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New Method for the Simultaneous Analysis of Types A and B Trichothecenes by Ultrahigh-Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry in Potato Tubers Inoculated with *Fusarium sulphureum*

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Supporting Information

ABSTRACT: A reliable and sensitive method for rapid simultaneous determination of two type A (T-2 and diacetoxyscirpenol) and two type B (3-acetyldeoxynivalenol and Fusarenon X) trichothecenes was developed and successfully applied for detecting trichothecenes in potato tubers by ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry. The established method was further evaluated by determining the linearity ($R \geq 0.9995$), recovery (113.28−77.97%), precision (relative standard deviation ≤ 5.89), and sensitivity (limit of detection, 0.002−0.005 μg/g; limit of quantitation, 0.005−0.015 μg/g). The method proved to be suitable for simultaneous determination of T-2, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X in potato tubers inoculated with *Fusarium sulphureum*. In addition, it was found that T-2, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X could be predominantly detected in the lesion, and the toxin could also be identified in tubers without any disease symptoms. The experimental results also indicated that the concentration of toxin in the susceptible cultivar (Longshu No. 3) was significantly higher than that in the resistant cultivar (Longshu No. 6).

KEYWORDS: *Mycotoxin, potato, Fusarium sulphureum, dry rot, analysis*

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced in agricultural commodities and processed food by phytopathogenic fungi under particular environmental conditions.[1] Trichothecenes form a large family of structurally related mycotoxins produced by various genera of fungi, such as *Fusarium*, *Myrothecium*, *Trichoderma*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*.[2] Among the above fungi, the species of *Fusarium* produces the most prominent trichothecenes.[3,4] There are more than 190 known trichothecenes.[5] According to their chemical structure, trichothecenes can be classified into four groups: types A, B, C, and D.[6] Their structure are shown in Figure 1. Types A and B are commonly found in cereal grains, animal feed, and human food made from contaminated grains. In addition, they were also found in potato tubers infected by *Fusarium* spp.[7−9]

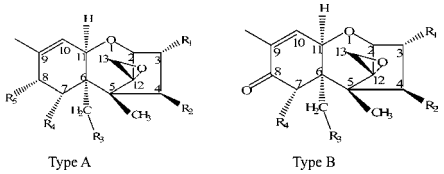

Figure 1. Chemical structure of types A and B trichothecenes. T-2 (type A), **1**: R_1, OH; R_2, OAc; R_3, OAc; R_4, H; and R_5, $OCOCH_2CH(CH_3)_2$. Diacetoxyscirpenol (type A), **2**: R_1, OH; R_2, OAc; R_3, OAc; R_4, H; and R_5, H. 3-Acetyldeoxynivalenol (type B), **3**: R_1, OAc; R_2, H; R_3, OH; and R_4, OH. Fusarenon X (type B), **4**: R_1, OH; R_2, Oac; R_3, OH; and R_4, OH.

China is the largest potato producer in the world, with an annual yield of about 80 million tons, accounting for 25% of the total production.[10] Because 90% of potato tubers need to be stored for vegetable and industrial materials, the postharvest loss accounts for nearly 25−30% of potato tuber production each year in northwest areas of China.[11] Many pathogens result in the loss of harvested potato tubers; among them, *Fusarium* spp., pathogens of tuber dry rot, play the most predominant role and cause about 20−25% of postharvest loss.[12] *Fusarium sulphureum* is a typical tuber dry rot pathogen during potato storage in China.[13−15] Besides causing the loss in production of tubers, *Fusarium* spp. also produce trichothecenes. These mycotoxins have potential health consequences to the consumers and may have immunosuppressive effects on animals and humans.[2] This is because of their multiple inhibitory effects on eukaryotic cells, including inhibition of protein, DNA, and RNA synthesis, mitochondrial function, and effects on cell division and membrane function.[16] The main symptoms are skin and gastrointestinal irritation, necrosis, hematological disorders, diarrhea, vomiting and feed refusal, decreased body weight gain, damage to the hematopoietic systems in bone marrow, spleen, thymus, and lymph nodes, and immunological alterations.[17]

