Determination of Residues of Carbamates and Their Metabolites in Ginger by Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry

Application Note

Food

Authors

Huali He[1], Xiaomin Xu[2], Meiling Lu[3], Weimin Mo[1], and Yiping Ren[2]

[1]Zhejiang University of Technology

[2]Zhejiang Provincial Center for Disease Control and Prevention

[3]Agilent Technologies (China) Limited, Beijing

Abstract

This application note describes a method for the simultaneous determination of 26 carbamates and their metabolite residues in ginger by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) based on the work published by He, *et al*. [1]. The ginger samples were initially extracted using acetonitrile, followed by cleanup using NH_2 solid phase extraction (SPE) column. The samples were then enriched by nitrogen evaporation, redissolved in a mixture of acetonitrile:water (1:1, v:v), and analyzed by UHPLC-ESI-MS/MS. With matrix-matched standard calibration for quantitation, the method showed excellent linearity, with correlation coefficients of ≥0.99 for all compounds in the examined concentration range. The limits of detection and limits of quantitation were in the ranges of 0.050–2.0 µg/kg and 0.20–5.0 µg/kg, respectively. At spiking levels of 10, 30, and 100 µg/kg, the recoveries for all compounds ranged from 70.9–119% with RSD of 1.0–11%. The method is simple, rapid, and sensitive, thus it can meet the requirements for the determination of carbamates and their metabolite residues in ginger.

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Introduction

Ginger is a widely used, spicy condiment. It is often used to make ginger tea, ginger wine, and ginger bread in many regions of the world. In China, ginger is commonly used as a condiment in daily cuisine, and as an herb medicine for protection from cold and phlegm cough, and so forth. To increase product yield and reduce cost, pesticides such as carbamates have often been used in ginger cultivation. Unfortunately, some carbamates, such as aldicarb, are highly toxic, and can oxidize quickly into more toxic metabolites including aldicard-sulfoxide and aldicarb sulfone, posing a potential threat to consumers. Currently, many countries and organizations have issued very strict regulations on the maximum residue levels (MRL) of this class of pesticides. The European Union regulates the total residues of aldicarb and its metabolites with an MRL of 0.05 mg/kg in ginger [2]. The same MRL has been tentatively adopted by Japan [3]. According to Chinese regulation GB2763-2014, the total residues for aldicarb and its metabolites in ginger should not exceed 0.03 mg/kg [4].

LC-MS/MS has widely been applied in the analysis of carbamates in various food matrixes, however, few reports have focused on carbamate metabolites, or in complicated matrixes such as ginger. Ginger is rich in volatile oils and spicy components such as gingerols, zingerone, and so forth. These interference compounds are difficult to remove during extraction and, thus, often lead to severe matrix suppression effect. This application note describes a sensitive and reliable method to determine the residue levels of carbamates and their metabolites in ginger.

Instrumental

Reagents and materials

Twenty-six carbamates and their metabolites were purchased from Sigma-Aldrich, Chem Service, or Dr. Ehrenstorfer, with purity higher than 96%. HPLC grade methanol, acetonitrile, hexane, ethyl acetate, and dichloromethane were obtained from Tedia. Formic acid (HPLC grade) was obtained from ROE, and other reagents were analytical grade and obtained from local vendors.

Sample preparation

Ten grams of minced sample was accurately weighed into a 50-mL centrifuge tube. Twenty milliliters of acetonitrile was then added. The mixture was homogenized for 1 minute. Two grams of NaCl was then added. The tube was vortexed for 1 minute, then centrifuged at 10,000 rpm for 5 minutes. The resulting supernatant solution (10 mL) was thoroughly mixed with 1 g of anhydrous Na_2SO_4. After sitting still, 2 mL of the supernatant solution was evaporated to near dryness under nitrogen. The residue was redissolved in 2 mL of dichloromethane:hexane (20:80), and then centrifuged at 3,000 rpm for 1 minute. The supernatant was kept for following cleanup.

