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Simultaneous determination of carbamate insecticides and mycotoxins in cereals by reversed phase liquid chromatography tandem mass spectrometry using a quick, easy, cheap, effective, rugged and safe extraction procedure

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ABSTRACT

A simple, sensitive and reliable analytical method was developed for the simultaneous determination of 22 carbamate insecticides and 17 mycotoxins in cereals by ultra high performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC–ESI-MS/MS). Carbamates and mycotoxins were extracted from cereal samples using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure without any further clean-up step. The extract was diluted with water containing 0.1% formic acid and 5.0 mM ammonium acetate, and analyzed by LC–MS/MS on a Waters Acquity BEH C_{18} column with water (0.1% formic acid, 0.50 mM ammonium acetate)/methanol as mobile phase with gradient elution. Matrix-matched calibration was used for quantification. Blank samples (rice, wheat and corn) were fortified at 5, 10 and 50 μg/kg except for five zearalenonic compounds at 25, 50 and 250 μg/kg, and recoveries were in the range of 70–120%. Relative standard deviations were lower than 20% in all cases. The LOQ values were in the range of 0.20–29.7 μg/kg. The method is suitable for the simultaneous determination of carbamate insecticides and mycotoxins in cereals. The total time required for the analysis of one sample, including sample preparation, was about 35 min.

1. Introduction

Cereals are the most important crops in the world for human diet. Among the cereals, corn, wheat and rice are the most important. However, the consumption of cereals is not free from the risk of exposure to harmful compounds, such as pesticides and mycotoxins [1]. In order to protect human health, the maximum residue limits (MRLs) of pesticides and mycotoxins have been established in food by the European Union, China, etc. [2–5].

Carbamate insecticides are widely used in agricultural environments to protect crops against a range of pests; whereas mycotoxins are secondary metabolites of fungal origin, and they were found in different relevant food crops, especially in cereals and cereal products. To control and monitor the occurrence of carbamate insecticides and mycotoxins in food, it is necessary to develop accurate analytical methods for their identification and quantification. Undoubtedly, multi-residue analytical methods are the best strategy for monitoring purposes. They allow for the analysis of a number of compounds in a single operation, as well as decrease the cost of analysis. Therefore, a large number of multi-residue analytical methods have been established for the determination of carbamate insecticides and mycotoxins since the 1980s [6–13]. Especially in recent years, many liquid chromatography tandem mass spectrometry (LC–MS/MS) methods have been developed for simultaneous determination of multi-class mycotoxins in food [13–23].

However, the confirmatory method has not been reported for simultaneously determine carbamate insecticides and mycotoxins in cereals. In the area of analysis of pesticides and mycotoxins, only three papers have been focused on a simultaneous determination in food. Lacina et al. developed a LC–MS/MS method with QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method to evaluate 22 mycotoxins and 222 pesticides in cereals [24]. Mol et al. developed a generic extraction method for contaminants to evaluate 36 mycotoxins and 136 pesticides in food and feed, and proposed three new extractions/"dilute-and-shoot" type methods [1]. However, the LC–MS/MS methods developed by Lacina et al. and Mol et al. often were chosen as a kind of screening method. Recently, Aguilera-Luiz et al. have successfully developed a confirmatory LC–MS/MS method to determine 42 pesticides and six mycotoxins in milk samples [25].

It is well known that LC with fluorescence detection after derivatization was the most widely used quantitative method

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for carbamate insecticides and mycotoxins in the past due to their physical chemical properties, such as polarity [26,27]. Now, LC–MS/MS is an excellent alternative technique for the analysis of polar substances like carbamate insecticides and mycotoxins, because no derivatization step is required and higher selectivity and sensitivity can be acquired. To reduce the cost of sample analysis and to increase sample throughput, it is essential to develop simultaneous determination method for carbamate insecticides and mycotoxins by LC–MS/MS. Electrospray ionization (ESI) positive ion mode had been used to analyze carbamate insecticides and mycotoxins, but factors, that can affect ESI efficiency and chromatographic behavior include mobile phase composition and type of column, have to studied again to determine simultaneously carbamate insecticides and mycotoxins with good sensitivity. QuEChERS method was originally developed for the extraction of pesticides from fruits and vegetables [29], has been employed for the extraction of mycotoxins in many matrices [19]. Nevertheless, its application in simultaneous determination of pesticides and mycotoxins is still very scarce, especially in cereals.

Here we developed a simple confirmatory LC–MS/MS method for the simultaneous determination of carbamate insecticides and multiclass mycotoxins in corn, wheat and rice with reverse phase system. To achieve the goal, a simple pretreatment procedure based on QuEChERS was established. The final optimized method was validated for selectivity, linearity, trueness, precision, limit of detection (LOD), and limit of quantification (LOQ).