Several methods have been developed for the analysis of trichothecenes.[18−20] However, most of them were based on the

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Table 1. Qualitative Ion Pair, Quantitative Ion Pair, Cone Voltage, Collision Energy, and Ionization Mode of Types A and B Trichothecenes

analyte	Q1/Q3 (m/z)	RT[a] (min)	DT[b] (s)	CV[c] U/v	CE[d] E/ev	ionization mode
Fusarenon X	399.34/353,[e] 399.34/263.39[f]	0.58	0.200	20	10.15	ESI^-
3-acetyldeoxynivalenol	337.4/173.3,[e] 337.4/307.2[f]	0.69	0.200	22	10.12	ESI^-
diacetoxyscirpenol	384.4/247.2,[e] 384.4/307.3[f]	1.03	0.200	20	13.12	ESI^+
T-2	489.3/245.2,[e] 489.3/387.1[f]	1.86	0.200	50	27.21	ESI^+

[a]RT = retention time. [b]DT = dwell time. [c]CV = cone voltage. [d]CE = collision energy. [e]Qualitative ion pair. [f]Quantitative ion pair.

extraction, cleanup, and detection of a single or group of trichothecenes (type A or B), instead of simultaneous analysis of types A and B trichothecenes. When types A and B are coexistent in the same fungal host, synergistic effects on human or animal health would occur. However, there is little information on such effects.[21] No information can be found on the accumulation of types A and B trichothecenes in rotted and asymptomatic tissue surrounding dry rot lesions caused by *F. sulphureum* in potato tubers. To simultaneously detect several different types of trichothecenes in a potato sample, it is necessary to develop a new analytical method.

The purpose of this study was to develop a rapid, reliable, and sensitive method for detecting T-2, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X trichothecenes, to find out the distribution of trichothecenes in rotted and asymptomatic tissue surrounding dry rot lesions, and further to supply occurrence data on their presence in potato tubers. The study is important in improvement of potato tuber storage and protection of the consumer from the risk of exposure to these trichothecenes but also provides scientific information in supporting the introduction of European Union (EU) limits for Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2.

MATERIALS AND METHODS

Reagents. T-2, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X (analytical standard) used as standards and high-performance liquid chromatography (HPLC)-grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). PriboFast M270 columns were supplied from Pribolab (Singapore). Other chemicals were of analytical grade and purchased from Tianjin Chemical Reagent Company (Tianjing, China). All reagents were used as obtained without further purification. Polytetrafluoroethylene filters (0.22 μm) were from Millipore (Bedford, MA).

Standard Solutions. All stock mycotoxin solutions of T-2, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X were prepared using acetonitrile. The ratio of acetonitrile/water in working solutions was 86:14 (v/v). Individual stock solutions of each mycotoxin were prepared at a concentration of 100 μg/mL and stored at −20 °C. They were brought to room temperature for 30 min before use. Individual standard working solutions with a concentration of 50, 10, 1, 0.1, and 0.01 μg/mL for Fusarenon X and T-2, 10, 3, 1, 0.1, and 0.03 μg/mL for 3-acetyldeoxynivalenol, and 10, 5, 1, 0.1, and 0.01 μg/mL for diacetoxyscirpenol were prepared by diluting each stock solution. For calibration sample preparation, blank potato sample extracts were fortified with an appropriate amount of a multi-analyte working standard solution.

Potato Samples. Potato tubers of susceptible cultivar Longshu No. 3 and resistant cultivar Longshu No. 6 were harvested from Weiyuan county in Gansu province of China on October 10, 2012. Tubers with the same size but without physical injuries or visible infection were packed in net bags, transported to the laboratory, and stored at 5−8 °C. Before treatment, the tubers were surface-sterilized in 0.5% sodium hypochlorite for 15 min, rinsed thoroughly with sterile water, and air-dried overnight.[13]

Pathogen. *F. sulphureum* isolate, the pathogen of dry rot of potato,[13] was provided by the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, China. The pathogen was grown on potato dextrose agar (PDA) for 7 days for further use.

