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Quantitative Analysis of 15 Carbamates in Vegetables Using DisQuE Cleanup and UHPLC with Mass Detection

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APPLICATION BENEFITS

- Sensitive and quantitative UHPLC-MS analysis of 15 carbamates at regulatory limits in compliance with the Chinese GB standard (NY/T-761 2008).
- Simple sample preparation protocol with no derivatization provides a more efficient workflow with reduced interferences and matrix effects. Excellent recoveries and reproducibility in complex food matrices.

WATERS SOLUTIONS

ACQUITY™ Arc™ System

ACQUITY QDa™ Mass Detector

CORTECS™ T3 Column

DisQuE™ QuEChERS dSPE

KEYWORDS

Carbamates, insecticide, mass detection, QuEChERS dSPE

INTRODUCTION

Carbamate pesticides are derived from carbamic acid and kill insects in a similar fashion as organophosphate insecticides. They are widely used in agricultural production and are thus transferred to food which necessitates a need for rapid analytical methods to screen and quantify carbamates in raw agricultural commodities, drinking and surface water, and soil. The general formula of the carbamates is shown in Figure 1, where "R" represents the alkyl or aryl groups.

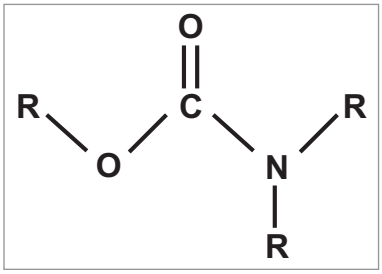

Figure 1. Structural formula of carbamates.

Currently, the official Chinese GB standard (NY/T-761 2008) used for carbamate analysis requires HPLC with post-column derivatization with fluorescence detection.[1] As routine testing requires the analysis of carbamate pesticides in a variety of complex food matrices, LC-MS/MS methodologies offer a confirmatory analysis following the AOAC standard 2007.01[2] or CEN standard method 15662:2008.[3]

Mass detection offers increased selectivity and sensitivity when compared to optical detectors such as ultraviolet (UV), photodiode array (PDA), fluorescence (FLR), refractive index (RI), or electrochemical detection for identifying target compounds in complex matrices. While mass spectrometry (MS), especially tandem quadrupole MS, has been used for trace detection of contaminants, the cost of MS/MS systems can be prohibitive for many routine analysis laboratories. Typical carbamates testing does not necessarily require the level of sensitivity offered by MS/MS systems to meet the regulatory residual limits of 10 µg/kg.

In this application note we show an efficient and cost effective UHPLC-MS method using Waters® ACQUITY Arc UHPLC System coupled with the ACQUITY QDa Mass Detector for the quantification of carbamate pesticides, as an alternative to post column derivatization used in the Chinese GB standard (NY/T-761 2008).[1] Figure 2 shows the structures of the targeted carbamates. The ACQUITY QDa Detector enables implementation of mass detection capabilities to an LC workflow that currently employs optical detectors. The selectivity of mass detection allows for low level detection of carbamates, enabling simpler sample preparation protocol for complex matrices when compared to HPLC-FLR method.

