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RESEARCH ARTICLE

Determination of multiple pesticide residues in teas by gas chromatography with accurate time-of-flight mass spectrometry

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In this work, gas chromatography tandem with electron ionization and full-scan high-resolution mass spectrometry with a time-of-flight mass analyzer was evaluated for analyzing pesticide residues in teas. The relevant aspects for mass spectrometry analysis, including the resolution and mass accuracy, acquisition rate, temperature of ion source, were investigated. Under acquisition condition in 2-GHz extended dynamic range mode, accurate mass spectral library including 184 gas chromatography detectable pesticides was established and retrieval parameters were optimized. The mass spectra were consistent over a wide concentration range (three orders) with good match values to those of NIST (EI-quadrupole). The methodology was verified by the validation of 184 pesticides in four tea matrices. A wide linear range (1–1000 μg/kg) was obtained for most compounds in four matrices. Limit of detection, limit of quantification, and limit of identification values acquired in this study could satisfy the requirements for maximum residue levels prescribed by the European Community. Recovery studies were performed at three concentrations (10, 50, and 100 μg/kg). Most of the analytes were recovered at an acceptable range of 70–120% with relative standard deviations ≤ 20% in four matrices. The potential extension of qualitative screening scope makes gas chromatography tandem with electron ionization and mass spectrometry with a time-of-flight mass analyzer a more powerful tool compared with gas chromatography with tandem mass spectrometry.

KEYWORDS
accurate mass spectral library, gas chromatography, pesticides, teas, time-of-flight mass analyzer

1 | INTRODUCTION

Among three major nonalcoholic drinks (tea, coffee, and cocoa) worldwide, tea is the most widely consumed beverage for both pleasure and therapeutic purposes [1–3]. During the cultivation of tea, the usage of various pesticides and related chemicals plays a significant role in killing pest, weeding control, and improving yield. However, pesticides applied during the growing stages or postharvest treatment may remain in the plant and expose potential risks to consumers' health [4, 5]. Thus, strict regulations on the maximum residue limits (MRLs) for pesticides in tea have been established by several countries and international organizations, e.g. EU, USA, and Japan. The EU Pesticides database [6], which is based on the European Community Regulation No. 396/2005, contains a list of 474 pesticide residues with their respective MRLs at various levels in tea [7]. The analytical methods for pesticide residues have been continually improved due to the

Article Related Abbreviations: d-SPE, dispersive solid-phase extraction; EDR, extended dynamic range; EIC, extracted ion chromatogram; GCB, graphitized carbon black; HRMS, high resolution mass spectrometry; LOI, limit of identification; ME, matrix effect; MRL, the maximum residue limit; MRM, multiple reaction monitoring; MWCNT, multiwalled carbon nanotube; n-MEW, narrow mass extraction window; PSA, primary secondary amine.

introduction of innovative technologies. One of the urgent goals for supervisory authority and private laboratories is to develop fast and effective methods that could achieve reliable results for a wide scope of pesticides and matrices and have excellent robustness for routine test with low detection limit, easy operation, and environmental friendly.

In analysis of pesticide residues, major progress in recent years was the introduction of GC-MS/MS and LC-MS/MS. They can significantly increase the analyzing speed and identify more than one hundred of pesticides compared to previous GC or LC methods, and have been widely used to analyze multiple pesticide residues in various matrices, such as water [8,9], animal tissues [10–12], plants and plant oils [13–16], and teas [17,18]. When operated in multiple reaction monitoring (MRM) mode, improved sensitivity and excellent selectivity can be achieved. However, the major limitation of the MRM method is that the acquisition time of each transition restricts the number of target analytes [19]. In MRM mode, nontargeted compounds even at high concentration will be ignored [20]. Moreover, reference standards have to be purchased for analyte qualification, which are crucial to identifying the suspected findings in routine target analysis. The present of high resolution mass spectrometry (HRMS), i.e. GC or LC coupled with TOF/MS, could be a powerful screening tool for chemical contaminant residues to overcome the major limitation of triple quadrupole mass spectrometer analysis.

GC-EI-TOF/MS is suitable for volatile compounds. This technique offers higher sensitivity in full spectrum acquisition scan mode than conventional GC-EI-MS due to its high mass analyzer efficiency [21]. Theoretically, GC-EI-TOF/MS can simultaneously screen unlimited number of compounds, without the need for preselection of analytes or reference standards, at high sensitivity with only single injection [22]. The high mass resolving capability and exact mass measurement provided by GC-EI-TOF/MS is suitable for the use of narrow mass extraction window (n-MEW) at selected *m/z* ions [23]. Thus, a large amount of isobaric background interferences will be filtered out, so it could remarkably improve the S/N and enhance the identification ability. Moreover, it is an attractive option to facilitate the identification process to build a wide range home-made library based on GC-EI-TOF/MS. To date, GC-EI-TOF/MS has been applied for screening pesticide residues in fruits and vegetables [24–26], dairy products [27], honey products [28–30], and environmental samples [31,32]. Nevertheless, reports on screening of multiple pesticide residues in tea are relatively rare.

An efficient and useful screening method should detect multiclasses pesticides in various kinds of matrices with high sensitivity. The screening method should be validated, which process is laborious and necessary to provide evidence of the applicability and robustness of the method, especially for complex matrices, such as teas. Until now few works have been reported on qualitative screening validation on pesticides in tea.

This work developed and validated a method for rapid simultaneous screening and identification of 184 pesticides in multiclasses in four kinds of teas using dispersive solid-phase extraction (d-SPE), followed by GC-EI-TOF/MS based on a home-made mass spectral library. TOF/MS acquisition conditions were optimized to improve the sensitivity and accuracy of pesticides detection. A mass spectral library was built and evaluated in terms of consistency and stability. The retrieval parameters were also optimized to increase the accuracy of pesticide residues identification. The performance of the proposed method was evaluated on linearity, LOD, LOQ, limits of identification (LOI), recoveries, precision, and matrix effect (ME). Moreover, this method was employed to screening of pesticide residues in 54 commercial available tea samples to demonstrate its applicability.

2 | MATERIALS AND METHODS

2.1 | Materials

Pesticide standards (≥ 95% purity) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock solution (1 mg/mL) of each pesticide standard was prepared in methanol, acetone, or toluene according to their solubility, and stored at 4°C in the dark. Mixtures (10 μg/mL) were prepared in methanol by mixing the appropriate quantities of individual stock solutions in a volumetric flask. Pesticide residue grade acetonitrile, acetone, methanol, and toluene were purchased from Fisher Scientific (Fair Lawn, NJ). d-SPE sorbents, including multiwalled carbon nanotubes (MWCNTs, 10–20 nm), primary secondary amine (PSA, 50 μm), and graphitized carbon black (GCB, 40 μm) were purchased from Tianjin Agela (Tianjin, China).

Various tea samples (16 green teas, 10 black teas, 13 oolong teas, and 15 dark teas) were bought from local supermarkets and stored at room temperature for a maximum of three months before analysis.

2.2 | Sample preparation

The tea matrix extract was prepared using a modified d-SPE method [33], and the specific steps are as follows: (1) 5 g sample was put in 10 mL of acetonitrile with 1% acetic acid v/v and homogenized at 15 000 rpm for 1 min, and centrifuged for 5 min at 4200 rpm; (2) 5 mL of supernatants was then transferred to a tube containing 45 mg of MWCNTs, 75 mg of PSA, and 25 mg of GCB; (3) the tube was vortexed for 1 min and centrifuged at 10 000 rpm for 5 min; (4) 4 mL of acetonitrile extract was evaporated to approximately 0.5 mL by rotor; (5) the extract were concentrated to dryness with nitrogen flow

and reconstituted with 0.5 mL of hexane; (6) and then filtered through a 0.22-μm Nylon membrane. The mixture (1 μL) was injected into a chromatograph with an autosampler.

2.3 | GC-EI-TOF/MS analysis

The GC-EI-QTOF/MS system consists of a 7890A gas chromatograph connected to a 7200 hybrid QTOF mass analyzer with an EI source (Agilent Technologies, Wilmington, DE, USA). A VF-1701 MS capillary column (30 m, 0.25 mm id, and 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) was employed as the analytical column. The oven temperature was held at 40°C for 1 min, then ramped at 30°C/min to 130°C, followed by a 5°C/min ramp to 250°C, and a 10°C/min ramp to 300°C (held for 5 min). The chromatography conditions were as follows: He (99.999%) flow rate was adjusted according to the retention time of heptachlor epoxide (22.153 min) before each batch, with an injection port temperature of 250°C, an injection volume of 1 μL (splitless), and a GC interface temperature of 280°C.