Tuber Inoculation. Sterilized potato tubers were inoculated according to the method by Delgado et al.,[7] with some modifications. A plug of potato tuber tissue (8 mm in diameter and 1 cm in depth) was removed aseptically from the stem end of each tuber with a cork borer, and the resulting cavity was inoculated with a mycelial plug (8 mm in diameter) from a 7 day *F. sulphureum* culture grown on PDA. The inoculated tubers were incubated in sealed plastic bags with a sterile moist paper towel for 60 days in the dark at 5−8 °C. The relative humidity was between 80 and 85%.[7] Subsequently, the rotted tissue and asymptomatic tissues were separately excised from each tuber and immediately stored at −80 °C until their analysis.

Sample Extraction and Cleanup from Potato. The method used for trichothecene extraction from inoculated potato samples was based on that described previously.[22,23] A total of 100 g of lesion tissue was initially ground in a mortar, and 5 g of milled sample was then added to a 50 mL centrifuge tube and homogenized for 3 min with 20 mL of a mixture of either methanol/acetonitrile (100:0, 86:14, 50:50, or 0:100, v/v), methanol/water (100:0, 90:10, 86:14, or 80:20, v/v), or acetonitrile/water (100:0, 90:10, 86:14, or 80:20, v/v), followed by centrifugation at 11000*g* for 5 min. The supernatant was filtered and placed into a 150 mL clean and dry distillation flask. The solid was extracted twice as above. The three supernatants were mixed and subsequently evaporated to approximately 3−4 mL using a rotary evaporator at 60 °C, and then the concentrated solution was passed through a PriboFast 270 column. The eluate was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 1 mL of the mobile phase with vortex mixing for 3 min. The resultant solution was filtered through a 0.22 μm filter and stored at 4 °C before it was injected into the ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC−MS/MS) system.

UHPLC−MS/MS Analysis. UHPLC analysis was performed with a Waters Acquity Ultra Performance LC system (Waters, Milford, MA). The column used was a 50 × 2.1 mm inner diameter, 1.7 μm, ACQUITY UPLC BEH C_{18}, with a 4 × 4 mm inner diameter guard column of the same material (Waters, Milford, MA). Mobile-phase A was acetonitrile, and mobile-phase B was 0.1% formic acid and 10 mM ammonium acetate in water.[22] The linear gradient program started at 35% of A and 65% of B and increased linearly to 90% A in 4.8 min. Afterward, it was linearly decreased to 35% of A within 0.2 min and maintained for 1 min. The flow rate was 0.3 mL/min, and the injection volume was 5 μL. The column and sample temperatures were maintained at 35 and 4 °C, respectively.

MS/MS detection was performed on an Acquity Quattro Premier XE triple-quadrupole mass spectrometer equipped with ESI^+ and ESI^- sources (Waters, Milford, MA). The conditions used for the ionization source were set as follows: capillary voltage, 3.2 kV; source temperature, 110 °C; and desolvation temperature, 350 °C. The cone and desolvation gas flows were 50 and 550 L/h, respectively. The conditions were optimized for each analyte during infusion. The parameters and collision energies of precursor and product ions selected for the analysis of types A and B trichothecenes are listed in