The NH_2 SPE column (Bond Elut-NH_2, p/n 12256045) was initially washed using 5 mL of dichloromethane and 5 mL of hexane sequentially to remove the impurity. The supernatant extract from above was then loaded onto the SPE column. The flowthrough was discarded. The column was then washed using 3 mL of dichloromethane:hexane (20:80) to remove interference. The retained target analytes were eluted out of the SPE column using 5 mL of dichloromethane:ethyl acetate:methanol (89:10:1). The eluate was further dried under nitrogen. The resulting residue was redissolved in 1 mL of acetonitrile:water (1:1), vortexed for 30 seconds, and filtered through 0.22 µm of membrane for LC-MS/MS analysis.

Instrumentation conditions

LC configuration and conditions

- Agilent 1290 Infinity UHPLC Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent Autosampler Thermostat (G1330B)
- Agilent Thermostatted Column Compartment SL (G1316B)

Column	Agilent ZORBAX Eclipse Plus C18 RRHT, 100 × 2.1 mm, 1.8 µm
Column temperature	30 °C
Injection volume	5 µL
Mobile phase	A) 5 mM Ammonium formate/0.02% formic acid in water B) Acetonitrile

Gradient elution profile is shown in Table 1.

Table 1. The Gradient Elution Profile

Time (min)	Sol. A (%)	Sol. B (%)	Flow rate (mL/min)
0.0	80	20	0.20
3.0	50	50	0.20
5.0	5	95	0.40
8.0	5	95	0.40
8.5	80	20	0.40
9.5	80	20	0.20

MS configuration and conditions

Agilent 6460 Triple Quadrupole Mass Spectrometer with Jet Stream ionization source

Ionization mode	Positive ionization
Scanning mode	Multiple reactions monitoring (MRM)
Capillary voltage	4,500 V
Nozzle voltage	500 V
Nebulizer pressure	25 psi
Dry gas temperature	325 °C
Dry gas flow rate	10 L/min
Sheath gas temperature	375 °C
Sheath gas flow rate	12 L/min

Results and Discussion

Optimization of LC-MS/MS conditions

Carbamates and their metabolites were initially infused into the MS spectrometer to optimize the acquisition parameters. An MS scan was first used to obtain the fragment voltage for precursor ions at which their highest intensity can be observed. Product ion scanning was then applied to optimize the collision energy for specific fragment ions at which their highest intensity can be reached. The optimized parameters for these target compounds are listed in Table 2. According to the structural properties of these compounds, the narrow bore reversed phase column, Agilent ZORBAX Ecplise Plus C18 was selected for separation. Formic acid and ammonium formate were added to mobile phase A to enhance the electrospray ionization efficiency, and to improve the peak shape, respectively. Under the optimized conditions, 26 compounds were eluted out of column within 6 minutes, and 10 minutes was sufficient to finish one cycle of analysis. Figure 1 shows the typical MRM chromatogram for each compound.

Table 2. The Retention Time and MRM Acquisition Parameters for 26 Compounds

No	Pesticide	t_R (min)	Precursor ions	Fragmenter voltage (V)*	Fragment ions	CE/V*
1	Aldicarb-sulfoxide	1.37	207	63	89,132	10, 5
2	Aldicarb-sulfone	2.25	223	90	76, 148	13, 5
3	Aminocarb	2.28	209	90	137, 152	22, 10
4	Methomyl	2.53	163	58	88,106	4, 8
5	Carbofuran-3-hydroxyl	3.16	238	80	181,163	5, 13
6	Dimetilan	3.34	241	90	72,196	15, 5
7	Methiocarb-sulfone	3.76	258	100	122,201	15, 5
8	Butocarboxim	3.86	213	75	75, 156	13, 5
9	Aldicarb	4.02	213	80	89, 116	14, 7
10	Metolcarb	4.28	166	73	109, 94	10, 35
11	Thiodicarb	4.33	355	80	88, 108	12, 12
12	Pirimicarb	4.45	239	100	72, 182	22, 12
13	Propoxur	4.46	210	65	111, 168	10, 5
14	Bendiocarb	4.48	224	70	167, 109	5, 15
15	Carbofuran	4.49	222	80	165, 123	5, 20
16	XMC	4.54	180	65	123, 95	8, 23
17	Carbaryl	4.58	202	58	145, 127	5, 30
18	Thiofanox	4.61	241	90	184, 57	5, 22
19	Ethiofencarb	4.66	226	58	107, 164	12, 5
20	3,4,5-Trimethacarb	4.76	194	73	137, 122	8, 30
21	Isoprocarb	4.76	194	78	95, 152	15, 5
22	Mercaptodimethur	4.97	226	75	169, 121	5, 15
23	Diethofencarb	5.01	268	75	226, 180	5, 15
24	Promecarb	5.03	208	65	109,151	12, 5
25	Fenoxycarb	5.07	302	80	88, 256	20, 8
26	Furathiocarb	5.79	383	100	195, 252	15, 8

