APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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Analysis of Target Pesticide Residues in Berries with LC/MS/MS Coupled with a QuEChERS Sample Preparation

Introduction

Pesticides are widely used in agriculture to protect plants from a variety of pests and to increase productivity. However, the extensive use of pesticides can pose a health risk to humans and this has led to worldwide stringent regulations, for maximum allowable limits for these residues in foods. Among the routinely used testing methods, LC/MS/MS has become the method of choice, due to its high sensitivity, reliability and accuracy.

In the present study, a unique laminar flow UPLC-ESI-MS/MS triple quad mass spectrometer was used to identify and quantitate 40 pesticides in four brands of non-organic berries. The QuEChERs extraction method proved both rapid and reliable for extracting pesticide residues in the heavily pigmented berry samples.

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Experimental

Hardware/Software

Chromatographic separation was conducted by a PerkinElmer Altus® A-30 UPLC® System and detection was achieved using a PerkinElmer QSight™ 220 MS/MS detector with dual ionization source. All instrument control, data acquisition and data processing was performed using the Simplicity 3Q™ software platform.

Method parameters

The LC method and MS source parameters are shown in Table 1.

Table 1. LC Method and General MS Conditions.

LC Method				
Column: PerkinElmer Brownlee Phenyl-Hexyl column, 2.7 µm, 2.1 x 100 mm				
Mobile Phase: Solvent A: 5 mM ammonium formate in water Solvent B: 5 mM ammonium formate in methanol				
	Time (min)	%A	%B	Flow rate (ml/min)
1	Initial	90	10	0.3
2	1	90	10	0.3
3	15	5	95	0.3
4	17	5	95	0.3
5	17.1	90	10	0.3
6	20	90	10	0.3
Oven Temp.: 40 °C				
Injection Volume: 20 µL				
General MS Conditions				
ESI voltage:	5000 V			
Drying gas:	120			
HSID Temp:	200 °C			
Entrance voltage:	30 V			
Source Temp:	325 °C			
Nebulizer gas:	350			
Detection Mode:	MRM Mode			

Solvents, Standards and Sample Preparation

Berry samples were obtained from a local grocery store in Ontario, Canada. Pesticide standards were obtained from ULTRA Scientific® (North Kingstown, RI). All solvents, reagents and diluents used were HPLC grade.

Samples were prepared using Supra-d™ QuEChERS kits (AOAC 2007.01 method). Briefly, samples were homogenized using a blender at high speed, and 10 g of homogenized samples were weighed and transferred to a 50 mL extraction tube containing 6 g of $MgSO_4$ and 1.5 g of sodium acetate. 10 mL of cold acetonitrile was then added and vortexed until the salt was completely mixed. The solution was then centrifuged at 3500 rpm for 5 minutes. 4 mL of supernatant was then transferred to a 15 mL clean-up tube (AOAC 2007.01 Clean-up Kits), vortexed for 3 minutes and centrifuged for 5 minutes.

0.1 mL of supernatant was transferred to a 1.5-mL centrifuge tube, diluted 10-fold with mobile phase A and then centrifuged at 4000 rpm for 5 minutes. The resulting supernatant was transferred to a 1.5 mL LC vial for direct LC/MS/MS analysis.

Optimizing MS/MS Parameters

Source parameters, including gas flows, source temperature and position settings, were optimized to achieve the best sensitivity. The Q1 and Q2 quadrupole peak widths were set at 0.7 amu. Multiple reaction monitoring mode (MRM) transitions are listed in Table 2.

Table 2. Optimized compound-dependent MS parameters for tested pesticides (partial list).

Compound	Precursor Ion	Product 1	CE1	Product 2	CE2
Atrazine	216.1	174.1	15	132.0	20
Azoxystrobin	404.1	372.1	18	344.1	34
Bifenazate	301.1	198.0	16	170.0	20
Boscalid	343.0	307.0	25	140.0	28
Cyprodinil	226.0	93.0	48	108.0	34
Flonicamid	230.1	203.1	20	174.0	20
Hexythiazox	353.0	228.0	20	168.0	34
Pyraclostrobin	388.0	194.0	16	163.0	36
Pyrimethanil	200.0	107.0	33	82.0	32
Thiamethoxam	292.0	211.0	18	181.1	28

Results and Discussion

Figure 1 shows example chromatograms of the pesticides analyzed in MRM mode at 1 ng/mL. All of the tested pesticides were detected with good signal to noise even at concentrations well below the regulatory limits.

The method showed excellent linearity ($R^2 \geq 0.99$) over three orders of concentration (0.1-100 ng/mL for most analytes). Some of the calibration curves are shown in Figure 2.

The limit of quantification (LOQ) was 0.1 ng/mL for most of the analytes, which is well below the regulatory limits of 10 ng/mL.