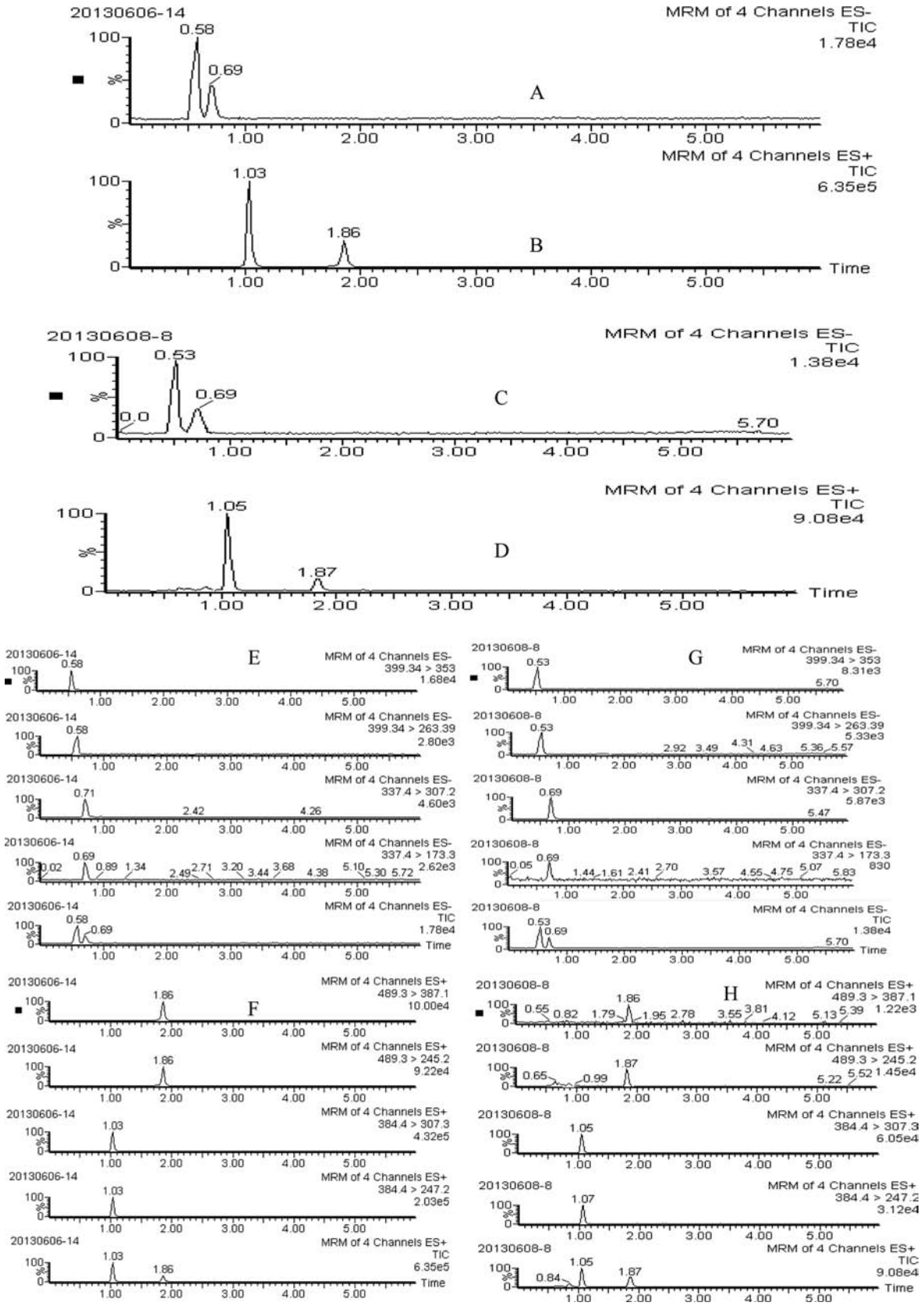

Figure 2. UHPLC−MS/MS chromatograms of (A and B) four trichothecene standards in the blank potato samples, (C and D) trichothecenes in the contaminated potato sample, (E and F) multiple-reaction monitoring (MRM) chromatograms of the blank potato samples containing fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 at 50, 50, 100, and 100 μg/kg, respectively, and (G and H) MRM chromatograms of the contaminated potato containing four trichothecenes at the respective concentrations of 174.2, 36.8, 33.8, and 56 μg/kg. The solution of the contaminated potato samples was detected after diluting 200 times.

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Table 1. Data acquisition and processing were performed using MassLynx, version 4.1, and Quanlynx (Waters, Milford, MA).

Validation of the Method. Method validation was based on the following criteria: selectivity, accuracy, precision (intra- and interday variability), linearity, and sensitivity.

