Rapid Quantitation and Identification of Carbendazim in Orange Juice Using the New AB SCIEX QTRAP® 4500 LC-MS/MS System

Fast method development in response to contaminated orange juice imports to the U.S.

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Introduction

Recent issues surrounding the presence of the fungicide Carbendazim in orange juice samples imported to the U.S. from Brazil, the biggest orange juice exporter in the world, have heightened the need for regulatory agencies and food manufacturers to begin proactive testing of orange juice to ensure product compliance to U.S. regulatory standards and overall consumer safety.

Carbendazim (a fungicide used to treat citrus trees in Brazil against diseases such as black spot), while approved for use in some countries, is not approved by the U.S. Environmental Protection Agency for use on oranges. The United States reportedly imports 15 percent of its orange juice supply, the majority of which comes from Brazil.[1] Given this volume of product imported, the detection of this substance has created cause for investigation and increased testing of orange juice shipments to the U.S. and throughout the world.

A fast, easy, and sensitive LC-MS/MS method was developed for the detection of Carbendazim in orange juice samples. The method utilizes a simple dilute-and-shoot approach, with UHPLC analysis using a Phenomenex Synergi-Fusion 2.5 µm column. This method, with minor adjustments, can be adapted for analysis using different AB SCIEX mass spectrometers, including the QTRAP® 4500 and 5500 LC-MS/MS systems.

Additionally, the acquisition method is amenable to extension for screening of up to 204 additional commonly used pesticides through incorporation of the iD*Quant*™ standards kit for pesticide analysis.

Experimental

Sample Preparation

The sensitivity and selectivity of the AB SCIEX Q TRAP® systems allow minimal sample preparation for this analysis. Orange juice samples were simply centrifuged at high speed, an aliquot of the supernatant was diluted 5-fold with water, and the sample was ready for LC-MS/MS analysis.

However, to achieve even lower limits of quantitation, samples may be prepared through an SPE clean-up procedure optimized for Carbendazim.[2]

LC

LC separation was achieved using the Eksigent ekspert™ ultraLC 100 with a Phenomenex Synergi-Fusion 2.5 um (2 x 50 mm) column with a gradient of water and methanol containing 10 mM ammonium formate at a flow rate of 0.5 mL/min. The injection volume was set to 10 µL.

MS/MS

The AB SCIEX QTRAP® 4500 and 5500 systems are highly suitable for this analysis allowing simultaneous quantitation using Multiple Reaction Monitoring (MRM) and identification based on Enhanced Product Ion (EPI) scanning with library searching. The Turbo V™ source was used with an Electrospray Ionization (ESI) source. Two selective MRM transitions were monitored for Carbendazim as outlined in Table 1. EPI spectra were acquired using dynamic fill time and Collision Energy Spread (CES) for highest spectral quality.

Table 1. MS/MS Parameters for Carbendazim using the AB SCIEX Q TRAP® 4500 system

MRM	Q1/Q3	DP (V)	CE (V)
Carbendazim 1	192/160	56	27
Carbendazim 2	192/132	56	41

Results and Discussion

First, limit of detection (LOD), limit of quantitation (LOQ), linearity, and reproducibility were evaluated using injections of the iD*Quant*™ Standards Kit for Pesticide Analysis ranging in concentration from 0.05 to 100 ng/mL.

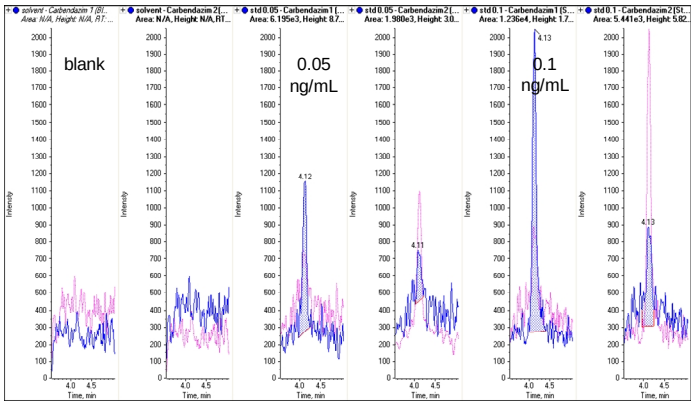

Figure 1. Determination of LOD and LOQ of detection of Carbendazim, LOD was found at 0.05 ng/mL and LOQ at 0.1 ng/mL using the AB SCIEX QTRAP® 4500 system

The LOD was determined based on Signal-to-Noise (S/N) calculated with an algorithm using 3x standard deviation. The S/N at a concentration of 0.05 ng/mL was 5. The LOQ was determined based on reproducibility. The coefficient of variation (%CV) at 0.1 ng/mL was 7.0% (Figure 1 and Table 2).

This level of sensitivity allows the direct injection of orange juice samples without using time-consuming and extensive sample cleanup. Juice samples were injected directly after centrifugation and a simple dilution to minimize any possible matrix effects.