The hybrid QTOF mass analyzer was operated in the single MS mode, and scan spectra were recorded in the *m/z* range from 50 to 600 units, with an acquisition rate of 2 spectra/s. The TOF mass analyzer was optimized as the 2-GHz extended dynamic range (EDR) mode. Mass detector condition was performed with an ion source temperature of 270°C and ionized by electron impact at 70 eV. Automated recalibration of the mass shaft was carried out every three injections, by infusion of PFTBA in the EI source.

2.4 | Screening strategy

Identification of pesticides was based on the presence of three diagnostic ions (the base peak of mass spectra (used as quantitative ion) and other two next more abundant ions (used as qualitative ions)), the ion ratios (respect to the quantitative ion), and the retention time. The spectra for peaks in the extracted ion chromatograms (EICs) were compared with those in the mass spectral library, and the retrieval parameters set for identification were as follows: a retention time window of ± 0.15 min, a mass error tolerance of 10 ppm for the three diagnostic ions, and two qualitative ions should be consistent with the ion ratios of standards (variation ≤ 30%). Supporting Information Table S1 summarizes the information of 184 pesticide compounds, including the retention times, quantitative ions, two qualitative ions, and ion ratios. From the data, there is no case that two pesticide compounds share the same retention times and masses of three most abundant diagnosed ions. Therefore, the retention time, the three most abundant diagnosed ions and ion ratios are useful combination in achieving separate identification of each compound.

3 | RESULTS AND DISCUSSIONS

3.1 | TOF mass acquisition parameters for residue analysis

The key TOF mass parameters for pesticide residues analysis include mass range, resolution, mass accuracy, acquisition rate, and temperature of ion source. The mass range was set at *m/z* 50–600, since there is no pesticide that is suitable for GC test, its molecular weight is beyond 600.

3.1.1 | Resolution and mass accuracy

High selective and sensitive determination technique is necessary to accurately detect the trace level of pesticides in tea samples. In TOF/MS system, selectivity depends on the mass resolution and accuracy of mass values assigned to fragment ions [34]. In 2-GHz EDR mode, the mass spectrometer could switch between low- and high-performance modes during acquisition and the resolution of TOF analyzer changed from 4000 to 10 000 along with the mass values varied from 100 to 500. The mass resolution values can be significantly improved when 4-GHz mode was adopted. The advantage of 2-GHz EDR mode are shown in Figure 1A and B. When the concentration of tolcofos-methyl (quantitative ion *m/z* 264.9850) and fenazaquin (quantitative ion *m/z* 145.1012) in black tea matrix are relatively high (300 μg/kg) in 4-GHz acquisition mode, their EIC peaks are splitted, the quantitative ions of tolcofos-methyl and fenazaquin are oversaturated, and the concentration is beyond the detector linear range. In addition, the response usually falls outside the extracted mass chromatogram window (10 ppm), which easily leads to false negative results. The saturated ions were flagged with asterisk mark (*), and the mass errors for quantitative ions of tolcofos-methyl and fenazaquin were of 12.4 and 13.8 ppm, respectively.

In 2-GHz EDR mode, the mass accuracy of the fragment ions was controlled by performing an internal mass calibration, which was investigated by injecting six times standards of 79 representative pesticides (100 μg/L): (1) a mass calibration of the TOF analyzer before each injection; (2) a mass calibration of the TOF analyzer for every three injections; and (3) the injections were performed one-by-one injection for every 4 h without mass recalibration. The results are shown in Supporting Information Table S2; average mass errors are 1.8, 2.2, and 4.0 ppm, respectively. In addition, a real-time calibration mode was also conducted by using an internal reference mass (IRM), where the introduction of IRM reduced the target compounds into the detector, which has an obvious negative impact in the achieved LODs, especially in low concentrations; hence, the data are not shown in Supporting Information Table S2. Considering the short stability of the mass shaft, mass calibration was conducted between every three injections.

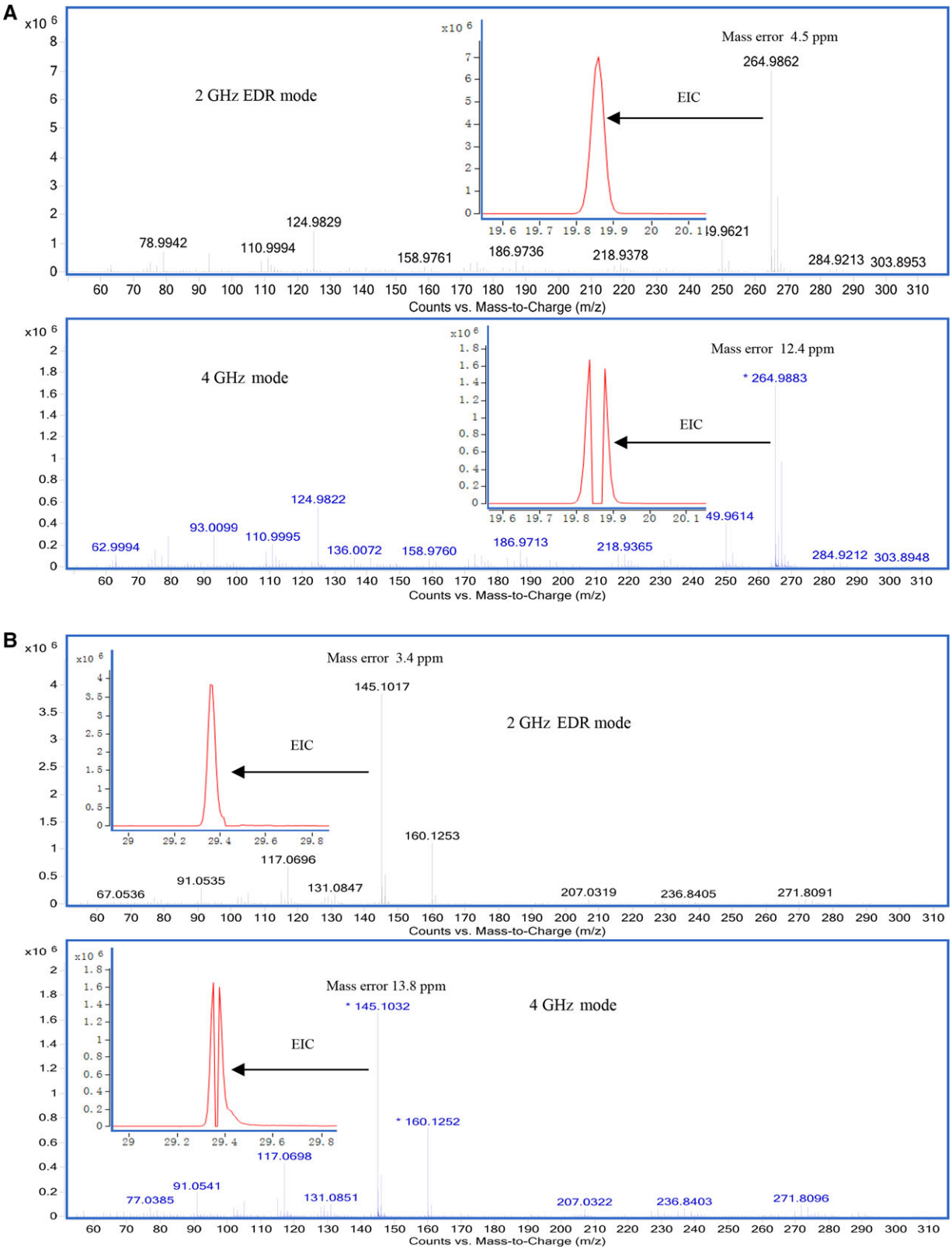

FIGURE 1 The spectra of (A) tolcofos-methyl (EIC for *m/z* 264.9850 embedded) and (B) fenazaquin (EIC for *m/z* 145.1012 embedded) with 2 GHz EDR mode and 4 GHz mode, in a black tea matrix spiked standard at 300 μg/kg