Recoveries of the pesticides were determined by spiking 10 µg/kg of pesticides in three different berry samples (raspberry, blackberry and blueberry) in triplicates. Both the mean recovery and reproducibility (RSD) were determined for each berry/analyte combination. The recoveries were between 70 and 115% with a RSD of <20% for the berry/analyte combinations.

Berry samples bought in local grocery stores were tested for pesticide residues using the developed method. Figures 3-5 show the chromatograms of berry samples with positive hits for the target pesticides. The calculated concentrations of those pesticides are listed in Table 3, which ranged from 4.5 to 447.3 µg/kg.

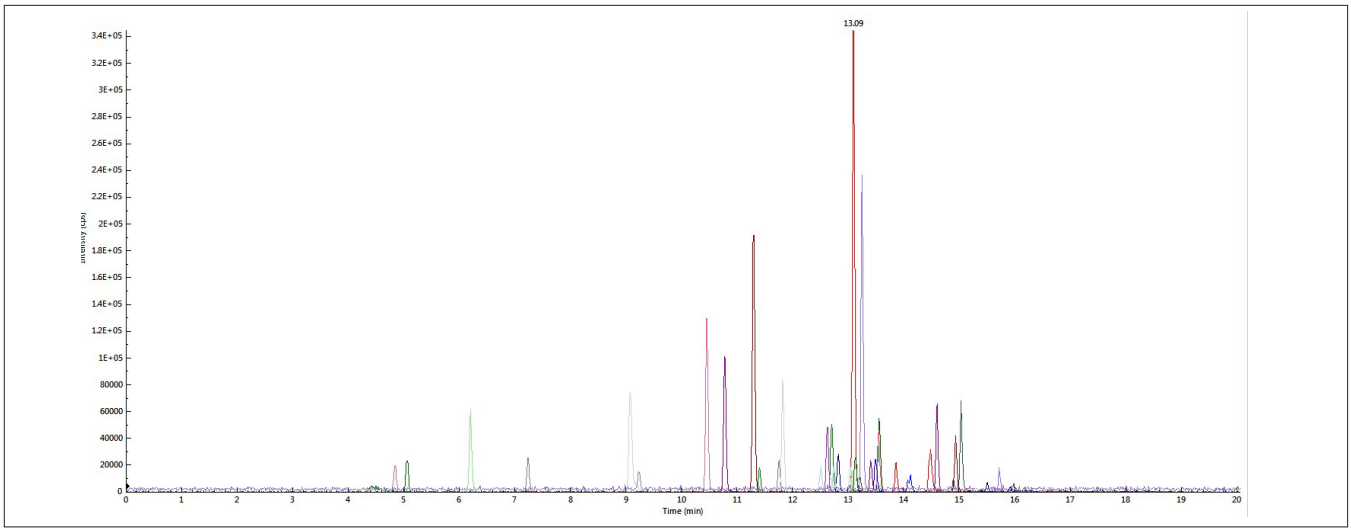

Figure 1. MRM chromatogram of pesticides at 10 ng/mL in neat solution.

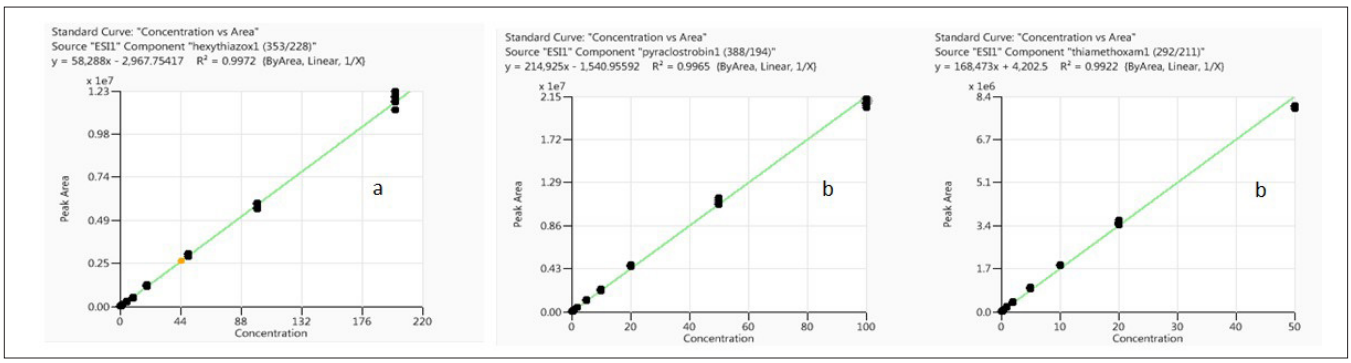

Figure 2. Calibration curves for hexythiazox (a), pyrochlostrobin (b), and thiamethoxam (c) with 6 injections at each concentration level (ng/mL).