2. Materials and methods

2.1. Reagents

Aflatoxin B_1 (AFB_1, 99%), aflatoxin B_2 (AFB_2, 99%), aflatoxin M_1 (AFM_1, 99%), aflatoxin M_2 (AFM_2, 99%), aflatoxin G_1 (AFG_1, 99%) and aflatoxin G_2 (AFG_2, 99%), DON (98%), T-2 (98%), OTA (99%), fumonisin B_1 (FB_1, 97%) and fumonisin B_2 (FB_2, 97%) were from Alexis Biochemicals (San Diego, USA). HT-2 (98%) was from Biopure Corporation (Tulin, Austria). α-Zearalanol (α-ZAL, 98%), β-zearalanol (β-ZAL, 98%), α-zearalenol (α-ZOL, 98%), β-zearalenol (β-ZOL, 98%), zearalenone (ZAN, 98%) were from National Measurement Institute of Australia (Sydney, Australia). Carbamate reference standards (purity higher than 98%) were from Dr. Ehrenstofer (Augsburg, Germany). Water was purified with a Milli-Q reverse osmosis system (Millipore, Milford, MA, USA). Methanol (LC grade) and acetonitrile (LC grade) were from Fisher Chemicals (Fairlawn, USA). Formic acid was from Tedia Company Inc. (Fairfield, USA). Acetic acid and ammonium acetate were analytical grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Standard solutions

Stock standard solutions of individual compounds (100 μg/mL) were prepared by exact weighing of the compound followed by dissolution in 100 mL (carbamate insecticides) or 10 mL (mycotoxins) of acetonitrile, and stored at −18 °C in the dark. Three multi-compound working solutions (1.25, 2.50, and 12.5 μg/mL for zearalenonic compounds and 0.25, 0.50 and 2.50 μg/mL for the rest of the compounds) were prepared by diluting stock standard solutions with acetonitrile.

2.3. Chromatographic conditions

A Waters Acquity UPLC instrument (Milford, MA, USA) was used in the present experiment. Separation was carried out on an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) maintained at 30 °C. The mobile phase consisted of solvent A (0.1% formic acid–0.50 mM ammonium acetate in water) and solvent B (methanol). Initial gradient conditions were set to 15% B and held for 1.5 min before incorporating a linear gradient increasing to 85% B at 7.5 min and held for 1.5 min. At 10.1 min the gradient was programmed to initial conditions to reequilibrate the column for 1.9 min (total run time 12 min). The flow rate was 0.20 mL/min. The injection volume was 10 μL in full loop injection mode.

2.4. Mass spectrometry conditions

Detection was carried out by a Waters Xevo™ TQ triple-quadrupole MS fitted with ESI probe operated in the positive ion mode except for α-ZOL and β-ZOL in the negative ion mode. The following parameters were optimal: capillary voltage, 2500 V; ion source temperature, 150 °C; desolvation gas temperature, 500 °C; desolvation gas flow rate, 1000 L/h of nitrogen. Detection was carried out in multiple reaction monitoring (MRM) mode. Argon was used as the collision gas, and the collision cell pressure was 3.2 mbar. Other parameters are shown in Table 1.

2.5. Sample preparation

A 5 g of homogenous representative sample was weighed in a 50 mL plastic centrifuge tube and 20 mL of methanol/water/acetic acid (74.25:24.75:1) were added. The samples were extracted in an ultrasonic water bath (300 mm × 150 mm × 150 mm, Kunshan Ultrasonic Instrument Co. Ltd., Jiangshu, China) for 10 min at room temperature. After addition of 1 g of NaCl and 5 g of $MgSO_4$, the mixture was shaken vigorously for 1 min. To separate aqueous and organic phase, the sample was centrifuged at 8000 rpm for 3 min. An aliquot of the upper organic phase (2.0 mL) was diluted with 2 mL of water containing 0.1% formic acid and 5 mM ammonium acetate. Prior to final instrumental analysis, sample solution was passed through the 0.20 μm filter (Jinteng, Tianjin, China).

2.6. Confirmation criteria

For confirmation of carbamate insecticides and mycotoxins in cereals, the following three criteria had to be met: (i) the retention time was within 2.5% of the external standard solution; (ii) the signal-to-noise ratio (S/N) for each diagnostic ion shall be ≥3:1; and (iii) the relative abundance of two transitions in the samples was within an acceptable range relative to the average external standards according to the European SANCO guideline 10684/2009 for LC–MS/MS methods [28].

2.7. Method validation

The validation study was performed on the basis of the European SANCO guideline 10684/2009 [28]. Analytical characteristics evaluated were linearity, selectivity, sensitivity, mean recovery (as a measure of trueness) and intra-day and inter-day precision (expressed as relative standard deviation, RSD).

Linearity was studied using matrix-matched standards, and analyzed each of them in triplicate at six concentrations (1.0, 2.0, 5.0, 10, 25 and 100 μg/L for zearalenonic compounds, 0.05, 0.25, 1.0, 2.0, 10 and 25 μg/L for carbamates except for methomyl, indoxacarb, pirimicarbdesmethyl, aldicarb sulfone and aldicarb sulfoxide, 0.20, 0.50, 1.0, 2.0, 10 and 25 μg/L for the rest of the compounds).

To verify the absence of interfering substances around the retention time of carbamate insecticides and mycotoxins, 10 blank samples for each kind of sample were analyzed.

LODs of the method were estimated with respect to signal of the chromatographic peak of analyte (signal to noise peak to peak ratio >3) in fortified samples at the lowest concentration, LOQs were

Table 1
Retention time (RT) and MS/MS parameters of the selected carbamate insecticides and mycotoxins.