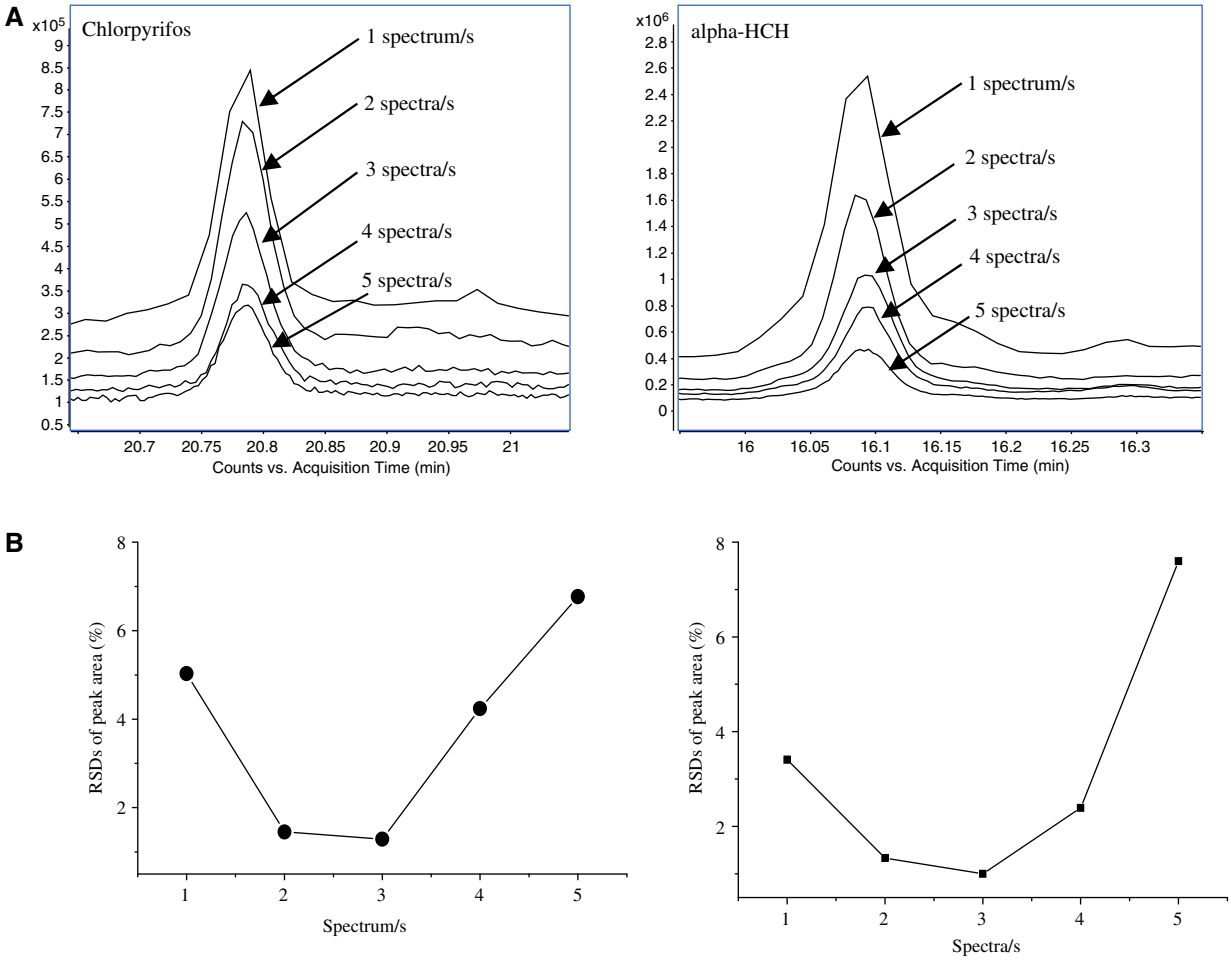

FIGURE 2 (A) The influence of acquisition rate (1–5 spectra/s) on peak shape and sensitivity, and (B) RSDs for peak area as a function of the acquisition rate ($n = 5$) for chlorpyrifos (*m/z* 196.9197) and alpha-HCH (*m/z* 180.9373) in solvent standard (100 µg/L)

3.1.2 | Acquisition rate

The number of mass spectra was obtained from a chromatographic peak based on (1) the number of fragment ions with overlapped chromatographic windows and (2) the acquisition rate of the TOF analyzer. In most situations, 3–8 mass spectra were recorded per peak using an acquisition rate of 1 spectrum/s. To assess the effect of this change in the performance of the GC-EI-TOF/MS instrument, the chromatograms of chlorpyrifos and alpha-HCH in different acquisition rates at 100 µg/L were investigated, as shown in Figure 2A. At higher acquisition rates, more mass spectra per peak could be collected at an expense of reducing the number of transients per mass spectra, which led to the decrease in intensity of detector response and S/N ratio. For detection, the high S/N is indispensable for acquiring low LOD. The low acquisition rate significantly led to poor peak shapes, which obviously cannot follow the Gaussian curve. The plot of RSDs for peak area versus acquisition rate for chlorpyrifos and alpha-HCH (Figure 2B) shows that low RSDs, which were shown acceptable precision in peak area measurements, are acquired when an acquisition rate of 2 spectra/s was adopted. No difference was found in the accuracy of mass assignation to MS fragments under different acquisition rates. As compromise, 2 spectra/s was selected as the TOF mass acquisition rate.

3.1.3 | Temperature of ion source

The effect of temperature of the ion source (210–290°C) on quantitative ion signal responses was also investigated. Figure 3 shows the plots for bifenthrin (*m/z* 181.1012), chlorothalonil (*m/z* 263.8811), and chlordane (*m/z* 372.8250) peak area versus ion source temperature. The responses of quantitative ion changed with the variation of temperature, although no regularity was found, and the changes depended on the analytes. The quantitative ion of bifenthrin (*m/z* 180.1012) showed an increase in response with the increase of ion source temperature, while chlorothalonil (*m/z* 263.8811) and chlordane (*m/z* 372.8250) exhibited slight change, and the ion of *m/z* 372.8250 exhibited the highest response when the ion source temperature was set at 250°C. Overall, the ion source temperature was set relatively higher not only for the enhancement of the most ions response, but was also for the ion source cleaning. However, excessively

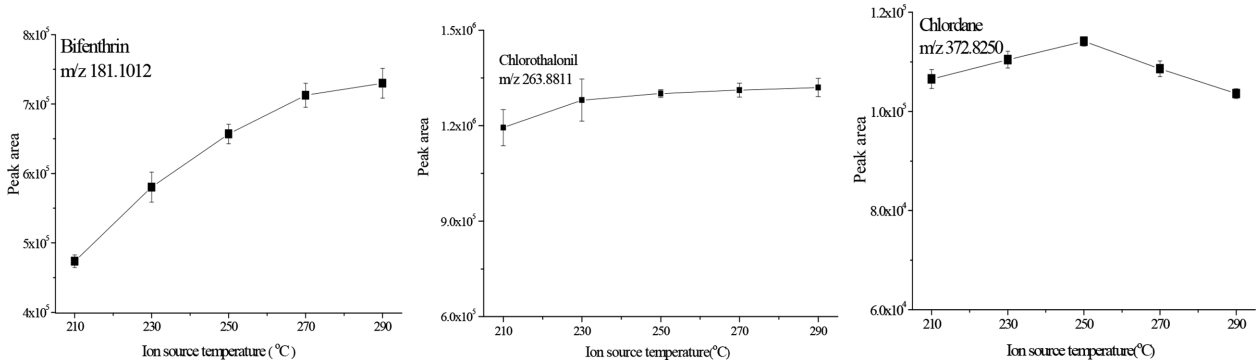

FIGURE 3 Peak area of 1 μL injection volume (100 μg/L) for bifenthrin (*m/z* 181.1012), chlorothalonil (*m/z* 263.8811), and chlordane (*m/z* 372.8250) in GC-TOF/MS at different ion source temperature setting

high temperature setting for a long time would damage the ion source. For these reasons, the ion source temperature was set at 270°C as the overall compromise in the final method.