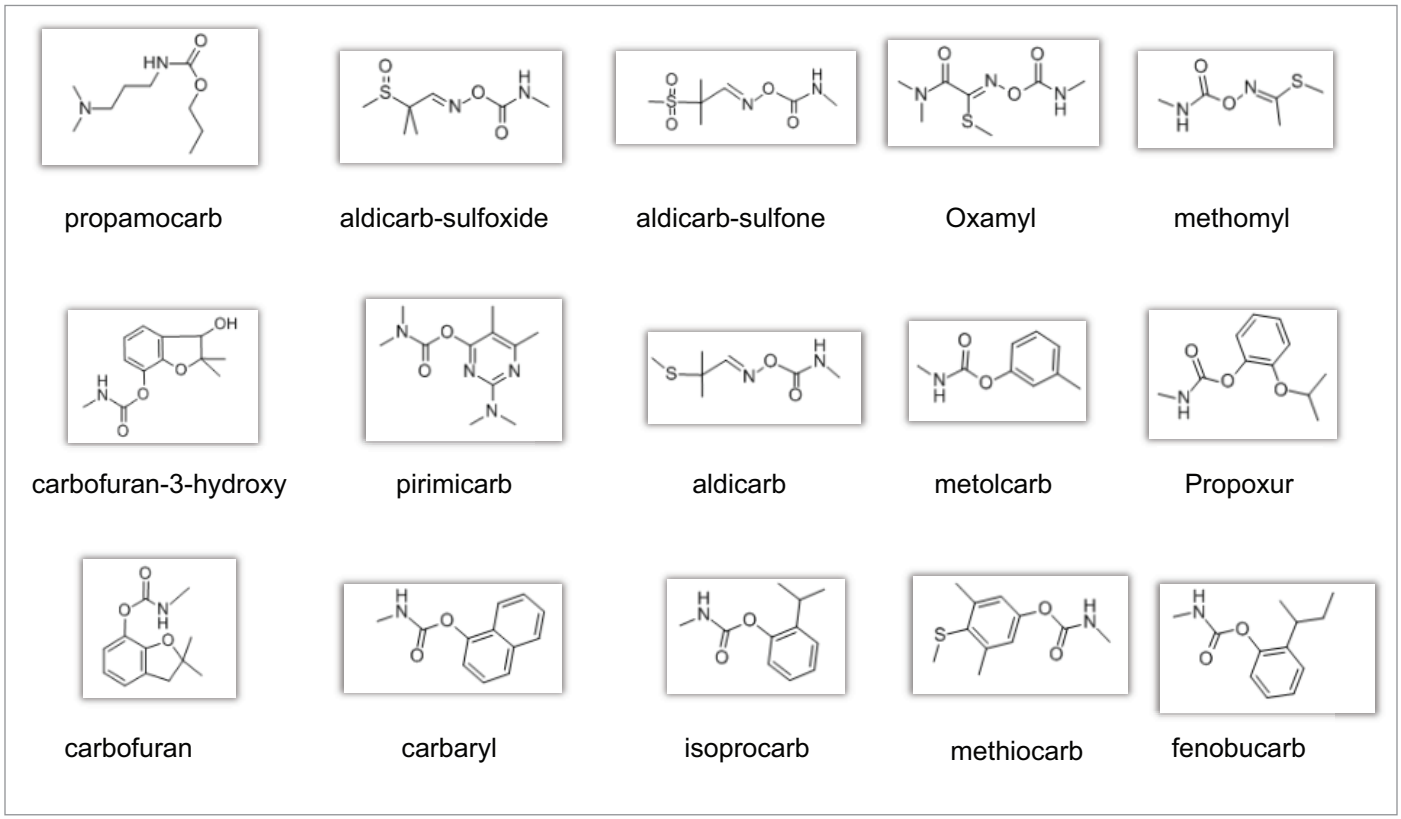

Figure 2. Structures of the carbamates analyzed in this study.

EXPERIMENTAL

UHPLC conditions

UHPLC system:	ACQUITY Arc
Column:	CORTECS T3 2.7 µm, 3 x 150 mm
Column temp.:	40 °C
Injection volume:	5 µL
Flow rate:	1.0 mL/min
Mobile phase A:	Water with 0.1% formic acid, 2 mM ammonium formate
Mobile phase B:	Methanol with 0.1% formic acid, 2 mM ammonium formate
Gradient:	5% B initial and hold to 1.0 min, linear gradient to 90% B at 5.0 min, hold to 8.0 min, back to 5% B immediately, hold and re-equilibrate until 12 min.

MS conditions

MS system:	ACQUITY QDa
Ionization mode:	ESI+
Capillary voltage:	0.5 kV
Source temp.:	120 °C
Probe temp.:	600 °C
Sampling rate:	10 Hz

MS cone voltage and ion masses monitored in this study are presented in Table 1.

Table.1 Analytes MS parameters and retention times.