Selectivity was assessed by checking the retention time of each mycotoxin in the sample to determine if it corresponded to the retention time in the matrix standards and existed for any interfering peaks.[24]

The linearity was evaluated using the matrix standards containing the four mycotoxins at the concentrations of 50, 10, 1, 0.1, and 0.01 μg/mL for Fusarenon X and T-2, 10, 3, 1, 0.1, and 0.03 μg/mL for 3-acetyldeoxynivalenol, and 10, 5, 1, 0.1, and 0.01 μg/mL for diacetoxyscirpenol. Each sample was analyzed with triplicate injections. The standard curves were calculated using linear regression of the peak area of each mycotoxin. Linearity was determined from the correlation coefficients (r) obtained using linear regression analysis of the concentration versus peak area for each mycotoxin. Standard concentrations were back-calculated using the standard regression curve, and the percent deviation was calculated.[24]

The accuracy was evaluated by the closeness of the standard samples to the actual known amount. The accuracy was expressed as a percentage of recovery, i.e., (concentration found/concentration added) × 100%.[24] The accuracy was tested on the healthy potato tubers spiked with the four analytes at high, intermediate, and low concentration levels (1, 0.1, and 0.01 μg/g for Fusarenon X, diacetoxyscirpenol, and T-2 and 3, 0.3, and 0.03 μg/g for 3-acetyldeoxynivalenol), and then the concentration of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 toxin were analyzed. Each level had three replicates, and the experiment was performed 3 times.

Precision was the degree of repeatability between samples analyzed on the same day (intraday) and 3 different days (interday) and was assessed by the coefficient of variation (CV).[22] Intraday precisions were determined on the basis of the validation samples at concentrations of 0.01, 0.1, and 1 μg/g by analyzing T-2 toxin, diacetoxyscirpenol, and Fusarenon X (0.03, 0.3, and 3 μg/g for 3-acetyldeoxynivalenol) on the same day. Each spiked level of each toxin was applied for three replicates, and the experiment was performed 3 times on the same day. Meanwhile, interday precision was checked once a day for 3 successive days.

The sensitivity of the analysis method was expressed by the limit of detection (LOD) and the limit of quantitation (LOQ) values for T-2 toxin, diacetoxyscirpenol, 3-acetyldeoxynivalenol, and Fusarenon X. The LOD was estimated from blank extract, spiked with a decreasing concentration of the analytes in the acetonitrile/water (86:14, v/v), where the response of the qualifier ion was equal to 3 times the response from the blank extract. Once evaluated, three samples were spiked at the estimated levels and extracted according to the proposed procedure. The LOQ was preliminarily estimated, in the same way as the LOD, but using signal-to-noise ratios of 10:1.[25]

Statistical Analysis. The statistical analyses were performed using SPSS, version 17.0 (SPSS, Inc., Chicago, IL). To test for the effect of the treatment, the data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered as significant. The data were expressed as the means [standard error (SE)].

RESULTS AND DISCUSSION

Extraction and Cleanup. To select for the optimal extraction, different extraction solvents or a mixture including methanol/acetonitrile (100:0, 86:14, 50:50, or 0:100, v/v), methanol/water (100:0, 90:10, 86:14, or 80:20, v/v), or acetonitrile/water (100:0, 90:10, 86:14, or 80:20, v/v) was tested. Among them, acetonitrile/water (84:16, v/v) exhibited the best extraction efficiency. These results are consistent with the previous studies, which also indicated that this extraction solvent composition resulted in a lesser co-extraction of the interfering compounds.[26,27] Centrifugation was used for removing macromolecules from the extracted solution. To improve the sensitivity of the sample, the extracted solution was subsequently evaporated to approximately 3−4 mL at 60 °C and further purified by passing through a PriboFast 270 column. This process was similar to the methods reported by Zou et al.[22]

Selectivity. The chromatograms of the blank sample spiked with Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 toxin mixed mycotoxins were shown in Figure 2. The retention times of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 toxin were 0.58, 0.69, 1.03, and 1.86 min, respectively, consistent with the individual blank sample (data not shown) and did not fluctuate significantly during the course of the testing process. The chromatogram background in the retention time domain of the analyses was used to evaluate the selectivity. No interfering peaks were found at the elution times of the analyses, and all of the peaks could be easily identified and accurately quantified. In addition, Table 1 showed the transitions monitored for Fusarenon X, 3-acetyldeoxynivalenol, T-2, and diacetoxyscirpenol as well as the ESI source conditions for each analyte. The most characteristic ion in the spectrum was selected for quantitation, and other ions were selected for the purpose of confirmation (Table 1). This result demonstrated that the method could successfully be applied for the analysis of the type A (T-2 and diacetoxyscirpenol) and type B (Fusarenon X and 3-acetyldeoxynivalenol) trichothecenes in the potato sample.

