LC/MS/MS of Fungicides and Metabolites in Orange Juice with Agilent Bond Elut Plexa and Poroshell 120

Application Note

Food Testing & Agriculture

Author

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Abstract

In January 2012, the US FDA announced that it began sampling import shipments of orange juice and will deny entry to shipments testing positive for carbendazim. This application note describes an analytical method for determination of carbendazim and 3 other fungicide residues in orange juice with easy sample preparation and LC/MS/MS detection. Developed for the 3 most important systemic fungicides, carbendazim, thiabendazole, and imazalil with its metabolite, this method can be easily expanded to include a broader range of pesticides. A simple and rapid extraction procedure provides clean extracts that without any preconcentration allow for detection of analytes at concentrations below 2 ppb. Quantification was performed using triphenyl phosphate as an internal standard. Excellent accuracy (% recovery) and precision (% CV) data were obtained for three concentration levels: 10, 50, and 250 ppb of each analyte.

Introduction

Fungicides are a group of pesticides used to inhibit fungal growth. Since fungicide residues in food present serious health concerns, they are regulated in Europe, United States, and many other countries. Fungicides carbendazim and thiabendazole belong to the benzimidazole class, and imazalil to the imidazole class of pesticides. Imazalil is easily metabolized to alpha-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol, and the sum of the parent compound and the metabolite is regulated in the United States.

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Benzimidazole fungicides are widely used in agriculture both for plant disease control and postharvest treatment of fruit. Carbendazim (MBC) is not registered for use in the US, but it is legally used in many other countries exporting their fruit and juice to the US. Also, carbendazim is a metabolite/degradate of the fungicide thiophanate-methyl (TM) which is registered for use on a number of crops and turf in the US, thus creating a potential for human exposure to MBC residues. Thiabendazole, as well as imazalil, is mostly used for postharvest treatment of fruit, including oranges, for storage and transportation, and both compounds are registered for use in the US.

The simple extraction procedure described here offers reproducible high recoveries of analytes of interest due to the advanced features of Agilent Bond Elut Plexa – a polymeric sorbent designed for improved analytical performance and ease-of-use. Along with superior retention of a broad range of analytes, Bond Elut Plexa provides fast and reproducible flow due to the narrow particle size distribution with no fines to cause blockages. Cleanliness of Bond Elut Plexa extracts results in low ion suppression, ensures extended HPLC column life and protects mass spectrometers from contamination.

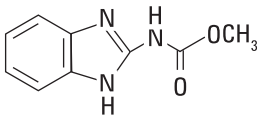
The superficially porous Agilent Poroshell 120 EC-C18 column with 2.7 µm particles ensures similar efficiency to sub-2 µm UHPLC columns but with about 40% less back pressure. During analysis, the actual pressure in the LC system does not exceed 400 bar.

Enhanced sensitivity of an Agilent 6460 Triple Quadrupole LC/MS system with the AJST electrospray source provides excellent detection of all four analytes of interest at 2 ppb. Because it is considerably below any existing testing requirements, our extraction method can be used with older and less sensitive models of mass spectrometers, such as the Agilent 6410 Triple Quadrupole LC/MS System. Users of MS detectors less sensitive than 6460 Triple Quadrupole could also consider preconcentration of their samples at a reconstitution step of the extraction procedure.

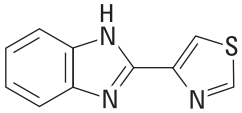
A previous method of imazalil and thiabendazole analysis in orange juice from Agilent [2] used Agilent Accubond C-18 SPE cartridges, an Agilent ZORBAX Eclipse XD-C8, 4.6 × 150 mm, 5 µm column and Agilent 1100 LC/MS Ion Trap system.

