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アプリケーションノート

QuEChERS Sample Preparation for LC-MS and LC-UV Determination of Carbendazim and Other Conazole Fungicides in Orange Juice

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Abstract

In this application note, we discuss sample preparation strategies for determination of conazole fungicides, including carbendazim, in orange juice. Using UPLC-MS/MS, five conazole fungicides, including carbendazim, were easily detected at 10 µg/L (ppb) in the QuEChERS extract of orange juice.

Benefits

- Rapid extraction of orange juice using proven QuEChERS methodology
- UPLC-MS/MS analysis in seven minutes with no further sample preparation required
- Straightforward SPE cleanup for LC-UV analysis
- Sub-ppb detection limits using UPLC-MS/MS
- Low ppb detection limits using LC-UV

Introduction

QuEChERS methodology is commonly used for sample preparation of fruits and fruit juices, prior to LC-MS or other analytical chromatography. Recently, a non-registered fungicide residue, carbendazim, was detected in orange juice purchased at a local market. This juice was produced from orange juice concentrate imported into the USA. In this application note, we discuss sample preparation strategies for determination of conazole fungicides, including carbendazim, in orange juice. First, we will focus on a sample preparation procedure using DisQuE products for QuEChERS suitable for many classes of pesticides and a wide variety of fruits, vegetables, and juices. Using UPLC-MS/MS, five conazole fungicides, including carbendazim, were easily detected at 10 µg/L (ppb) in the QuEChERS extract of orange juice. No further sample preparation was required. The QuEChERS extract can then be used as a precursor to further sample cleanup for targeted analysis of carbendazim and other conazole fungicides at ppb levels using LC with UV detection. Solid-phase extraction (SPE) with an Oasis MCX cartridge was used to reduce interference, and to concentrate the sample for this purpose. An incurred carbendazim sample was quantified at low ppb levels using LC with UV detection, applying this cleanup strategy. The Oasis MCX cleanup, although not required for the UPLC-MS/MS method, was shown to reduce matrix effects, and provide a cleaner extract. Thus, sample preparation using the DisQuE product for QuEChERS provides an extract suitable for direct analysis using UPLC-MS/MS and for HPLC/UV analysis, after enrichment and cleanup using mixed-mode SPE.

Experimental

UPLC conditions for Mass Spectrometry

System:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 100 mm, 1.7 µm (p/n 186002352)
Injection volume:	10 µL
Temp.:	40 °C
Mobile phase A:	0.1% NH_4OH in water
Mobile phase B:	0.1% NH_4OH in MeOH
Flow rate:	0.40 mL/min
Gradient:	10% B initial, linear gradient to 90% B in 4 min, hold for 5 min, back to 10% B for 5.1 min. Hold and re-equilibrate for 7 min.

HPLC conditions with XP Column for UV

System:	ACQUITY UPLC H-Class
Detection:	Photodiode Array (PDA)
Column:	XBridge C_{18} *XP* 4.6 x 100 mm, 2.5 µm (p/n 186006039)
Injection volume:	50 µL

Temp.:	40 °C
Mobile phase A:	20 mM Potassium phosphate in water (pH 6.8)
Mobile phase B:	Acetonitrile
Flow rate:	1.3 mL/min
Gradient:	25% B initial and hold for 3.4 min, then linear gradient to 65% B for 9.7 min, hold for 11.0 min, then linear gradient to 95% B for 11.7 min, hold for 13.0 min, then linear back to 25% B for 13.2 min. Hold and re-equilibrate for 18.3 min.

Sample description

The fungicides studied included the following: carbendazim, thiabendazole, imazalil, fenbuconazole, and difenoconazole. Structures for these analytes are shown in Figure 1. These compounds are bases that can be retained on Oasis MCX mixed-mode cation-exchange sorbent for SPE.

