



# Fast Determination of N-Methyl Carbamate Pesticides in Fruit and Vegetable Juice

## Application Note

Food Testing & Agriculture

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### Abstract

A method for the determination of N-methyl carbamate pesticides in fruit and vegetable juice uses an Agilent 6460 Triple Quadrupole LC/MS with an Agilent Poroshell 120 Bonus-RP LC column. At the same time, a sample preparation method using Agilent Bond Elut QuEChERS is optimized to extract 10 pesticides in juice samples. Results indicate that sample preparations using QuEChERS, and LC/MS/MS analysis with the Poroshell column, are suitable for determination of N-methyl carbamate compounds in juices. The 10 target compounds are well separated in multiple-reaction monitoring (MRM) mode and showed no interferences in the chromatogram. Method recoveries ranged from 74.4 to 111.0% with relative standard deviations between 1.4 and 4.1%.

### Introduction

N-methyl carbamate pesticides are widely used in agriculture, due to their broad range of activity to protect crops, fruits, and vegetables from a variety of pests. Because of their wide use, water sources can become polluted by carbamates through surface water runoff. Human and animal studies have suggested that long-term, low-level exposure to carbamates can cause adverse health effects, including nervous system and neuromuscular effects, and persistent neurobehavioral changes. Furthermore, human health effects from carbamates are considered to be additive, that is, the toxicity of a combination of two or more pesticides is equal to the sum of the toxicity of each individual of pesticide [1].



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To determine N-methyl carbamates in a sample, most of the current methods use HPLC, postcolumn derivatization and fluorescence detection because of the compounds low UV absorption. The procedure in US EPA Method 531.2 includes an HPLC method with postcolumn derivatization and fluorescence for carbamate analysis in water [2].

Advancements in LC/MS/MS have helped this technology gain wide acceptance for food analysis. Most food samples, including fruit juices and vegetable juices, are very complex. LC/MS/MS is particularly useful for analyzing sets of known target pesticides, due to its sensitivity and specificity in complex food matrixes.

This application note describes a simple, fast, and sensitive LC/MS/MS method for quick identification and quantitation of N-methyl carbamates in juice. Table 1 shows the details of the compounds. This method, using an Agilent Poroshell 120 Bonus-RP column and QuEChERS for sample preparation, can quantitatively monitor carbamate pesticides at a low level of less than 1 parts per billion (ppb).

## Materials and Methods

All reagents and solvents were HPLC or analytical grade. The N-methyl carbamate standard mixture (M-531M) was purchased from AccuStandard, Inc., New Haven, CT, USA. Juice samples were purchased from a local market. The mixture solution was prepared in acetonitrile at a concentration of 100 µg/mL and kept in the refrigerator (0 to 5 °C). Working solutions were prepared using the stock solution diluted with water:acetonitrile (10:90). The Flying Pigeon centrifuge was from Anting Scientific Instrument Co. Ltd, Shanghai, P. R. China.

Table 1. N-methyl carbamates used in this study.

No.	Compound	CAS no.	Structure
1	Aldicarb sulfoxide	1646-87-3	
2	Aldicarb sulfone	1646-88-4	
3	Oxamyl	23135-22-0	
4	Methomyl	16752-77-5	
5	Carbofuran-3-hydroxy	16655-82-6	
6	Aldicarb	116-06-3	
7	Propoxur	114-26-1	
8	Carbofuran	1563-66-2	
9	Carbaryl	63-25-2	
10	Methiocarb	2032-65-7	

## Conditions

Column: Agilent Poroshell 120 Bonus-RP, 2.1 × 100 mm, 2.7 μm (p/n 695768-901)

Sample prep: Agilent QuEChERS AOAC Extraction Kit (p/n 5982-5755CH), Agilent QuEChERS Dispersive Kit, Fruits and Vegetables (p/n 5982-5058CH)

Eluent: A: water + 0.05% formic acid + 5 mM ammonium acetate  
B: acetonitrile + 0.05% formic acid