Name	SIR, *m/z*	Cone voltage (V)	Ion monitored
Propamocarb	189.20	10	$[M+H^+]$
Aldicarb-sulfoxide	132.00	20	fragment
Aldicarb-sulfone	148.00	20	$[M+H^+]$
Oxamyl	237.06	5	$[M+H^+]$
Methomyl	163.10	5	$[M+H^+]$
Carbofuran-3-hydroxy	163.00	20	fragment
Pirimicarb	239.20	10	$[M+H^+]$
Aldicarb	116.00	10	fragment
Metolcarb	166.10	10	$[M+H^+]$
Propoxur	210.08	5	$[M+H^+]$
Carbofuran	222.30	10	$[M+H^+]$
Carbaryl	144.90	20	fragment
Isoprocarb	194.10	10	$[M+H^+]$
Methiocarb	169.00	20	$[M+H^+]$
Fenobucarb	208.30	10	$[M+H^+]$

Sample preparation

Sample extraction

Weigh 15 g of the sample into 50-mL tube, add 10 mL of water. Note that the amount of water added depends on the water content of the sample (e.g. it is not necessary to add any water to a sample when its water content is more than 80%). Add 15 mL of 1% acetic acid in acetonitrile (ACN), and shake for 1 min. Add contents of DisQuE Pouch (p/n 186006812) and shake vigorously for 1 min, then centrifuge at 3000 rcf for 10 min. Transfer 1 mL of the supernatant into the DisQuE QuEChERS 2-mL Tube (p/n 186008071) for cleanup. Vortex tube for 1 min and centrifuge again at 3000 rcf for 10 min. Dilute the supernatant four times with water, filter the diluents, and transfer to the vial for UHPLC-MS analysis using the ACQUITY Arc System and ACQUITY QDa Detector.

Preparation of standards

Matrix-matched calibration curves allow for accurate quantification of carbamates fortified in the matrix at regulatory limits. Calibration curves ranged from 1 to 100 µg/L for each target compound. Linearity for target analytes is shown in Table 2.

RESULTS AND DISCUSSION

OPTIMIZATION OF INSTRUMENT CONDITIONS

Optimal source conditions were investigated for carbamates before sample analysis. Ion masses monitored for each carbamate during the analysis were optimized and are listed in Table 1. Carbamate pesticides are generally labile and are prone to in-source fragmentation. For example, aldicarb sulfone forms three major ions (*m/z* 148, 223, and 240) during SIR (Selected Ion Recording) optimization. Ion mass *m/z* 148 was chosen for analysis because it is less affected by matrix interferences and provides good sensitivity to meet the performance requirements of the method, as shown in Figure 3.

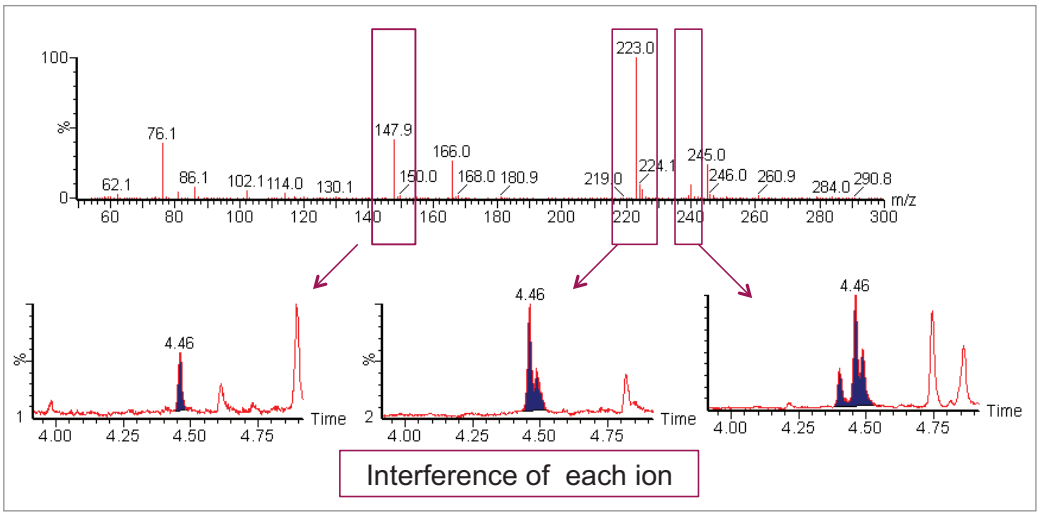

Figure 3. Selection of ion masses with minimal matrix interference.

