

Trace Analysis of Fenbutatin Oxide in Soil and Plant- and Animal-Derived Foods Using Modified QuEChERS Coupled with HPLC-MS/MS

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ABSTRACT: A modified QuEChERS method in combination with high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was first developed for the determination of fenbutatin oxide in six types of samples (soil, tobacco, rice, milk, pork liver, and pork). Fenbutatin oxide was extracted with acetonitrile containing 1% formic acid (v/v) and purified by dispersive solid-phase extraction using primary secondary amine (PSA) and quantitatively analyzed by HPLC-MS/MS. In the range of 0.005–1 mg/kg, a good linear relationship exists between the concentration of fenbutatin oxide and the peak area, giving a coefficient of determination (R^2) of >0.99. The recoveries of fenbutatin oxide at three spiked levels were 79.04–97.12% with the relative standard deviations (RSDs) of 3.30–10.96%, and the limit of quantification (LOQ) was 0.007 mg/kg. In addition, the developed method is consistent with the reference method ($R^2 = 0.9896$, $n = 40$). The method is demonstrated to be convenient and reliable for the routine monitoring of fenbutatin oxide in soil and plant- and animal-derived foods.

1. INTRODUCTION

Pesticides have been playing a key role in agricultural pest management in recent decades. Although the use of pesticides brings enormous benefits, pesticide residues in soil and plants may exist in grain or feed, leading to the accumulation of animal products.^{1,2} Pesticide residues could cause adverse health effects of the consumer through the food chain, including the dysfunction of nervous and reproductive systems.³ Therefore, potentially hazardous pesticides in the environment and food products have aroused considerable worldwide interest and become a growing public concern.

Fenbutatin oxide, bis[tris(2-methyl-2-phenylpropyl)tin] oxide is one of the organotin compounds, which is often used as acaricide (via contact and stomach actions).⁴ Because of its extremely high octanol–water partition coefficient ($\log K_{ow}$ 12.8), negligible vapor pressure, and chemical stability, fenbutatin oxide is considered as a persistent compound in the environment and the treated food stuffs.^{5,6} In southern China, fenbutatin oxide is often used in tobacco/

rice rotation farmlands to control red spider mites, so its residues may be transferred to animal-derived products. Therefore, the detection and monitoring of fenbutatin oxide residues in the environment and food products including animal origin products has become an extremely demanding task for the assurance of food safety.

At present, fenbutatin oxide residues are mainly detected with gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).^{5,6} Due to the low volatility of fenbutatin oxide, fenbutatin oxide derivatives that are volatile and thermally stable are required for the separation in GC.⁷ However, the low yields of the derivatization process often lead