Compound	RT (min)	Cone voltage (V)	Quantification transition (*m*/*z*)	Confirmation transitions (*m*/*z*)	Dwell time (s)
Methomyl	5.80	44	162.97 > 106.94 (16)	162.97 > 135.00 (12)	0.05
Metolcarb	7.02	18	166.03 > 108.96 (10)	166.03 > 90.92 (22)	0.05
Isoprocarb	8.23	22	194.03 > 94.89 (14)	194.03 > 137.01 (8)	0.025
Carbaryl	7.69	20	201.97 > 126.92 (26)	201.97 > 144.95 (8)	0.025
Fenobucarb	8.79	22	208.03 > 94.90 (14)	208.03 > 151.80 (8)	0.025
Promecarb	9.01	18	208.10 > 109.08 (16)	208.10 > 151.08 (8)	0.025
Aldicarb	6.72	8	208.20 > 88.91 (10)	208.20 > 116.04 (6)	0.025
Propoxur	7.36	18	210.03 > 92.94 (24)	210.03 > 110.97 (14)	0.025
Carbofuran	7.43	14	222.03 > 122.97 (20)	222.03 > 165.03 (12)	0.025
Bendiocarb	7.43	16	224.10 > 109.01 (16)	224.10 > 167.09 (8)	0.025
Dioxacarb	5.76	18	224.13 > 123.01 (14)	224.13 > 167.08 (8)	0.025
Ethiofencarb	7.89	14	225.96 > 106.99 (14)	225.96 > 169.03 (6)	0.025
Methiocarb	8.95	18	226.15 > 169.10 (10)	226.15 > 121.12 (18)	0.050
3-Hydroxycarbofuran	5.76	22	238.03 > 163.01 (14)	238.03 > 181.04 (10)	0.025
Thiodicarb	7.69	18	355.11 > 107.96 (14)	355.11 > 162.97 (8)	0.05
Indoxcarb	10.06	32	528.29 > 149.95 (24)	528.29 > 202.99 (40)	0.05
DON	3.96	20	297.27 > 249.07 (10)	297.27 > 231.01 (14)	0.25
Pirimicarb-desmethyl	4.76	10	225.16 > 136.95 (22)	225.16 > 168.08 (14)	0.025
Aldicarb sulfone	3.69	24	223.00 > 85.90 (12)	223.00 > 148.00 (10)	0.25
Aldicarb sulfoxide	3.18	18	207.03 > 131.93 (6)	207.03 > 88.96 (14)	0.25
AFM_2	6.07	36	331.20 > 273.08 (22)	331.20 > 259.03 (22)	0.05
AFG_2	6.37	44	331.20 > 189.01 (42)	331.20 > 245.04 (30)	0.05
AFG_1	6.67	42	329.17 > 214.44 (34)	329.17 > 243.01 (26)	0.05
AFM_1	6.41	36	329.12 > 273.08 (24)	329.12 > 229.09 (38)	0.05
AFB_2	6.89	46	315.26 > 259.08 (28)	315.26 > 287.12 (24)	0.05
AFB_1	7.11	46	313.24 > 241.09 (38)	313.24 > 213.11 (46)	0.05
Methiocarb sulfone	6.00	20	258.09 > 106.99 (38)	258.09 > 122.03 (24)	0.05
Methiocarb sulfoxide	5.55	24	242.15 > 122.03 (28)	242.15 > 185.05 (12)	0.05
Pirimicarb	6.18	8	239.10 > 71.91 (18)	239.10 > 182.06 (16)	0.025
α-ZAL	8.90	12	323.30 > 189.08 (18)	323.30 > 287.22 (12)	0.15
β-ZAL	8.26	12	323.30 > 189.08 (18)	323.30 > 287.22 (12)	0.15
ZAN	9.08	18	319.33 > 187.12 (20)	319.33 > 203.13 (24)	0.15
OTA	9.15	25	404.50 > 239.10 (25)	404.50 > 358.12 (14)	0.05
HT-2	8.20	36	447.38 > 285.07 (20)	447.38 > 345.11 (18)	0.05
T-2	8.76	42	489.41 > 327.12 (24)	489.41 > 387.15 (22)	0.05
FB_2	9.09	48	706.58 > 336.33 (38)	706.58 > 354.29 (32)	0.20
FB_1	8.31	50	722.70 > 334.28 (42)	722.70 > 352.29 (36)	0.20
α-ZOL	8.97	46	319.24 > 174.20 (28)	319.24 > 187.82 (30)	0.10
β-ZOL	8.52	46	319.24 > 174.20 (28)	319.24 > 187.82 (30)	0.10

determined as signal of the chromatographic peak of analyte to noise peak to peak ratio >10.

The recoveries and repeatabilities (intra-day and inter-day) of the method were determined with blank samples (rice, wheat, corn) fortified at three levels (25, 50 and 250 μg/kg for zearalenonic compounds, 5.0, 10 and 50 μg/kg for the rest of the compounds). Incubation period of fortified samples was 30 min. The intra-day repeatabilities were assessed by performing six repetitions of each level during a single day, and the inter-day repeatabilities were assessed by six repetitions at 25 μg/kg (zearalenonic compounds) or 5.0 μg/kg (the rest of the compounds) per day over three different days. Recoveries were calculated employing external calibration method.

3. Results and discussion

3.1. LC–MS/MS optimization

Working solutions of 2.0 μg/mL were infused to optimize the MS–MS parameters of carbamate insecticides and mycotoxins and were used to select the appropriate diagnostic ions. The ESI positive and negative modes were evaluated. The ESI positive mode was effective for all of carbamates and mycotoxins. The ESI negative mode was effective for the ionization of five zearalenonic compounds. Initially, we had used the ESI positive ion mode to analyze all of the compounds. However, it was difficult to quantify the α-ZOL and β-ZOL at 25.0 μg/kg fortification level. Finally, α-ZOL and β-ZOL were analyzed in the negative mode.

Each compound was detectable in the form of $[M+H]^+$ or $[M-H]^-$ ions, except for aldicarb, HT-2 and T-2. Aldicarb was detected as ammonium adduct ions $[M+NH_4]^+$, and HT-2 and T-2 were detected as sodium adduct ions $[M+Na]^+$. One precursor ion and two transitions were selected for the identification and quantification of all of the compounds. The optimal parameters for each compound are shown in Table 1.