Experimental

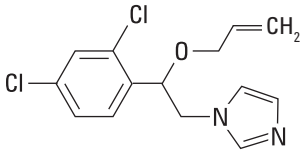
Analytes



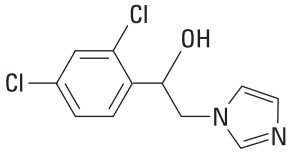
Carbendazim $C_9H_9N_3O_2$
m.w. 191.19
pKa 4.5 LogP 1.5



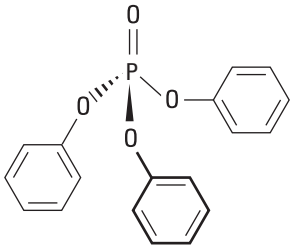
Thiabendazole $C_{10}H_7N_3S$
m.w. 201.25
pKa 4.7 LogP 2.4



Imazalil $C_{14}H_{14}Cl_2N_2O$
m.w. 297.18
pKa 6.5 LogP 3.8



Imazalil metabolite $C_{11}H_{10}Cl_2N_2O$
m.w. 257.12
LogP 2.6



Triphenyl phosphate $C_{18}H_{15}O_4P$
m.w. 326.28
LogP 4.7

Figure 1. Analyte structures and properties.

Pesticide standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). An internal standard (triphenyl phosphate) was from Agilent Technologies (Wilmington, DE, USA). Original stock solutions (0.25 mg/mL for carbendazim and 1 mg/mL for the other 3 analytes and internal standard) were prepared in methanol and stored at -20 °C.

Materials and instrumentation

SPE: Agilent Bond Elut Plexa cartridges 30 mg, 3 mL (part number 12109303)

Agilent vacuum manifold VacElut 20 (part number 12234100)

Agilent stopcock valves (part number 12234520)

Agilent silanized autosampler vials 2-mL (part number 5183-2072)

Agilent screw caps for AS vials (part number 5182-0717)

LC: Agilent Poroshell 120 EC-C18 2.1 × 50 mm, 2.7 µm column (part number 699775-902)

Agilent 1260 Infinity LC system (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, G1330B thermostat)

MS: Agilent 6460A Triple Quadrupole mass spectrometer with AJST electrospray ionization source

Sample preparation

Pretreatment

Spike 0.5 mL of juice with TPP (internal standard) at 50 ppb; use of 12 x 75 mm glass tubes is recommended. Dilute 1:3 with HPLC grade water, vortex. Centrifuge 15-20 min at 6000 rpm.

Extraction

1. Condition Bond Elut Plexa column with 0.5 mL methanol – soak, then let drip
2. Load sample/supernatants
3. Wash 1: 1 mL water
4. Wash 2: 1 mL of 30:70 methanol: water
5. Dry 5-10 min under vacuum (10-15 in Hg)
6. Elute with 1 mL 80 ethyl acetate: 20 IPA. Soak, then let eluate drip into collection vials. When dripping ceases, apply low vacuum (2-3 in Hg).
7. Evaporate under stream of nitrogen to dryness at 55 °C.
8. Reconstitute in 0.5 mL initial mobile phase (10% methanol, 90% water, 0.1% formic acid).

LC/MS/MS

LC conditions

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in methanol

Injection volume: 5 µL

Flow rate: 0.5 mL/min

Gradient

Time (min)	% B
0.0	10
0.5	10
2.0	50
3.0	95
7.0	95
7.1	10

Stop time: 7.2 min

Post time: 2.5 min

Max pump pressure: 400 bar

Needle wash: Flush port 75 methanol: 25 water for 10 s

Disable overlapped injection

No automatic delay volume reduction

MS conditions

ES source parameters

Ionization mode: Positive

Capillary voltage: 2,800 V

Drying gas flow: 12 L/min

Drying gas temperature: 350 °C

Nebulizer gas: 40 psi

Sheath gas flow: 12 L/min

Sheath gas temperature: 300 °C

Nozzle voltage: 0 V

MS parameters

Scan type:	Dynamic MRM
Pre-run script:	SCP_MSDiverterValveToWaste() {MH_Acq_Scripts.exe}
Time segments #1:	1.5 min - diverter valve to MS
Delta EMV:	(+) 400 V