MATRIX EFFECTS AND LINEARITY

Matrix effects were measured by comparing areas of post-spiked sample and solvent standard, where the spiked level was equal to 10 µg/kg, as shown in Table 2.

Table 2. Matrix effect on target analytes in corn powder, cabbage and tomato matrices. Negative results displayed in parentheses represent matrix suppression and positive values represent matrix enhancement.

	Matrix effect corn powder (%)	Matrix effect Chinese cabbage (%)	Matrix effect tomato (%)	Standard curve R^2
Aldicarb	(0.0)	3.3	(2.7)	0.9995
Aldicarb sulfoxide	6.3	(2.3)	(0.4)	0.9996
Carbaryl	9.0	2.2	(3.8)	0.9992
Aldicarb sulfone	3.5	12.7	1.0	0.9994
Carbofuran-3-hydroxy	6.1	5.1	(26.2)	0.9993
Methomyl	3.5	7.6	0.3	0.9995
Metolcarb	1.5	5.9	(0.1)	0.9994
Methiocarb	3.6	4.5	(2.4)	0.9995
Propamocarb	9.2	5.2	(31.7)	0.9996
Isoprocarb	6.4	(0.5)	(73.9)	0.9995
Fenobucarb	(0.9)	4.1	(5.9)	0.9996
Propoxur	18.5	6.6	5.9	0.9982
Carbofuran	1.2	7.4	(22.8)	0.9990
Oxamyl	5.9	11.0	(2.3)	0.9994
Pirimicarb	(1.3)	13.5	1.9	0.9995

ANALYTE RECOVERY AND PRECISION

Analyte recovery was determined by spiking carbamate standards into the blank matrices (corn, cabbage, and tomato). The analytes were spiked at concentrations of 5.0 µg/kg, 10.0 µg/kg, and 20.0 µg/kg. Each level of spiking was repeated in three replicates. All of the samples were processed according to the method described previously. The concentrations were calculated using a matrix-matched calibration curve. The average recoveries and precision for each spiking level are listed in Table 3.

Table. 3 Recoveries and reproducibility for carbamates from corn, Chinese cabbage, and tomato. ND represents recoveries that were not determined due to high incurred residues.

	Corn				Chinese cabbage				Tomato			
	Recovery (%)			RSD (%)	Recovery (%)			RSD (%)	Recovery (%)			RSD (%)
	5 µg/kg	10 µg/kg	20 µg/kg		5 µg/kg	10 µg/kg	20 µg/kg		5 µg/kg	10 µg/kg	20 µg/kg	
Aldicarb	92.1	92.8	94.5	1.4	107.9	105.3	102.6	2.5	90.9	93.7	95.3	2.4
Aldicarb sulfoxide	82.6	84.7	91.1	5.2	86.9	88.1	92.8	3.4	88.1	90.5	91.5	1.9
Carbaryl	90.8	93.3	96.6	3.1	108.5	103.0	104.3	2.8	88.9	95.3	97.9	4.9
Aldicarb sulfone	72.5	81.6	91.6	11.7	99.1	94.4	89.8	5.0	83.3	91.3	91.5	5.3
Carbofuran-3-hydroxy	87.0	93.3	95.3	4.7	83.3	96.2	102.1	10.2	ND	80.3	91.5	9.2
Methomyl	77.4	88.7	93.1	9.3	100.0	97.1	100.1	1.7	83.5	89.7	94.0	5.9
Metolcarb	73.2	88.8	93.8	12.6	101.1	103.2	103.0	1.1	85.6	93.9	96.2	6.1
Methiocarb	88.7	95.6	96.4	4.5	100.7	103.3	106.2	2.6	89.5	92.2	97.5	4.4
Propamocarb	73.1	73.8	76.4	2.4	92.7	81.6	79.8	8.3	85.2	75.6	78.7	6.1
Isoprocarb	91.4	93.7	98.1	3.6	112.2	109.7	106.8	2.5	ND	ND	ND	ND
Fenobucarb	88.5	92.6	96.6	4.4	107.5	108.6	108.1	0.5	98.0	99.4	100.9	1.4
Propoxur	94.7	95.7	96.2	0.8	104.5	103.1	107.1	1.9	88.5	90.5	94.1	3.1
Carbofuran	83.3	92.4	98.8	8.5	95.4	99.0	106.2	5.5	71.3	84.7	94.8	14.1
Oxamyl	86.5	90.9	94.3	4.3	ND	93.0	93.3	0.2	88.2	95.6	96.8	0.9
Pirimicarb	88.6	95.4	95.7	4.3	124.5	112.5	110.3	6.6	100.2	97.2	97.4	1.7

