

Tyler Trent¹, Rick Jordan²¹Teledyne Tekmar, 4736 Socialville Foster Road; Mason, Ohio 45040, USA; tyler.trent@teledyne.com²Pacific Agricultural Laboratory, 12505 N.W Cornell Road; Portland, Oregon 97229, USA

Application Note

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Abstract

The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method was developed for the determination of pesticide residues in agricultural commodities. Since development, QuEChERS has been applied for the extraction of many different classes of pesticides, with one exception, very polar non-QuEChERS-amenable pesticides. In 2008 at EPRW in Berlin the “Quick Method for Analysis of Residues of numerous Highly Polar Pesticides in Food of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination” (QuPpe Method) was presented.

The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40 for the extraction of highly polar pesticides using the QuPpe Method. Liquid Chromatography coupled to triple-quadrupole mass spectrometry (LC-MS/MS) was employed for the detection of highly polar pesticides in agricultural commodities. Quantification was based on matrix-matched calibration curves for each commodity. The AutoMate-Q40 streamlines the QuPpe Method while providing suitable analytical results falling in the method guidelines (range of 70-120% and RSD <20%) for all target compounds.

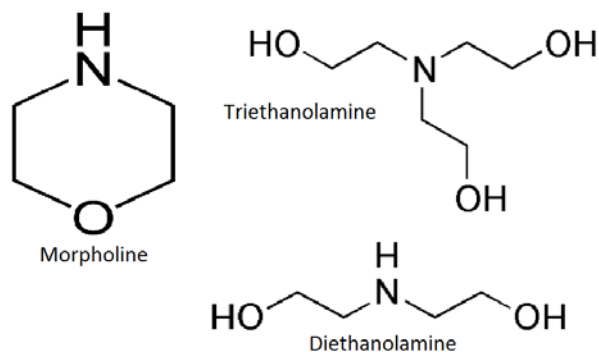
Introduction

In the global market for fresh fruit the United States is a major exporter of apples and orange fruits to various countries. However, exporters need to be mindful of the maximum residue levels (MRLs) of pesticides and food additives for various countries.¹ Since many of these fruits need to be shipped over a long distances a coating is applied post-harvest with a glazing agents to shield their surfaces from insects and fungi.² Three components of these coatings, Morpholine, Diethanolamine (DEA), and Triethanolamine (TEA) have come under scrutiny by the European Union (EU) due to the toxicological concerns with these compounds inherent tendency to oxidize to nitroso compounds which are known carcinogens.² Although the U.S. packing lines used for export have been cleaned and free of waxes containing these compounds, inadvertent residues at low levels can occur.³



Currently there is a validated method in the EU “Quick Method for Analysis of Residues of numerous Highly Polar Pesticides in Food of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination (QuPpe-Method)” to determine Morpholine, DEA, and TEA (Figure 1) residues on waxed commodities. The QuPpe-Method was designed for the residue analysis of very polar, non-QuEChERS amenable, compounds.

Figure 1 Chemical structure



The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40 for the extraction of highly polar compounds using the QuPPE-Method M7. Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (LC-MS/MS) was used to analyze the samples prepared with the AutoMate-Q40. Quantification was based on matrix-matched calibration curves for apples and oranges. QC samples were evaluated at levels of 0.01, 0.05 and 0.1 mg/kg to ensure the precision and accuracy of the AutoMate-Q40.

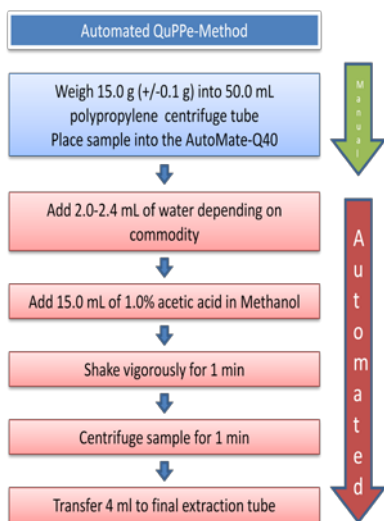
Experiment - Instrument Conditions

Sample Preparation/Extraction

Apple and orange samples were milled to reduce particle size and improve homogeneity. The samples were then stored at -20 °C until the samples were extracted.