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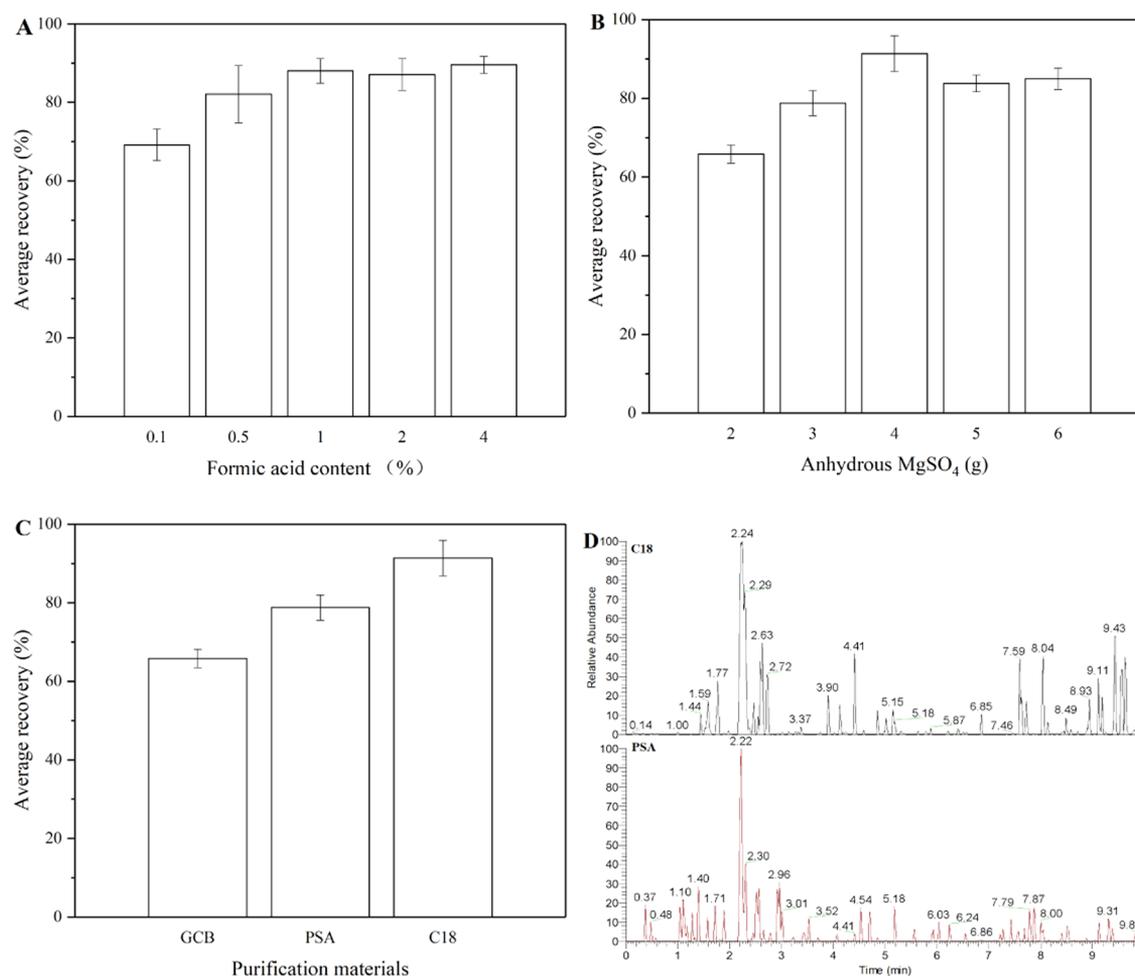


Figure 1. Experimental optimization of the extraction agent (A), water absorbent (B), and purification agent (C, D).

to an underestimation of the fenbutatin oxide concentration.^{5,6} The drawback of derivatization can be eliminated by high-performance liquid chromatography (HPLC), but HPLC suffers from insufficient sensitivity. In recent years, the integrated approach of QuEChERS and HPLC-tandem mass spectrometry (HPLC-MS/MS) has been proved to be a rapid, highly selective, and sensitive method for the determination of pesticide residues in different matrices.^{8–11} To the best of our knowledge, there are only two existing reports that QuEChERS coupled with the HPLC-MS/MS method was used to determine multiresidues including fenbutatin oxide in plant samples, i.e., citrus and peppers.^{12,13} However, no report is available on the determination of fenbutatin oxide residues in animal-derived foods. The obstacle may be due to the fact that they are rich in fat, protein, and other lipophilic compounds, which are easily coextracted with the target analytes.¹⁴

The objective of this study is to develop an effective approach for the trace quantification of fenbutatin oxide in six types of samples (soil, tobacco, rice, milk, pork liver, and pork). The parameters of QuEChERS (extraction and purification) and HPLC-MS/MS (determination) were optimized. The linear range, sensitivity, accuracy, and precision of the method were evaluated. This study establishes a new method for the determination of fenbutatin oxide residues in soil and plant- and animal-derived foods.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. Fenbutatin oxide standard (purity > 99.7%) was obtained from Aladdin Reagent Co., Ltd. (China). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific. Analytical grade sodium chloride (NaCl), anhydrous magnesium sulfate (MgSO₄) formic acid, and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Primary secondary amine (PSA) was obtained from Tianjin Agela Technologies (China). C₁₈ and graphitized carbon black (GCB) were obtained from Shanghai Macklin Biochemical Co., Ltd. (China).