The LC conditions such as the stationary phase and mobile phase composition were investigated after optimization of MS parameters. Initially, Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) and Acquity HSS T3 column (2.1 mm × 100 mm, 1.8 μm) were investigated. Under the same LC gradient program and mobile phase composition, the MS signals for FB_1 and FB_2 were decreased by the factor 8–10 using Acquity HSS T3 column when compared to Acquity BEH C_{18} due to the much shorter retention time of FB_1 and FB_2. Therefore, Acquity BEH C_{18} column was selected as the analytical column. During optimization of mobile phase composition, the sensitivities of carbamate insecticides and mycotoxins using water/acetonitrile were lower than using water/methanol except for OTA. The same results had been found by Liu et al. [6] and Tamura et al. [14]. Therefore, 5.0 mM ammonium acetate in water/methanol and 0.1% formic acid in water/methanol were evaluated as mobile phases. Good sensitivity can be obtained for carbamate insecticides, aflatoxins, zearalenonic compounds, DON, HT-2, T-2 and OTA when 5.0 mM ammonium acetate in water/methanol was used as mobile phase. However, the sensitivities of FB_1 and FB_2 were poor. The better responses had been acquired for carbamate insecticides, HT-2, T-2, FB_1 and FB_2 when 0.1% formic acid in water/methanol was used as mobile phase, but

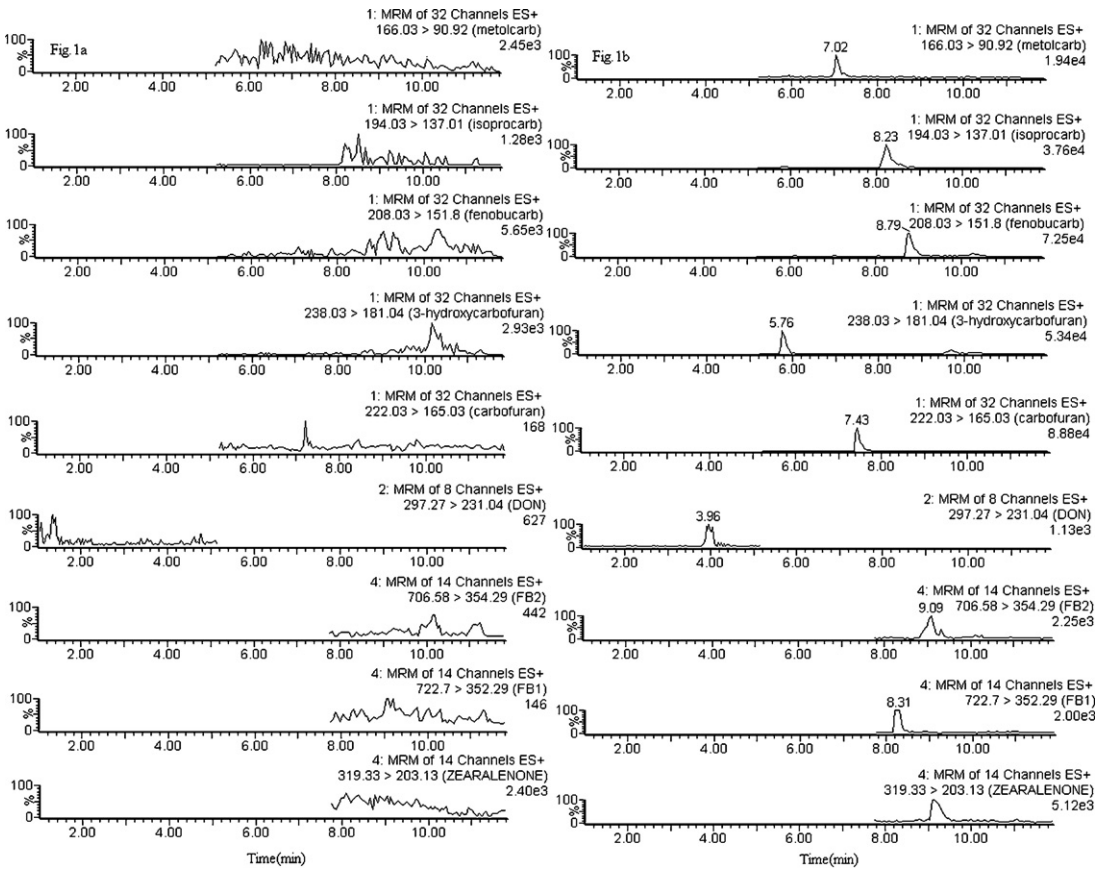

Fig. 1. The extracted qualitative transition chromatograms of rice blank sample (a) and rice fortified sample ((b) 25 μg/kg for zearalenone and 5.0 μg/kg for other compounds).

worse responses had been acquired for aflatoxins and DON. In order to enhance the sensitivity to all of the compounds, 0.1% formic acid–0.5 mM ammonium acetate–water/methanol was selected as the mobile phase. At the same time, the chromatogram was advantageously segmented in five parts to get the maximum sensitivity for all of the compounds (Fig. 1).

3.2. Sample preparation

The most important step is to establish a suitable extraction and clean-up procedure for development of multi-residue methods, especially when the different types of substances such as pesticides and mycotoxins are analyzed.