The linearity obtained for both MRM transitions for Carbendazim are shown in Figure 2. Results showed linearity with regression coefficients of > 0.999, sufficient to analyze for Carbendazim in juice samples, particularly at the FDA action level of 10 parts per billion (ppb)[3] and the EU maximum residue level of 200 mg/kg.[4-5]

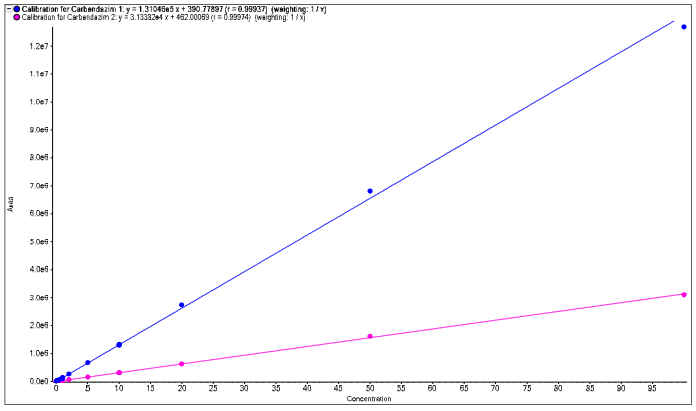

Figure 2. Linear range of the detection of Carbendazim from 0.05 to 100 ng/mL with an r > 0.999 for both MRM transitions

Table 2. Reproducibility and accuracy over the entire linear range when quantifying Carbendazim

Concentration (ng/mL)	# of injection	Accuracy (%)	% CV
0.050	1	88.6	-
0.100	3	98.0	7.0
0.200	1	109.0	-
0.500	1	100.1	-
1.000	10	98.0	3.6
2.000	1	104.7	-
5.000	1	104.0	-
10.00	3	100.1	0.4
20.00	1	104.5	-
50.00	1	104.1	-
100.0	1	96.8	-

Reproducibility was investigated by repeated injections of spiked juice at a concentration of 1 ng/mL. Both MRM transitions showed excellent %CV as shown in Figure 3.

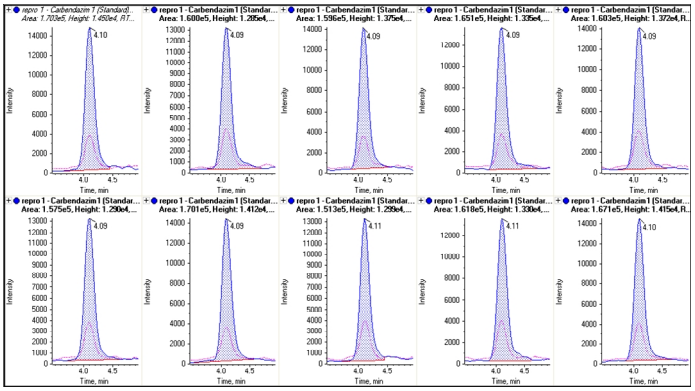

Figure 3. Reproducibility at 1 ng/mL with a %CV of 3.6 and 5.7%, respectively, for both MRM transitions

Several orange juice samples were purchased from a local store and analyzed by the method described. The MRM chromatograms of two samples are shown in Figure 4. When quantified against the standard calibration curve and corrected for dilution, the samples were determined to contain 13 ng/mL and 67 ng/mL of Carbendazim, respectively.

The MRM ratio of quantifier and qualifier transitions was used to identify Carbendazim in both samples. The 'Multicomponent' query in MultiQuant™ software automatically calculates and compares MRM ratios for identification and flags samples with a concentration of the targeted analytes above a specific concentration

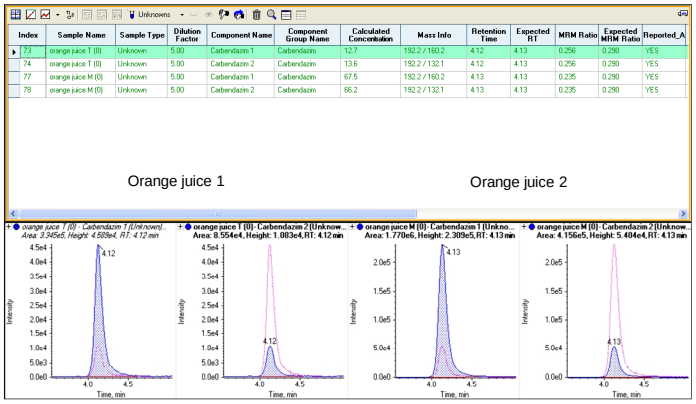

Figure 4. Quantitation and identification of Carbendazim in store bought orange juice using 'Multicomponent' query in MultiQuant™ software

To further confirm the identification of Carbendazim in both samples, the automatically collected EPI spectra were evaluated with a search against our pesticide MS/MS library (iMethod™ application pesticide LC-MS/MS library version 1.1). The results revealed a library FIT of 93% and 97%, respectively, for the MS/MS spectrum (Figures 5 and 6), further verifying the presence of Carbendazim in the juice sample, adding an extra level of confidence in the results.