2.2. Instruments and Parameter Setting. A TSQ Quantum Ultra (Thermo Fisher) equipped with a Hypersil Gold C₁₈ column (3.0 μ m \times 2.1 mm \times 100 mm) was used for the analysis of fenbutatin oxide residues. The mobile phase was composed of methanol (A) and a 0.1% (v/v) formic acid solution in water (B). The flow rate was 0.25 mL/min with the following gradient elution program (0 min, 60% A; 4.0 min, 95% A; 6.0 min, 95% A; 8.0 min, 60% A; 10.0 min, 60% A; and 10.0 min, 10% A). The injection volume was 10 μ L.

The determination of fenbutatin oxide was performed in the multiple-reaction monitoring (MRM) mode with the positive electrospray ionization (ESI+) source. The nebulizer gas was nitrogen with a temperature of 350 $^{\circ}$ C. The nebulizer gas flow was set at 8 mL/min with a pressure of 35 psi. The cone voltage was 129 V with the MRM transitions of m/z 518.96 \rightarrow

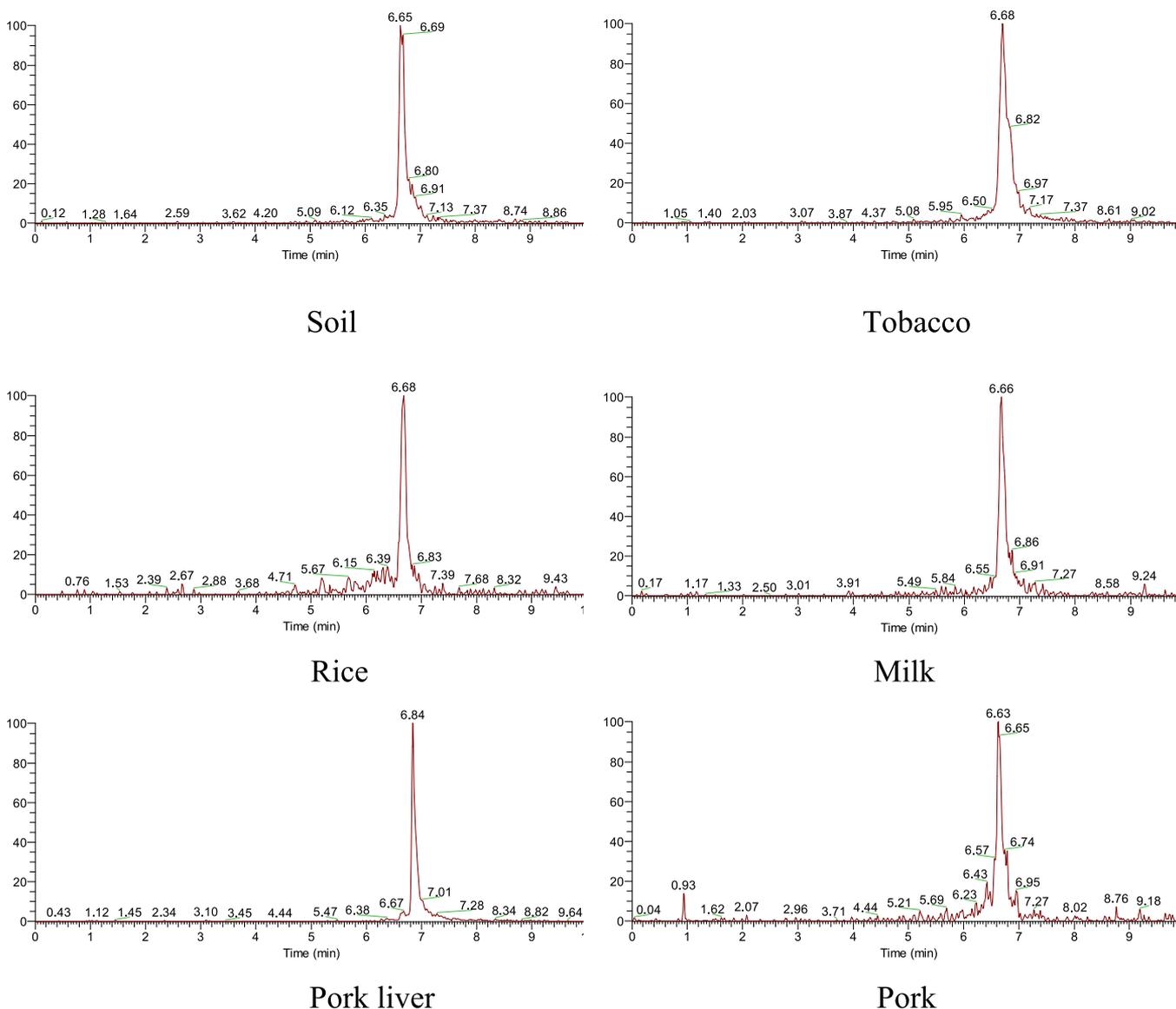


Figure 2. Representative chromatograms of fenbutatin oxide in six samples.

463.04 (collision energy, 21 eV) and 518.96 \rightarrow 350.91 (collision energy, 35 eV), respectively. The MRM transitions of m/z 518.96 \rightarrow 463.04 were used for quantitative analysis.

2.3. Sample Extraction and Purification. Ten grams of homogenized samples were placed into a 50 mL polypropylene conical bottom centrifuge tube. For wetting purpose, 5 mL of water was added to the soil and rice samples. After 20 mL of acetonitrile containing 1% formic acid (v/v) was added, the sample was vortexed for 2 min. Anhydrous $MgSO_4$ (4 g) and NaCl (2 g) were subsequently added, and the resulting sample was immediately vortexed vigorously for 1 min and centrifuged for 5 min at 4000 rpm.