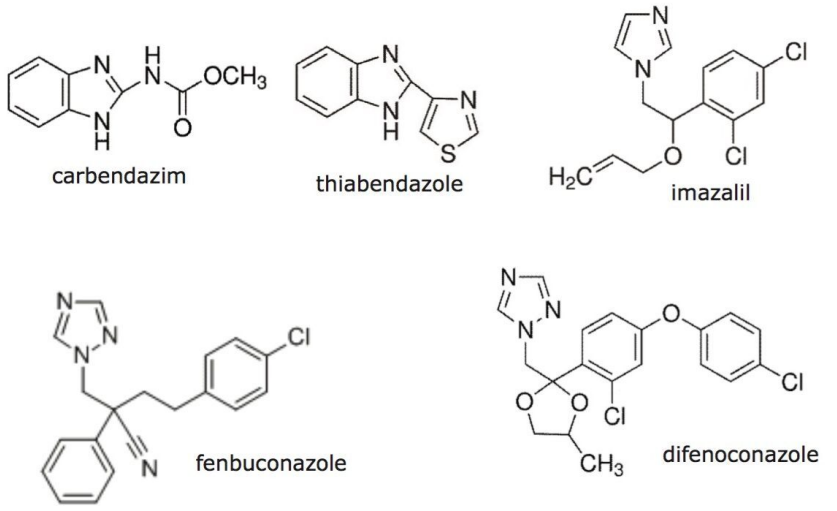

Figure 1. Structures of conazole fungicides.

Sample preparation

Initial extraction using QuEChERS

Place 15-mL orange juice sample into a 50-mL centrifuge tube. Add 15 mL of 1% acetic acid in acetonitrile (ACN), and shake the tube for 1 min. Add contents of DisQuE pouch for AOAC QuEChERS method (p/n 186006812), and shake vigorously for 1 min. Then centrifuge for 5 min at 3000 rpm. For UPLC-MS/MS analysis without SPE, dilute 0.5 mL of supernatant to 1.0 mL with water. The protocol for the initial QuEChERS extraction is shown in Figure 2.

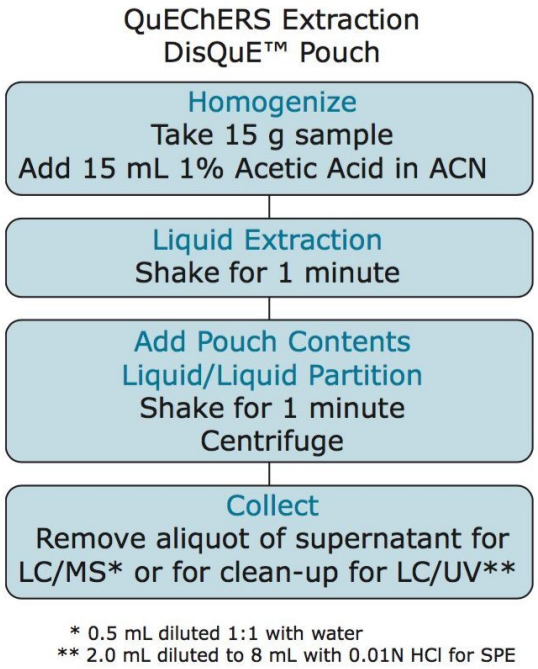

Figure 2. QuEChERS extraction using DisQuE product for AOAC method (p/n 186006812).

SPE cleanup

SPE with the Oasis MCX cartridge provides cleanup for basic compounds, such as conazole fungicides. For LC/UV analysis (or if cleanup is desired for UPLC-MS/MS analysis), take a 2-mL aliquot of supernatant from QuEChERS extract, add 6 mL 0.01 M aqueous HCl, and mix well. Proceed to the SPE cleanup protocol using a 3-cc Oasis MCX cartridge, shown in Figure 3. The QuEChERS extract is diluted with aqueous acid to enhance mixed-mode SPE retention, while the aqueous dilution enhances reversed phase retention.