The QuEChERS method was developed for the determination of pesticides in fruit and vegetable samples with primary and secondary amine (PSA) as the base sorbent [29]. However, PSA sorbent will remove FB_1, FB_2 and OTA from samples due to ion exchange. So, PSA purification was not applicable in our procedure. In relation to the use of acidified acetonitrile and the partition step, the reported recoveries of FB_1, FB_2 and OTA were very low [1]. In order to acquire satisfied recoveries for all of the compounds, four extraction solvents (a mixture of acetonitrile/water (80:20, v/v), a mixture of methanol/water (75:25, v/v), a mixture of acetonitrile/water (80:20, v/v) with 1% acetic acid, and a mixture of methanol/water (75:25, v/v) with 1% acetic acid) were evaluated in wheat with salt addition after extraction according to Section 2.5 at fortification level of 50 μg/kg. The results are shown in Fig. 2 for all of the compounds in wheat. It can be observed that the best results were obtained for most of the compounds with the methanol/water (75:25) with 1% acetic acid. Therefore, methanol/water (75:25) with 1% acetic acid was used in this study.

Finally, Fig. 1 shows two typical chromatograms of a blank rice sample and the blank sample fortified with 25 μg/kg (zearalenonic compounds) and 5.0 μg/kg (the rest of compounds). It can be observed that the optimized extraction procedure coupled to LC–MS/MS provides a clean chromatogram without interferences for selected compounds. Furthermore, complete resolution was not obtained for some compounds but MS/MS detection allows the selective analysis of all of the compounds.

3.3. Evaluation of matrix effects

The post-extraction spiked method was utilized for the evaluation of the matrix effects in this study. To evaluate matrix effect, six concentrations (0.50, 1.0, 2.0, 10, 25 and 100 μg/L) were analyzed in solvent and matrix-matched standards in rice, wheat and corn samples. Table 2 shows slope ratios matrix/solvent for each compound. According to Frenich et al., signal suppression or enhancement effect was considered tolerable if the value was between 0.8 and 1.2 [19]. It can be observed that there was a strong matrix effect for about half of the compounds evaluated. Moreover, different slope ratio values were acquired for some of the compounds at rice, wheat and corn. In order to compensate the matrix effects, matrix-matched calibration standard curves were used for quantification.

3.4. Confirmation

At least two product ions are required for confirmation according to the requirements of European SANCO guideline 10684/2009 [28]. In our study, two different transitions were detected for each compound in the MRM mode. The retention time of each compound in fortified samples was within 2.5% of the external standards. Ion ratios of the product ions for quantification and confirmation were

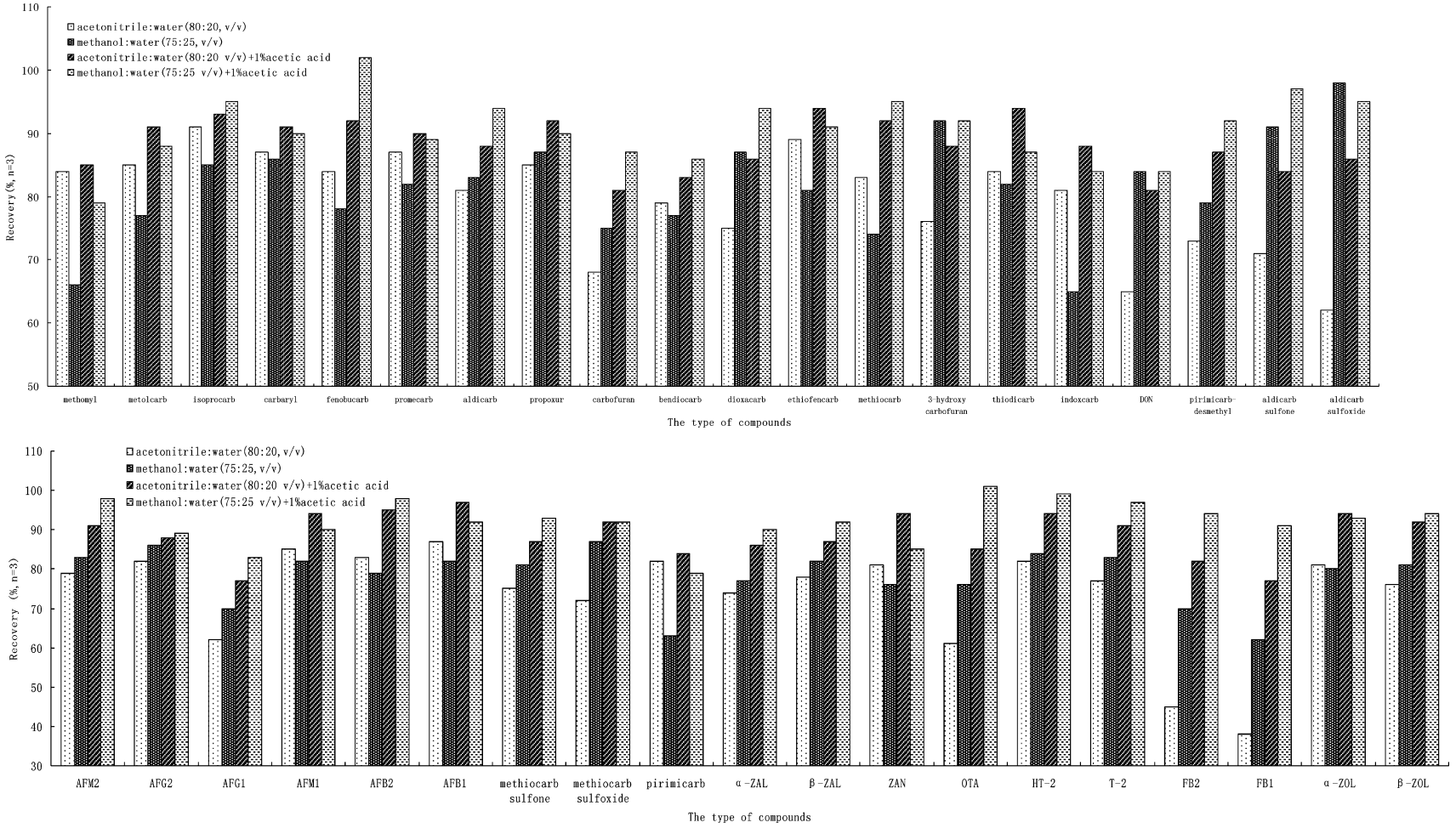

Fig. 2. Effect of type of solvent on the extraction recovery of carbamate insecticides and mycotoxins in wheat.

