concentrations ranging from 0.25 µg/kg to 200.0 µg/kg, with most spiked at 5.0, 10.0, and 20.0 µg/kg. The spiked samples were then subjected to extraction and cleanup, following the sample preparation procedure in the next section.

Sample preparation

The sample was subjected to the QuEChERS extraction and cleanup procedure shown in Figure 1. Briefly, ten grams of homoginized sample was mixed with acetonitrile/acetic acid at a ratio of (99:1). One gram of NaAc and 4 g $MgSO_4$ were then added to the mixture. The resultant mixture was shaken for 3 minutes, followed by centrifugation at 4,200 rpm for 5 minutes. Ten mL of the supernatant solution was transferred to a clean centrifuge tube. Then, PSA sorbent (300 mg) was added to the centrifuge tube. The tube was shaken for 3 minutes, followed by centrifugation at 4,200 rpm for 5 minutes. The 5 mL of resultant supernatant solution was dried by nitrogen evaporation. The residue was dissolved in 1 mL of a solution containing water/acetonitrile/acetic acid (79.9:20:0.1), filtered through a 0.22-µm membrane, and subjected to UHPLC-Q-TOF/MS and MS/MS analysis.

Instrumentation and software

The method was developed using an Agilent high resolution LC/MS system consisting of:

- Agilent 1290 Infinity Binary Pump with a built-in degasser
- Agilent 1290 Infinity Autosampler with thermostat
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity Diode Arrray Detector
- Agilent 6530 Accurate-Mass Q-TOF Mass Spectrometer with dual JetStream ESI source
- Agilent MassHunter Acquisition software for TOF and Q-TOF B.05.01.
- Agilent MassHunter Qualitative and Quantitative Analysis Software B.05.01
- Agilent MassHunter Personal Compound Database and Library B.04.0.

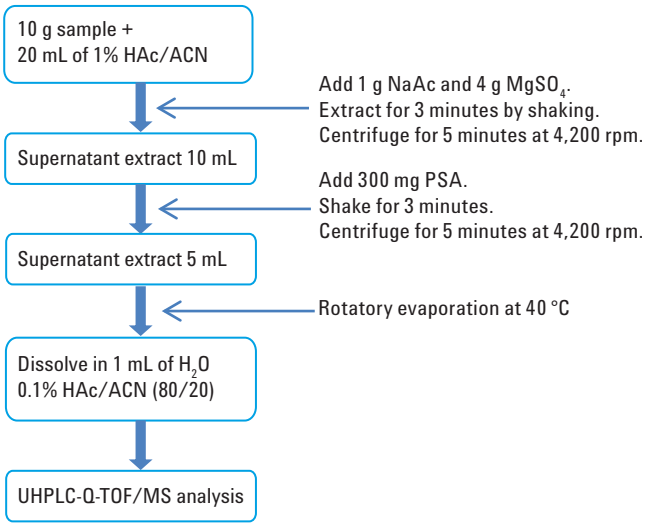


Figure 1. Optimized QuEChERS extraction and cleanup procedures.

Instrumental conditions

LC system

Column	Agilent ZORBAX SB C18, 2.1 mm × 100 mm, 3.5 µm
Mobile phase	A) Milli-Q water containing 5 mmol/L ammonium acetate and 0.1% formic acid B) acetonitrile
Flow rate	0.4 mL/min
Column temperature	40 °C
Injection volume	10 µL with 5 second backflush
Run time	23 minutes
Post time	4 minutes
Gradient elution profile	0–3 minutes: %B increasing from 1% to 30% 3–6 minutes: %B increasing from 30% to 40% 6–9 minutes: %B maintaining at 40% 9–15 minutes: %B increasing from 40% to 60% 15–19 minutes: %B increasing from 60% to 90%
Mass system	Agilent Accurate Mass Q-TOF 6530A
Ionization source	Dual JetStream ESI
Polarity	positive ionization
Capillary voltage	4,000 V
Nebulizer gas	N_2 (45 psi)
Drying gas (N_2) temperature	325 °C
Drying gas flow rate	10 L/min
Sheath gas (N_2) temperature	325 °C
Sheath gas flow rate	11 L/min
Fragmentor voltage	140 V
Acquisition mode	MS and target MS/MS
Scanning *m/z* range	100–1,600 for MS, and 50–1,000 for MS/MS
Acquisition rate	4 spectra/sec for MS, and 3 spectra/sec for MS/MS
Reference masses	*m/z* 121.0509, *m/z* 922.0098