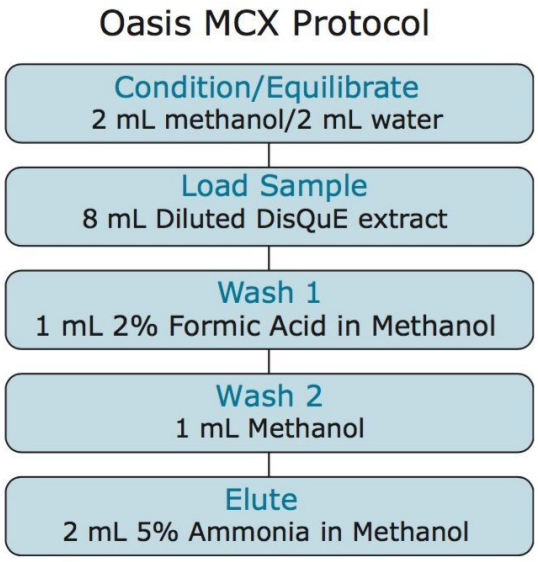

Figure 3. Oasis MCX SPE protocol for this study using a 3-cc cartridge (p/n 186000253).

MS conditions

Mass spectrometer: Waters Xevo TQ

Table 1 summarizes the cone and collision parameters, and MRM transitions used in this study obtained with the Xevo TQ Mass Spectrometer.

Fungicides	MRM Transitions	Cone (V)	Collision (eV)
Carbendazim	192.00 > 132 .00	30	30
	192.00 > 160.00	30	25
Difenoconazole	406.10 > 110.89	20	55
	406.10 > 250.87	20	30
Fenbuconazole	337.17 > 69.84	25	30
	337.17 > 124.83	25	30
Imazalil	297.10 > 68.90	35	20
	297.10 > 158.87	35	20
Thiabendazole	202.04 > 130.91	45	30
	202.04 > 174.93	45	25

Table 1. MRM transitions, cone voltages, and collision cell energies for this study.

Results and Discussion

The QuEChERS extraction procedure was effective for the extraction of conazole fungicides, prior to UPLC-MS/MS analysis. One of the fungicides, carbendazim, was not registered for use in orange juice, but was identified as an incurred residue in store-bought orange juice samples. Therefore, performance for this compound was verified at the 1-ppb level using mass spectrometry, and at the 10-ppb level using LC/UV. The results of this study show that QuEChERS sample preparation, using the DisQuE pouch, is not only effective for UPLC-MS/MS analysis of conazole fungicides, but with proper cleanup, low ppb levels of the fungicides can be determined using LC with UV detection.

The results of this study also illustrate the important conclusion that the QuEChERS procedure is a very effective sample preparation technique for many pesticides and related compounds in a wide variety of matrices. The level of subsequent cleanup that is required is dictated entirely by the needs of the analyst, as well as the sensitivity and specificity of the detection technique. The analysis presented in this study revealed that SPE cleanup, although recommended, is not required for effective UPLC-MS/MS analysis. On the other hand, SPE using a subsequent Oasis MCX cartridge step is required for effective LC/UV determination of carbendazim at low ppb levels.

UPLC-MS/MS with no SPE cleanup

Figure 4 shows a reconstructed UPLC-MS/MS chromatogram obtained from the analysis of an orange juice sample spiked at 10 ppb of each fungicide (1 ppb for carbendazim). Using external standard calculation, correlation (r^2) was 0.995 or better for all conazole fungicides for a five-point matrix-matched curve (data not shown). Table 2 summarizes the results obtained from the spiking experiments.