Results and Discussion

All four fungicides of interest and an internal standard (TPP) are efficiently retained by Bond Elut Plexa – a polar-enhanced styrene-divinylbenzene neutral polymeric sorbent. This sorbent is the best choice for non-ionic extraction of a wide range of acidic, neutral and basic analytes. We developed a very simple extraction method where dilution of juice with water is the only pretreatment required. Centrifugation is recommended with most orange juices if loading and washing of samples by gravity flow is desired. With centrifuged samples, vacuum is only needed to dry the column before elution and to maximize recovery at the end of the elution step. Gravity flow on Bond Elut Plexa generally occurs at a slower rate than vacuum-induced flow, and thus provides more time for interaction with the sorbent. Without centrifugation, vacuum has to be applied at all steps of the extraction including loading, washing and elution. This should be done very gradually, aiming to achieve a 1-2 mL/min dripping rate from the SPE cartridge.

An increase in methanol concentration at the 2nd wash step from 5% (generic method) to the 30% recommended here allows to significantly reduce matrix interferences. This results in enhanced sensitivity of LC/MS/MS detection for earlier eluting compounds: by 20% for carbendazim and by 60% for thiabendazole, and by 5-7% for imazalil metabolite. Imazalil peak areas remain unchanged. Combination eluent (80 ethyl acetate: 20 IPA) compared to 100% methanol only slightly improves recovery of the four fungicides but gives about a two-fold increase in recovery of the highly hydrophobic internal standard (TPP).

A list of dynamic MRM transitions for four fungicides and internal standard is given in Table 1, showing firstly a quantifier, and secondly a qualifier transition.

The Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm column provides separation of 4 fungicides and TPP within 5 minutes. Figure 2 shows chromatograms of quantifier transitions of 4 fungicides in orange juice spiked at 2 ppb, with TPP as internal standard at 50 ppb. Signal-to-noise ratio for the peaks of 3 major fungicides is higher than 150:1, and it is 49:1 for the imazalil metabolite peak. Obviously, a detection limit for each of the analytes will be at ppt level. Each sample run begins with diverting a first portion of flow (0 to 1.5 minutes) to waste to minimize source contamination.

Table 1. Dynamic MRM transitions.

Compound name	ISTD?	Prec ion	MS1 res	Prod ion	MS2 res	Frag (V)	CE (V)	Ret time	Ret window	Polarity
Carbendazim	☐	192.1	Unit	160.1	Wide	95	15	2.1	2	Positive
Carbendazim	☐	192.1	Unit	132.1	Wide	95	35	2.1	2	Positive
Thiabendazole	☐	202	Unit	175	Wide	110	27	2.4	2	Positive
Thiabendazole	☐	202	Unit	131.1	Wide	110	35	2.4	2	Positive
Imazalil metabolite	☐	257	Unit	125	Wide	110	32	2.9	2	Positive
Imazalil metabolite	☐	257	Unit	69	Wide	110	22	2.9	2	Positive
Imazalil	☐	297.1	Unit	159	Wide	130	23	3.6	2	Positive
Imazalil	☐	297.1	Unit	69	Wide	130	22	3.6	2	Positive
Triphenyl phosphate	☑	327.1	Unit	77	Wide	170	40	4	2	Positive
Triphenyl phosphate	☑	327.1	Unit	152	Wide	170	45	4	2	Positive