Results and Discussion

Optimization of sample preparation

We selected 75 representative pesticides from various classes of the total 281 pesticides for optimization of the QuEChERS protocol. The average recovery and the percentage of compounds with recovery within 60–130% were used to evaluate the performance of the QuEChERS protocol. As shown in Figure 2, a spiking time of 0.5 hours or 12 hours displays similar average recovery, and a 2-minute extraction time is sufficient for best recovery (A). By examining the extraction solvent components, it was found that acetonitrile with 1% acetic acid was best for extraction with 96% compounds having average recovery above 80% (B). PSA was tested as the appropriate sorbent during cleanup, and 300 mg of PSA showed the highest recovery for the representative pesticides (C). Redissolvation solvent components can also affect the recovery of the analytes. Investigation of a wide range of the solvent composition demonstrated that acetonitrile/water containing 0.1% HAc (20:80) provides the best recovery. The optimized QuEChERS procedure was then established as shown in Figure 1.

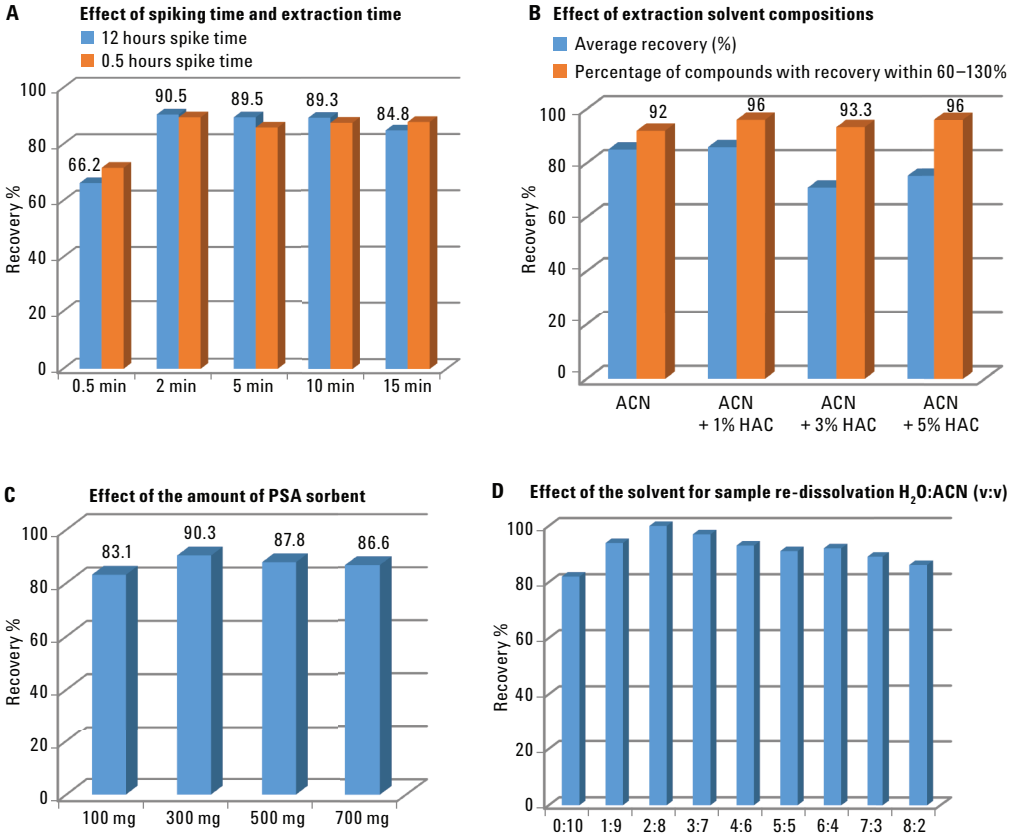
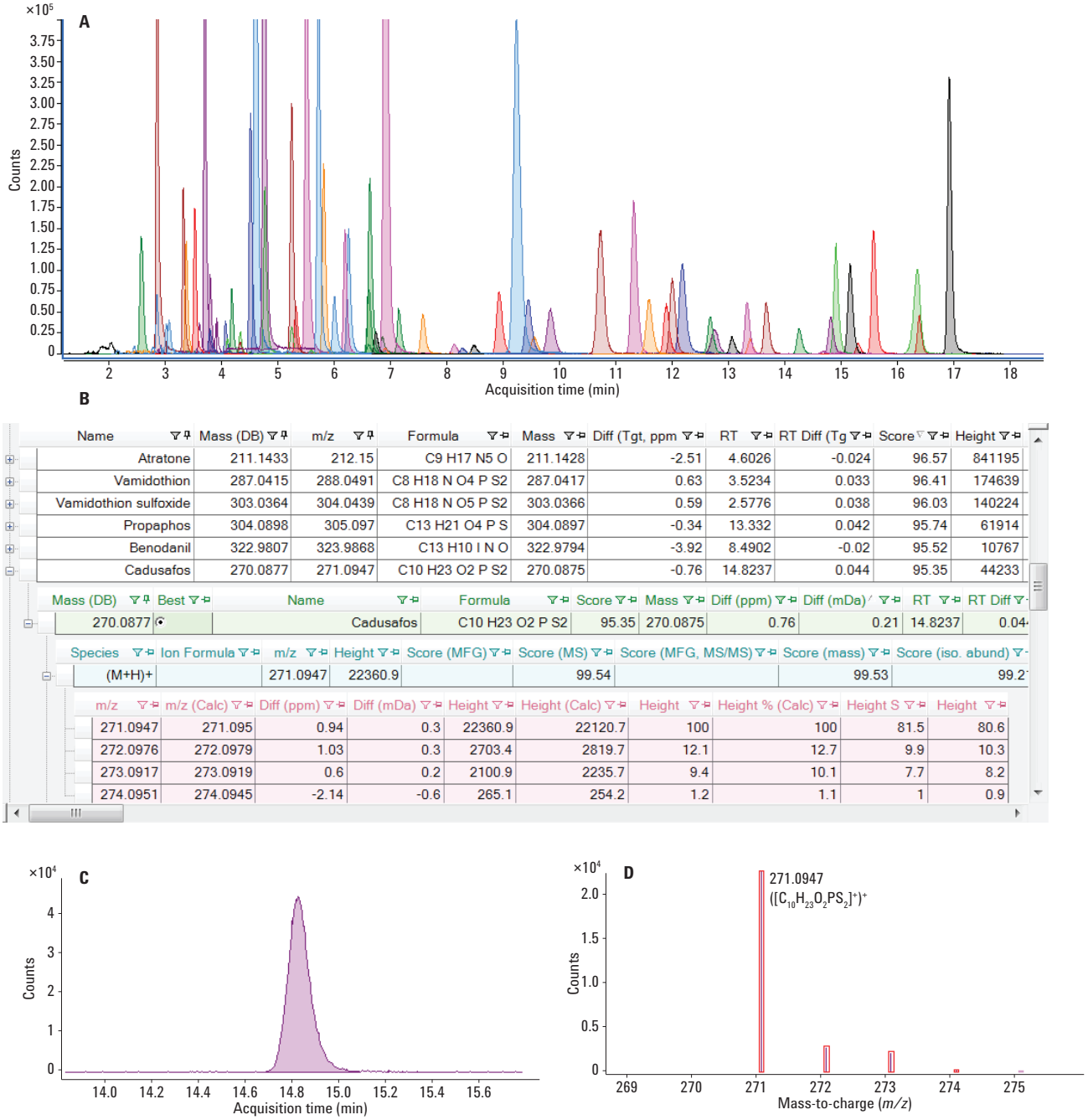


Figure 2. Optimization of QuEChERS extraction and cleanup protocol including the effects of the spiking time and the extraction time (A), the extraction solvent (B), the amount of PSA sorbent (C), and the redissolvation solvent (H_2O:ACN) for the sample before LC/MS analysis (D).