Figure 2 shows the sample preparation and extraction steps that are needed to extract Morpholine, DEA, and TEA residues from apples and oranges. The following extraction was performed at Pacific Agricultural Laboratory (PAL).

Figure 2 Automated QuPPE-Method Extraction on the AutoMate-Q40



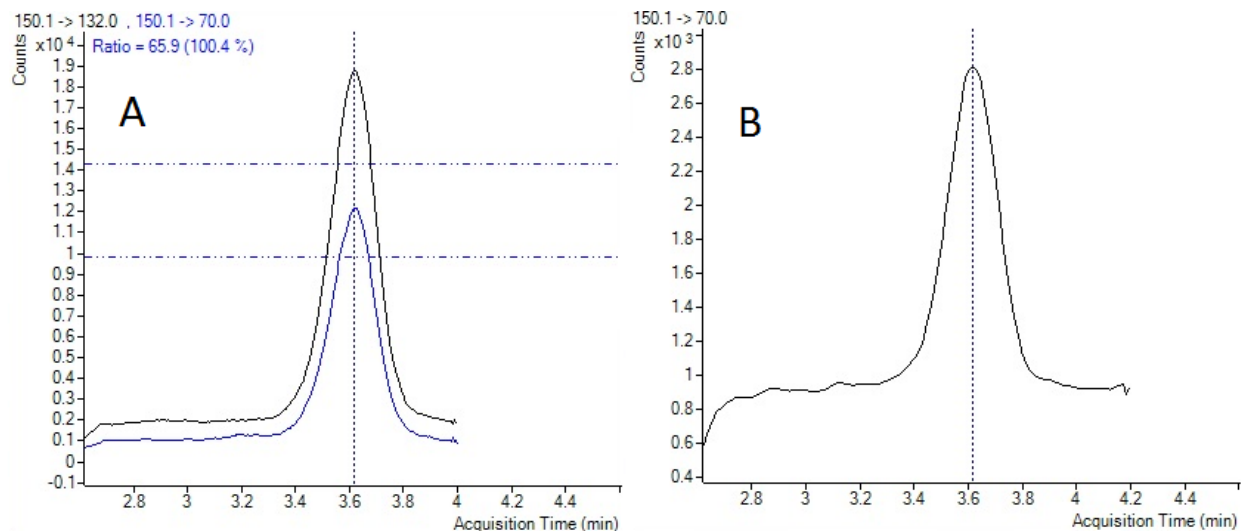
Sample Analysis

At PAL, sample analysis was conducted using an Agilent 1260 HPLC system coupled to an Agilent 6460 tandem mass spectrometer (MS/MS) via electrospray ionization (ESI). See Table I for LC-MS/MS instrument-specific conditions. The mass transitions for each analyte are presented in Table II. Typical chromatograms of standards can be seen in Figures 3-5.

Table I LC-MS/MS Parameters	
Agilent 1260 HPLC	
Column	Sielc Primesep-A, 2.1 mm x 100mm, 5µm (HILIC)
Column Flow	0.55 mL/min
Injection Vol.	4.0 µL
Mobile Phase 80%A/20%B	A: 20mM Ammonium formate, 0.1% formic acid in 95:5 water/methanol
	B: 20mM Ammonium formate, 0.1% formic acid in 95:5 methanol/water
Agilent 6460 Triple Quadrupole	
Gas Temp	300.0 °C
Drying gas (N ₂)	9.0 L/min
Nebulizer Pressure	45.0 psi
Capillary Voltage	4000.0 V
Sheath Gas Temp	350 °C
Sheath Gas (N ₂)	11.0 L/min