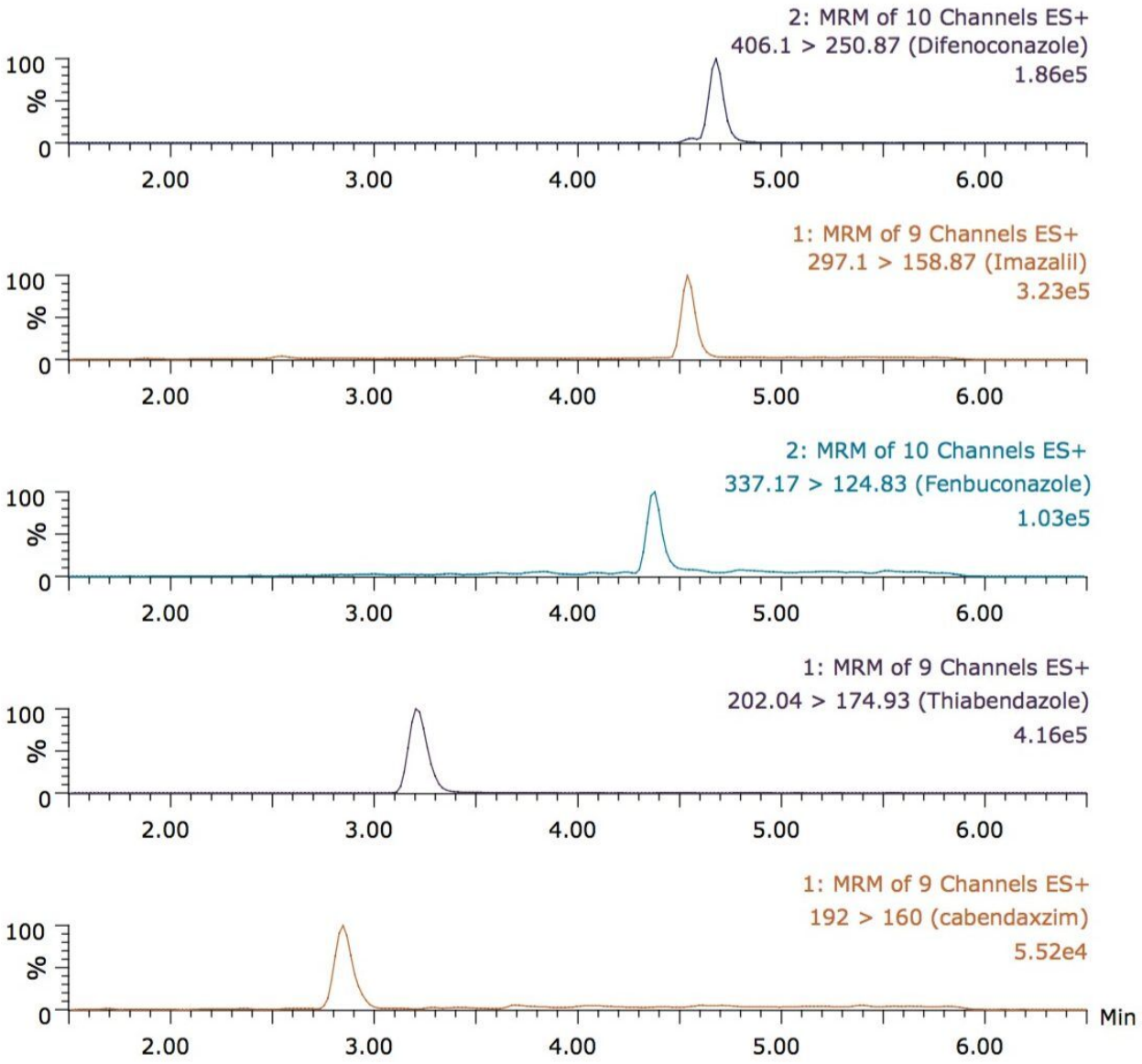

Figure 4. UPLC-MS/MS chromatogram obtained from DisQuE pouch extract of orange juice spiked at 10 ng/g (ppb) of each fungicide (except 1 ppb carbendazim).

Compound Conazole Fungicides	Spike Level (ppb)	% Recovery (%RSD)	% Suppression
Carbendazim	10	98.7 (1.3)	16.1
Thiabendazole	100	94.9 (2.3)	28.9
Imazalil	100	97.3 (0.9)	4.0
Difenoconazole	100	96.4 (0.6)	9.7
Fenbuconazole	100	97.6 (1.6)	8.6
Carbendazim	1	94.5 (4.2)	11.2
Thiabendazole	10	97.8 (4.1)	34.0
Imazalil	10	104 (3.7)	15.2
Difenoconazole	10	92.6 (6.5)	12.1
Fenbuconazole	10	95.2 (4.9)	5.0

Table 2. Summary of recovery data for conazole fungicides (n = 4) using the DisQuE pouch for AOAC QuEChERS. Ion suppression is significantly reduced (<2%), if Oasis MCX cleanup is employed (data not shown).