The supernatant (1.5 mL) was transferred into a single-use centrifuge tube that was prefilled with 25 mg of PSA and 150 mg of anhydrous $MgSO_4$. The sample was vortexed for 2 min and then centrifuged for 5 min at 10 000 rpm. The supernatant of the prepared sample was filtered through a 0.22 μm nylon syringe filter and transferred to an autosampler vial for HPLC-MS/MS injection.

2.4. Standard Curve and the Spiking Experiment. Individual stock standard solutions were prepared by dissolving

the fenbutatin oxide standard in acetonitrile to obtain 100 mg/L solutions. Working standard solutions were prepared by diluting the stock standard solution with a blank sample extract or acetonitrile to the appropriate concentrations. All of the standard solutions were stored at -20 $^{\circ}C$ in the dark. Six different samples were added with the reserve standard solution of fenbutatin oxide to make the concentration 0.02, 0.1, and 0.5 mg/kg, respectively. After standing for 0.5 h, the samples were analyzed according to the abovementioned procedure. Five replicates were measured for each concentration of each sample.

2.5. Method Validation. Soil, tobacco, rice, milk, pork liver, and pork samples were collected randomly and locally (three samples for each). The established method and the method specified for SN/T 4558-2016 (China) were used for the determination of fenbutatin oxide in real samples. The recovery rate was determined for the samples in which fenbutatin oxide was not detected.

