Strengthen the Food Safety Barrier! Efficient and Accurate Detection Scheme for 9 Carbamate Pesticides and Their Metabolites in Plant-Based Foods

Preface

Currently, pesticide residues in vegetables are mainly from carbamates and organophosphates. Common carbamate pesticides include aldicarb sulfoxide, aldicarb sulfone, methomyl, carbofuran, etc. This type of pesticide inhibits acetylcholinesterase in the body, preventing the breakdown of acetylcholine, leading to its accumulation in tissues and causing poisoning.

Reported methods for detecting carbamate pesticides mainly include chromatography, spectrophotometry, and mass spectrometry. Spectrophotometry uses spectral characteristics for qualitative and quantitative analysis of known substances. Although simple, it is susceptible to interference. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) can also be used, offering high sensitivity but requiring complex operations and expensive instruments. This application refers to GB23200.112-2018 National Food Safety Standard — Determination of 9 Carbamate Pesticides and Their Metabolites in Plant-Based Foods by Liquid Chromatography–Post-Column Derivatization Method. The method combines liquid chromatography with post-column derivatization and fluorescence detection, which improves sensitivity and provides strong anti-interference capability.

Experimental Section

1. Instruments and Equipment

Leaps HPLC system: dual ternary gradient pump P60, autosampler A10C, column oven C10, fluorescence detector D50, post-column derivatization device R10 (500 μL reactor).

2. Solution Preparation

Sodium hydroxide solution (0.05 mol/L): Weigh 2.0 g NaOH, dilute to 1000 mL with purified water.

Sodium tetraborate decahydrate solution (4 g/L): Weigh 4.0 g, dilute to 1000 mL with purified water.

OPA reagent: Dissolve 100.0 mg o-phthalaldehyde in 10 mL methanol. Add 3 mL 2-mercaptoethanol to 10 mL sodium tetraborate solution. Mix both solutions into 980 mL sodium tetraborate solution, sonicate to homogenize.

Standard stock solution (10 μg/mL): Take 1 mL mixed carbamate pesticide standard (GB23200.112-2018), dilute to 10 mL in a brown volumetric flask with methanol.

Calibration curve: Dilute stock solution with methanol-water (1:1) to obtain 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/mL solutions.

3. Chromatographic Conditions

Mobile phase: A: Water; B: Methanol

Column: Lotus C8, 4.6 × 250 mm, 5 μm

Injection volume: 30 μL

Column oven temperature: 42 ℃

Fluorescence detector: excitation wavelength 330 nm, emission wavelength 465 nm

Hydrolysis solution: 0.05 mol/L NaOH, flow rate 0.3 mL/min, hydrolysis temperature 100 ℃

Derivatization solution: OPA reagent, flow rate 0.3 mL/min, room temperature

Gradient program: (same as original table).

Time (min)	Flow rate (mL/min)	A% (Water)	B% (Methanol)
0.01	1.0	80	20
15	1.0	66	34
38	1.0	55	45
47	1.0	45	55
52	1.0	20	80
52.1	1.0	0	100
55	1.0	0	100
55.1	1.0	80	20

65	1.0	80	20

4. Experimental Results

1. Standard Chromatogram

The standard chromatogram shows that all 12 carbamate compounds were baseline separated.

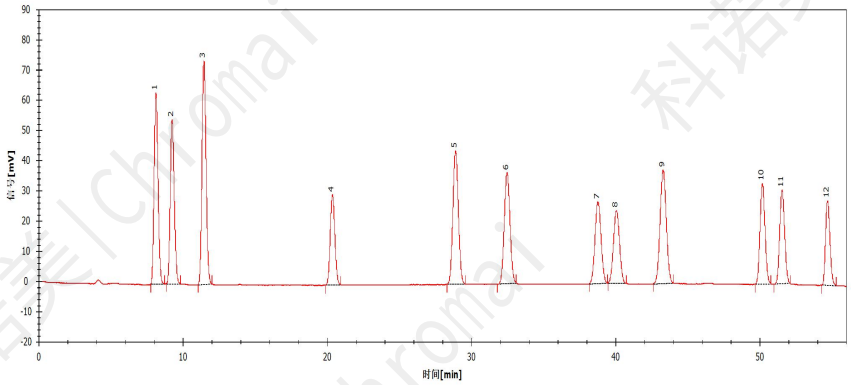

Figure 1. Typical chromatograms of 12 carbamate pesticides

（1-(1 – Thiodicarb sulfoxide; 2 – Thiodicarb sulfone; 3 – Methomyl; 4 – Carbaryl triol; 5 – Thiodicarb; 6 – Carbofuran; 7 – Fenobucarb; 8 – Carbaryl; 9 – Methomyl naphthyl; 10 – Isoprocarb; 11 – Mixture carbamates; 12 – Butocarboxim)

2. Linearity and Detection Limit

Standard solutions with concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/mL were injected sequentially from low to high concentration. Standard curves were plotted using concentration versus peak area. The correlation coefficients for all carbamates within the linear range were greater than 0.9999.