Conazole fungicide recovery was measured, based on the results obtained from 3 replicates prepared in the orange juice matrix. The recoveries ranged from 92% to 110% and %RSD was under 7%, as shown in Table 2. The recovery experiments were performed by comparing the MRM peak area of fungicides spiked into the juice sample, prior to QuEChERS extraction with the MRM peak area of fungicides spiked into the supernatant after QuEChERS extraction. Matrix effects (% suppression) were calculated by comparing the MRM peak area of fungicides in the supernatant after the QuEChERS extraction with the MRM peak area of the equivalent fungicide standards prepared in 50:50 acetonitrile/water.

LC/UV with SPE cleanup

Recovery was similar to the UPLC-MS/MS for samples spiked at the 100 ppb level. Figure 5 illustrates the determination of imazalil, fenbuconazole, and difenoconazole using LC/UV with the *XP* column. These compounds lack the useful 280- to 300-nm chromophore seen with carbendazim and thiabendazole; however, good sensitivity is reached at 220 nm. This is due, in large part, to the use of a UV-transparent mobile phase (potassium phosphate).

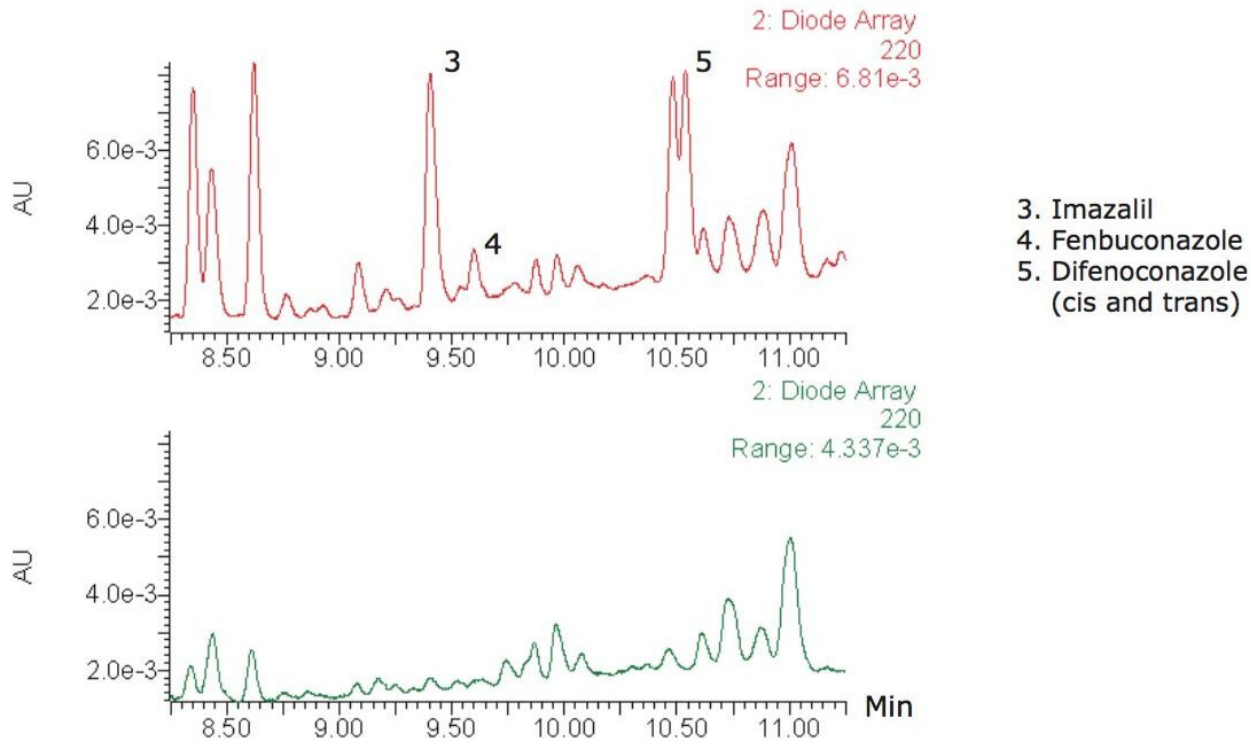

Figure 5. LC/UV (220 nm) chromatograms obtained on an XP column from a DisQuE pouch extract of orange juice spiked at 100 ng/g (ppb) after Oasis MCX cleanup.

