

# Determination of Highly Polar Pesticide Residues in Food of Plant Origin by an Automated QuPpe Method

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## Abstract

The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method has been applied for the extraction of many different classes of pesticides, with one exception, very polar non-QuEChERS-amenable pesticides. In 2008, an alternative simple and quick method to extract highly polar pesticides in food was presented, the "QuPpe-Method". In this poster we propose the automation of this extraction method with the use of a new dedicated instrument. The automatic system used 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.

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.

## Introduction

Since in the global market many of the fresh 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. 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.<sup>1</sup> Currently there is a validated method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPpe-Method).<sup>2</sup>

The aim of this study is to evaluate the performance and versatility of an automatic system for the extraction of highly polar compounds using the QuPpe-Method M7 to determine morpholine, DEA, and TEA residues in fruits.<sup>2</sup> Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (LC-MS/MS) was used to analyze the samples prepared with the automatic system.

## Experimental

### 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 1 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).

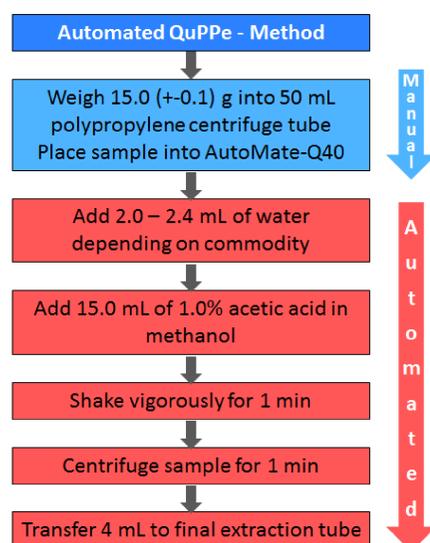


Figure 1. Automated QuPpe-Method Extraction on the AutoMate-Q40

## Sample analysis

The sample analysis was conducted using an HPLC system coupled to an triple quadrupole mass spectrometer (MS/MS) via electrospray ionization (ESI). The LC-MS/MS instrument conditions are presented in Table 1. The mass transitions for each analyte are presented in Table 2.

Table 1. LC-MS/MS Parameters

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



Table 2. 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

Table 3 shows that when using the AutoMate-Q40 to extract highly polar residues from apples and oranges recoveries range from 70.00% to 116.0%, and the results have excellent precision ranging from 1.72% to 14.29%.

Table 3. 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		106.00	5.66		102.00	6.86	
	0.05	116.00	1.72		112.00	1.79		108.00	1.79	
	0.01	100.00	10.0	0.0039	100.00	10.00	0.0036	110.00	9.09	0.0043
Orange	0.10	96.00	4.17		83.00	4.82				
	0.05	100.00	4.00		82.00	2.44				
	0.01	110.00	9.09	0.0034	70.00	14.29	0.0037			

\*MDL (Method Detection Limit) = Stdev of concentration found in 0.01 mg/kg spike [Student t99 (n-1), for seven reps (n=7) = 3.143]

## 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.<sup>3</sup> 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).

## References

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## Results

Typical chromatograms of standards can be seen in Figure 2. A precision and accuracy study was performed at PAL 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.

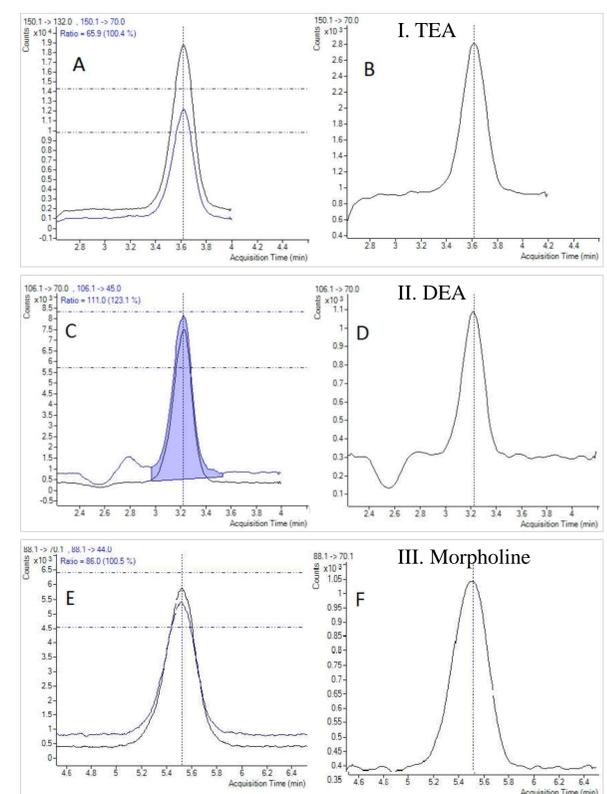


Figure 2. Extracted Ion Chromatograms (IC). I: (A) MRM transition of 0.05 µg/g of TEA; (B) IC of 0.01 µg/g of TEA; II: (C) MRM transition of 0.05 µg/g of DEA; (D) IC of 0.01 µg/g of DEA; III: (E) MRM transition of 0.05 µg/g of morpholine; (F) IC of 0.01 µg/g of morpholine.