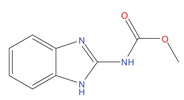
High Throughput LC-MS-MS Analysis of Carbendazim in Orange Juice

William Hedgepeth, Jeffrey H. Dahl, and Kenichiro Tanaka Shimadzu Scientific Instruments, Columbia, Maryland

Introduction

Highly sensitive analytical methods are necessary to prevent contaminated produce from entering the food supply. In order to help move fresh imports through customs without delay, methods must be rapid and cost effective, in order to test imports thoroughly. LC-MS offers the highly sensitive, robust measurement needed, while ultra high performance liquid chromatography enables high speed analysis. We developed an ultra fast UHPLC-MS-MS method for analysis of carbendazim in orange juice using a new high speed autosampler, the Nexera SIL-30AC$_{MP}$ in tandem with a newly developed high sensitivity triple quadrupole mass spectrometer, the LCMS-8040.

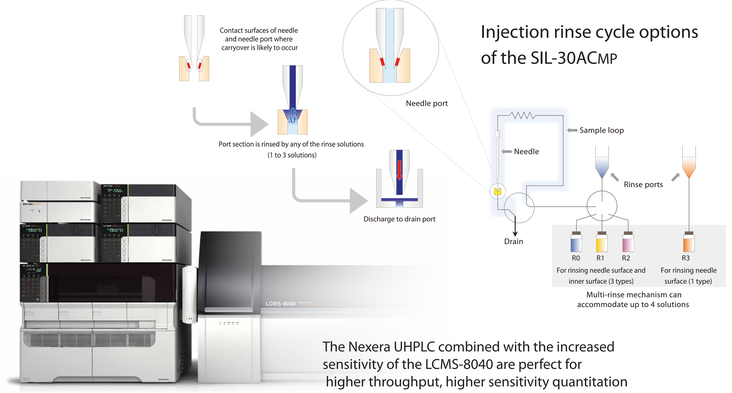


Carbendazim

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

Method

LC-MS-MS was performed using a Shimadzu Nexera UHPLC with a new high throughput autosampler, the Nexera SIL-30AC$_{MP}$. A binary gradient of 5 mM ammonium acetate and methanol was used at a flow rate of 0.75 mL/min with a Phenomenex Kinetex XB-C18 (2.6 µm, 2.1 × 30 mm) at 40 °C. Electrospray ionization in positive mode was used on a new fast-scanning, high sensitivity triple quadrupole mass spectrometer, the LCMS-8040. Various brands of orange juice were obtained and diluted 50-fold and filtered prior to injection; no other sample preparation was required. Due to the fast injection speed of the Nexera UHPLC system and the fast data acquisition of the LCMS-8040, the runtime was reduced to only 0.8 min.

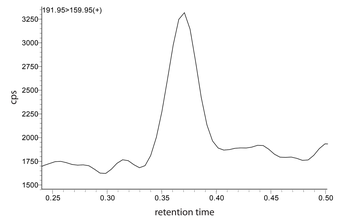


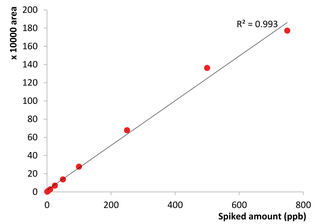
Injection rinse cycle options of the SIL-30AC$_{MP}$

The Nexera UHPLC combined with the increased sensitivity of the LCMS-8040 are perfect for higher throughput, higher sensitivity quantitation

Results and Discussion

Carbendazim was detected in several commercial brands of orange juice, all at levels below the FDA regulatory cutoff of 10 ng/mL and the health cutoffs established by the US EPA and European Union (80 and 200 ng/mL, respectively). The limit of detection of carbendazim in orange juice was approximately 0.4 ppb and the limit of quantitation was approximately 1 ppb. The sample preparation required only dilution and filtration, which enabled rapid sample analysis.



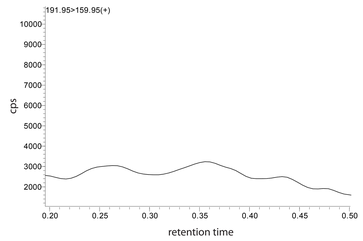


Chromatogram of orange juice spiked with 1 ppb carbendazim and calibration curve

Brand	Measured Carbendazim
HT *	ND
OV **	ND
GV	1.0 ppb
MM	8.1 ppb
T1	4.5 ppb
T2	1.1 ppb

* Generic store brand
** Organic certified

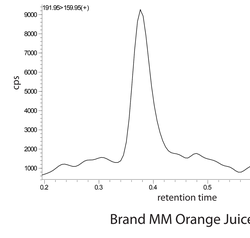
Table of brands tested



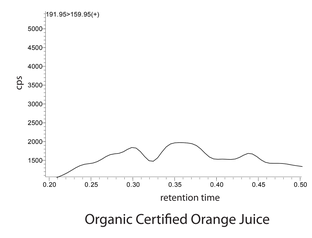
Orange juice matrix blank injected after 1 ppm standard

Levels of carbendazim measured in different orange juice brands and an orange juice blank matrix.

The calibration curve ranged from 1 ppb up to 750 ppb. The curve was linear up to 500 ppb, while above 500 ppb some slight saturation was observed. Among the six brands of juice tested were popular name brands as well as a generic store brand and an organic certified orange juice. The detected amounts of carbendazim ranged from 1 ppb up to 8.1 ppb, while no carbendazim was detected in either the generic store brand or the organic certified juice.



Brand MM Orange Juice

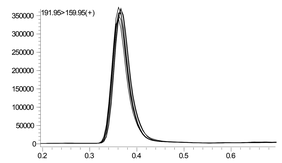


Organic Certified Orange Juice

Representative chromatograms of two brands of orange juice. The MM brand contained 8.1 ppb carbendazim, while none was detected in the certified organic orange juice.

The Nexera UHPLC pumps enabled a fast gradient with a runtime of under 1 minute, while the SIL-30AC$_{MP}$ autosampler enabled injection cycle times of just 7 seconds. The improved ion optics of the LCMS-8040 made it possible to achieve better sensitivity with dilute-and-shoot sample preparation. Not only were the runs rapid, but the retention times and peak areas were extremely reproducible as well, with relative standard deviations of just 0.55 and 2.98% respectively.

File Name	Retention Time	Peak Area
CarbRSD-01.lsd	0.361	861474
CarbRSD-02.lsd	0.363	843203
CarbRSD-03.lsd	0.363	843727
CarbRSD-04.lsd	0.366	884182
CarbRSD-05.lsd	0.365	910001
CarbRSD-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.

We found carbendazim levels remained consistent in orange juice samples even several months past their expiration dates under refridgerated storage conditions. This demonstrates the ability of carbendazim to persist in the food supply and underscores the need for thorough food safety screening.

Conclusion

Carbendazim was detected in four commercial brands of orange juice at low ppb levels. Levels found were below FDA limits for a positive test. The calibration curve was linear up to 500 ppb, and the method was sensitive enough to detect carbendazim below the most stringent regulatory threshold. No carryover was detected and the method run time was under one minute, thanks to the extremely high speed autosampler which completed each injection cycle in just 7 seconds. Sample preparation only required dilution and filtration for rapid, efficient analysis.