Analysis of an incurred sample

Figure 6 shows LC/UV chromatograms obtained from the analysis of an incurred orange juice sample. This illustrates the profound level of cleanup imparted on the sample using the Oasis MCX cartridge. The top chromatogram was obtained with no SPE cleanup, while the bottom chromatogram was obtained after cleanup with the Oasis MCX cartridge. Figure 7 shows the LC/UV chromatograms prepared using QuEChERS and Oasis MCX, and the UPLC-MS/MS chromatograms obtained from analysis of the same incurred sample, without cleanup, using the Oasis MCX cartridge (QuEChERS only). The results are in very good agreement, with 11 ppb carbendazim detected by LC/UV and 13 ppb detected by UPLC-MS/MS. None of the other fungicides were identified in the tested orange juice samples. Figure 8 shows the UV spectrum obtained from the incurred sample, compared with the reference UV spectrum obtained from a ppb standard, confirming the presence of the residue in the orange juice sample.

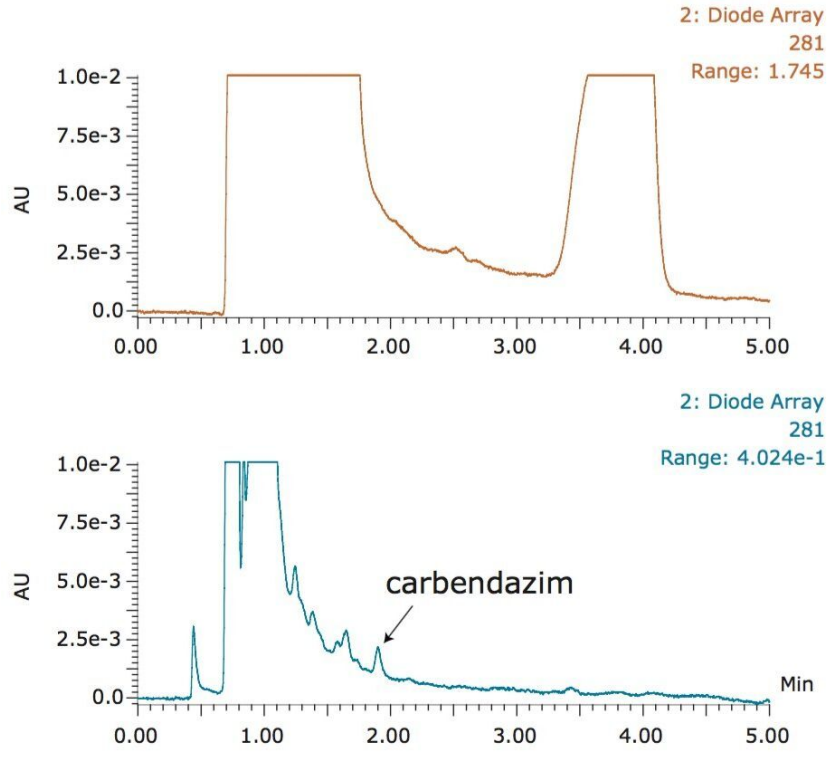

Figure 6. LC/UV chromatograms (281 nm) obtained from a DisQuE pouch extract of an orange juice sample with incurred carbendazim. The top chromatogram was obtained with no SPE cleanup, while the bottom chromatogram was obtained after cleanup with the Oasis MCX cartridge.