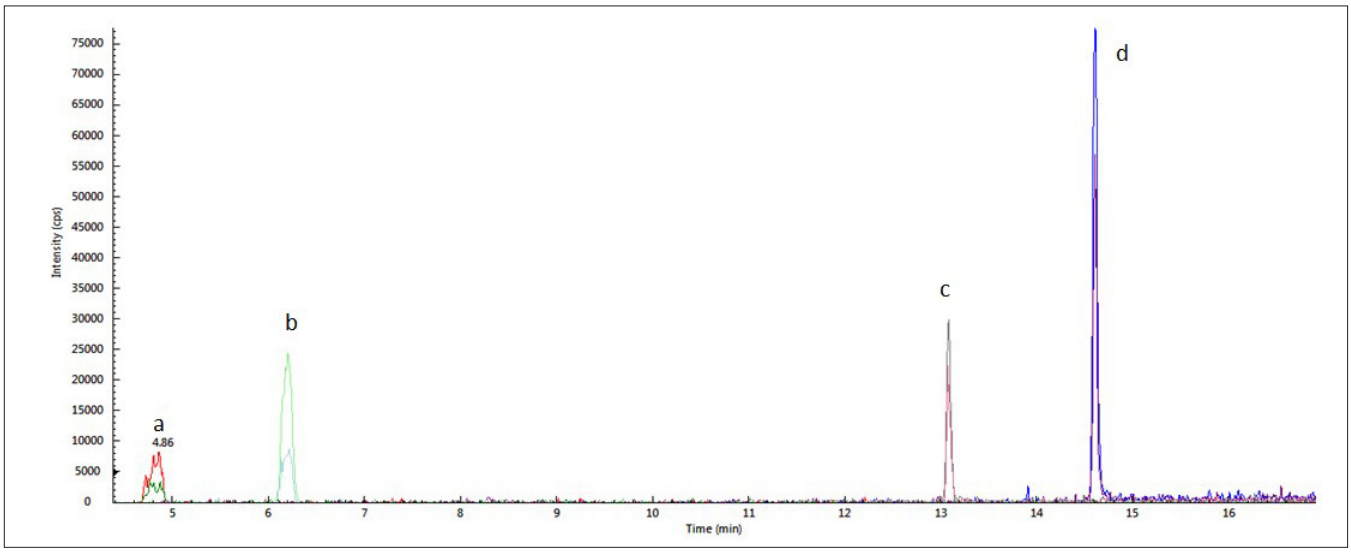

Figure 3. Pesticides identified and quantified from brand A blueberry. The pesticides are flonicamide (a), thiamethoxam (b), pyrimethanil (c), and bifenazate (d).