TYPICAL CHROMATOGRAM

An example chromatogram was acquired and is shown in Figure 4, where the corn powder was fortified with carbamate standards at 5.0 µg/kg following the regulatory limits. Satisfactory sensitivity was observed for detection of each carbamate by UHPLC-MS.

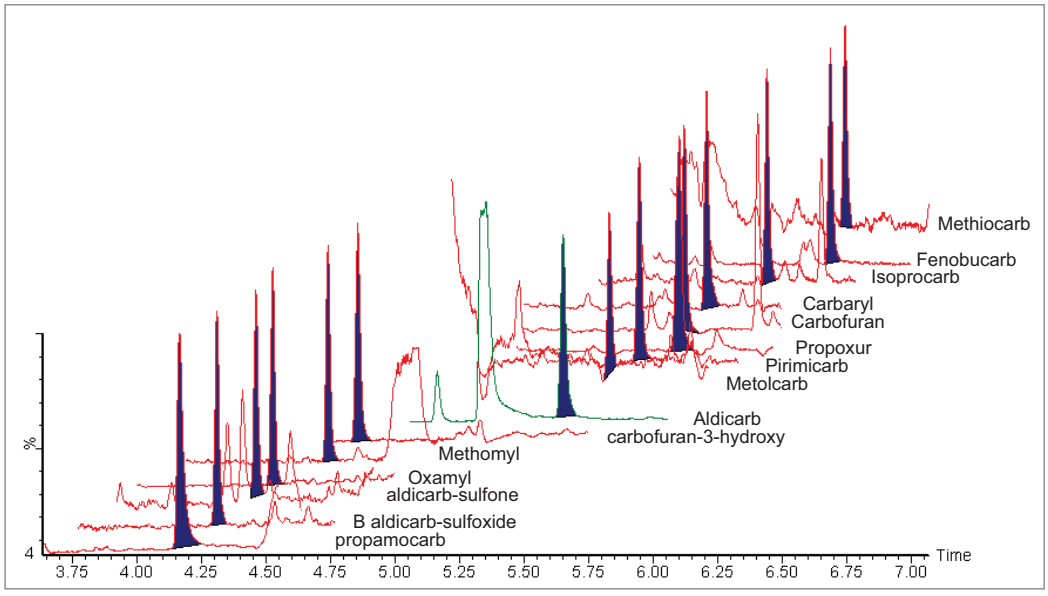

Figure 4. Typical chromatogram of carbamates at 5 µg/kg spiked in corn powder.

CONCLUSIONS

- An accurate and robust method has been developed for the reliable quantitative analysis of 15 carbamate pesticides in vegetables. This method has been proven to achieve levels of detection that meets the regulatory requirements of Chinese GB standard (NY/T-761 2008).
- Using DisQuE dSPE cleanup with a "dilute and shoot" sample preparation procedure, all analytes were detected using the ACQUITY QDa Mass Detector.
- No derivatization or time-consuming sample preparation was required, thus allowing for the rapid quantitation of carbamate pesticides on a single detector.

References

1. NY/T-761 2008: Pesticide multiresidue screening methods for the determination of organophosphorus pesticides, organochlorine pesticides, pyrethroid pesticides and carbamate pesticides in vegetables and fruits.
2. AOAC Official Method 2007.01: Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate using gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry.
3. CEN Standard Method 15662:2008: Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS-method.

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