2.6. Data Analysis and Evaluation. All data were processed and analyzed by Excel 2016 and SPSS 25.0. The limit of detection (LOD) was defined as the concentration

Table 1. Overview of the Standard Curve, Sensitivity, and Matrix Effect of Fenbutatin Oxide in Different Samples

samples	range (mg/L)	regression equation	R ²	LOD (mg/kg)	LOQ (mg/kg)	matrix effect
soil	0.005–1	$y = 3.22e07x + 2.71e05$	0.9994	0.002	0.007	0.93
tobacco		$y = 3.70e07x + 5.99e05$	0.9953	0.002	0.007	1.06
rice		$y = 3.52e07x + 1.87e05$	0.9980	0.002	0.007	1.01
milk		$y = 4.42e07x + 4.64e05$	0.9987	0.002	0.007	1.27
pork liver		$y = 4.48e07x - 7.96e05$	0.9941	0.002	0.007	1.29
pork		$y = 3.53e07x + 7.13e05$	0.9981	0.002	0.007	1.01

corresponding to 3 times signal to noise ratio, and the limit of quantitation (LOQ) was defined as the concentration corresponding to 10 times signal to noise ratio. The accuracy of the method was evaluated by the recovery rates. The precision was evaluated by the relative standard deviation (RSD) of the recovery rates. The results obtained from different methods were compared by a linear regression equation.

3. RESULTS AND DISCUSSION

3.1. Experimental Optimization. **3.1.1. Extraction Agent.** Methanol and acetonitrile are two commonly used solvents for the extraction of pesticide residue.¹⁵ As fenbutatin oxide is insoluble in methanol, so acetonitrile was selected as the extractant. Considering that fenbutatin oxide was relatively stable in an acidic environment, the addition of formic acid was expected to improve the extraction efficiency.⁴ Therefore, the relationship between the extraction efficiency and the acetonitrile/formic acid ratio was investigated. With the increase in the formic acid ratio concentrations, the recovery rates increase; however, when the content of formic acid exceeds 1%, the extraction efficiency reaches a plateau (Figure 1A). Therefore, acetonitrile containing 1% formic acid was selected as the extraction agent.

3.1.2. Water Absorbent. Anhydrous MgSO₄ is usually used as a water absorbent in the QuEChERS method. The use of a water absorbent along with the salting-out effect of NaCl leads to that the pesticide can be dissolved in an organic phase.¹⁶ The amount of anhydrous MgSO₄ plays an important role in the extraction of pesticides. Our results are shown in Figure 1B. When the amount of anhydrous MgSO₄ was 4 g, the highest recovery rate was obtained. In addition, excessive anhydrous MgSO₄ was prone to cause solidification and caking. Therefore, the amount of anhydrous MgSO₄ was selected as 4 g.

3.1.3. Purification Agent. The impurities, such as pigment, fat, fatty acid, and sugar in the extract of complex samples can be removed by purification, which is beneficial for reducing the interference in the detection.¹⁷ GCB, PSA, and C₁₈ are usually used as purifying agents in the QuEChERS method. As shown in Figure 1C, the recovery rate was found less than 70% for the samples purified by GCB. GCB has a symmetrical structure, while fenbutatin oxide also has a symmetrical structure and an aromatic ring. As a result, there may be a possibility for adsorption between GCB and fenbutatin oxide. With the purification by PSA and C₁₈, the recovery of fenbutatin oxide was found over 80%, which meets the requirements of pesticide residue detection. However, the matrix effect of C₁₈ was stronger than that of PSA. Therefore, PSA was selected as the purification agent.

3.1.4. Optimization of Instrument Parameters. The mobile phase plays an important role in the separation and peak shape of the target.¹⁸ Methanol/water and acetonitrile/

water are commonly used in LC. Compared with acetonitrile, methanol can significantly improve the response of fenbutatin oxide in MS, which may be related to the higher ionization efficiency of methanol.¹⁸ However, when methanol/water was used as a mobile phase, a poorly shaped peak was observed (tailing phenomenon). When 0.1% formic acid was added to the mobile phase system, the peak shape of fenbutatin oxide was obviously improved. Besides, the instrument and the column could be protected. Furthermore, the MS parameters, such as collision energy and cone hole voltage, were optimized, by which the chromatograms of fenbutatin oxide in typical matrix samples were obtained (Figure 2). A symmetrical peak and a stable baseline were conceived.