* The fragment ion and CE on the left of the corresponding columns were used for quantitation.

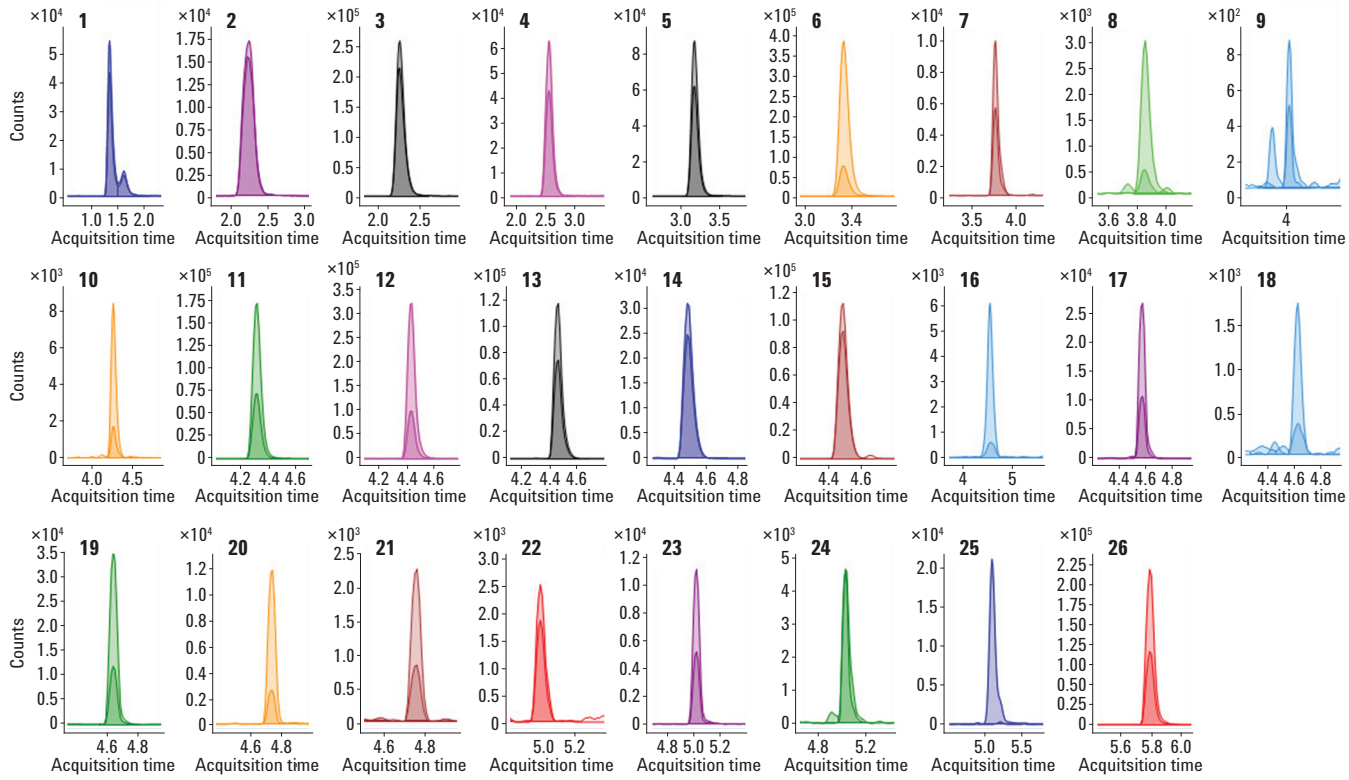

Figure 1. Typical MRM chromatograms for 26 carbamates and their metabolites. The concentration of each compound was 20 µg/L. The ordering number for each compound is listed in Table 1.