Injection volume: 5 μL

Flow rate: 0.5 mL/min

Gradient: Time (min) % B  
0 5  
10 90  
12 90

Temperature: 40 °C

Stop time: 12 minutes

Postrun: 2 minutes

Instrument: Agilent 1200 Infinity Series, G1379B degasser, G1312B binary pump SL, G1367C autosampler SL, G1316B thermostatted column compartment SL

## MS conditions

Instrument: Agilent 6460 Triple Quadrupole LC/MS

Mode: ESI, positive, MRM

Gas temperature: 350 °C

Gas flow: 7 L/min

Nebulizer: 45 psi

Sheath gas temperature: 350 °C

Sheath gas flow: 11 L/min

Capillary: positive 3,500 V

Nozzle voltage: 0 V

## MRM conditions

Compound	Precursor ion	Product ion	Dwell	Fragmentor	Collision energy	Polarity
3-Hydroxy carbofuran	238.1	220	20	80	5	Positive
3-Hydroxy carbofuran	238.1	163.1	20	80	10	Positive
Oxamyl	237.1	90.1	20	80	10	Positive
Oxamyl	237.1	72	20	80	10	Positive
Methiocarb	226.1	169.1	20	80	5	Positive
Methiocarb	226.1	121.1	20	80	10	Positive
Aldicarb sulfone	223.1	148	20	80	5	Positive
Aldicarb sulfone	223.1	76	20	80	5	Positive
Carbofuran	222.1	165.1	20	120	10	Positive
Carbofuran	222.1	123	20	120	15	Positive
Propoxur	210.1	168.1	20	80	5	Positive
Propoxur	210.1	111	20	80	10	Positive
Aldicarb sulfoxide	207.1	132.1	20	80	5	Positive
Aldicarb sulfoxide	207.1	89	20	80	5	Positive
Carbaryl	202.1	145.1	20	80	5	Positive
Carbaryl	202.1	117.1	20	80	10	Positive
Methomyl	163.1	106	20	80	5	Positive
Methomyl	163.1	88	20	80	5	Positive
Aldicarb	116.1	89	20	80	5	Positive
Aldicarb	116.1	70	20	80	5	Positive

## Sample preparation

The sample preparation procedure included sample extraction/partitioning and dispersive-SPE cleanup. The juices were purchased from a local supermarket and stored at 4 °C. A 15 g ( $\pm$  0.05 g) sample of juice was weighed into a 50 mL centrifuge tube. Samples were then fortified with appropriate QC spiking solutions. Tubes were vortexed for 10 seconds to mix, followed by adding 15 mL ACN + 1% acetic acid to each tube. The tubes were then capped and shaken by hand for 20 seconds. An Agilent QuEChERS AOAC extraction salt packet was added to each tube. Sample tubes were capped tightly and shaken vigorously for 1 minute by hand. Tubes were then centrifuged at 4,000 rpm for 5 minutes at 4 °C.

An 8 mL aliquot of the upper ACN layer was transferred to an Agilent QuEChERS dispersive-SPE 15 mL tube for general fruits and vegetables. This tube contained 400 mg PSA and 1,200 mg anhydrous  $MgSO_4$ . The tubes were tightly capped and vortexed for 1 minute. The tubes were then centrifuged at 4,000 rpm for 5 minutes at 4 °C. A 4 mL volume of extract was transferred into another tube and dried by  $N_2$  at 35 °C. Samples were reconstituted into 2 mL 10% ACN in water. After vortexing and sonicating for 10 minutes, the sample was filtered through an Agilent 0.22  $\mu m$  cellulose acetate spin filter (p/n 5185-5990). The clear filtered sample was transferred to an autosampler vial. The samples were then capped and vortexed thoroughly for HPLC analysis.

## Results and Discussion

### Separation

Poroshell analytical columns made with superficially porous particles have nearly identical efficiency to sub-2  $\mu m$  totally porous columns and can be used to provide similar fast and high resolution analyses at much lower system backpressure. Agilent Bonus-RP phases have a polar alkyl-amide embedded group inserted into the hydrophobic C14 alkyl chain, for minimum interaction of polar samples with silanols, providing symmetrical peak shape. It also provides unique selectivity compared to alkyl-only phases.

