Application Note : 1304

High-Throughput Determination of Carbendazim in Orange Juice using Strong Cation Exchange SPE and LDTD–MS/MS

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Introduction

Carbendazim is a fungicide used in many countries to preserve agricultural crops. The use of carbendazim on oranges is not approved by the Environmental Protection Agency (EPA) due to its known effects on male fertility. However, the Food and Drug Administration (FDA) has received reports of the presence of carbendazim in imported orange juices.

FDA refuses shipments of any orange juice showing detectable amounts (> 10 ng/mL). Most analytical methods have an LOD around 1 ng/mL, but require LC runtime of minutes. We developed a method using strong cationic exchange (SCX) SPE cartridges and LDTD-APCI-MS/MS for the determination of carbendazim in orange juice in less than 10 seconds.

Solid-Phase Extraction Cartridge

The Silia*PrepX*™ SCX cartridge available from SiliCycle® was used for the sample extraction procedure.

Figure 1: Silia*PrepX* SCX cartridge

Table 1: Silia*PrepX* SCX product number

Silia*PrepX* SCX Formats		
Formats	Qty/Box	Product Number
Silia*PrepX* Polymeric SPE Cartridges		
1 mL/30 mg	100	SPE-P0005-01AA
3 mL/60 mg	50	SPE-P0005-03BB
6 mL/100 mg	30	SPE-P0005-06C
6 mL/200 mg	30	SPE-P0005-06G
6 mL/500 mg	30	SPE-P0005-06P
Silia*PrepX* Polymeric 96-Well Plates		
2 mL/10 mg	1	96W-P0005-1A
2 mL/30 mg	1	96W-P0005-AA

LDTD-MS/MS System

Figure 2: LDTD system on Thermo Vantage Mass Spectrometer

Sample Method

Extraction procedure

1ml of orange juice was centrifuged (2 min/14,000 rpm)

Loading solution :	500 µL of supernatant
	1 mL acetic acid (10%)
	50 µL carbendazime-d4 (400 ng/mL in MeOH)
Cartridge:	Silia*PrepX* SCX (1 mL/30 mg)
Activation:	1 mL MeOH
	1 mL acetic acid (10%)
Load:	1.5 mL of loading solution
Wash 1:	1 mL Acetic acid (10%)
Wash 2:	1 mL MeOH
Elution:	1.5 mL MeOH/NH_4OH (95/5) (v/v)

Eluate was evapored to dryness

Reconstitution with 1.5 mL of MeOH/Water (75/25) with HCl (1 mM)

Spot 2 µL in LazWell plate.

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	45
	5.1	0
	8	0

MS/MS Method

	Transition	CE	S-Lens
Carbendazime	192->160	15	80
Carbendazime-d4	196->164	15	80
Mode:	Positive		

Results and Discussion

Linearity results

As shown in Figure 3, excellent linearity ($r^2 > 0.99$) with no signs of carryover effect was achieved within the quantification range (0.5 to 250 ng/mL).

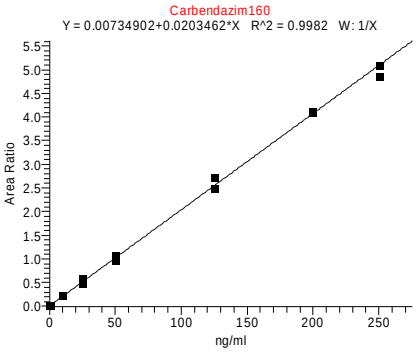

Figure 3: Typical standard curve

Accuracy and precision

As shown on Table 2, the intra-run accuracy and precision are between 99.4 to 113.6% and 3.9 to 15.1%.

Table 2: Intra-run precision and accuracy

	LLOQ	Low-QC	Mid-QC	High-QC	ULQC
Conc. (ng/mL)	0.5	1.5	75	175	250
N	6	6	6	6	6
Mean (ng/mL)	0.50	1.70	78.67	194.39	260.25
%RSD	15.1	3.9	6.3	5.1	4.1
%Nom	99.4	113.6	104.9	111.1	104.1

Recovery

Recovery of 93% ± 2 was obtained for all concentrations within the linear range using the Silia*PrepX* SCX cartridges.

Matrix effect

Matrix effect was also evaluated by adding a known concentration (10 ng/mL) of carbendazim in different orange juice brands. As shown in Table 3, no matrix effect was observed using four different orange juice brands.

Table 3: Matrix effect evaluation

	Brand 1	Brand 2	Brand 3	Brand 4
Conc. (ng/mL)	10	15.5[1]	10	10
N	3	3	3	3
Mean (ng/mL)	10.80	16.60	10.72	11.20
%RSD	3.9	5.2	3.7	2.2
%Nom	108.0	107.1	107.2	112.0

1. Concentration of 5.5 ng/mL was detected in this juice before carbendazim spiking.

LC-MS/MS vs LDTD-MS/MS correlation data of real sample analysis.

Four different orange juice brands were extracted and analyzed using LDTD-MS/MS and LC-MS/MS technologies. As shown in Table 4, same results were obtained using both techniques.

Table 4: Comparison sample analysis LDTD-MS/MS vs LC-MS/

Orange Juice Identification	LDTD-MS/MS Results	LC-MS/MS Results
Brand 1	< LLOQ	< LLOQ
Brand 2	5.5 ± 0.2	5.6 ± 0.1
Brand 3	< LLOQ	< LLOQ
Brand 4	< LLOQ	< LLOQ

As a confirmation analysis, series of carbendazim additions were done for Brand 2. As shown in Figure 4, same concentration was obtained using serial carbendazim addition.

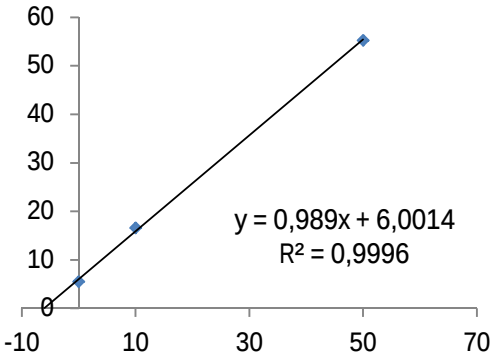

Figure 4: Serial carbendazim addition curve spiked in orange juice Brand 2

Conclusions

The solid phase-extraction using Silia*PrepX* SCX cartridges ensure accurate and precise results with a linear standard curve ($r^2 > 0.99$) for carbendazim extraction in orange juice.

Good correlation between LDTD-MS/MS and LC-MS/MS result.

A fast analysis can be reached using LDTD-MS/MS system. LDTD provides the high-throughput analysis (at least 10 times faster comparatively to LC-MS/MS) of carbendazim in orange juice in 9 seconds sample-to-sample.

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