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High Throughput Analysis of Carbendazim in Orange Juice

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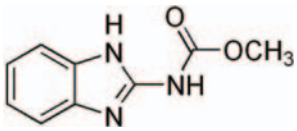
Introduction

Carbendazim is a broad spectrum fungicide used in cereal and fruit crops. Although its use is banned in the US, there are a number of countries that still legally use Carbendazim to help control molds such as black spot. Recently, orange juice imports have tested positive for Carbendazim. As of late March, 30 out of 144 lots had tested positive (≥ 10 ppb) and been detained by the FDA. A number of commercial orange juices were tested from the local area and found to contain Carbendazim in low ppb levels. A novel high-throughput LC-MS/MS method was developed that allows the analysis of Carbendazim to be completed in one minute to facilitate the testing of multiple samples.



Sample Preparation

Standards: An orange juice blend was spiked to obtain varying levels from 1 to 1000 ppb of Carbendazim. The standard was then diluted 50x with solvent (65/35 H_2O/MeOH), centrifuged, and filtered.

Samples: Several local orange juice samples were diluted 50x with solvent, centrifuged, and injected.

Fugicides help protect oranges (citrus sinensis) from disease, such as black spot mold

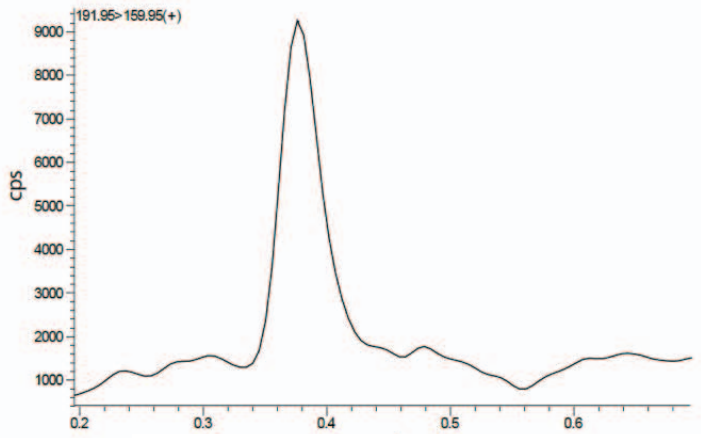
Method

Column	: Phenomenex Kinetex XB-C18 2.6 um 2.1 × 30 mm
Column temp	: 40°C
A	: 5 mM Ammonium Acetate
B	: MeOH
Gradient linear	: Initial 35%B, 0.3min 90%B, 0.31 min 35%B
Flow rate	: 0.75 mL/min
Injection volume	: 25 uL
Runtime	: 1.0 minute
LC-MS/MS	: 195.95-159.95 MRM+
Dwell time	: 100 msec Drying gas: 15 L/min
Nebulizing gas	: 2 L/min
DL	: 250°C
Block	: 400°C

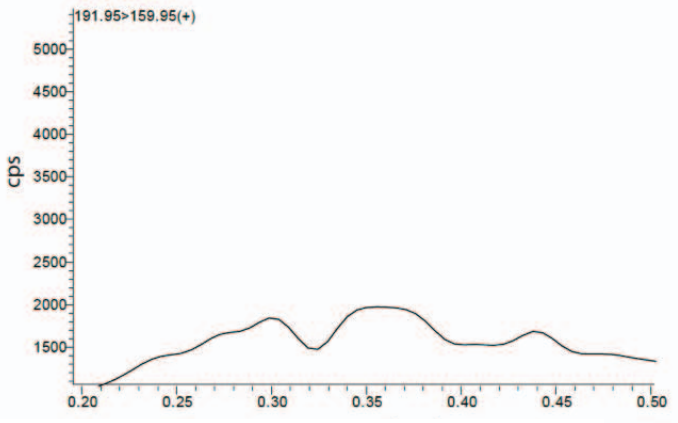
Instrumentation

- UHPLC: Shimadzu Nexera UHPLC system with SIL-30ACMP autosampler and CTO-30AS column oven.
- MS: Shimadzu 8040 LC-MS/MS DUIS source