3.2 | Home-made mass spectral library

3.2.1 | Setting up of the mass spectral library

To evaluate of the GC-EI-TOF/MS analysis system, 184 pesticides suitable for GC detection, which are typically found in teas with MRLs established by Japan and EU legislation, were selected to create the mass spectral library. Each pesticide standard at concentration of 1 μg/mL was injected into the instrument system to obtain the corresponding full-scan mass spectrum. As a kind of hard ionization technique, there are numerous fragment ions generated at EI source, among which the molecular ion might not be present or display low response in most cases. Therefore, each spectrum was carefully checked to verify that the molecular theoretical exact mass and the measured exact mass are matched. To guarantee accurate mass assignment of each diagnostic ion, the tool was applied, which is buttoned as "generate formula from spectrum peak" embedded in the MassHunter® Qualitative Analysis software. We selected three ions with relative abundance higher than 10% of the base peak as diagnostic ions. Information containing the name, retention time, and the theoretical exact mass of diagnostic ions were sent to PCDL manager® software to create the .cdb format mass spectral library and linked to the instrument software to conduct an automatic search of the pesticides from tea matrices extraction. The created library can be easily extended to include more pesticide compounds using the above procedure.

3.2.2 | Spectra consistency and mass accuracy VS pesticide standard concentration

Spectra consistency and mass accuracy are necessary in a wide concentration range in the pesticide residue analysis of actual samples. In EU, the MRLs for pesticide residues in teas range from 10 μg/kg to 50 mg/kg. For some pesticides in the spectral library, the detector signal of GC-EI-TOF/MS is prone to be saturated at high concentration level, leading to inaccurate results, as discussed in Section 3.1.1. In this study, standard solutions of 2′4-DDE in hexane (from 1 μg/L to 5 mg/L) were investigated. Consistent spectra were obtained in a wide concentration range, 1 μg/L and 5 mg/L spectra are shown in Figure 4. Mass deviations for all ions were within 5 ppm over the entire range at high and low concentrations. Thus, mass accuracy of diagnosed ions is not affected by concentration of pesticide in this range.

The data can also be used to evaluate the linear range of detection and the consistency of ion ratios. The linear range of detection was assessed through a parameter named "response factors (RF)," which is calculated by dividing the peak area over the pesticide concentration. The concentration levels and relative abundance of qualitative ions to the quantitative ion (*m/z* 245.9998) are supplied in Supporting Information Table S3. The data showed that the system was linear from 1 μg/L to 5 mg/L, and ion ratios remain consistently. Spectra library can be applied for pesticides retrieval in the above concentration range. At 10 mg/L, diagnostic ions ratio keeps consistent, however, depressed RF and larger ion mass deviation were observed due to saturation of detector, and in this case, dilution is needed for precise mass spectra library screening and pesticides quantitation.

3.2.3 | Comparability to NIST spectra

An advantage of using EI source in GC-MS is the availability of mass spectra in the NIST library with nominal mass for more than 240 000 compounds. In practice, until now there is no commercial mass spectral library for EI-TOF/MS, in which case the nominal mass NIST library is an alternative to verify comparability with other EI spectra. In this study, we obtained the HRMS spectra of pesticides by injecting pesticide standards and subtracting their background. The HRMS spectra were automatically searched in the NIST library, and the latter subsequently converted the HRMS spectra into nominal

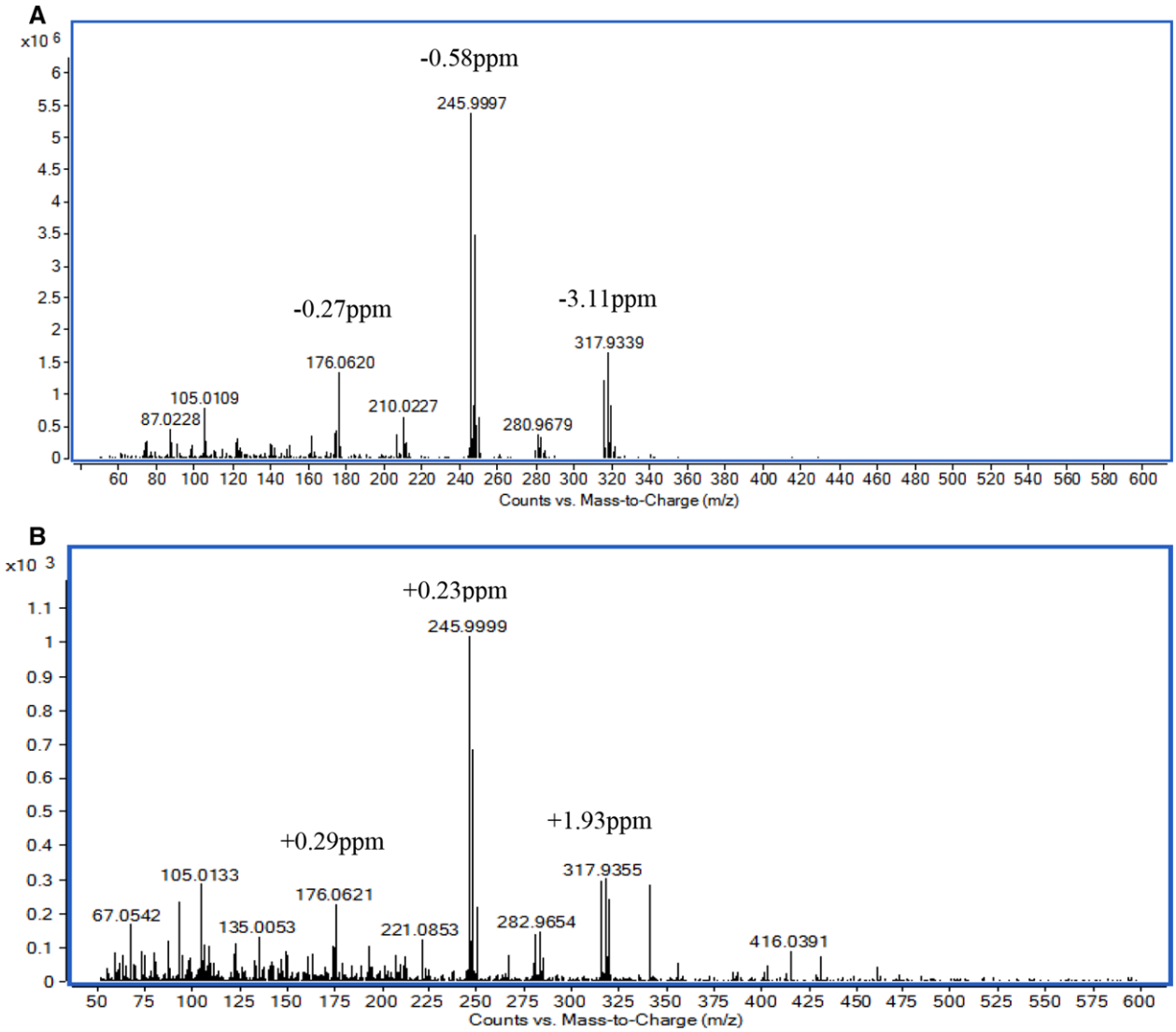

FIGURE 4 GC-EI-TOF accurate mass spectra for standards of 2′4-DDE in hexane with two concentrations. (A) 5 mg/L, (B) 1 μg/L

mass spectra. Generally, the spectra of GC-EI-TOF/MS were very similar to that of the NIST, as shown in Figure 5.

For some special pesticides, a more detailed comparison was conducted to determine some differences, such as the abundance of some ions compared with that of the NIST spectra (e.g., ethoprophos in Figure 6). The most abundant ion of the NIST spectrum was *m/z* 158, followed by *m/z* 97 and 200, whereas *m/z* 97 is the most abundant ion in the EI-TOF/MS spectra, followed by *m/z* 158. Similar phenomenon was found for methabenzthiazuron, as shown in Supporting Information Figure S1. It may be the reason that transmitting path of the ions in TOF is different with that of low-resolution mass spectrometry (LRMS). The ion abundance ratio was not affected by acquisition parameter settings and matrix types. Although difference between EI-TOF/MS spectra and EI-quadrupole spectra was observed for these two pesticides, there is no significant impact found on the automated search results. Thus, the EI-TOF/MS spectral library can also work for LRMS.

