Determination of Carbendazim in Orange Juice by QuEChERS and LC/MS/MS Detection

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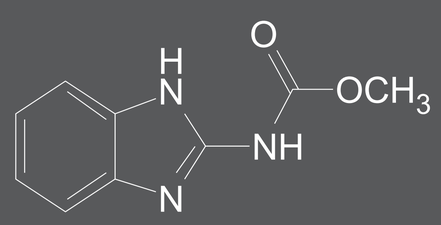
INTRODUCTION

Carbendazim is a broad-spectrum fungicide with a planar structure that is widely used to control fungi and molds in crops and fruits. It is permitted for use in the European Union (EU) and many other countries, but it is not registered for use in the United States. A beverage manufacturer reported the detection of carbendazim in its orange juice products in early 2012, thereafter, the US FDA set the rejection limit of carbendazim at 0.01 mg/kg in imported orange juice products.

Orange juice is a complex matrix that contains organic acids, sugars, orange oil, and carotenoids. In this study the QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) procedure is used to extract carbendazim from orange juice into acetonitrile (MeCN). The co-extractives are cleaned up using dispersive solid phase extraction (dSPE) with anhydrous magnesium sulfate (MgSO4), primary secondary amine (PSA) and endcapped C18. $MgSO_4$ absorbs remaining water residue in the extract, PSA removes organic acids and sugars, while endcapped C18 removes orange oil and carotenoids, resulting in clean extract for LC/MS/MS analysis.

Structure of Carbendazim



EXPERIMENTAL

Apparatus

50 mL centrifuge tubes and salts packed in Mylar pouch (4000 mg magnesium sulfate, 1000 mg sodium chloride, 500 mg sodium citrate dibasic sesquihydrate, and 1000 mg sodium citrate tribasic dehydrate) (UCT Part#: ECQUEU750CT-MP)

2 mL centrifuge tubes with 150 mg anhydrous magnesium sulfate and 50 mg PSA (UCT Part#: CUMPS2CT)

2 mL centrifuge tubes with 150 mg anhydrous magnesium sulfate, 50 mg PSA and 50 mg C18 (UCT Part#: CUMPSC18CT)

2 mL centrifuge tubes with 150 mg anhydrous magnesium sulfate, 25 mg PSA and 7.5 mg GCB (UCT Part#: ECQUEU42CT)

QuEChERS EXTRACTION

Add 10 mL orange juice to 50 mL centrifuge tubes, spike with 10, 50, and 250 ng/mL of carbendazim standard for fortified samples.

Vortex for 30 sec and equilibrate for 15 min.

Add 10 mL of MeCN, vortex for 30 sec.

Add salts packed in Mylar pouch, shake vigorously for 1 min.

Centrifuge at 5000 rpm for 5 min, the upper layer extract is ready for cleanup.

Photograph of orange juice sample extracted with QuEChERS

dSPE CLEANUP

Graphitized carbon black (GCB) is often used to cleanup pigmented samples, however it also removes compounds with planar structures, such as carbendazim and thiabendazole. In this study, dSPE cleanup with different sorbents are compared.

Transfer 1 mL of the orange juice extract to 2 mL dSPE tubes.

Three different dSPE were tested:

dSPE (1): 150 mg MgSO4 and 50 mg PSA (CUMPS2CT)
dSPE (2): 150 mg MgSO4, 50 mg PSA, and 50 mg C18 (CUMPSC18CT)
dSPE (3): 150 mg MgSO4, 25 mg PSA, and 7.5 mg of GCB (ECQUEU42CT)

Shake for 30 seconds and centrifuge at 10,000 rpm for 5 min.

Transfer 0.5 mL of the cleaned extract into 2 mL auto-sampler vial, add 25 µL of 1 ppm triphenyl phosphate (TPP) as internal standard (IS), the extract is ready for LC/MS/MS analysis.

Photos of orange juice extracts cleaned up with three different dSPE

dSPE (1) dSPE (2) dSPE (3)

The extract with dSPE (1) (PSA) is quite yellowish, indicating that the carotenoids are not efficiently cleaned up with PSA. Extracts cleaned up with dSPE (2) (PSA and C18) and dSPE (3) (PSA and GCB) generated similar colorless extracts. Because GCB adversely affects the recovery of carbendazim, a planar pesticide, dSPE (2) with PSA and C18 was selected to cleanup orange juice samples. Carotenoids, which contain long hydrocarbon chains in their structures are retained onto non-polar endcapped C18, resulting in sufficient cleanup without compromising the recovery of carbendazim.