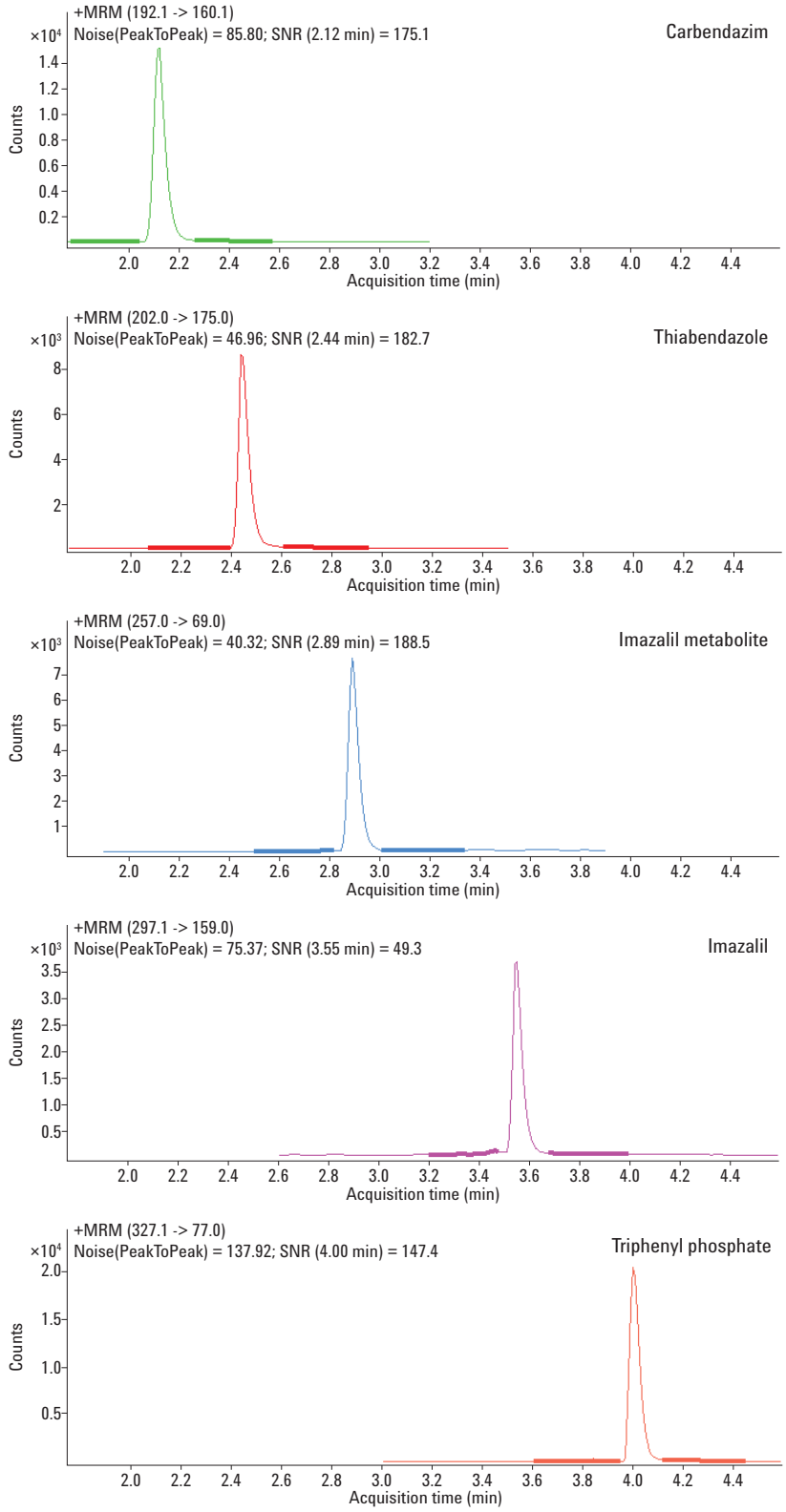


Figure 2. MRM extracted ion chromatograms for four fungicides (2 ppb) and TPP (50 ppb) in orange juice extract. Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm column. Noise regions are shown in bold.