Table 1. Linearity and Detection Limits

No.	Compound	Linear Range (μg/mL)	线性方程	Correlation Coefficient (R)	LOD (μg/mL)
1	Aldicarb sulfoxide	0.02~1.0	y=1196.3x+1.6860	0.9999	0.0026

2	Aldicarb sulfone	0.02~1.0	y=1042.8x-0.6515	0.9999	0.0033
3	Methomyl	0.02~1.0	y=1463.0x-0.0941	0.9999	0.0024
4	3-Hydroxycarbofuran	0.02~1.0	y=672.7x+0.0347	0.9999	0.0053
5	Aldicarb	0.02~1.0	y=1175.0x+0.1018	0.9999	0.0042
6	Oxamyl	0.02~1.0	y=1029.4x+0.6467	0.9999	0.0045
7	Propoxur	0.02~1.0	y=789.5x+1.5616	0.9999	0.0057
8	Carbofuran	0.02~1.0	y=706.5x-0.5810	0.9999	0.0060
9	Carbaryl	0.02~1.0	y=1139.2x-2.3958	0.9999	0.0043
10	Isoprocarb	0.02~1.0	y=800.5x+1.4746	0.9999	0.0045
11	Methiocarb	0.02~1.0	y=754.6x-0.4662	0.9999	0.0046
12	Butocarboxim	0.02~1.0	y=598.16x+1.9821	0.9999	0.0057

3. Repeatability

A solution with a concentration of 0.2 μg/mL was injected six times, and the repeatability of retention time and peak area was calculated. The results are shown in Table 2. The RSD% of retention times for all 12 compounds was less than 0.15%, and the RSD% of peak areas was less than 2%.

Table 2. Repeatability Results

No.	Compound	Retention Time RSD%	Peak Area RSD%
1	Aldicarb sulfoxide	0.11%	0.84%
2	Aldicarb sulfone	0.07%	1.94%
3	Methomyl	0.11%	1.53%
4	3-Hydroxycarbofuran	0.05%	1.18%
5	Aldicarb	0.06%	1.17%

6	Oxamyl	0.07%	1.43%
7	Propoxur	0.08%	1.41%
8	Carbofuran	0.05%	1.67%
9	Carbaryl	0.06%	1.56%
10	Isoprocarb	0.04%	1.99%
11	Methiocarb	0.03%	1.38%
12	Butocarboxim	0.02%	1.71%

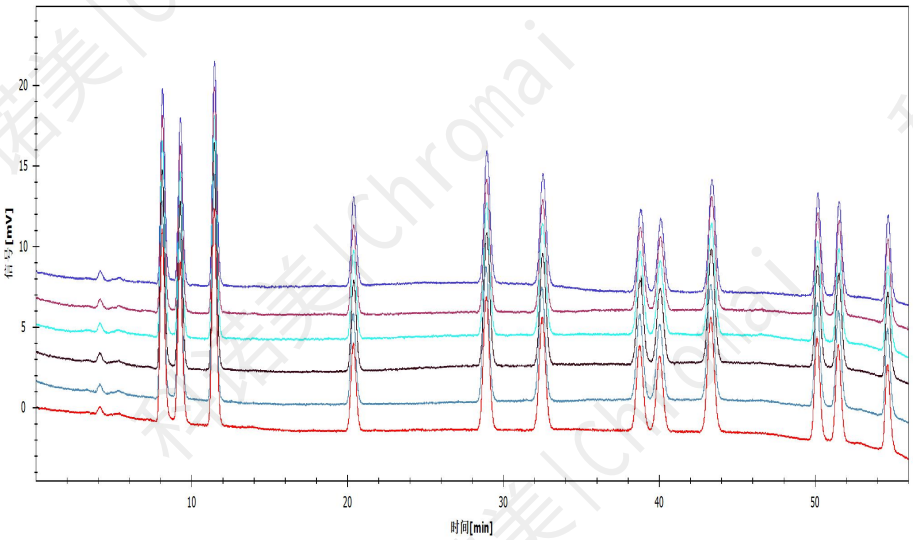

Figure 2. Repeatability chromatograms of 12 carbamate pesticides

Conclusion

In this application, the Chromai LEAPS UHPLC system, coupled with the R10 column and post-column derivatization device, was used to determine the residues of nine carbamate pesticides and their metabolites in plant-derived foods. All compounds showed correlation coefficients greater than 0.9999 within the linear range; the RSD% of retention times was less than 0.15%, and the RSD% of peak areas was less than 2.0%. The detection limits were below 0.006 μg/mL. This method is highly automated, stable, and sensitive, with simple operational procedures, making it suitable for determining carbamate pesticide residues in plant-derived foods.