3.2. Standard Curve and Sensitivity. Under the optimal conditions, the standard curve was obtained by plotting the concentration of different matrix standard solutions with the area of the chromatographic peak. The linear fitting results showed that there was a good linear relationship between the concentration of fenbutatin oxide and the peak area (Table 1). The LOD and LOQ of fenbutatin oxide in different samples were 0.002 and 0.007 mg/kg. According to the GB 2763-2019, the minimum MRL of fenbutatin oxide is 0.5 mg/kg in plant-derived foods and 0.05 mg/kg in animal-derived foods.¹⁹ Therefore, the sensitivity of the established method could meet the detection requirements.

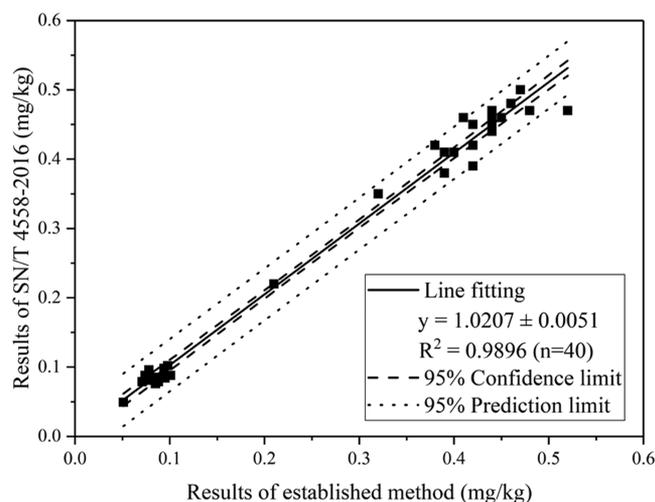
3.3. Matrix Effect. The matrix effect has a great influence on the quantification of pesticides in HPLC-MS/MS.²⁰ Compared with the response of the standard in a solvent, the matrix effect showed signal suppression and enhancement.²¹ The matrix effect was evaluated by calculating the slope ratio of the matrix/solvent-matched standard curve (Table 1). A minimal matrix effect was found for most samples, but milk and pork liver samples showed significant matrix enhancement. Therefore, the employment of the matrix-matched standard curve will eliminate the matrix effect and obtain a more realistic determination.

3.4. Evaluation of Accuracy and Precision. At three concentration levels, the average recoveries of fenbutatin oxide in six kinds of samples were 87.87–101.67% and RSD were 2.34–8.94% (Table 2). The method has high accuracy and precision, which can fully meet the detection requirements of fenbutatin oxide in different samples.¹⁹

3.5. Method Validation. Eighteen real samples were randomly collected from tobacco/rice rotation fields or local markets and detected by the established method in this work and the reference method (SN/T 4558-2016).²² Fenbutatin oxide residues were detected in only four samples. After spiking 0.1 and 0.5 mg/kg fenbutatin oxide to the sample, the results of our method were consistent with those of the reference method (Figure 3). The linear regression equation was $y = 1.0207x \pm 0.0051$ ($R^2 = 0.9896$, $n = 40$), which confirms the accuracy and reliability of our method.

Table 2. Recovery Rates of Fenbutatin Oxide in Different Samples

sample	concentration (mg/kg)	recovery range (%)	average recovery (%)	RSD
soil	0.02	85.80–96.10	92.20	4.40
	0.10	91.60–101.90	97.12	4.42
	0.50	83.60–105.10	91.70	9.10
tobacco	0.02	80.10–95.12	87.74	7.24
	0.10	75.60–88.50	82.84	6.72
	0.50	74.00–82.11	79.04	4.37
rice	0.02	81.10–96.30	88.42	6.83
	0.10	79.40–94.50	84.82	7.44
	0.50	82.10–95.60	90.34	5.78
milk	0.02	81.80–96.10	88.20	6.51
	0.10	88.90–104.60	95.12	7.40
	0.50	80.30–105.10	89.70	10.96
pork liver	0.02	70.60–82.70	79.64	6.48
	0.10	81.40–91.40	87.44	4.60
	0.50	72.00–84.00	78.82	5.74
pork	0.02	74.70–87.60	80.82	6.68
	0.10	77.40–94.50	86.42	8.14
	0.50	83.80–90.90	87.78	3.30

**Figure 3.** Correlation between the established method in this work and the reference method (SN/T 4558-2016) in terms of detecting fenbutatin oxide in real samples ($n = 40$).