3.3 | Retrieval parameters of TOF spectral library

3.3.1 | MEW setting

In residue analysis based on accurate mass measurement, analyte ions are extracted from the full-scan data with a given MEW in MassHunter® Qualitative Analysis Software. The demand for applying MEW is that the analyte ions are separated from the background chemical noise of various sources, such as matrix coextracts, contaminants from chromatographic column, and ion source. A narrower MEW results in higher selectivity. The enhancement in selectivity contributed to the S/N increase, thereby improving LODs. Supporting Information Figure S2 shows the changes of S/N for quantitative ions of dichlorvos (*m/z* 109.0049) and bupirimate (*m/z* 208.1444) at four MEWs (5, 10, 20, and 50 ppm) in green tea, oolong tea, black tea, and puer tea matched standards at 10 μg/kg. For example, bupirimate in green tea

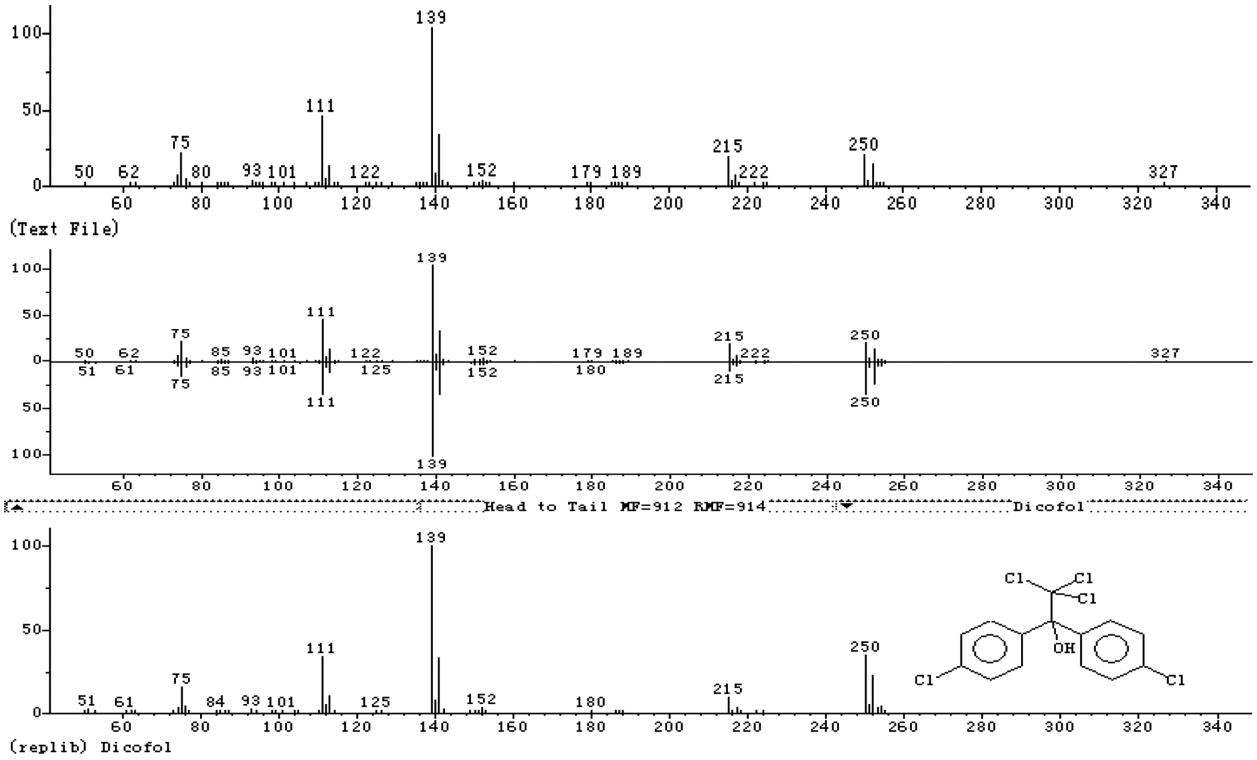

FIGURE 5 Example output automatic search GC-EI-TOF mass spectrum in NIST library: dicofol; the HRMS spectrum (upper spectrum) was converted by the software into a nominal mass spectrum

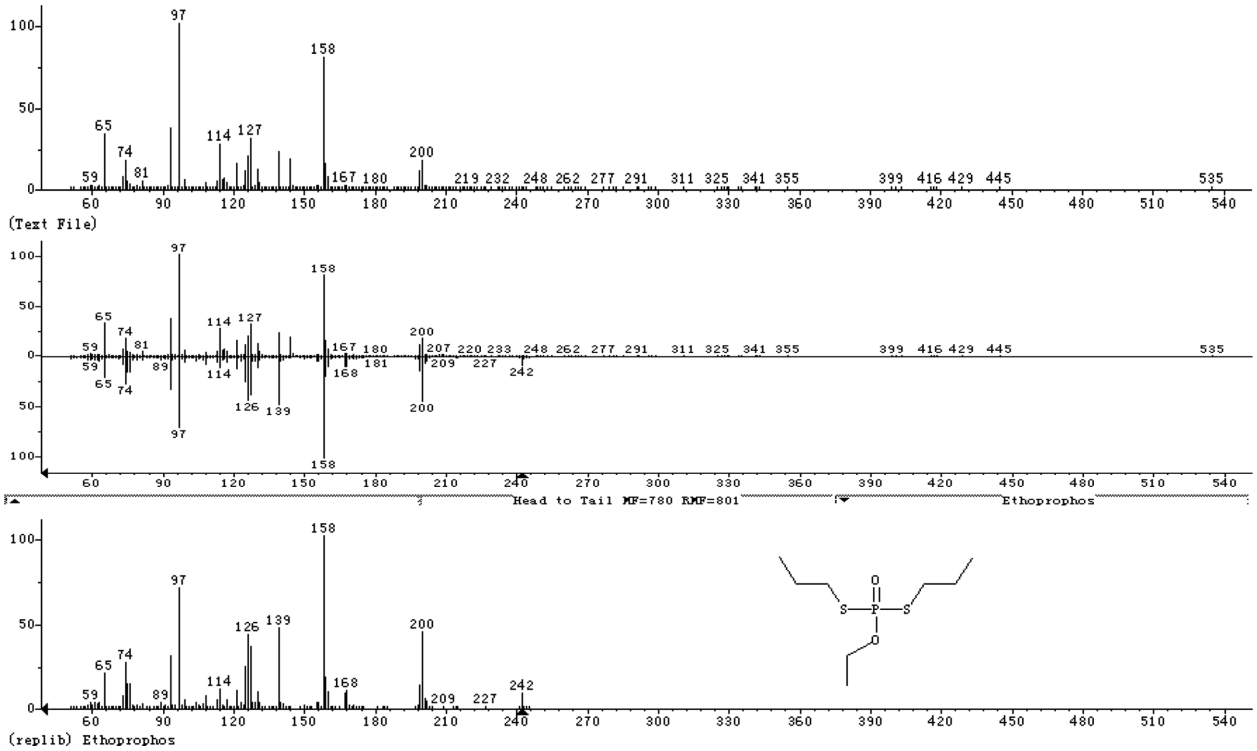

FIGURE 6 GC-EI-TOF accurate mass spectrum for ethoprophos (upper spectrum); GC-EI-MS nominal mass spectrum for ethoprophos (lower spectrum) from NIST; the differences between GC-EI-TOF mass spectrum and GC-EI-MS mass spectrum (middle spectrum)

(Supporting Information Figure S2B), the S/N increased from 47.5 to 306.1 when the mass window was set from 50 ppm to 10 ppm, whereas the S/N is decreased to 49.9 as the mass window continued to narrow to 5 ppm. The phenomenon of peak split may be caused by the large effect of coelution interferences on the ion *m/z* 208.1444 or the drifting of the quality shaft of time-of-flight tube, which resulted in mass deviation of some acquisition points by greater than 5 ppm. In other word, too narrow MEW setting (5 ppm) had negative effect on accurate quantitative purpose. For dichlorvos in green tea (Supporting Information Figure S2A), the S/N is 70.1 with a mass window of 50 ppm, the S/N increased to 192.1 for all MEWs of 20, 10, and 5 ppm. The selectivity of these two pesticides in green tea, as well as in oolong tea, black tea, and puer tea, was improved by narrowing the MEW from 50 to 10 ppm.