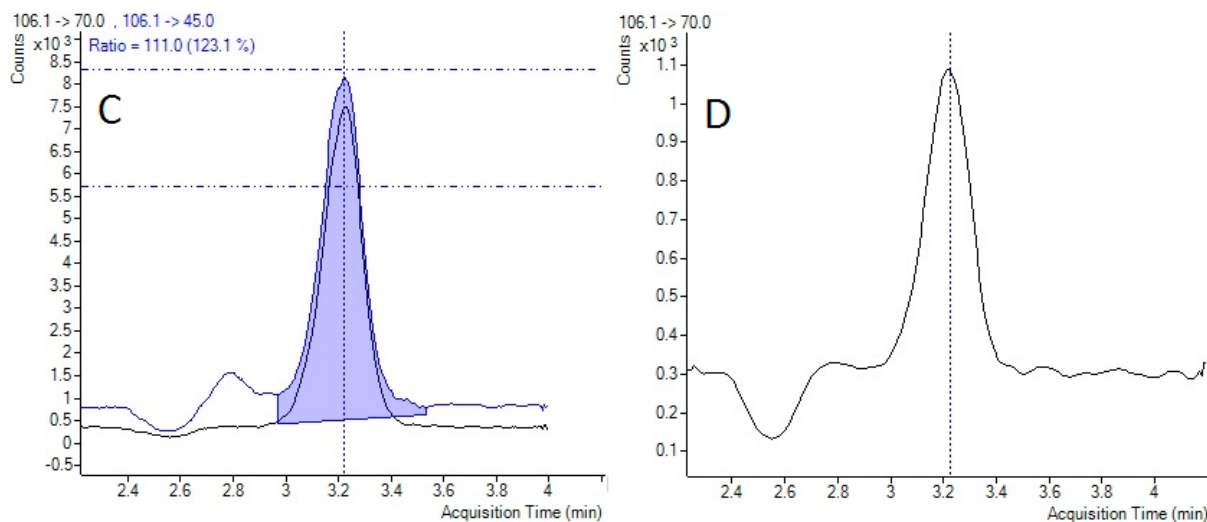
Table II MS/MS Acquired Mass Transitions			
Compound	Precursor Ion (m/z)	Quantization Product Ion (m/z)	Confirmation Product Ion (m/z)
Morpholine	88.1	70.1	44.0
Diethanolamine (DEA)	106.0	70.0	45.0
Triethanolamine (TEA)	150.0	70.0	132.0

Figure 3 Extracted Ion Chromatograms of TEA



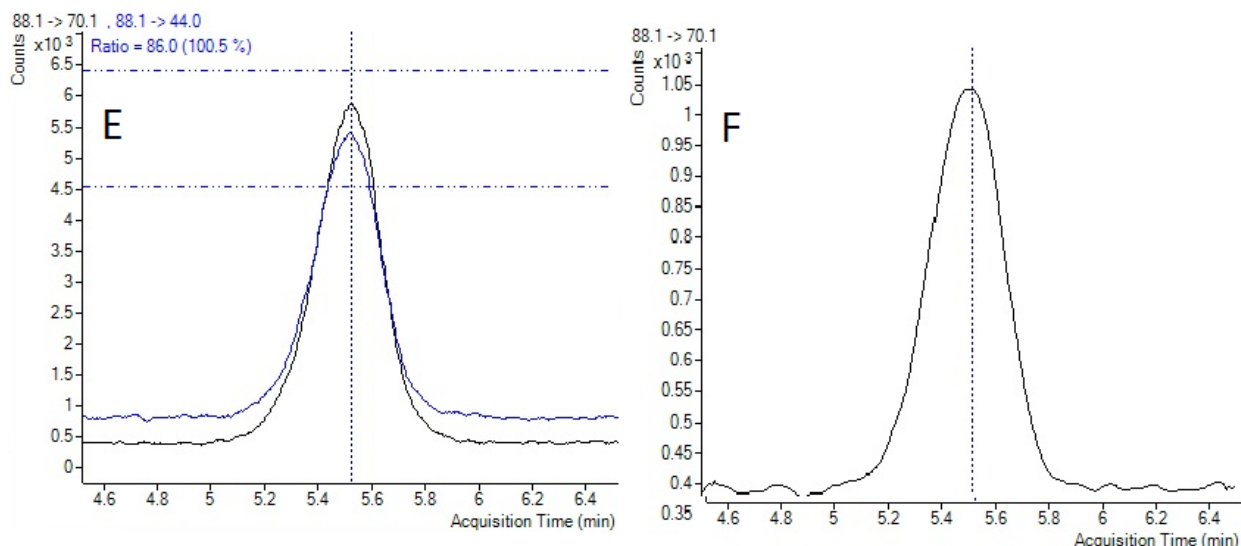
Note: (A) MRM transition of 0.05 ug/g of TEA (B) Extracted ion chromatogram of 0.01 ug/g of TEA