In the SN/T 4558-2016 method, the sample needs to be digested by HCl–tetrahydrofuran and then extracted, and the derivatization using ethyl magnesium bromide is needed.²² Our method eliminates the procedure of digestion and derivatization, which greatly improves the detection efficiency without undermining the accuracy. Related studies reported that the use of QuEChERS coupled with the HPLC-MS/MS method for the determination of multiresidues including fenbutatin oxide in plant-derived foods (citrus, pepper) achieved good results.^{12,13} However, our method can be applied not only to the determination of fenbutatin oxide in plant-derived foods but also in animal-derived foods that are rich in fat, protein, and other lipophilic compounds.

4. CONCLUSIONS

In this paper, we established a method for the determination of fenbutatin oxide using a modified QuEChERS coupled with HPLC-MS/MS. Under the optimized detection conditions, the

sensitivity, precision, and accuracy of the method can meet the requirements of residue detection, and our method shows good consistency with the reference method. This method can be applied to the determination of fenbutatin oxide residues in soil and plant- and animal-derived foods.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Li, R.; He, L.; Zhou, T.; Ji, X.; Qian, M.; Zhou, Y.; Wang, Q. Simultaneous determination of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol in duck muscle by modified QuEChERS coupled to gas chromatography tandem mass spectrometry (GC-MS/MS). *Anal. Bioanal. Chem.* **2014**, *406*, 2899–2907.
- (2) Castillo, M.; Carbonell, E.; González, C.; Miralles-Marco, A. Pesticide Residue Analysis in Animal Origin Food: Procedure Proposal and Evaluation for Lipophilic Pesticides. In *Pesticides-Recent Trends in Pesticide Residue Assay*; InTech, 2012.
- (3) Zheng, W.; Park, J.-A.; Zhang, D.; Abd El-Aty, A. M.; Kim, S.-K.; Cho, S.-H.; Choi, J.-M.; Shim, J.-H.; Chang, B.-J.; Kim, J.-S.; Shin, H.-C. Determination of fenobucarb residues in animal and aquatic food products using liquid chromatography-tandem mass spectrometry coupled with a QuEChERS extraction method. *J. Chromatogr. B* **2017**, *1058*, 1–7.
- (4) Jacobson, R. J.; Croft, P.; Fenlon, J. Response to fenbutatin oxide in populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) in UK protected crops. *Crop Prot.* **1999**, *18*, 47–52.
- (5) Montes, R.; Canosa, P.; Lamas, J. P.; Piñeiro, A.; Orriols, I.; Cela, R.; Rodríguez, I. Matrix solid-phase dispersion and solid-phase microextraction applied to study the distribution of fenbutatin oxide in grapes and white wine. *Anal. Bioanal. Chem.* **2009**, *395*, 2601–2610.
- (6) Canosa, P.; Montes, R.; Lamas, J. P.; Garcia-Lopez, M.; Orriols, I.; Rodríguez, I. Pressurized liquid extraction followed by gas chromatography with atomic emission detection for the determi-

nation of fenbutatin oxide in soil samples. *Talanta* **2009**, *79*, 598–602.

(7) Morabito, R.; Massanisso, P.; Quevauviller, P. Derivatization methods for the determination of organotin compounds in environmental samples. *TrAC, Trends Anal. Chem.* **2000**, *19*, 113–119.

(8) Steinborn, A.; Alder, L.; Spitzke, M.; Dörk, D.; Anastassiades, M. Development of a QuEChERS-based method for the simultaneous determination of acidic pesticides, their esters, and conjugates following alkaline hydrolysis. *J. Agric. Food Chem.* **2017**, *65*, 1296–1305.