Identification of pesticides with database and library

A pesticide database was customized that contained the compound name, formula, accurate monoisotopic molecular weight, and retention time under the LC conditions. It was used for preliminary pesticide identification. Incorporating the database into the Agilent MassHunter Find-by-Formula algorithm (FBF) allowed automatic identification of the individual compounds. As shown in Figure 3, using the FBF combined with the pesticide database, a group of the representative 75 pesticides spiked in the apple matrix were rapidly



Name	Mass (DB)	m/z	Formula	Mass	Diff (Tgt, ppm	RT	RT Diff (Tg	Score	Height
Atratone	211.1433	212.15	C9 H17 N5 O	211.1428	-2.51	4.6026	-0.024	96.57	841195
Vamidothion	287.0415	288.0491	C8 H18 N O4 P S2	287.0417	0.63	3.5234	0.033	96.41	174639
Vamidothion sulfoxide	303.0364	304.0439	C8 H18 N O5 P S2	303.0366	0.59	2.5776	0.038	96.03	140224
Propaphos	304.0898	305.097	C13 H21 O4 P S	304.0897	-0.34	13.332	0.042	95.74	61914
Benodanil	322.9807	323.9868	C13 H10 I N O	322.9794	-3.92	8.4902	-0.02	95.52	10767
Cadusafos	270.0877	271.0947	C10 H23 O2 P S2	270.0875	-0.76	14.8237	0.044	95.35	44233

Mass (DB)	Best	Name	Formula	Score	Mass	Diff (ppm)	Diff (mDa)	RT	RT Diff
270.0877	●	Cadusafos	C10 H23 O2 P S2	95.35	270.0875	0.76	0.21	14.8237	0.04

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MS)	Score (MFG, MS/MS)	Score (mass)	Score (iso. abund)
(M+H)+		271.0947	22360.9		99.54		99.53	99.2

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height	Height % (Calc)	Height S	Height
271.0947	271.095	0.94	0.3	22360.9	22120.7	100	100	81.5	80.6
272.0976	272.0979	1.03	0.3	2703.4	2819.7	12.1	12.7	9.9	10.3
273.0917	273.0919	0.6	0.2	2100.9	2235.7	9.4	10.1	7.7	8.2
274.0951	274.0945	-2.14	-0.6	265.1	254.2	1.2	1.1	1	0.9

Figure 3. Separation and identification of one group of 75 pesticides in apple matrix with a spiking level of 10 µg/kg. (A) The overlapped extracted ion chromatograms using the Find by Formula (FBF) algorithm with the accurate mass database, (B) List of identified pesticides and the searching score, (C) The chromatogram of a typical pesticide (cadusafos), and (D) its relevant mass spectrum with background removal.

extracted and assigned. Most compounds were separated with high column efficiency and excellent peak shapes (Figure 3-A). Each compound was identified based on its retention time (Figure 3C), accurate mass, its isotopic abundance, and isotopic spacing (Figure 3D). The overall identification results are shown in Figure 3B.

The 281 pesticides (four groups) in apple matrix at medium spiking level (mostly 10 µg/kg), had mass accuracies within ± 3 ppm, and the MS searching scores all exceeded 90%. For real sample screening, when the use of accurate mass with retention time is insufficient (< 80) for confirmation of the compound, target MS/MS acquisition is used, and the customized accurate mass library is applied for compound identification using the Agilent MS/MS library search algorithm provided by Agilent MassHunter Qualitative Software 5.0. Figure 4A shows an excellent match of the cadusafos spectrum in matrix with that spectrum stored in the library. However, during real sample analysis, matrix effect can be very severe when the target compound is at a very low level. The MS score and MS/MS matching score can be low, and, therefore, cannot meet the confirmation threshold automatically. A manual check of the matching spectra with background removal can improve the confirmation confidence (Figures 4B and 4C).