Table 2
Matrix effect, determination coefficients (R^2), limit of detection (LOD) and limit of quantification (LOQ) obtained for the carbamate insecticides and mycotoxins in cereal samples evaluated.

Analyte	Rice				Wheat				Corn			
	Slope ratio	R^2	LOD (μg/kg)	LOQ (μg/kg)	Slope ratio	R^2	LOD (μg/kg)	LOQ (μg/kg)	Slope ratio	R^2	LOD (μg/kg)	LOQ (μg/kg)
Methomyl	2.5	0.9916	1.0	2.7	1.9	0.9933	1.2	3.5	2.1	0.9984	1.4	3.2
Metolcarb	1.6	0.9978	0.4	1.2	1.7	0.9994	0.4	1.2	1.4	0.9985	0.5	1.4
Isoprocarb	1.2	0.9995	0.2	0.5	1.1	0.9987	0.2	0.5	1.3	0.9997	0.1	0.4
Carbaryl	1.0	0.9989	0.07	0.2	1.2	0.9991	0.08	0.2	0.9	0.9969	0.08	0.2
Fenobucarb	0.9	0.9965	0.2	0.5	1.1	0.9982	0.2	0.4	1.3	0.9991	0.1	0.4
Promecarb	0.7	0.9992	0.1	0.3	0.9	0.9997	0.1	0.3	1.2	0.9966	0.08	0.2
Aldicarb	1.8	0.9966	0.6	0.6	1.6	0.9976	0.2	0.7	1.9	0.9984	0.2	0.6
Propoxur	1.3	0.9990	0.5	1.4	0.9	0.9961	0.6	1.8	1.1	0.9979	0.3	1.5
Carbofuran	1.3	0.9996	0.2	0.6	1.6	0.9998	0.2	0.5	1.2	0.9987	0.2	0.7
Bendiocarb	1.4	0.9987	0.2	0.6	1.2	0.9935	0.3	0.8	1.6	0.9974	0.2	0.5
Dioxacarb	1.9	0.9991	0.2	0.5	1.6	0.9977	0.2	0.6	1.4	0.9920	0.2	0.7
Ethiofencarb	0.9	0.9983	0.3	0.8	1.2	0.9994	0.2	0.6	0.8	0.9965	0.3	0.9
Methiocarb	1.1	0.9995	0.1	0.4	1.3	0.9991	0.1	0.4	1.1	0.9972	0.2	0.5
3-Hydroxycarbofuran	1.3	0.9979	0.5	1.4	1.4	0.9998	0.5	1.4	1.2	0.9994	0.3	1.5
Thiodicarb	1.2	0.9992	0.1	0.4	1.1	0.9986	0.1	0.4	0.9	0.9978	0.2	0.5
Indoxcarb	0.9	0.9917	1.1	3.0	1.2	0.9967	0.9	2.5	1.0	0.9954	1.0	2.9
DON	1.6	0.9894	1.8	5.0	2.1	0.9952	1.5	4.1	2.5	0.9967	1.2	3.5
Pirimicarbdesmethyl	0.8	0.9938	1.2	3.7	1.3	0.9914	1.0	3.1	1.1	0.9987	1.1	3.3
Aldicarb sulfone	1.1	0.9996	0.5	1.6	1.0	0.9994	0.6	1.8	0.8	0.9997	0.7	2.0
Aldicarb sulfoxide	1.0	0.9912	1.0	2.7	1.2	0.9932	0.8	2.5	0.8	0.9990	1.1	3.4
AFM_2	1.8	0.9926	0.5	1.3	1.6	0.9910	0.5	1.5	2.2	0.9943	0.4	1.2
AFG_2	0.8	0.9990	0.8	2.4	1.3	0.9964	0.6	1.8	1.5	0.9936	0.5	1.4
AFG_1	1.0	0.9945	1.7	4.8	1.2	0.9957	1.4	4.1	1.3	0.9971	1.3	3.8
AFM_1	2.4	0.9992	0.3	1.0	2.0	0.9987	0.5	1.3	1.4	0.9976	0.6	1.7
AFB_2	2.4	0.9963	0.7	2.0	1.8	0.9995	0.9	2.7	1.5	0.9981	1.1	3.2
AFB_1	1.6	0.9958	0.7	2.1	2.3	0.9977	0.5	1.6	1.7	0.9964	0.7	2.0
Methiocarbsulfone	1.4	0.9993	0.3	1.0	1.2	0.9989	0.3	1.5	0.8	0.9998	0.6	1.8
Methiocarbsulfoxide	1.5	0.9988	0.2	0.5	1.1	0.9997	0.2	0.7	1.0	0.9962	0.3	0.8
Pirimicarb	2.0	0.9971	0.1	0.3	1.5	0.9998	0.1	0.4	1.3	0.9990	0.2	0.5
α-ZAL	2.2	0.9937	8.2	24.5	1.9	0.9934	8.6	28.5	2.0	0.9847	8.1	26.8
β-ZAL	1.2	0.9912	7.4	22.1	1.6	0.9920	5.6	18.5	1.3	0.9963	7.0	21.0
ZAN	2.4	0.9980	6.7	20.1	1.7	0.9968	8.9	29.7	1.8	0.9924	8.1	26.9
OTA	0.8	0.9994	0.5	1.7	0.9	0.9987	0.5	1.5	0.9	0.9982	0.5	1.5
HT-2	1.1	0.9954	1.5	4.4	1.3	0.9962	1.4	3.9	1.3	0.9948	1.3	4.0
T-2	1.4	0.9972	0.7	2.0	1.1	0.9943	0.8	2.5	1.3	0.9959	0.7	2.2
FB_2	1.2	0.9947	0.6	1.8	1.4	0.9965	0.6	1.9	1.7	0.9987	0.5	1.5
FB_1	1.3	0.9975	0.4	1.3	1.7	0.9974	0.3	1.0	1.6	0.9963	0.4	1.1
α-ZOL	0.9	0.9964	5.6	16.9	1.0	0.9989	5.3	17.4	0.9	0.9992	5.6	18.5
β-ZOL	0.9	0.9959	7.3	21.7	0.9	0.9967	6.0	19.8	0.9	0.9981	6.7	22.4