Then, the mass accuracy for each ion of the 184 analytes was investigated for the four matrices spiked at 100 μg/kg, and 10 ppm was set as the MEW. The results showed that 92.4, 92.9, 94.6, and 95.7% of the investigated pesticides were identified in green tea, oolong tea, black tea, and puer tea, respectively. In other word, the false-negative rate of pesticide detection is reduced to less than 8%. In the final analysis, a MEW of ± 10 ppm was used for data processing.

3.3.2 | Retention time windows setting

In complex matrices, numerous matrix interferences are apt to cause false-positive results, especially in retention time, which is very close to the target compound. Thus, setting a suitable retention time window is necessary to exclude the interferences. To set a reasonable retention time window, experiments were conducted under the appointed chromatographic and MS conditions, and four analyses were made on the four different tea samples spiked with 184 pesticides (100 μg/kg). The deviations of retention time of EICs for 184 quantitative ions are shown in Supporting Information Table S4, in which the retention time deviation of over 85% of the pesticides was less than 0.1 min in different tea matrices. Moreover, the maximum retention time deviation was 0.136 min, which observed for ethofumesate in black tea. No other compound whose retention time deviation exceeded 0.13 min for all the four kinds of teas. The stable retention time of pesticide compounds is mainly attributed to the robust electronic pressure controller as an important part of the gas chromatographic system and the retention time locking function by using heptachlor epoxide before each batch. Therefore, we finally set the optimal retention time window at ± 0.15 min, and the interferences can be excluded maximum.

3.4 | Performance of the analytical procedure

The performance of this method was evaluated in terms of linear range, LODs, LOQs, LOIs, accuracy, precise, and ME.

3.4.1 | Linear range, LODs, LOQs, and LOIs

The linear range was studied from 1–1000 μg/kg in all four tea matrices. The linear equation was acceptable when the coefficient of determination (R^2) ≥ 0.995. The results are shown in Supporting Information Table S5. Most of the pesticides showed good linearity in their individual linear range, with R^2 meeting the established criterion, except for chlorfenapyr and deltamethrin in green tea and oolong tea. Narrow linear range (500–1000 μg/kg, data not shown) was acquired for only two compounds because of their high LOQs (500 μg/kg).

In this study, the LOD, LOQ, and LOI were determined by analyzing spiked extracts (1, 2, 5, 10, 20, 50, 100, and 500 μg/kg) of four matrices. LOD was defined as the level that corresponded to three times S/N in the EIC of the quantitative ion. The LODs acquired from the 184 investigated pesticides in four matrices are listed in Supporting Information Table S5 and summarized in Table 1. For more than 95% of the pesticides in different tea matrices, the LOD was ≤ 50 μg/kg.

TABLE 1 The LOD, LOQ, and LOI for 184 pesticides spiking in four kinds of tea matrices

	Green tea	Oolong tea	Black tea	Puer tea
LOD				
μg/kg	Number of pesticides			
≤1	32	20	31	29
2	19	13	29	14
5	44	43	35	49
10	47	53	53	42
50	40	47	34	46
100	2	8	2	4
500	0	0	0	0
LOQ				
μg/kg	Number of pesticides			
≤1	9	5	13	12
2	18	13	28	17
5	38	31	46	34
10	63	61	37	56
50	49	57	53	57
100	5	15	7	8
500	2	2	0	0
LOI				
μg/kg	Number of pesticides			
≤1	17	14	12	15
2	21	14	23	17
5	35	33	37	39
10	52	57	51	54
50	46	50	51	48
100	11	14	10	11
500	2	2	0	0

LOQ was defined as the lowest point in linear range with RF deviation ≤ 20%. The LOQs obtained were ≤ 10 μg/kg for 69.5, 59.8, 67.4, and 64.7% of 184 pesticides for green tea, oolong tea, black tea, and puer tea, respectively. Most of the pesticides can excellently meet the MRLs, but a few exceptions were observed: endrin and oxadixyl in green tea, azinphos-ethyl, endrin, flucythrinate, oxadixyl, phosphamidon, pyridalyl, spirodiclofen, and tolylfluanid in oolong tea, endrin, and oxadixyl in black tea, and endrin, iprovalicarb, oxadixyl, phosphamidon, and triazophos in puer tea (Supporting Information Table S5). The response is relatively low on the detector for these compounds, and the matrix interference is different in different tea type.

In SANTE/11945/2015, LOIs was required that the deviation of ion ratio of the qualitative and quantitative ion of analytes should be less than 30% comparing to the reference ion ratio. Except for the ion ratio criterion, the guidance document also sets regulations refer to retention time deviation (≤ 0.1 min from reference retention time) and mass deviation (≤ 5 ppm). In our study, slightly broad condition was set due to the complexity of the tea matrices and to reduce the false negative possibility. Retention time deviation of 0.15 min and mass deviation of 10 ppm were selected as the optimized conditions. The default reference ion ratio was the standard compound in solvent at 1000 μg/kg, which is used for mass spectral library establishment. Solvent standards were adopted to minimize the risk of contribution of matrix ions to the signal of the quantitative or qualitative ions. No obvious difference was found between LOIs and LOQs for the number of pesticides in different teas (Table 1), which indicated that reliable ion ratio was easily obtained at the concentration equal to LOQ. Overall, the GC-EI-TOF/MS can serve as a reliable and powerful tool for identification and quantification of pesticide residues in tea matrices.

3.4.2 | Accuracy and precision

Accuracy and precision were evaluated using green tea, oolong tea, black tea, and puer tea spiked at three different concentration levels: 10, 50, and 100 μg/kg. The spiked tea samples were processed in five parallel runs, and the pesticides in tea sample extracts were determined by external calibration. The method performance results are shown in Supporting Information Table S6. At 10 μg/kg, 92.2, 96.3, 92.7, and 95.1% of the detectable pesticides in green tea, oolong tea, black tea, and dark tea had recovery within the EU guidelines for pesticide residue analysis [35], that is, between 70 and 120% with precision ≤ 20%. At 50 and 100 μg/kg, the values were also more than 90% for different tea matrices. For some pesticides, such as 1-naphthyl acetamide, acibenzolar-s-methyl, chlorothalonil, pyrimethanil, and chlorbenside, the recovery was below 70% due to the loss in the pretreatment process and adsorption by purification materials. Although in these cases, the good repeatability could still be achieved, they could be quantified accurately by the correction of recovery.

3.4.3 | MEs

ME is classified as signal enhancement or suppression of an analyte, and this phenomenon is due to the coelution of matrix components and it exerts an important influence on quantitative analysis of pesticides [36]. In this study, MEs were assessed based on the instrumental response of the pesticide (100 μg/kg) obtained in matrix extracts and solvents for green, oolong, black, and puer teas. The ME of each pesticide was calculated by the following equation: ME = [(A1-A2)/A2] × 100%, where A1 and A2 are the peak areas measured for spiked extracts and solvent standard with the same concentration of the same tea sample. Moreover, the analytes were classified into three groups: matrix suppression effect (ME lowers than –20%), weak matrix effect (ME between –20% and 20%), and matrix enhancement effect (ME higher than 20%). Results are shown in Figure 7. Fewer pesticides showed matrix enhancement effect in black tea and puer tea than in green tea and oolong tea. The 74.5 and 79.9% of the pesticides exhibited weak matrix effect in black tea and puer tea, respectively, but 62.0 and 59.2% of the pesticides descend into the same range in green tea and oolong tea, respectively. No pesticides were subjected to matrix suppression effect in the four tea matrices. It should be paid attention to that the strongest matrix enhancement effect (approximately 80%) that occurred in EPTC and phosmet in green tea. Base on the above reasons, matrix-matched standard calibration curves were applied to compensate for matrix effect and to get the accurate quantitative result.