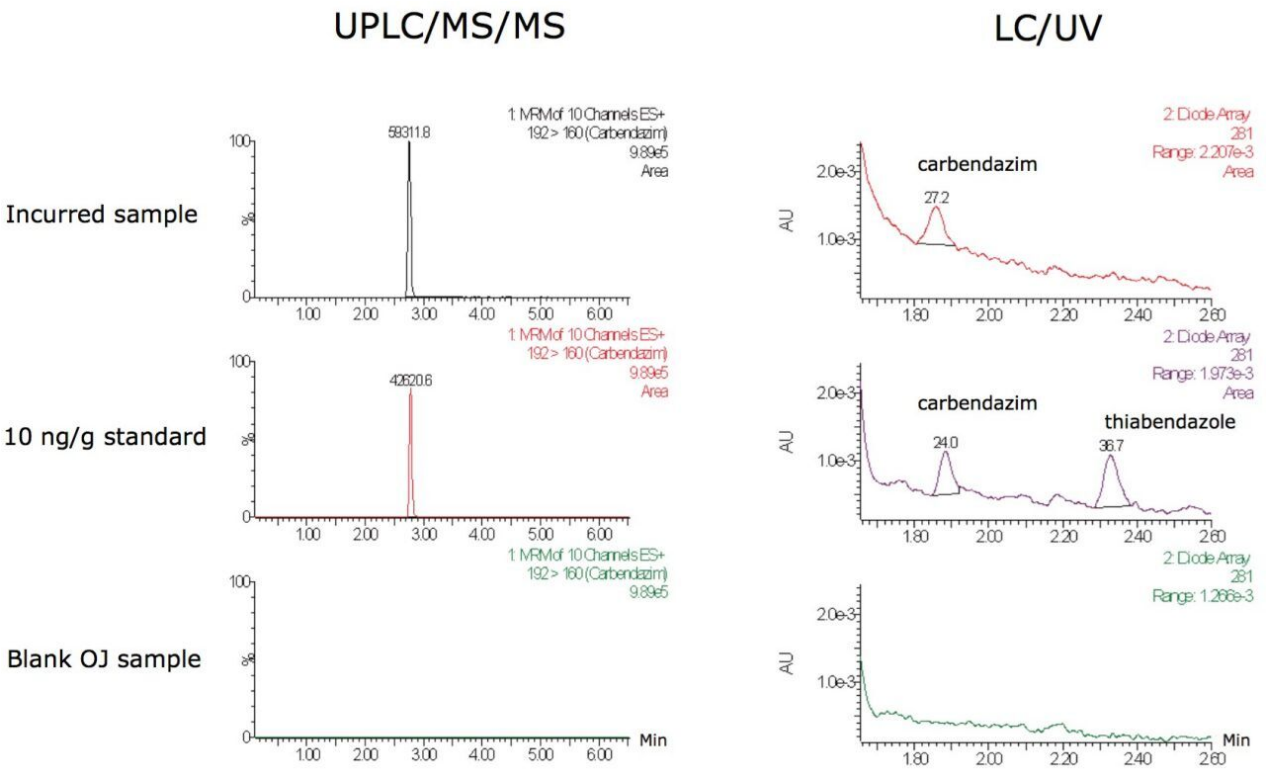

Figure 7. Comparison of UPLC-MS/MS and LC/UV for commercial orange juice with incurred carbendazim.