Figure 1 shows the 50 ppb standard carbamate mixture separated on a Poroshell 120 Bonus-RP column. All 10 compounds were well separated in 7 minutes with symmetrical peak shapes using a simple gradient. Many published methods developed on traditional 5  $\mu m$  columns need more than 30 minutes for this separation [3].

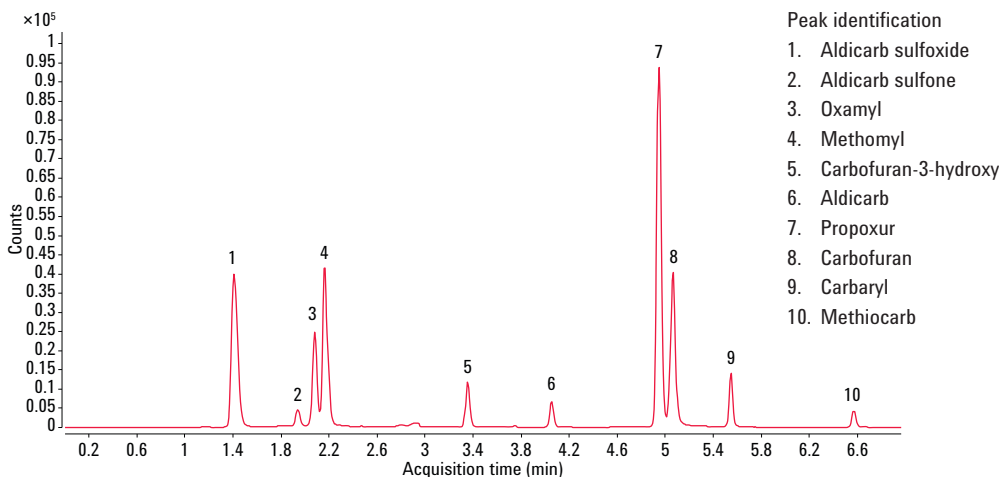


Figure 1. TIC MRM chromatogram of 50 ppb standard carbamates in 10% acetonitrile solution on an Agilent Poroshell 120 Bonus-RP column.

To study the system's suitability for the juice samples, the standard mixture was spiked into the blank sample after the QuEChERS extraction method. Figure 2 and Figure 3 show the dMRM chromatograms of blank sample with 10 ppb spiked into the blank sample. All compounds could be identified and quantified in dMRM mode. The estimated limit of detection using dMRM for the 10 compounds was below 0.1 ppb.

### Recovery and repeatability

The precision of the method was determined in terms of the recovery of spiked standards in juice samples at 0.01 mg/kg. Each analysis was repeated six times. The data in Table 2 demonstrate excellent recovery and reproducibility for the QuEChERS method for N-methyl carbamate pesticide determination in juices.

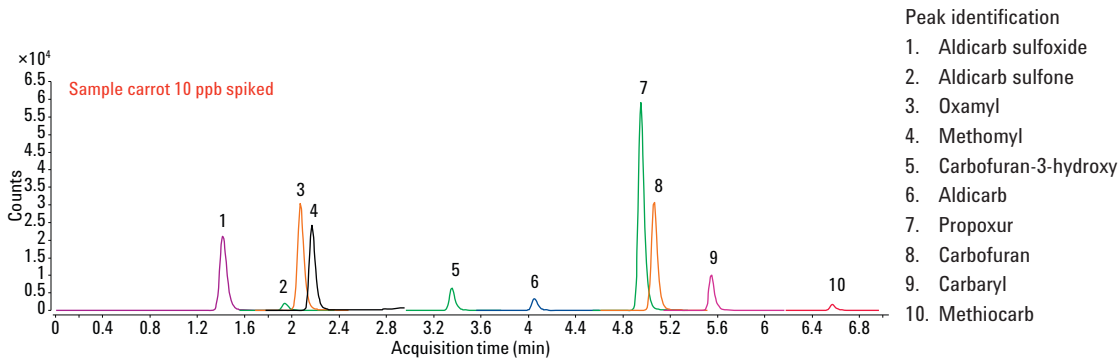


Figure 2. dMRM chromatogram of 10 ppb carbamates spiked in blank carrot juice.