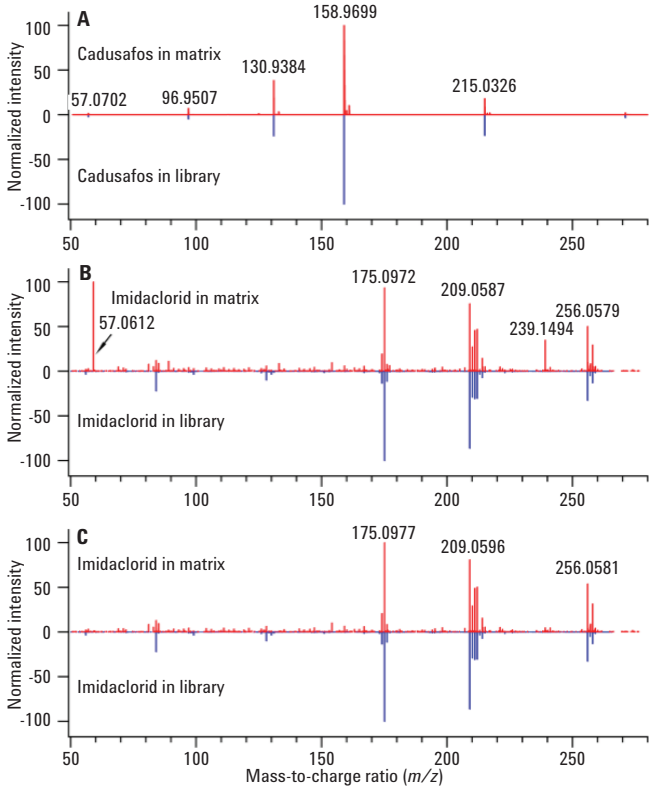


Figure 4. Screening using the 'Identify-by-Library' algorithm enhanced the compound confirmation confidence. (A) a typical library matching result for cadusafos in apple matrix (matching score = 98.6), (B) poor matching of imidaclorid in apple matrix without background subtraction (matching score = 49.9), (C) improved matching for imidaclorid in apple matrix after background subtraction (matching score = 68.5).

Matrix effect

The compounds present in matrix can coelute with target compounds, and suppress or enhance the ionization of target compounds. We tested a series of concentrations of 281 target compounds (mostly at 2.5, 5.0, 10.0, 20.0, 50.0, and 100.0 µg/kg) prepared in solvent or matrix extract, and obtained the calibration curves. The effect of suppression or enhancement was then evaluated. As shown in Figure 5, 11 pesticides display matrix enhancement effect in the three matrixes, and all the others showed matrix suppression effect. The matrix effect in apple and tomato was relatively lower as most compounds (approximately 90%) showed matrix suppression within 0–50%. In comparison, cabbage matrix can suppress 80% of pesticides with a suppression degree higher than 20%, and among which about 20% of compounds can be suppressed more than 50%. Hence, matrix-matched calibration curves are required to avoid quantitation bias.

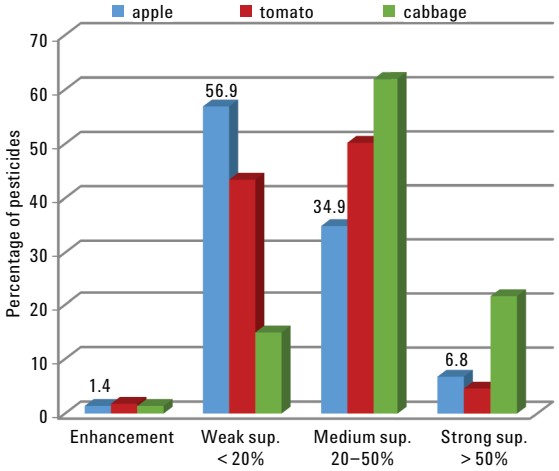


Figure 5. Distribution of matrix effects for 281 pesticides spiked in the matrices of apple, tomato, and cabbage. The suppression or enhancement percentage (X) was calculated using the formula below [6]: $X = (1–Slope_{matrix}/Slope_{solvent}) \times 100\%$.

Method performance

Linearity and sensitivity

Method performance was further evaluated using the matrix-matched calibration. As shown in Figure 6, all the pesticides display linear regression coefficients (R^2) of 0.985 or above, among which up to 95.7% of 281 pesticides have $R^2 \geq 0.99$ (A). In addition, all the 281 pesticides have LOQs less than 10 µg/kg (B).