Table 3
Recoveries and relative standard deviations (RSDs) obtained for the carbamate insecticides and mycotoxins in cereals by LC–MS/MS.

Analyte	Rice[a]			Wheat			Corn			Inter-day RSDs (wheat)
	5 μg/kg[b]	10 μg/kg	50 μg/kg	5 μg/kg	10 μg/kg	50 μg/kg	5 μg/kg	10 μg/kg	50 μg/kg	
Methomyl	81 (11)	95 (8)	86 (7)	79 (8)	85 (6)	92 (6)	87 (13)	78 (8)	83 (5)	13
Metolcarb	99 (7)	89 (6)	92 (6)	84 (6)	91 (5)	91 (4)	87 (6)	85 (7)	94 (4)	11
Isoprocarb	88 (6)	82 (5)	90 (4)	84 (7)	85 (6)	84 (5)	89 (5)	91 (4)	85 (4)	8
Carbaryl	100 (4)	87 (5)	93 (5)	92 (6)	94 (6)	91 (5)	95 (6)	93 (5)	90 (3)	9
Fenobucarb	78 (6)	82 (6)	84 (7)	87 (7)	83 (4)	84 (5)	86 (7)	83 (6)	86 (2)	10
Promecarb	84 (8)	88 (7)	91 (5)	87 (7)	89 (6)	80 (5)	83 (4)	81 (3)	84 (3)	8
Aldicarb	79 (7)	77 (8)	83 (6)	83 (9)	81 (7)	86 (4)	90 (5)	86 (5)	94 (4)	11
Propoxur	89 (7)	92 (4)	87 (5)	86 (10)	94 (8)	92 (5)	86 (7)	92 (5)	82 (5)	13
Carbofuran	75 (4)	81 (6)	84 (6)	78 (8)	76 (6)	87 (4)	85 (6)	73 (3)	86 (3)	9
Bendiocarb	101 (6)	94 (4)	97 (3)	91 (7)	93 (6)	86 (4)	96 (5)	97 (4)	91 (3)	10
Dioxacarb	93 (8)	87 (9)	88 (6)	90 (9)	100 (8)	103 (5)	87 (5)	90 (6)	85 (4)	13
Ethiofencarb	86 (7)	84 (6)	95 (4)	92 (6)	88 (7)	101 (5)	88 (6)	89 (3)	104 (3)	9
Methiocarb	95 (4)	93 (5)	101 (5)	81 (8)	91 (6)	90 (3)	87 (8)	101 (5)	87 (4)	9
3-Hydroxycarbofuran	110 (9)	102 (7)	95 (5)	98 (7)	98 (7)	96 (6)	105 (9)	97 (6)	92 (5)	10
Thiodicarb	100 (4)	94 (6)	103 (4)	85 (6)	92 (5)	94 (6)	92 (8)	93 (5)	96 (4)	8
Indoxcarb	111 (7)	103 (6)	90 (5)	81 (9)	118 (5)	99 (4)	117 (10)	102 (6)	94 (4)	11
DON	81 (13)	84 (11)	88 (7)	93 (12)	84 (10)	96 (7)	89 (11)	93 (8)	84 (6)	16
Pirimicarb-desmethyl	116 (8)	101 (7)	96 (5)	97 (10)	102 (9)	93 (6)	97 (8)	92 (7)	88 (5)	14
Aldicarb sulfone	80 (6)	92 (7)	87 (4)	93 (8)	91 (7)	85 (5)	86 (9)	90 (7)	101 (4)	11
Aldicarb sulfoxide	72 (8)	81 (6)	86 (5)	88 (9)	79 (8)	83 (6)	80 (12)	86 (9)	83 (6)	10
AFM_2	84 (7)	89 (6)	85 (4)	94 (6)	81 (6)	89 (4)	83 (11)	92 (6)	85 (4)	9
AFG_2	116 (13)	91 (10)	90 (7)	86 (12)	95 (7)	89 (7)	91 (7)	103 (7)	86 (5)	16
AFG1	84 (14)	88 (10)	89 (6)	76 (10)	101 (6)	87 (5)	87 (16)	85 (11)	91 (6)	18
AFM_1	81 (9)	82 (5)	87 (5)	94 (14)	90 (6)	102 (7)	91 (6)	86 (5)	95 (5)	13
AFB_2	101 (8)	95 (7)	88 (5)	92 (11)	89 (8)	92 (6)	96 (9)	91 (8)	90 (5)	13
AFB_1	81 (10)	80 (7)	90 (5)	76 (8)	88 (5)	90 (6)	81 (8)	86 (8)	94 (6)	10
Methiocarb sulfone	99 (8)	103 (6)	93 (7)	96 (9)	102 (7)	87 (5)	93 (7)	84 (6)	91 (5)	11
Methiocarb sulfoxide	91 (6)	96 (4)	90 (5)	95 (6)	106 (5)	86 (3)	84 (6)	89 (5)	85 (4)	8
Pirimicarb	82 (8)	90 (6)	95 (3)	87 (7)	84 (5)	97 (4)	92 (6)	90 (5)	84 (4)	8
α-ZAL	102 (15)	88 (13)	86 (8)	91 (13)	99 (9)	107 (6)	97 (8)	106 (10)	97 (6)	17
β-ZAL	107 (12)	91 (10)	94 (7)	99 (14)	94 (11)	105 (7)	94 (9)	103 (8)	91 (5)	19
ZAN	115 (11)	104 (8)	89 (7)	106 (12)	106 (7)	94 (6)	101 (9)	99 (7)	83 (4)	15
OTA	89 (8)	99 (10)	103 (6)	83 (12)	101 (7)	92 (7)	95 (10)	87 (6)	85 (5)	16
HT-2	87 (6)	83 (5)	81 (4)	91 (11)	86 (8)	83 (6)	84 (7)	92 (6)	79 (4)	17
T-2	95 (7)	87 (4)	86 (4)	104 (9)	81 (6)	102 (5)	94 (6)	79 (7)	84 (6)	12
FB_2	88 (8)	86 (7)	83 (5)	101 (8)	86 (4)	94 (5)	106 (6)	89 (6)	86 (4)	13
FB_1	83 (8)	82 (5)	87 (4)	82 (11)	89 (5)	96 (4)	85 (5)	87 (7)	94 (4)	15
α-ZOL	104 (13)	91 (11)	95 (8)	92 (12)	92 (7)	88 (5)	103 (15)	96 (11)	90 (6)	17
β-ZOL	96 (14)	87 (10)	92 (7)	97 (13)	101 (9)	97 (6)	93 (11)	87 (7)	96 (5)	16