Accuracy and Precision. An accuracy test was performed with healthy tubers, employing the method of standard addition. Except for T-2 toxin (0.01 μg/g) (Table 2), the average recoveries were higher than 80%, suggesting that only a small amount of analytes was lost during the extraction process. Usually, lower than 80% average recoveries indicates that the method loss a large number of samples and is not reliable.[22]

Table 2. Recoveries for Fusarenon X, 3-Acetyldeoxynivalenol, Diacetoxyscirpenol, and T-2 in Validation Samples [Mean ± Percent Relative Standard Deviation (% RSD); n = 3]

analytes	spiking levels (μg/g)	recovery (%)
Fusarenon X	0.01	90.72 ± 2.29
	0.10	113.28 ± 2.45
	1.00	106.25 ± 2.85
3-acetyldeoxynivalenol	0.03	88.73 ± 3.05
	0.30	99.78 ± 3.76
	3.00	98.63 ± 2.81
diacetoxyscirpenol	0.01	88.49 ± 1.85
	0.10	100.16 ± 1.22
	1.00	95.12 ± 4.62
T-2	0.01	77.97 ± 4.64
	0.10	91.50 ± 2.99
	1.00	81.80 ± 8.25

For intra- and interday precision tests, healthy tubers were also selected and spiked with the standard analytes at high, intermediate, and low concentration levels (see Table 1 of the Supporting Information). The coefficients of variation were less than 6%. The method meets the requirements established by the EU [Commission Regulation (EC) No. 401/2006, 2006].[28]

Linearity and Detectability. A good linearity relationship and high coefficient ($r \geq 0.9995$) were obtained over the

Table 3. Trichothecenes Distributed in Potato Tubers Inoculated with *F. sulphureum* and Stored at 5−8 °C for 60 Days[a]

cultivar	tissue	Fusarenon X (μg/g)	3-acetyldeoxynivalenol (μg/g)	diacetoxyscirpenol (μg/g)	T-2 (μg/g)
Longshu No. 3	lesion	36.89 ± 1.96 a	6.86 ± 0.51 a	6.73 ± 0.16 a	1.01 ± 0.11 a
	asymptomatic tissue	0.51 ± 0.07 c	0.30 ± 0.10 c	0.10 ± 0.01 c	0.16 ± 0.00 c
Longshu No. 6	lesion	20.78 ± 2.26 b	1.98 ± 0.28 b	0.50 ± 0.08 b	0.91 ± 0.14 b
	asymptomatic tissue	0.16 ± 0.02 c	0.02 ± 0.01 d	0.00 ± 0.00 d	0.00 ± 0.00 d

[a]The data were based on three replicates of 10 tubers each. Values followed by the same letter are not significantly different according to the least significant difference test ($p < 0.05$).

concentration range of 0.01−50 μg/mL for Fusarenon X and T-2, 0.03−10 μg/mL for 3-acetyldeoxynivalenol, and 0.01−10 μg/mL for diacetoxyscirpenol, respectively (see Table 2 of the Supporting Information).

The sensitivity was expressed by the LOD and the LOQ for the four trichothecenes. LOD and LOQ were estimated on the basis of signal-to-noise ratios of 3:1 and 10:1, respectively.[29] The LODs and LOQs of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were 0.002, 0.005, 0.002, and 0.002 μg/g and 0.005, 0.015, 0.005, and 0.005 μg/g, respectively. Because of the high sensitivity of the UHPLC−MS/MS technique, the LOQs of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were lower than those of the common technique [for instance, HPLC−ultraviolet (UV) and thin-layer chromatography (TLC)] and also lower than the maximum residue limits established by the EU [Commission Regulation (EC) No. 1881/2006, 2006].[30] The LOQ of the four trichothecenes was slightly higher than that reported by Wang et al.,[2] which may indicate that matrix effects are apparent in potato tubers and corn-based products. The present result indicated that the proposed method was suitable for simultaneous detection of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 toxin in dry rotted potato samples.