Optimization of sample extraction and cleanup procedure

Selection of the extraction solvent depends upon the properties of the target compounds and the sample matrix. Acetonitrile is commonly used for the extraction of pesticides from food matrixes due to its good solubility for pesticides. The addition of NaCl into the extraction solvent can further improve the partition of pesticides into the acetonitrile layer, enhancing the recovery.

The commonly used cleanup procedure in pesticide analysis includes both SPE and DSPE. Since ginger is a complicated matrix that contains abundant interference compounds, a NH_2 SPE column was selected for cleanup. It was found that 5 mL of dichloromethane:hexane (20:80) and 5 mL of dichloromethane:ethyl acetate:methanol as washing solvent and elution solvent, respectively, can significantly decrease the matrix interference effect.

Method performance

Linearity and limit of detection

A ginger sample with negligible carmabates and their metabolites was selected as the blank matrix, and used for preparation of matrix-matched calibration solutions. Within the examined concentration range of 0.20 to 5.0 × 10^2 µg/L, the correlation coefficients for all compounds were higher than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) were determined as the level at which the signal-to-noise ratio (S/N) for the quantifying MRM of the compounds reached 3 and 10, respectively, in the matrix. As shown in Table 3, the LOD for these compounds ranged from 0.050 to 2.0 µg/kg, while the LOQs were within 0.50–5.0 µg/kg.

Table 3. The Linearity, LOD, and LOQ of Each Compound in the Examined Concentration Range

No.	Linear range (µg/L)	Correlation coefficient (R^2)	LOD (µg/kg)	LOQ (µg/kg)
1	0.50–2.0 × 10^2	0.9997	0.20	0.50
2	0.50–2.0 × 10^2	0.9982	0.20	0.50
3	0.20–2.0 × 10^2	0.9951	0.10	0.20
4	0.20–2.0 × 10^2	0.9981	0.10	0.20
5	0.20–2.0 × 10^2	0.9940	0.10	0.20
6	0.20–2.0 × 10^2	0.9994	0.10	0.20
7	1.0–5.0 × 10^2	0.9992	0.50	1.0
8	2.0–5.0 × 10^2	0.9986	1.0	2.0
9	5.0–5.0 × 10^2	0.9985	2.0	5.0
10	1.0–5.0 × 10^2	0.9989	0.50	1.0
11	0.20–2.0 × 10^2	0.9985	0.050	0.20
12	0.20–2.0 × 10^2	0.9955	0.050	0.20
13	0.20–1.0 × 10^2	0.9962	0.10	0.20
14	0.20–1.0 × 10^2	0.9961	0.10	0.20
15	0.50–1.0 × 10^2	0.9977	0.20	0.50
16	2.0–2.0 × 10^2	0.9976	1.0	2.0
17	2.0–2.0 × 10^2	0.9984	0.50	2.0
18	5.0–2.0 × 10^2	0.9983	2.0	5.0
19	0.50–2.0 × 10^2	0.9990	0.20	0.50
20	2.0–2.0 × 10^2	0.9969	1.0	2.0
21	5.0–5.0 × 10^2	0.9968	2.0	5.0
22	5.0–5.0 × 10^2	0.9985	2.0	5.0
23	0.50–5.0 × 10^2	0.9996	0.20	0.5
24	2.0–5.0 × 10^2	0.9995	1.0	2.0
25	0.20–1.0 × 10^2	0.9958	0.050	0.20
26	0.20–1.0 × 10^2	0.9930	0.10	0.20

Recovery and precision

Three levels, 10, 30, and 100 µg/kg of the target compounds were spiked into the blank ginger matrix with six replicates at each level. The spiked samples were then thoroughly mixed, and rested for 10 minutes. Then, they were analyzed using the developed approach. Figure 2 shows that the spiking recoveries for each compound under three levels ranged from 70.9 to 119%, and the relative standard deviation ranged from 1.0–11%. This shows that the developed method is accurate and reproducible, and, thus, can meet the requirements for trace analysis of these compounds in ginger.