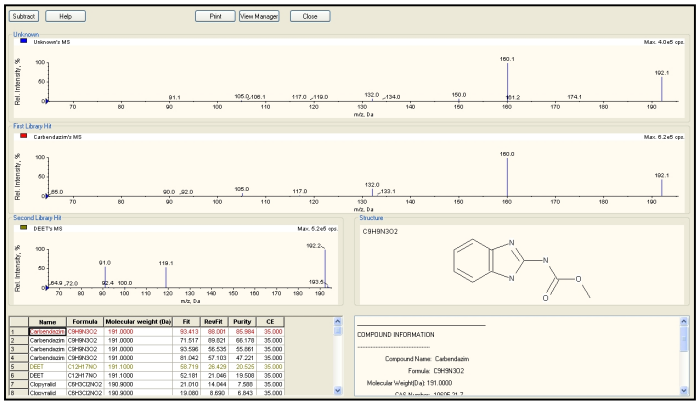

Figure 5. Library search of automatically collected EPI spectra of the orange juice sample 1 identifying 13 ng/mL Carbendazim with a library FIT of 93%

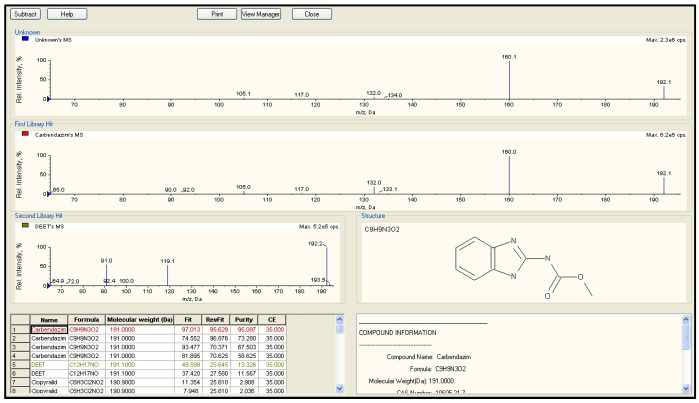

Figure 5. Library search of automatically collected EPI spectra of the orange juice sample 2 identifying 67 ng/mL Carbendazim with a library FIT of 97%

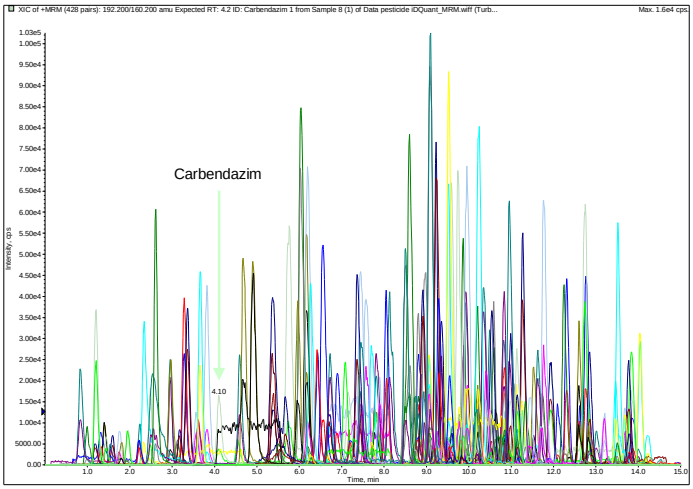

Figure 7. Comprehensive pesticide screening using LC-MS/MS and the iDQuant™ standards kit for pesticide analysis

Summary

The method and data presented here showcase the fast, easy, and accurate solutions for the analysis of Carbendazim in orange juice by LC-MS/MS. The AB SCIEX QTRAP® 4500 and 5500 systems provide excellent sensitivity and selectivity for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

The approach also lends itself to be extended for the screening of many different pesticides through the use of the iD*Quant*™ kit for pesticide analysis and MS/MS library searching, which would be ideal to identify any additional potential contaminants that could arise in the future (Figure 7).

References

1 http://www.ers.usda.gov/publications/aer827/aer827e.pdf
2 http://www.absciex.com/applications/food-and-beverage-testing/carbendazim-contamination-in-orange-juice
3 http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/ucm287783.htm
4 Regulation (EC) No 396/2005 'on maximum residue levels of pesticides in or on food and feed of plant and animal origin' with amendment (EC) No 299/2008
5 Regulation (EC) No 149/2008 'Annexes II setting maximum residue levels' with amendment (EC) No 559/2011

Additional resources and information

For more details on free iMethod™ applications for the analysis of Carbendazim in orange juice, as well as for details on iMethod™ applications for general pesticide screening, visit www.absciex.com or contact us as support@absciex.com.

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