Figure 4 Extracted Ion chromatograms of DEA



Note: (C) MRM transition of 0.05 ug/g of DEA (D) Extracted ion chromatogram of 0.01 ug/g of DEA

Figure 5 Extracted Ion chromatograms of Morpholine



Note: (E) MRM transition of 0.05 ug/g of Morpholine (F) Extracted ion chromatogram of 0.01 ug/g of Morpholine

Results

Automating the QuPPE extraction, enables fast, easy, reliable and more highly reproducible extractions. The AutoMate-Q40 offers labor savings, and improves the repeatability and consistency between samples.

A precision and accuracy study was performed at Pacific Agricultural Laboratory using the AutoMate-Q40. The system was able to fortify the apple and orange samples at 0.01, 0.05, and 0.10 mg/kg using the AutoMate-Q40's ability to make standard additions. All control samples used for this study showed no significant residue for the apple samples but there was TEA present in the orange samples, so no validation was performed in the orange for TEA.

Table III Analytical Results for the QuPPE M7 Method										
Matrix	Fortification Level mg/kg	Morpholine			Diethanolamine (DEA)			Triethanolamine (TEA)		
		Recovery %	RSD %	MDL*	Recovery %	RSD %	MDL*	Recovery %	RSD %	MDL*
Apple	0.10	113.00%	6.20	0.0039	106.00%	5.66	0.0036	102.00%	6.86	0.0043
	0.05	116.00%	1.72		112.00%	1.79		108.00%	1.79	
	0.01	100.00%	10.0		100.00%	10.00		110.00%	9.09	
Orange	0.10	96.00%	4.17	0.0034	83.00%	4.82	0.0037			
	0.05	100.00%	4.00		82.00%	2.44				
	0.01	110.00%	9.09		70.00%	14.29				
Calculated Method Detection Limit (MDL) = Stdv of concentration found in 0.01 mg/kg spike * Student t99 (n-1), for seven reps (n=7), Student t= 3.143										

Table III shows that when using the AutoMate-Q40 to extract highly polar residues from apples and oranges recoveries ranging from 70.00% to 116.0%. Table III also shows that the results have excellent precision ranging from 1.72% to 14.29%.

Conclusion

Automation of the QuPPE extraction method produced reliable results for the spiked samples, which compared favorably with those from the existing manual procedure. There was excellent agreement between results for the analysis of samples prepared manually and using the automated system.⁴ Automating the procedure led to improved repeatability, a reduction in the likelihood of human error and the potential for significant labor savings.

Precision and accuracy were assessed for the two commodities analyzed. Results for the automated procedure were well within the criteria set forth in this study: Average recoveries for the range of commodities were between 70.0% and 116.0% with good precision (ca. 6% RSD).

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