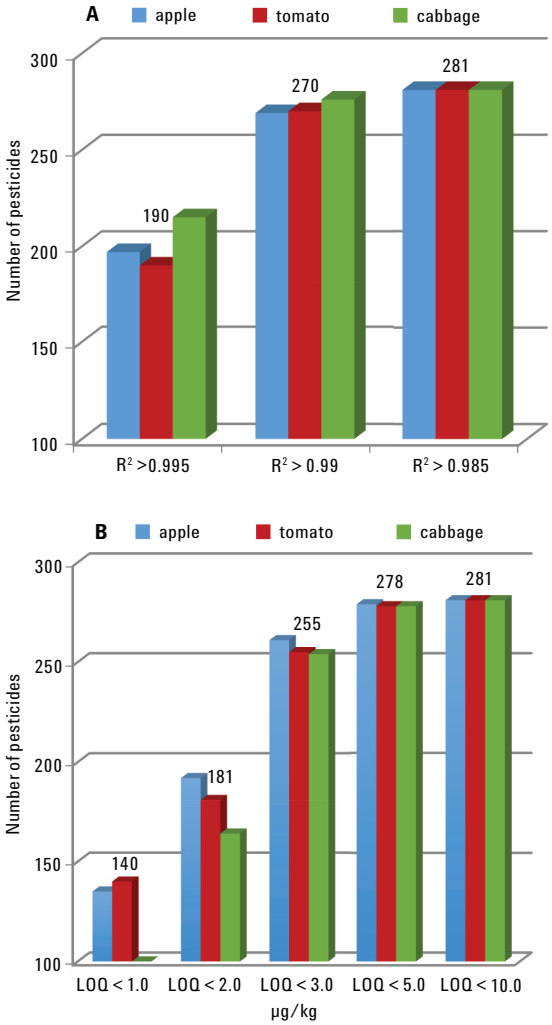


Figure 6. Summary of method performance in term of linearity and LOQ. (A) distribution of pesticides with linearity (R^2); (B) distribution of pesticides with LOQ range.

Recovery and precision

Each pesticide was spiked into apple, tomato, and cabbage matrixes respectively, at low, medium, and high levels (mostly at levels of 5.0, 10.0, and 20.0 µg/kg) to test the method accuracy and precision. For the pesticides with spiking level higher than LOQ, more than 98.5% of them had recovery within 70–120% over the three spiking levels (Figure 7A). All RSDs (n = 5) were within 20% (Figure 7B).

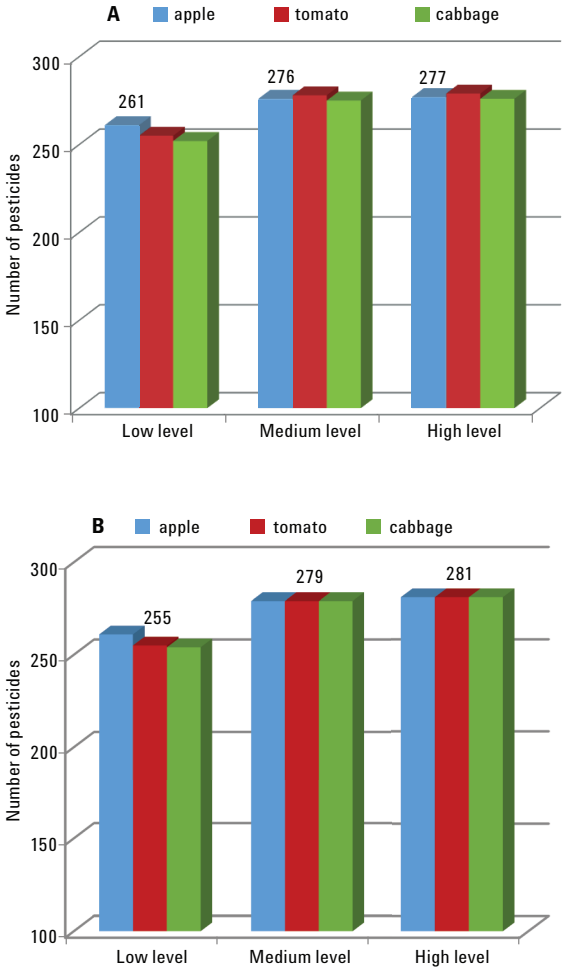


Figure 7. Summary of method performance in terms of recovery and precision. (A) Distribution of pesticides with recovery within 70–120%; (B) Distribution of pesticides with RSD below 20%.