Analysis of Inoculated Potato Tubers. The concentration of trichothecenes in the lesion of potato tubers inoculated with *F. sulphureum* was significantly higher than that in the adjacent asymptomatic tissue (Table 3). For instance, the levels of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were 68, 22, 67, and 6 times higher, respectively, in the lesion of tubers (cv. Longshu No. 3) than those in the adjacent asymptomic tissue. The concentrations of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were 122, 196, 50, and 90 times higher, respectively, in the lesion of tubers (cv. Longshu No. 6) than those in the adjacent asymptomic tissue. The results were similar to those in the report by Delgado et al.,[7] who found that the trichothecenes deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol were detected predominantly in the rotted tissue of potato tubers inoculated with *Fusarium graminearum* rather than in the adjacent asymptomatic tissue. The higher concentration of trichothecenes in the lesion of potato tubers was attributed to a large number of the pathogens in the lesion, which could produce and accumulate more mycotoxins.[7] The trichothecenes of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were also detected in the adjacent asymptomic tissue. The trichothecenes, as fat-soluble and lower molecular weight substances, could penetrate and diffuse in the intercellular space of the tuber tissue and move further. A similar result had been found by Lafont et al.,[31] who indicated lower amounts of trichothecenes (diacetoxyscirpenol, DON, and T-2 toxin) in the adjacent asymptomatic tissue than in the lesion.

The accumulation level of toxin in the susceptible cultivar (Longshu No. 3) was significantly higher than that in the resistant cultivar (Longshu No. 6). For instance, the concentrations of Fusarenon X, 3-acetyldeoxynivalenol, and diacetoxyscirpenol in the susceptible cultivar were 1.8, 3.5, and 13.5 times higher, respectively, in the lesion and 3.2, 31, and 10 times higher, respectively, in the adjacent asymptomatic tissue than those in the resistant cultivar. *In vivo* tests also showed that the lesion diameter of susceptible cultivar potato slices inoculated with *F. sulphureum* was far greater than that of the resistant cultivar. The result indicated that the resistant cultivar had a significantly stronger resistance to the strain of *F. sulphureum* than the susceptible cultivar. There were no significant differences in the concentration of T-2 toxin between the resistant cultivar and the susceptible cultivar. The result was similar to the observations by El-Hassan et al.,[9] who found that concentrations of T-2 were 0.57 and 0.36 μg/g in the lesion of potato tubers cv. Spunta infected by *F. graminearum* and *Fusarium solani*, respectively.

Except for T-2 toxin, limit standards of Fusarenon X, 3-acetyldeoxynivalenol, and diacetoxyscirpenol have not been established. The contents of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 in potato tubers inoculated with *F. sulphureum* were 0.16−36.89, 0.02−6.86, 0.10−6.73, and 0.16−1.01 μg/g, respectively. The results showed that high contents of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 were detected in inoculated potato tubers. The levels of the concentrations in the lesion were much higher than those in grain-based food and feed, and the concentrations in the asymptomatic tissue were equivalent to those of grain-based food and feed, which were much higher than the maximum residue limits established by the EU [Commission Regulation (EC) No. 1881/2006, 2006].[30] The high levels of Fusarenon X, 3-acetyldeoxynivalenol, diacetoxyscirpenol, and T-2 in the adjacent asymptomatic tissue of potato tubers inoculated with *F. sulphureum* could cause potential health consequences to the consumers.[7]

■ ASSOCIATED CONTENT

Supporting Information

Intra- and interday precision for Fus-X, DAS, 3ADON, and T-2 in validation samples (Table 1) and linearity and detectability of Fus-X, 3ADON, DAS, and T-2 toxin (Table 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

UHPLC−MS/MS, ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry

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