Positive Sample and Matrix Blank

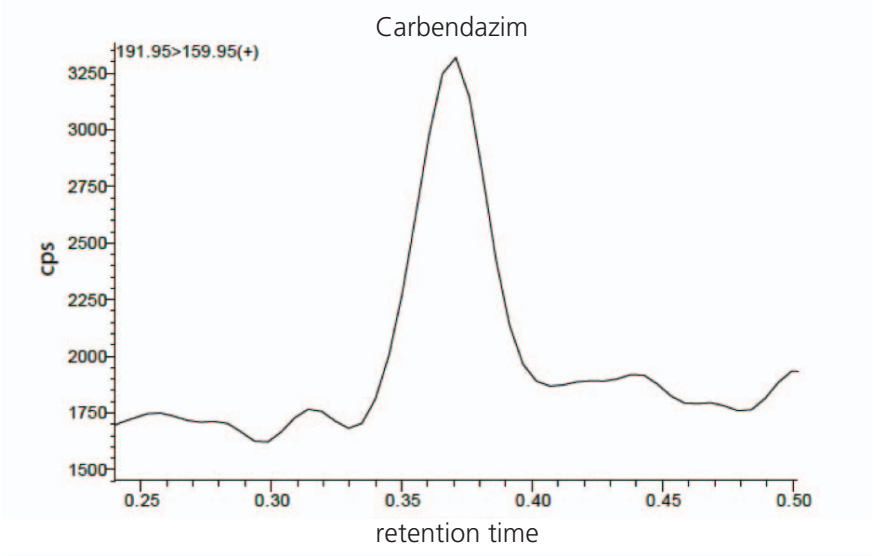


Brand MM Orange Juice

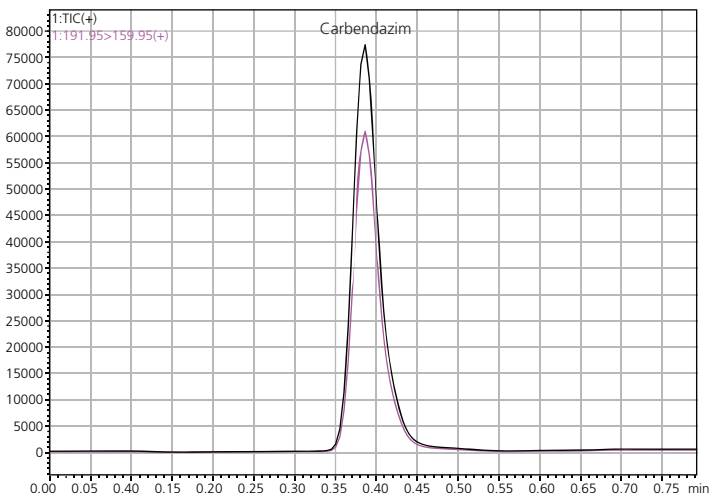


Organic Certified Orange Juice

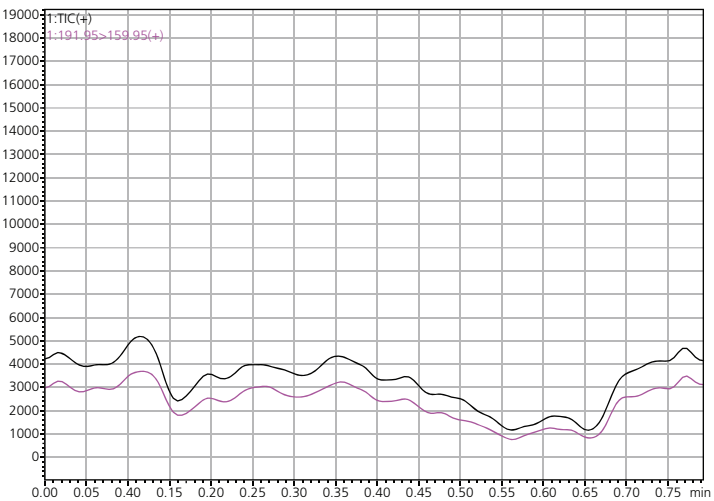
Carbendazim 1 ppb std diluted 50x



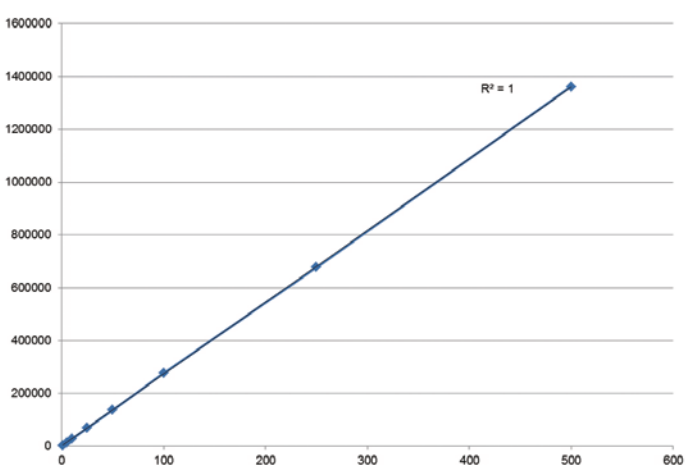
Carbendazim 500 ppb std



Carbendazim blank after 1000 ppb std



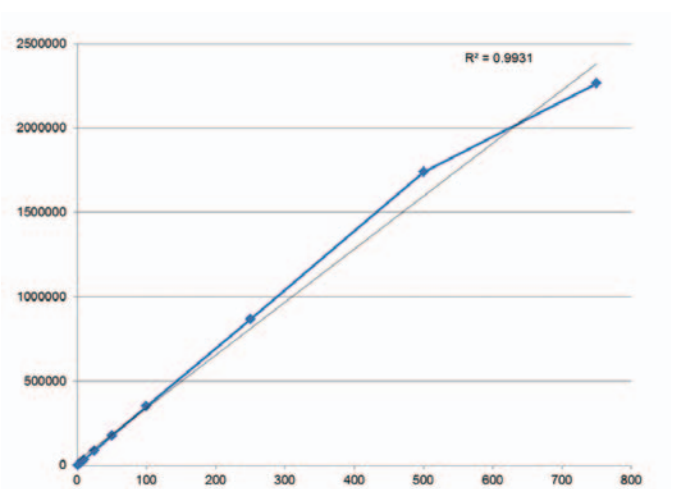
Carbendazim Calibration Curve (1 to 500 ppb)



Carbendazim ppd

ppd	191.95-159.95 area
1	2923
5	14204
10	28160
25	69258
50	136848
100	276748
250	678063
500	1361833

Carbendazim Calibration Curve (1 to 750 ppb)

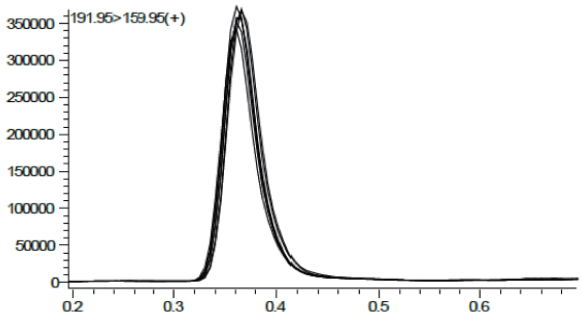


Carbendazim ppd

ppd	191.95-159.95
1	2923
5	14204
10	28160
25	69258
50	136848
100	276748
250	678063
500	1361833
750	1771800

Carbendazim LCMS/MS RSD Data

File Name	Retention Time	Peak Area
CarbRS-01.lsd	0.361	861474
CarbRS-02.lsd	0.363	843203
CarbRS-03.lsd	0.363	843727
CarbRS-04.lsd	0.366	884182
CarbRS-05.lsd	0.365	910001
CarbRS-06.lsd	0.361	878459
Average	0.363	870174
%RSD	**0.55%**	**2.98%**



The retention times and peak areas were very reproducible even without using stable isotope labeled standards.

Detected Carbendazim Levels in OJ

Samples:

HT: < 1 ppb

OV: < 1 ppb

GV: 1.0 ppb

MM: 8.1 ppb

T1: 4.5 ppb

T2: 1.1 ppb

Results

- Four commercial brands of orange juice tested positive for Carbendazim at low ppb levels. Levels found were below FDA limits for a positive test.
- A blank injected after the 1 ppm standard without rinsing the autosampler showed no carryover.
- The use of UHPLC with a high-speed injector (injection speed 7 seconds) allowed an analysis to be completed in a one-minute timeframe.

Summary

- A novel high-speed reversed phase UHPLC method was developed for the analysis of Carbendazim. Four commercial brands of orange juice from the local area tested positive for Carbendazim at low ppb levels.
- An organic brand of orange juice did not contain any detectable levels of Carbendazim and was used to prepare the matrix matched calibration curve. Good linearity was obtained from low ppb levels up to a 500 ppb concentration. A blank injected after the 1 ppm standard showed no carryover. Concentrations above 500 ppb showed a saturation effect with these conditions. Carbendazim levels of 1ppb could also be detected by fluorescence, but multiple interfering peaks were observed.
- The use of UHPLC with a high-speed injector allowed multiple analyses to be completed with a runtime of less than one minute for each analysis.
- Carbendazim levels remained consistent in samples two months past expiration dates under refrigerated storage conditions.

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