Real sample analysis

The developed method was applied to the analysis of 15 samples randomly obtained from local vendors. Two samples were found to be positive for carmabates. Carbofuran and its metabolite were detected in one sample with carbofuran at 1.2 µg/kg and 3-hydroxyl-carbofuran at 1.6 µg/kg; in the other sample, although no aldicarb was detected, its metabolite, aldicarb sulfone was determined at 5.3 µg/kg. During ginger production, some of the carbamate pesticides can be preburied under the soil. Under the impact of the soil environment, partial or total carbamates may be transformed into metabolites and absorbed by the plants, hence, it is essential to monitor both the prototype and metabolites of the pesticides in agricultural products.

Conclusion

In this application note, a method for simultaneous detection of 26 carmabates and their metabolites were developed by integration of NH_2-SPE cleanup with UHPLC-MS/MS analysis. The developed method allows removal of most interferences coextracted from the complicated ginger matrix, has high throughput and high sensitivity, and, thus, meets the requirement for routine screening of the common carbamates and their metabolites in ginger.

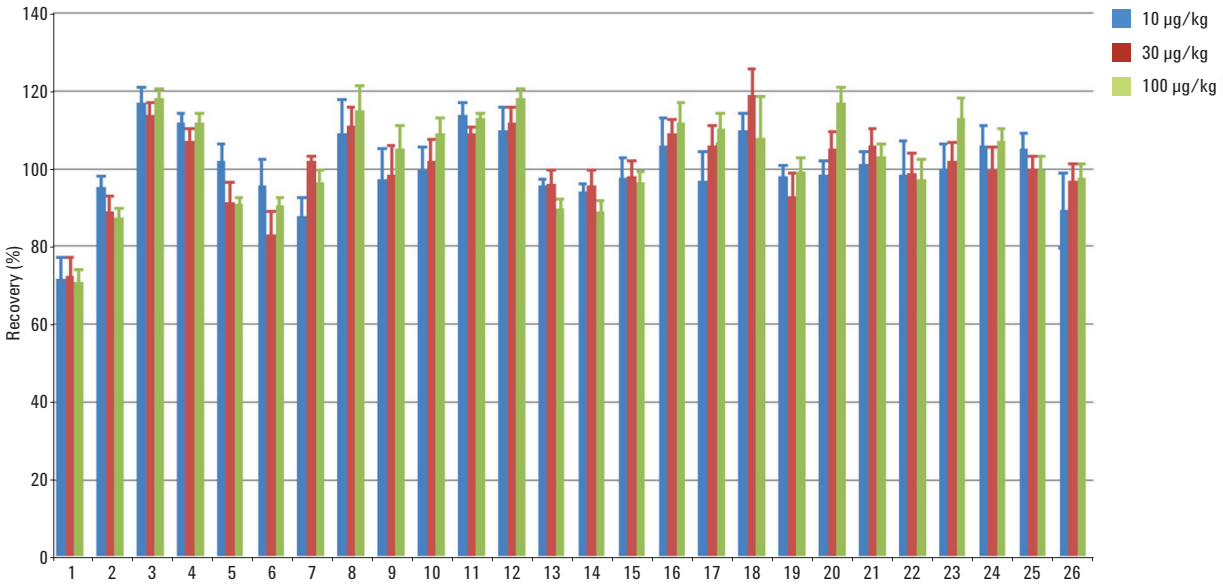

Figure 2. The recovery and standard deviation for each compound spiked at three levels in the blank ginger matrix. The upper error bar is the standard deviation of six replicates at each spiking level (n = 6). The number of compounds is listed in Table 1.

References

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