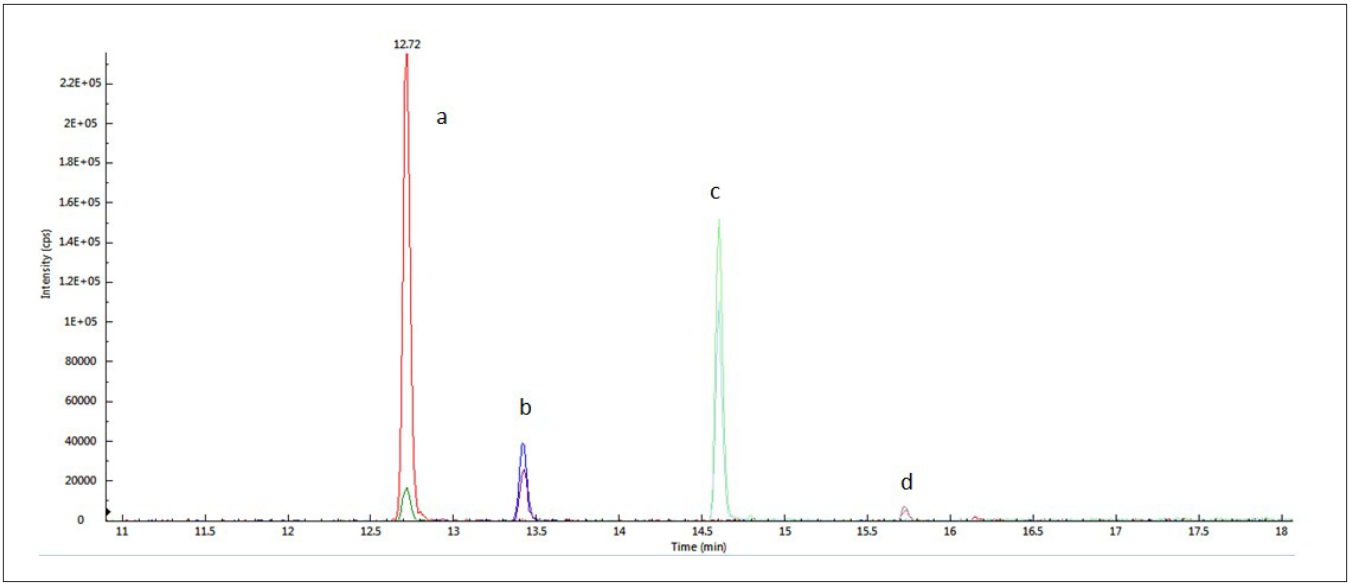

Figure 4. Pesticides identified and quantified from brand B blueberry. The pesticides are boscalide (a), cyprodinil (b), pyrochlostrobin (c), and hexythiazox (d).

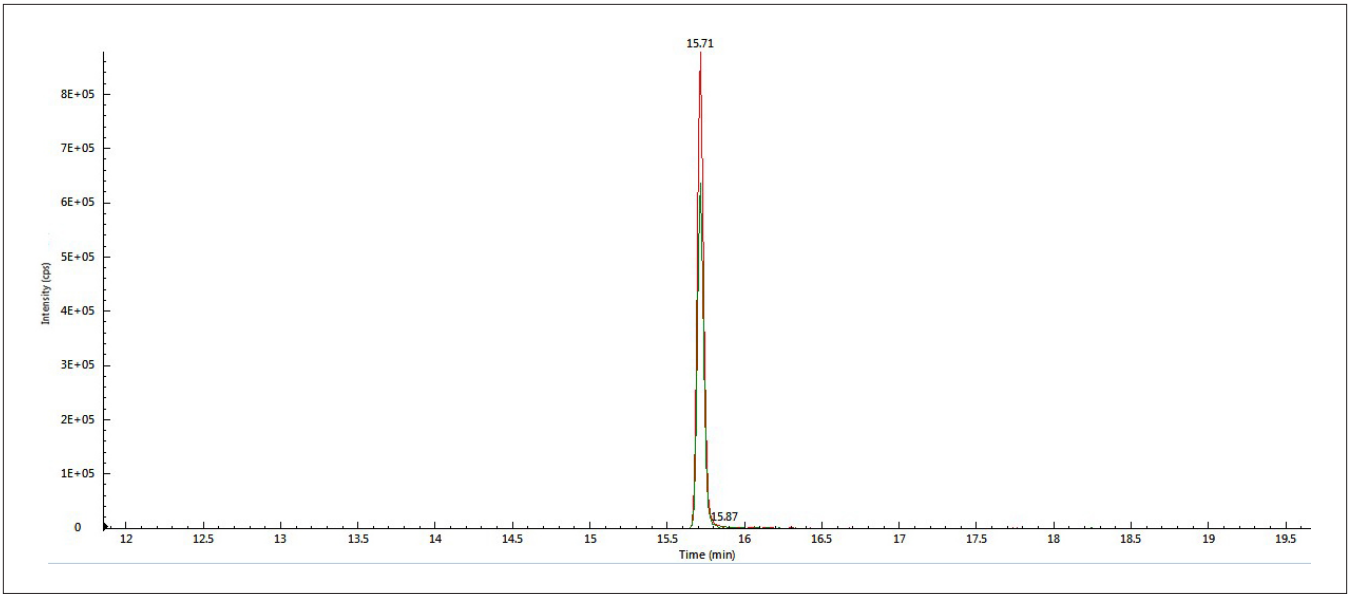

Figure 5. Pesticides identified and quantified from brand D blackberry. Only hexythiazox was detected.

Table 3. Summary of Pesticides found in berry samples.

	Pesticide	Concentration (µg/kg)
Blueberry (Brand A)	Pyraclostrobin	11.8
	Flonicamid	9.7
	Bifenazate	12.6
	Thiamethoxam	9.5
	Pyrimethanil	11.8
Blueberry (Brand B)	Cyprodinil	16.8
	Pyraclostrobin	23.2
	Boscalid	51.1
	Hexythiazox	4.5
Blueberry (Brand C)	N/A	--
Blackberry (Brand D)	Hexythiazox	447.3

Conclusions

A LC/MS/MS method for multi-pesticide residue analysis in berries was developed using a PerkinElmer Altus UPLC® system coupled to a QSight 220 triple-quad mass spectrometer.

The simple/routine sample preparation approach used in this work provides the following advantages: 1. dilution of the QuEChERS extract with water makes the sample extract more compatible with typical reversed phase separation, leading to reduced solvent effects; 2. dilution also helps to reduce any potential matrix effects, leading to more accurate and reproducible results.

These results demonstrated this methods applicability and effectiveness in detecting and quantitating pesticides lower than 10 parts per billion (ppb), per regulatory limits set by the EU directive 91/414/EEC.

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