(9) Zhou, L.; Luo, F.; Zhang, X.; Jiang, Y.; Lou, Z.; Chen, Z. Dissipation, transfer and safety evaluation of emamectin benzoate in tea. *Food Chem.* **2016**, *202*, 199–204.

(10) Chen, H.; Marín-Sáez, J.; Romero-González, R.; Frenich, A. G. Simultaneous determination of atropine and scopolamine in buckwheat and related products using modified QuEChERS and liquid chromatography tandem mass spectrometry. *Food Chem.* **2017**, *218*, 173–180.

(11) Li, R.; Liu, T.; Cui, S.; Zhang, S.; Yu, J.; Song, G. Residue behaviors and dietary risk assessment of dinotefuran and its metabolites in *Oryza sativa* by a new HPLC–MS/MS method. *Food Chem.* **2017**, *235*, 188–193.

(12) Morales, A.; Ruiz, I.; Oliva, J.; Barba, A. Determination of sixteen pesticides in peppers using high-performance liquid chromatography/mass spectrometry. *J. Environ. Sci. Health, Part B* **2011**, *46*, 525–529.

(13) Sun, D.; Pang, J.; Jiao, B.; Zhao, Q.; Zhou, Z. Multiresidue method for the simultaneous determination of 16 acaricides by modified quick, easy, cheap, effective, rugged, and safe extraction and ultra-high performance liquid chromatography coupled with tandem mass spectrometry in citrus. *J. Sep. Sci.* **2015**, *38*, 3845–3851.

(14) Li, M.; Liu, X.; Dong, F.; Xu, J.; Kong, Z.; Li, Y.; Zheng, Y. Simultaneous determination of cyflumetofen and its main metabolite residues in samples of plant and animal origin using multi-walled carbon nanotubes in dispersive solid-phase extraction and ultrahigh performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **2013**, *1300*, 95–103.

(15) Perestrelo, R.; Silva, P.; Porto-Figueira, P.; Pereira, J. A. M.; Silva, C.; Medina, S.; Câmara, J. S. QuEChERS-Fundamentals, relevant improvements, applications and future trends. *Anal. Chim. Acta* **2019**, *1070*, 1–28.

(16) Bruzzoniti, M. C.; Checchini, L.; De Carlo, R. M.; Orlandini, S.; Rivoira, L.; Del Bubba, M. QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: a critical review. *Anal. Bioanal. Chem.* **2014**, *406*, 4089–116.

(17) Li, J.; Zhang, J.; Liu, H.; Wu, L. A comparative study of primary secondary amino (PSA) and multi-walled carbon nanotubes (MWCNTs) as QuEChERS absorbents for the rapid determination of diazepam and its major metabolites in fish samples by high-performance liquid chromatography-electrospray. *J. Sci. Food. Agric.* **2016**, *96*, 555–560.

(18) Santana-Mayor, Á.; Socas-Rodríguez, B.; Herrera-Herrera, A. V.; Rodríguez-Delgado, M. Á. Current trends in quechers method. A versatile procedure for food, environmental and biological analysis trac-special issue “green extraction techniques”. *TrAC, Trends Anal. Chem.* **2019**, *116*, 214–235.

(19) *China: National Food Safety Standard-Maximum Residue Limits for Pesticides in Food*; GB 2763-2019, China's National Health Commission, 2019.

(20) 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.

(21) Tölgyesi, A.; Sharma, V. K.; Fekete, S.; Fekete, J.; Simon, A.; Farkas, S. Development of a rapid method for the determination and confirmation of nitroimidazoles in six matrices by fast liquid chromatography-tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2012**, *64–65*, 40–48.

(22) *Determination of Cyhexatin (Azocyclotin) and Fenbutatin Oxide in Foods for Export*; SN/T 4558-2016, 2016.