3.4.4 | Screening of samples

The proposed method was applied to 54 tea samples (16 green teas, 10 oolong teas, 13 black teas, and 15 puer teas) purchased from local supermarkets. Concentrations measured for the analytes are summarized in Supporting Information Table S7. A total of 16 pesticides were present in 46 samples, with the maximum individual concentration of 632.7 μg/kg for bifenthrin in puer tea (sample 9). Importantly, 70.4% of the samples showed at least one pesticide with concentration above 10 μg/kg and 25.9% stayed above 100 μg/kg. Bifenthrin was the most widely detected pesticide in various categories of tea samples, and acrinathrin and chlorpyrifos were also frequently detected in green and oolong teas. In completely fermented teas (black tea and puer tea), most pesticide residues might be decomposed during fermentation under relative high temperature and humidity. More attention should be given to individual residues that exceeded the MRL prescribed by EU; for instance, acrinathrin and cypermethrin in green tea (sample 9) and oolong tea (sample 5), and bifenthrin in puer tea (sample 9). Thus, monitoring the level of pesticide residues

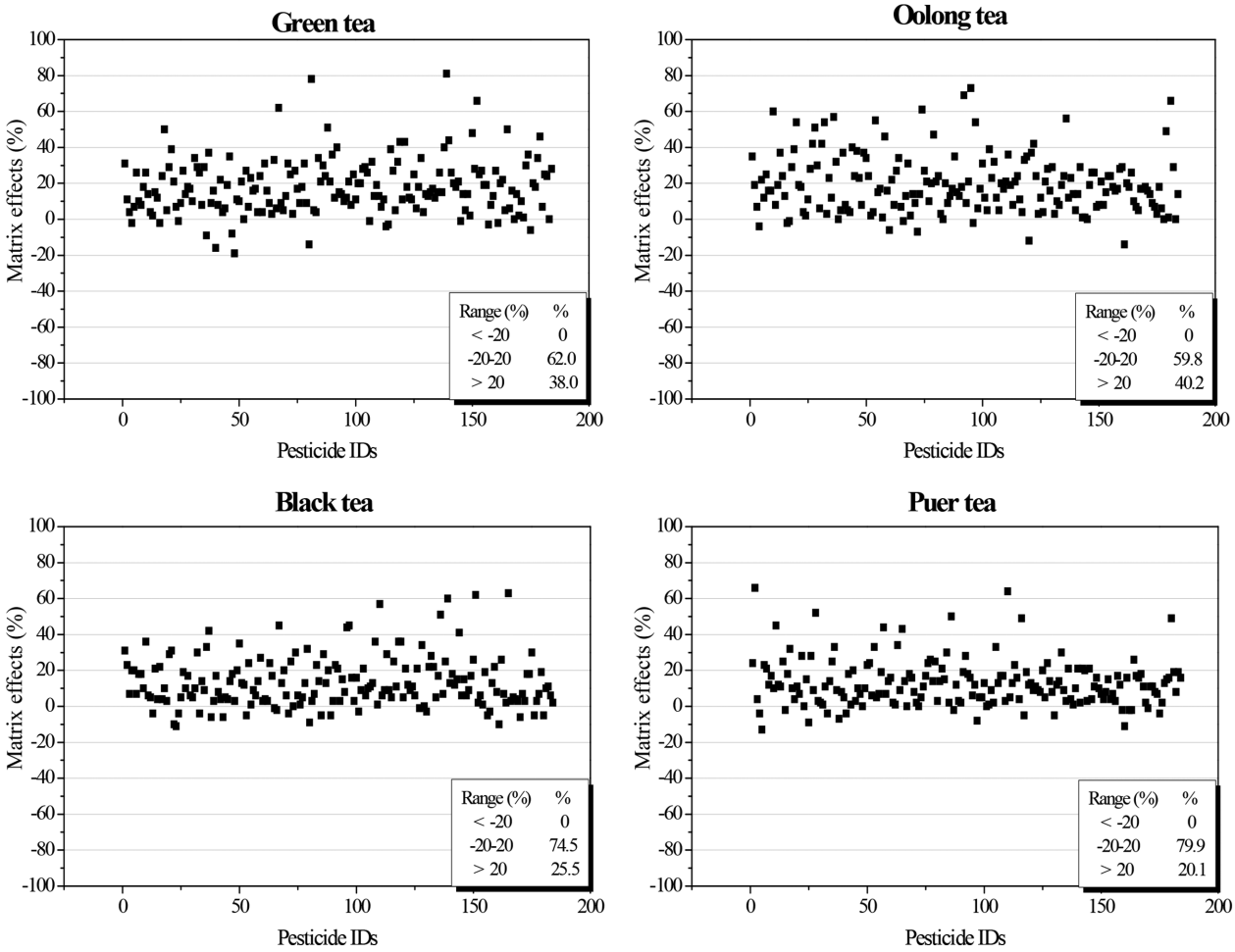

FIGURE 7 GC-TOF MS matrix effect. The 184 pesticides were prepared in sample extracts (four different tea matrices) and solvent at a concentration of 100 μg/kg (chlorfenapyr and deltamethrin were preparedat a concentration of 500 μg/kg) equivalent in sample

in tea is necessary to ensure consumers' health and to guide tea farmers to use pesticides scientifically and rationally. The results confirmed the feasibility of the proposed method for multiple pesticide residues screening and quantitation in tea samples.

4 | CONCLUDING REMARKS

This study is the first time to adopt a new qualitative screening strategy for pesticides in teas using GC-EI-TOF/MS technique combined with a home-made mass spectral library. Under the optimized acquisition condition, the home-made spectral library has been built and validated in different tea matrices. The developed technique was used for simultaneous determination of 184 pesticides in tea matrices. The accuracy of pesticide detection result is satisfactory when the optimized retrieval parameters of the TOF mass spectral library are applied.

The LODs, LOQs, and LOIs of this method were investigated and discussed. Results showed that it was an accurate, reliable, and low-cost method, and could be used for routine screening of pesticide residues in tea samples. This method was employed on commercial samples; the result showed that several pesticides included in mass spectral library were detected in the tea samples, wherein 16 out of the 184 pesticides were found.

In addition, the characterization of TOF analyzer is the application of full-scan acquisition with high sensitivity and mass accuracy. This feature facilitates an easier and more accurate qualitative analysis because the monitoring of diagnosed ions of analytes need not be predefined before data acquisition.

This developed method is very useful in the detection of the trace targeted chemical residues in complex matrices. Further, it could be easily extended to include more analytes. It can improve not only the ability of multiple pesticide residues screening, but also the performance of detection method. It has the advantage of cost-, labor-, and time- saving, high speed and high efficiency. Meanwhile, it can help to solve food safety problems in pesticide residues to make early detection, early warning, and early management. It also can

facilitate food safety supervision and make food safety from passive rectification to prevention.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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REFERENCES