[a] Repeatability values, expressed as RSD, are given in brackets ($n = 6$).
[b] 25, 50 and 250 μg/kg for zearalenonic compounds.

calculated and they can reach the requirements of European SANCO guideline 10684/2009. So, all of the compounds can meet all three of the above confirmation requirements in trueness and precision experiment.

3.5. Method validation

3.5.1. Linearity

The calibration graph was obtained by plotting the peak area of quantification transition versus compound concentration in 1.0–100 μg/L for zearalenonic compounds, 0.15–25 μg/L for other mycotoxins, methomyl, indoxacarb, pirimicarbdesmethyl, aldicarb sulfone and aldicarb sulfoxide, and 0.05–25 μg/L for other carbamates. From Table 2, good linear relationships and coefficients of determination ($R^2 \geq 0.99$) were obtained.

3.5.2. Selectivity

The selectivity was evaluated by analyzing 10 blank samples for each kind of cereals. No interfering peaks had appeared in blank samples at the same elution time as the target compounds.

3.5.3. Recovery and precision

All of the compounds were spiked into blank samples at three different concentrations. The results are summarized in Table 3. The mean recoveries, repeatability, and within-reproducibility varied from 72 to 118%, from 2 to 15% (intra-day relative standard deviations (RSDs)), and from 8 to 19% (inter-day RSDs), respectively. Therefore, good recoveries (70–120%) from cereal samples were obtained throughout the developed method, indicating the suitability of the proposed extraction procedure for the simultaneous extraction of carbamate insecticides and mycotoxins from cereal samples.

3.5.4. LOD and LOQ

As it is shown in Table 2, the ranges of LODs and LOQs were 0.07–8.9 μg/kg and 0.2–29.7 μg/kg for all of the compounds in three kinds of cereal samples, respectively. These LOQ values for all of the compounds are lower than their MRLs in corn, rice and wheat. Moreover, it was found that the LODs and LOQs are different for some of the compounds in the three matrices. However, the largest LODs and LOQs are less than twice of the smallest LODs and LOQs for the same compound in the three matrices.

3.6. Applications of the method to real samples

The developed method was applied to cereal samples. Eighty-two cereals samples (32, 26 and 24 samples for corn, rice and wheat, respectively) from local markets were analyzed in June 2011. Carbamates and mycotoxins were not detected in 41 samples. DON was detected in all of wheat samples ranged from 54 to 1750 μg/kg. ZAN

was detected in two rice and one corn samples ranged from 25.4 to 49.7 μg/kg. FB_1 and FB_2 were detected in five rice samples and seven corn samples ranged from 1.8 to 56.2 μg/kg. No other mycotoxins were detected in these samples. Five carbamates (metolcarb, fenobucarb, isoprocarb, carbofuran and 3-hydrocarbofuran) were detected in seven samples.

4. Conclusion

In the present study, a fast and sensitive method was developed for the simultaneous determination of carbamates and mycotoxins in three kinds of cereal samples (corn, rice and wheat) by LC–MS/MS with QuEChERS. This method was validated with fortified blank samples and satisfactory recoveries were obtained. The LODs and LOQs were found to be sufficiently low to determine the residue of carbamates and mycotoxins in cereal samples.

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