Real sample analysis

By screening 30 randomly selected samples (10 for each matrix), 13 pesticides were detectable from 17 samples (Table 1). Among these samples, carbendazin was detected in nine apple samples and six tomato samples, with one apple sample containing a level of 46.5 µg/kg, and such a level is lower than the EU MRL (100 µg/kg), the strictest one for apple matrix among EU, Japan, and China. Tebuconazole was detected in three apple samples, with two at levels (70.6 and 24.6 µg/kg) higher than 20 µg/kg, the strictest MRL in apple regulated by EU. Methamidophos was detected in one cabbage sample with the level as high as 82.2 µg/kg, which is within the regulations of Japan and China, but is much higher than the MRL of 10 µg/kg in cabbage regulated by the EU. Difenoconazole was shown slightly higher than 10 ug/kg in one apple sample, which is within MRL regulations of Japan, China, and EU. Rotenone was detected in one tomato sample at a level (12.9 µg/kg) slightly higher than 10 µg/kg. The other seven pesticides were detected with levels lower than 10 µg/kg. As a whole, five pesticides in seven individual samples exceeded 10 µg/kg, and two pesticide residues in three samples exceeded the strictest MRL regulated by EU.

Table 1. Qualitative and quantitative measurements of real samples.

No.	Name	TOF-score	Q-TOF-score	Level (µg/kg)
AP-1	Carbendazim	99.6	95.6	46.5
	Tebuconazole	73.1	63.4	1.9
AP-2	Carbendazim	98.2	90.1	1.0
	Acetamiprid	93.8	74.6	2.8
	Tebuconazole	75.6	88.4	70.6
	Difenoconazole	90.9	91.3	13.2
AP-4	Carbendazim	98.1	73.4	0.5
AP-5	Carbendazim	99.2	85.8	0.6
AP-6	Carbendazim	99.5	92.0	1.2
AP-7	Carbendazim	83.9	84.7	0.3
	Acetamiprid	95.6	56.8	1.5
AP-8	Carbendazim	99.1	88.6	0.9
AP-9	Carbendazim	96.9	85.8	0.6
AP-10	Carbendazim	97.1	84.2	0.6
	Imidacloprid	95	68.5	–
	Tebuconazole	75.6	86.3	24.6
TO-1	Rotenone	94.7	70.9	12.9
TO-3	Acetamiprid	97.5	75.5	4.7
	Buprofezin	95.3	87.2	1.3
	Carbendazim	84.6	70.9	0.9
	Metalaxyl	94.3	84.7	1.6
TO-4	Carbendazim	84.7	73.9	0.8
	Flusilazole	75.7	77.8	–
TO-5	Acetamiprid	98.6	83.1	16.1
	Carbendazim	87.7	70.0	0.7
TO-7	Carbendazim	93.9	80.7	1.1
TO-10	Carbendazim	92.4	71.3	2.8
	Ethirimol	81.6	80.8	1.1
CA-1	Methamidophos	99.3	79.8	82.2
CA-2	Phorate sulfoxide	94.8	86.6	2.0
	Azoxystrobin	98.8	84.3	1.3

Conclusion

A QuEChERS extraction and cleanup protocol combined with a UHPLC-Q-TOF-MS(/MS) method has been developed for the high-throughput screening of 281 pesticides in food matrix including apple, tomato, and cabbage. Accurate mass database and MS/MS spectra library were applied for pesticide confirmation with high confidence. Matrix-matched calibration demonstrated good linearity and high sensitivity. The spiking recovery and precision were primarily within 70–120%, and below 20% respectively. It suggests that the developed method can meet the requirement for quantitative screening of pesticides at a level of 10 µg/kg, at which most EU MRLs are set. The method has the advantage of being both qualitative and quantitative, and has been successfully applied for real sample screening.

References

1. Z. Zhao, *et al*. *Chin. J. Chromatogr*. **31** (2013): 372.
2. The International maximum residue level database. [2012-12-08]. http://www. Mrldatabase.com/results.cfm.
3. M. Anastassiades, S.J Lehotay, D. Štajnbaher, F.J. Schenck, *J. AOAC Int*., 2003, **86**: 412-431.
4. I. Ferrer, E.M. Thurman, *J. Chromatogr. A*., 2007, **1175**: 24-37.
5. U. Koesukwiwat, S.J. Lehotay, S. Miao, N. Leepipatpiboon. *J. Chromatogr*., 2010, **1217**:6692-6703.
6. L. Polgára, J.F. García-Reyesb, P. Fodora, etc. *J. Chromatogr. A*., 2012, **1249**: 83-91.
7. B. Kmellár, *et al*., *J. Chromatogr. A.*, 2008, **1215**:37-50.

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