1. Korir, M. W., Wachira, F. N., Wanyoko, J. K., Ngure, R. M., Khalid, R., The fortification of tea with sweeteners and milk and its effect on in vitro antioxidant potential of tea product and glutathione levels in an animal model. *Food Chem*. 2014, *145*, 145–153.
2. Martínez-Domínguez, G., Romero-González, R., Frenich, A. G., Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Food Chem*. 2016, *197*, 907–915.
3. Hou, X., Lei, S., Qiu, S., Guo, L., Yi, S., Liu, W., A multi-residue method for the determination of pesticides in tea using multi-walled carbon nanotubes as a dispersive solid phase extraction absorbent. *Food Chem*. 2014, *153*, 121–129.
4. Hou, X., Zheng, X., Zhang, C., Ma, X., Ling, Q., Zhao, L., Ultrasound-assisted dispersive liquid–liquid microextraction based on the solidification of a floating organic droplet followed by gas chromatography for the determination of eight pyrethroid pesticides in tea samples. *J. Chromatogr. B* 2014, *969*, 123–127.
5. Chen, L., Chen, J., Guo, Y., Li, J., Yang, Y., Xu, L., Fu, F., Study on the simultaneous determination of seven benzoylurea pesticides in Oolong tea and their leaching characteristics during infusing process by HPLC–MS/MS. *Food Chem*. 2014, *143*, 405–410.
6. European Commission. Plants, EU Pesticides Database, Search pesticide residues, http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN (accessed 08.09.2017).
7. EU Commission Regulation (EC). No. 396/2005 of the european parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending council directive 91/414/EEC, Implemented by 10/10/2015.
8. Margoum, C., Guillemain, C., Yang, X., Coquery, M., Stir bar sorptive extraction coupled to liquid chromatography-tandem mass spectrometry for the determination of pesticides in water samples: method validation and measurement uncertainty. *Talanta* 2013, *116*, 1–7.
9. Ferreira, J. A., Ferreira, J. M. S., Talamini, V., de Fátima Facco, J., Rizzett, T. M., Prestes, O. D., Adaime, M. B., Zanella, R., Bottoli, C. B. G., Determination of pesticides in coconut (*Cocos nucifera* Linn.) water and pulp using modified QuEChERS and LC–MS/MS. *Food Chem*. 2016, *213*, 616–624.
10. Wong, J. W., Zhang, K., Tech, K., Hayward, D. G., Makovi, C. M., Krynitsky, A. J., Schenck, F. J., Banerjee, K., Dasgupta, S., Brown, D., Multiresidue pesticide analysis in fresh produce by capillary gas chromatography−mass spectrometry/selective ion monitoring (GC-MS/SIM) and−tandem mass spectrometry (GC-MS/MS). *J. Agric. Food Chem.* 2010, *58*, 5868–5883.
11. Gan, J., Lv, L., Peng, J., Li, J., Xiong, Z., Chen, D., He, L., Multi-residue method for the determination of organofluorine pesticides in fish tissue by liquid chromatography triple quadrupole tandem mass spectrometry. *Food Chem*. 2016, *207*, 195–204.
12. Kadar, A., Peyre, L., de Souza, G., Wortham, H., Doumenq, P., Rahman, R., An accurate and robust LC-MS/MS method for the quantification of chlorfenvinphos, ethion and linuron in liver samples. *Chemosphere* 2017, *184*, 20–26.
13. Anagnostopoulos, C., Miliadis, G. E., Development and validation of an easy multiresidue method for the determination of multiclass pesticide residues using GC–MS/MS and LC–MS/MS in olive oil and olives. *Talanta* 2013, *112*, 1–10.
14. Chamkasem, N., Ollis, L. W., Harmon, T., Lee, S., Mercer, G., Analysis of 136 pesticides in avocado using a modified QuEChERS method with LC-MS/MS and GC-MS/MS. *J. Agric. Food Chem.* 2013, *61*, 2315–2329.
15. Chen, Y., Lopez, S., Hayward, D. G., Park, H. Y., Wong, J. W., Kim, S., Wan, J., Reddy, R. M., Quinn, D. J., Steiniger, D., Determination of multiresidue pesticides in botanical dietary supplements using gas chromatography–triple–quadrupole mass spectrometry (GC-MS/MS). *J. Agric. Food Chem.* 2016, *64*, 6125–6132.
16. Machado, I., Gérez, N., Pistón, M., Heinzen, H., Cesio, M. V., Determination of pesticide residues in globe artichoke leaves and fruits by GC–MS and LC–MS/MS using the same QuEChERS procedure. *Food Chem*. 2017, *227*, 227–236.
17. Chang, Q. Y., Pang, G. F., Fan, C. L., Chen, H., Yang, F., Li, J., Wen, B. F., High-throughput analytical techniques for the determination of the residues of 653 multiclass pesticides and chemical pollutants in tea, part VII: A GC-MS, GC-MS/MS, and LC-MS/MS study of the degradation profiles of pesticide residues in green tea. *J AOAC INT*. 2016, *99*, 1619–1627.
18. Jiao, W., Xiao, Y., Qian, X., Tong, M., Hu, Y., Hou, R., Hua, R., Optimized combination of dilution and refined QuEChERS to overcome matrix effects of six types of tea for determination eight neonicotinoid insecticides by ultra-performance liquid chromatography–electrospray tandem mass spectrometry. *Food Chem*. 2016, *210*, 26–34.
19. Aceña, J., Heuett, N., Gardinali, P., Pérez, S., Suspect screening of pharmaceuticals and related bioactive compounds, their metabolites and their transformation products in the aquatic environment, biota and humans using LC-HR-MS techniques. *Compre Anal. Chem.* 2016, *71*, 357–378.
20. Lehotay, S. J., Mastovska, K., Amirav, A., Fialkov, A. B., Martos, P. A., de Kok, A., Fernández-Alba, A. R., Identification and confirmation of chemical residues in food by chromatography-mass

spectrometry and other techniques. *Trends Anal. Chem.* 2008, *27*, 1070–1090.

21. Wang, X., Wang, S., Cai, Z., The latest developments and applications of mass spectrometry in food-safety and quality analysis. *Trends Anal. Chem.* 2013, *52*, 170–185.
22. García-Reyes, J. F., Hernando, M. D., Molina-Díaz, A., Fernández-Alba, A. R., Comprehensive screening of target, non-target and unknown pesticides in food by LC-TOF-MS. *Trends Anal. Chem.* 2007, *26*, 828–841.
23. Hernández, F., Portolés, T., Pitarch, E., López, F. J., Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology. *Trends Anal. Chem.* 2011, *30*, 388–400.
24. Zhang, F., Yu, C., Wang, W., Fan, R., Zhang, Z., Guo, Y., Rapid simultaneous screening and identification of multiple pesticide residues in vegetables. *Anal. Chim Acta*. 2012, *757*, 39–47.
25. Valles, N. B., Uclés, S., Besil, N., Mezcua, M., Fernández-Alba, A. R., Analysis of pesticide residues in fruits and vegetables using gas chromatography-high resolution time-of-flight mass spectrometry. *Anal. Methods* 2015, *7*, 2162–2171.
26. Koesukwiwat, U., Lehotay, S. J., Miao, S., Leepipatpiboon, N., High throughput analysis of 150 pesticides in fruits and vegetables using QuEChERS and low-pressure gas chromatography–time-of-flight mass spectrometry. *J. Chromatogr. A* 2010, *1217*, 6692–6703.
27. Hayward, D. G., Pisano, T. S., Wong, J. W., Scudder, R. J., Multiresidue method for pesticides and persistent organic pollutants (POPs) in milk and cream using comprehensive two-dimensional capillary gas chromatography−time-of-flight mass spectrometry. *J. Agric. Food Chem.* 2010, *58*, 5248–5256.
28. Hakme, E., Lozano, A., Gómez-Ramos, M. M., Hernando, M. D., Fernández-Alba, A. R., Non-target evaluation of contaminants in honey bees and pollen samples by gas chromatography time-of-flight mass spectrometry. *Chemosphere* 2017, *184*, 1310–1319.
29. Portolés, T., Ibáñez, M., Sancho, J. V., López, F. J., Hernández, F., Combined use of GC-TOF MS and UHPLC-(Q) TOF MS to investigate the presence of nontarget pollutants and their metabolites in a case of honeybee poisoning. *J. Agric. Food Chem.* 2009, *57*, 4079–4090.
30. Gómez-Ramos, M. M., García-Valcárcel, A. I., Tadeo, J. L., Fernández-Alba, A. R., Hernando, M. D., Screening of environmental contaminants in honey bee wax comb using gas chromatography–high-resolution time-of-flight mass spectrometry. *Environ. Sci. Pollut. R* 2016, *23*, 4609–4620.
31. Casado, J., Rodríguez, I., Carpinteiro, I., Ramil, M., Cela, R., Gas chromatography quadrupole time-of-flight mass spectrometry determination of benzotriazole ultraviolet stabilizers in sludge samples. *J. Chromatogr. A* 2013, *1293*, 126–132.
32. Pitarch, E., Portolés, T., Marín, J. M., Ibáñez, M., Albarrán, F., Hernández, F., Analytical strategy based on the use of liquid chromatography and gas chromatography with triple-quadrupole and time-of-flight MS analyzers for investigating organic contaminants in waste water. *Anal. Bioanal. Chem*. 2010, *397*, 2763–2776.
33. Zhao, P., Wang, L., Jiang, Y., Zhang, F., Pan, C., Dispersive cleanup of acetonitrile extracts of tea samples by mixed multiwalled carbon nanotubes, primary secondary amine, and graphitized carbon black sorbents. *J. Agric. Food Chem*. 2012, *60*, 4026–4033.
34. Schymanski, E. L., Singer, H. P., Longrée, P., Loos, M., Ruff, M., Stravs, M. A., Vidal, C. R., Hollender, J., Strategies to characterize polar organic contamination in wastewater: exploring the capability of high resolution mass spectrometry. *Environ. Sci. Technol.* 2014, *48*, 1811–1818.
35. EU Commision No. SANTE/11945/2015 Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Implemented by 01/01/2016.
36. Li, Y., Chen, X., Fan, C., Pang, G., Compensation for matrix effects in the gas chromatography–mass spectrometry analysis of 186 pesticides in tea matrices using analyte protectants. *J. Chromatogr. A* 2012, *1266*, 131–142.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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