INSTRUMENTAL

LC: Thermo Accela equipped with PAL auto-sampler
Guard column: Restek C18, 2.1*20 mm
Analytical column: Sepax HP-C18, 2.1*100 mm, 3 µm, 120 Å
Column temperature: ambient (about 20 ºC)
Injection volume: 10 µL at 15 ºC
Mobile phase:
A: 0.1% formic acid in water
B: 0.1% formic acid in methanol
Flow rate: 200 µL/min
Gradient program:

Time (min)	%A	%B
0	50	50
3	0	100
8	0	100
9	50	50
14	50	50

MS/MS: Thermo TSQ Vantage triple quadrupole
Ion source: heated ESI positive
Spray voltage: 3000 V
Sheath gas pressure (N2): 40 psi
Auxiliary gas pressure (N2): 10 psi
Ion transfer capillary temperature: 350 °C

Table 1: SRM transitions

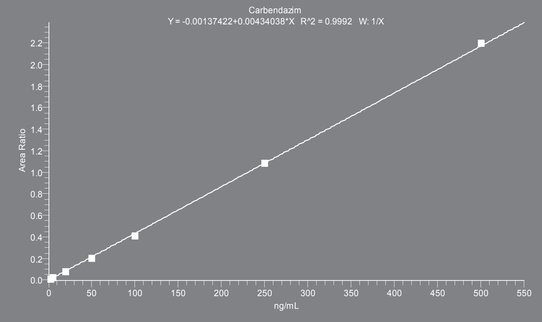
Compound	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-Lens	Dwell time (s)
Carbendazim	192.093	132.080	29	160.080	17	81	0.20
TPP (IS)	327.093	77.020	37	152.070	33	98	0.10

RESULTS

Matrix matched calibration

Calibration curves were obtained by analysis of matrix matched standards, which were prepared by spiking appropriate amounts of carbendazim to blank orange juice extracts after dSPE cleanup with PSA and C18. Seven matrix matched calibration standards at 2, 5, 20, 50, 100, 250, and 500 ng/mL were analyzed. The response was linear over the calibration range with correlation coefficients (R^2) of 0.9992. The limit of detection and quantification (LOD and LOQ) were estimated to be 1 and 2 ng/mL with signal to noise (S/N) value higher than 3 and 10 respectively.

Matrix matched calibration curve



Recovery study

Orange juice samples fortified with carbendazim at three levels were extracted in four replicates. Excellent recoveries and relative standard deviation (RSD%) were achieved.

Table 2: Recovery and Reproducibility Data

Fortified concentration (ng/mL)	Recovery%	RSD% (n=4)
10	96.6	4.5
50	100.2	3.4
250	103.7	2.1

Application to real samples

Six orange juice samples were tested in triplicates with this method. The detected carbendazim concentrations are listed in Table 3. Among the six samples tested, only Sample 3 was detected with a positive result at 5.3 ng/mL.

Table 3: Orange samples tested and the detected concentrations of carbendazim

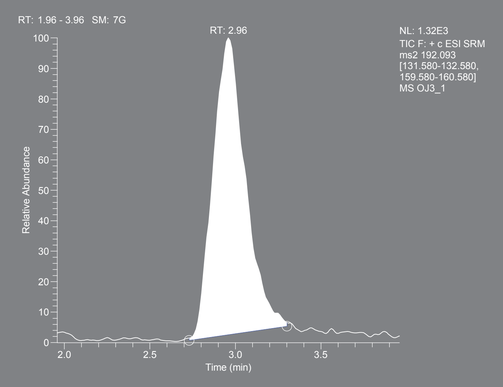
Samples	Detected
Sample 1	< LOQ
Sample 2	< LOQ
Sample 3	5.3 ng/mL
Sample 4	< LOQ
Sample 5	< LOQ
Sample 6	< LOQ

Chromatograms:

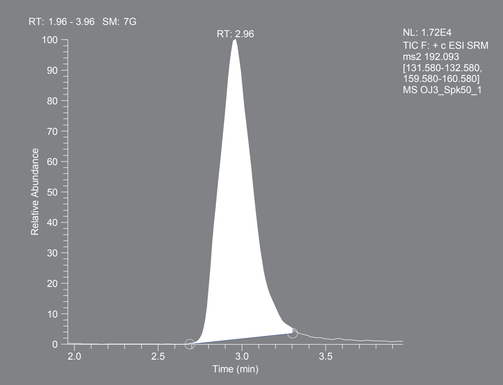
The chromatograms of Sample 3 and Sample 3 fortified with 50 ng/mL of carbendazim are shown in Figure 1.

Figure 1. Chromatograms of carbendazim in (a) Sample 3 and (b) Sample 3 fortified with 50 ng/mL of carbendazim

(a) Sample 3



(b) Sample 3 fortified with 50 ng/mL of carbendazim



CONCLUSIONS

A simple, fast, inexpensive, and effective method using QuEChERS extraction and dSPE cleanup with PSA and endcapped C18 was developed for the extraction and cleanup of carbendazim in orange juice. Clean extract, excellent recoveries (96.6, 100.2, and 103.7 % for three fortified levels at 10, 50, and 250 ng/mL), good reproducibility (RSD ≤ 4.5 %, n=4), linearity range of 2-500 ng/mL with R^2 of 0.9992, and low LOQ (2 ng/mL) were achieved. Among the six orange juice samples tested in this study one was detected positive with 5.3 ng/mL of carbendazim. The detected concentration was far below the EU's maximum allowable level of 200 ng/mL in orange juice.

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