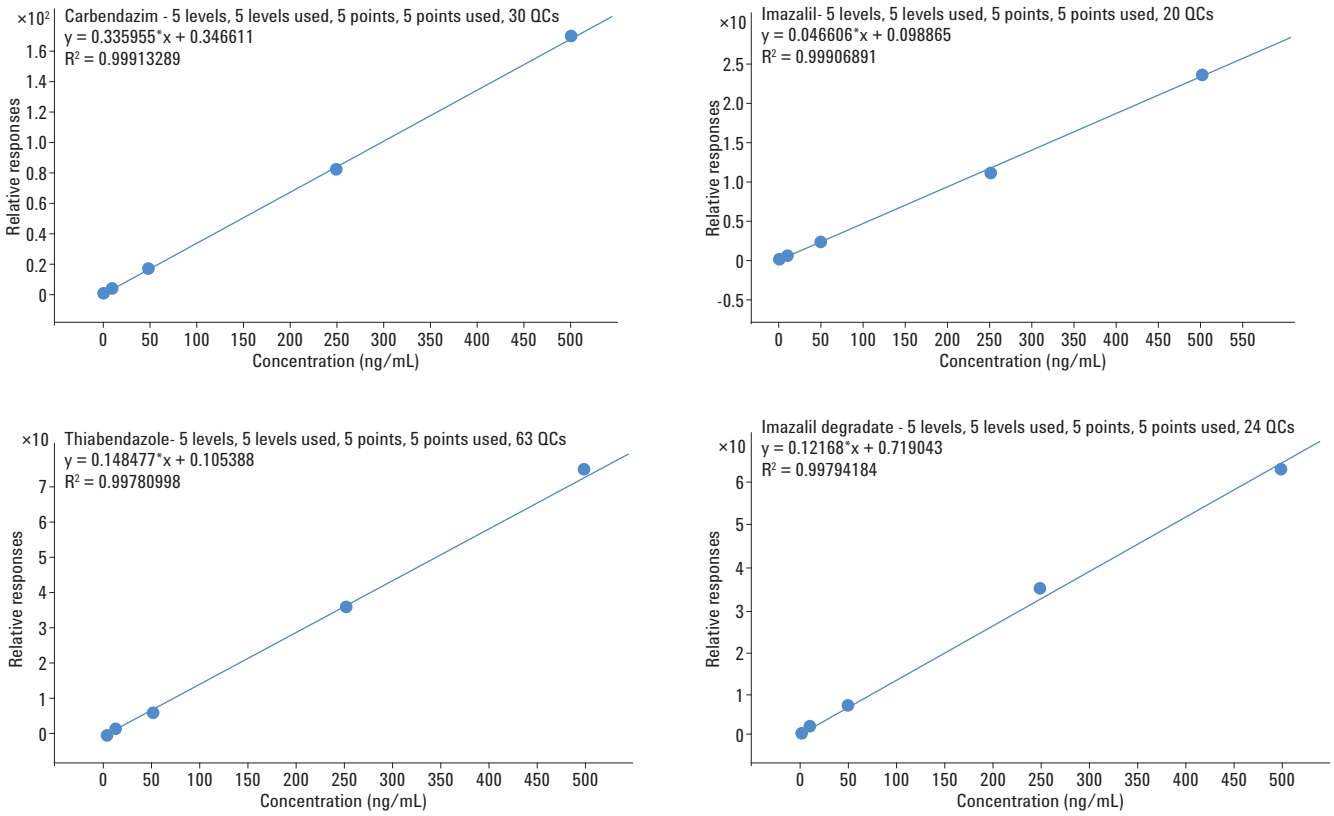


Figure 3. Example calibration curves for four fungicides in orange juice extract. Calibration range 2.0 to 500 ppb. All fits are linear, $R^2 \geq 0.998$.

Table 2. Method performance for fungicides in orange juice, n = 5.

		10 ppb		50 ppb		250 ppb	
Compound name	**R^2**	**Accuracy, (%)**	**CV, (%)**	**Accuracy, (%)**	**CV, (%)**	**Accuracy, (%)**	**CV, (%)**
Carbendazim	0.999	103.5	1.7	100.1	0.7	99	2.5
Thiabendazole	0.998	101.2	1.4	96.3	3.2	99.9	2.9
Imazalil	0.999	105.3	1.3	100.2	0.9	101.2	1.8
Imazalil degradate	0.998	101.7	3.5	103.3	2.8	101.6	3.1

Conclusions

Solid phase extraction procedure coupled with LC/MS/MS detection described here is simple and robust, providing highly accurate and reproducible results. The properties of the Bond Elut Plexa sorbent allow expansion of this method for a multiresidue pesticide analysis. Separation of the analytes of interest is completed within 5 minutes and can be performed on most of the Agilent LC systems.

MS source parameters developed for the Agilent 6460 Triple Quadrupole can be easily modified for use with other Agilent Triple Quadrupole LC/MS systems. Preconcentration at the reconstitution step can be applied when the extraction method is used with detectors of lower sensitivity.

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