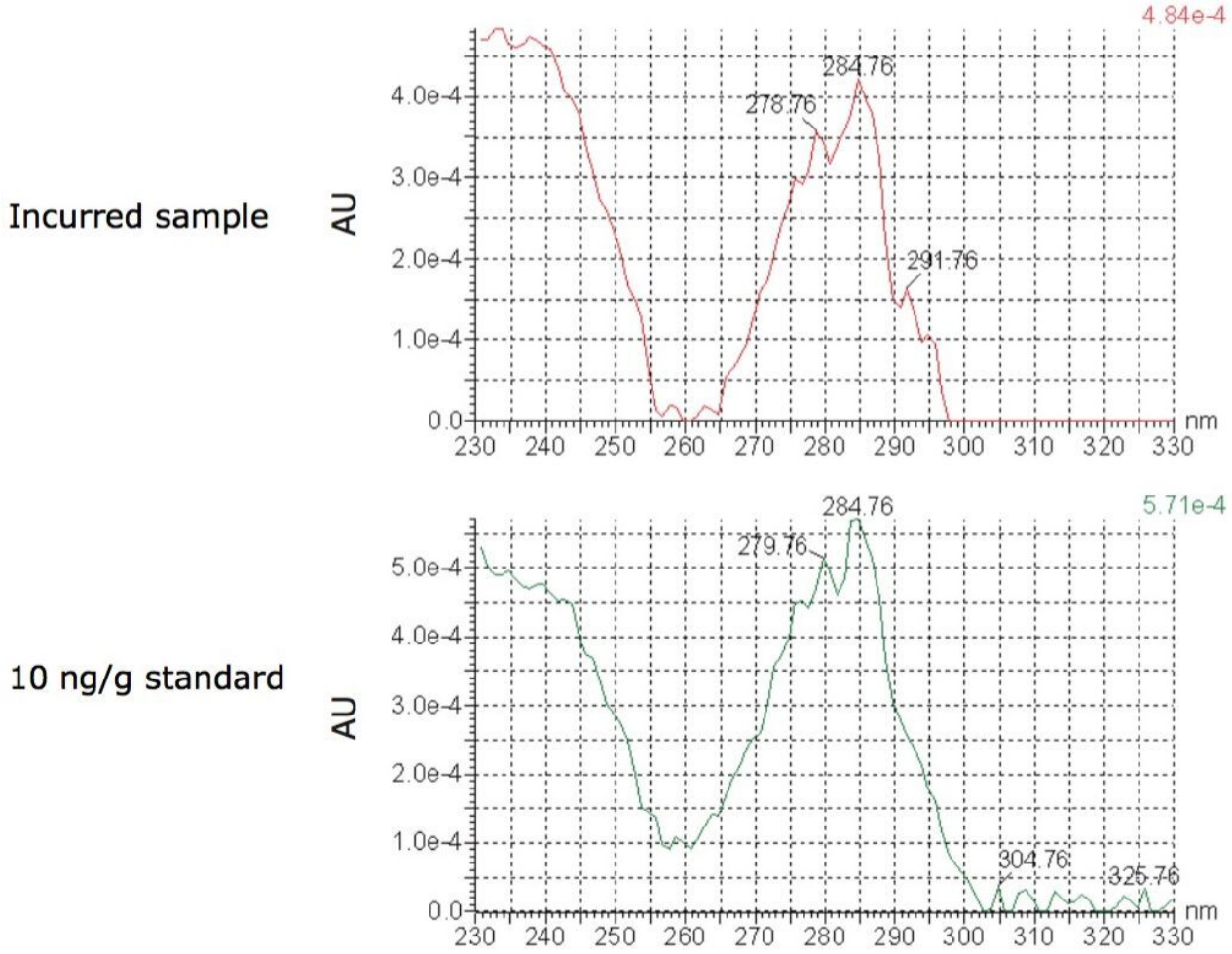

Figure 8. UV spectrum obtained from the incurred sample and the reference standard at 10 ng/g.

Conclusion

- The DisQuE pouch (QuEChERS) is effective for extraction of conazole fungicides from orange juice.
- The methodology provides high-throughput and low detection limits with a quick, simple, robust, and safe analysis.
- The QuEChERS extract can be readily utilized for UPLC-MS/MS analysis without further cleanup.
- A straightforward Oasis MCX SPE protocol provides effective sample cleanup and enrichment for low ppb detection limits for carbendazim using UV detection.
- Although not required, the Oasis MCX SPE cleanup gives improved performance for UPLC-MS/MS

analysis.

- An orange juice sample with incurred carbendazim was analyzed using LC/UV after SPE cleanup, and the results agreed with UPLC-MS/MS analysis.

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