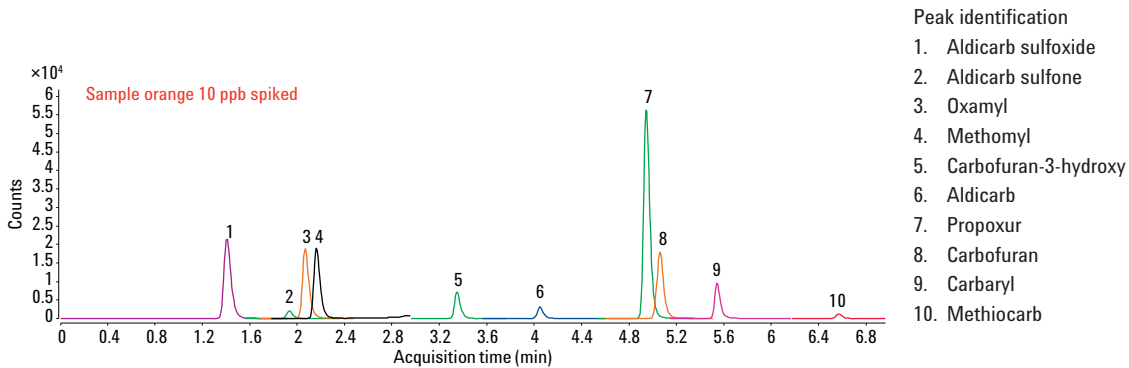


Figure 3. dMRM chromatogram of 10 ppb carbamates spiked in blank orange juice.

Table 2. Recoveries and RSDs of carbamates in juices.

Compound	Spiked level ( $\mu\text{g}/\text{kg}$ )	Recovery (%)		RSD (%) (n = 6)	
		Orange	Carrot	Orange	Carrot
Aldicarb sulfoxide	10	98.8	101.7	3.1	2.9
Aldicarb sulfone	10	99.0	104.0	2.5	2.3
Oxamyl	10	108.4	111.0	3.6	3.2
Methomyl	10	97.4	103.8	3.7	3.6
Carbofuran-3-hydroxy	10	99.6	97.5	3.0	2.1
Aldicarb	10	95.8	99.1	2.9	3.8
Propoxur	10	97.9	98.4	2.8	2.4
Carbofuran	10	98.0	99.1	3.1	2.9
Carbaryl	10	90.3	94.6	2.0	1.4
Methiocarb	10	76.5	74.4	4.1	3.7

## Conclusions

The Agilent QuEChERS buffered extraction AOAC kit and the Agilent dispersive-SPE kit for general fruits and vegetables provide a simple, fast and effective method for the purification of N-methyl carbamate pesticides in juice. Compared to other sample pretreatment methods, such as liquid-liquid extraction and solid-phase extraction, the QuEChERS method is easier to handle, faster, labor-saving, and cheaper. The recovery and reproducibility, based on matrix spiked standards, were acceptable for this type of pesticide residue determination in juice.

The Agilent 6460 Triple Quadrupole LC/MS with the Agilent Poroshell 120 Bonus-RP column resolved 10 carbamates in 7 minutes, compared with traditional methods of 30 minutes or more, and all the compounds were well separated from the matrix, providing high sensitivity. This method is suitable for the analysis of N-methyl carbamate pesticide determination in juices at low  $\mu\text{g}/\text{kg}$  levels.

## References

1. Z. Yang. A Simple and Ultra-Sensitive LC/MS/MS Method for Analysis of N-Methyl Carbamates in Water. Application note, Agilent Technologies, Inc., Publication number 17.r1.
2. Anon. Measurement of N-methylcarbamoyloximes and N-methylcarbamates in Water by Direct Aqueous Injection HPLC with Postcolumn Derivatization. EPA Method 531.2., Revision 1.0. Environmental Protection Agency, Washington, DC, USA (September 2001).
3. M. Hartz, E. George, J. Thoma, J. J. Sullivan, S. Aamodt, M. J. Lang. The Determination of N-Methylcarbamate Pesticides by HPLC EPA Method 531.1. Application note, Agilent Technologies, Inc., Publication number 25.

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