

Application Note

Tyler Trent, Applications Sales Specialist, Teledyne Tekmar

Page | 1

Abstract

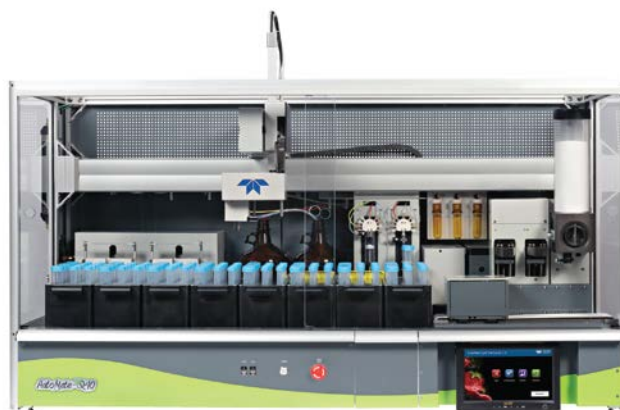
The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method was developed for the determination of pesticide residues in agricultural commodities. Since its development, QuEChERS has been modified to accommodate numerous matrices, including fruit. The simplicity of QuEChERS has led to a rise in its popularity and use, driving a need for the automation of this manual extraction technique. By using the Teledyne Tekmar AutoMate-Q40, the two-part QuEChERS method is efficiently conducted from the liquid extraction to the clean-up step.

This application note will evaluate the performance and versatility of the AutoMate-Q40. Liquid Chromatography coupled to triple-quadrupole mass spectrometry (LC-QqQ) was employed for the analysis of the LC-amenable pesticides in grapes. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. The use of the AutoMate-Q40 to perform the QuEChERS method produced sound analytical results falling in the method guidelines (range of 70-120% and RSD <20%) for the majority of the target compounds.

Introduction

Recent regulations on food analysis require screening for pesticides using confirmation techniques, such as LC-QqQ. With the ever-increasing amount of pesticides being employed, 500 or more pesticides must be analyzed on a wide range of commodities.^{1,2,3}

This application note will utilize the Teledyne Tekmar AutoMate-Q40, an automated QuEChERS extraction platform, for the determination of pesticides in grapes. Quantification was based on matrix-matched calibration curves with the use of an internal standard to ensure method accuracy. Quality Control (QC) samples were evaluated at levels of 10.0, 20.0 and 40.0 ng/mL to ensure the precision and accuracy of the AutoMate-Q40.



Experimental Instrument Conditions

Organic, pesticide-free grapes, purchased from an Ohio organic market, were used to develop this method and for method validation. Samples were stored at 4 °C until the time of extraction.

Figure 1 shows the sample preparation and extraction steps utilized for this analysis. The AutoMate-Q40 used a modified AOAC multiresidue analysis.² For this application note, the AutoMate-Q40 used 5.0 g of AOAC QuEChERS extraction salts (MgSO₄ and NaOAc). The AutoMate-Q40 also used the AOAC version of MgSO₄ (1200.0 mg), and PSA (400.0 mg) for the dSPE clean-up step.

Sample analysis was conducted using a Shimadzu Nexera UHPLC system coupled to an AB Sciex 4500 QTrap tandem mass spectrometer (QqQ) via electrospray ionization (ESI). For separation of the compounds of interest, a Phenomenex® Kinetex™ 2.6 µm Biphenyl (50 x 2.1mm) column was used. Table I and Table II demonstrate the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 2 shows the scheduled MRM chromatogram spiked at 400.0 ng/mL.

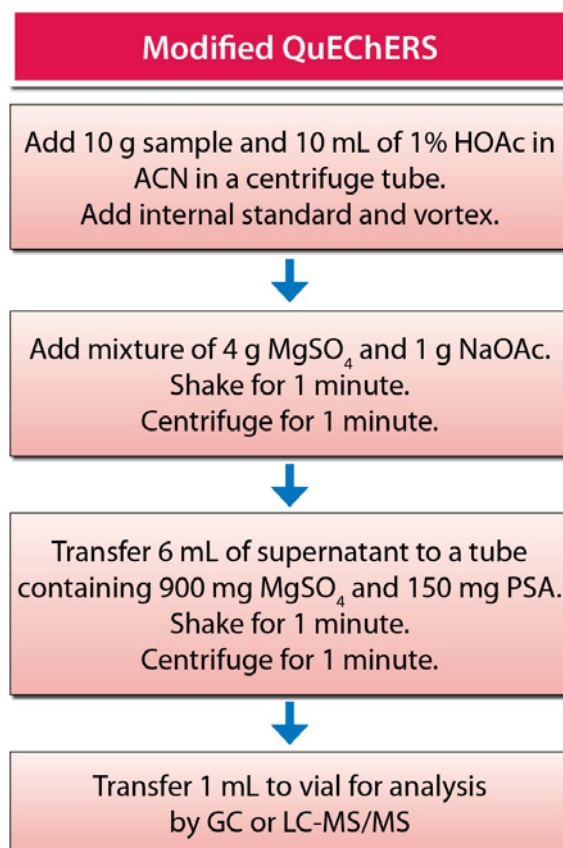
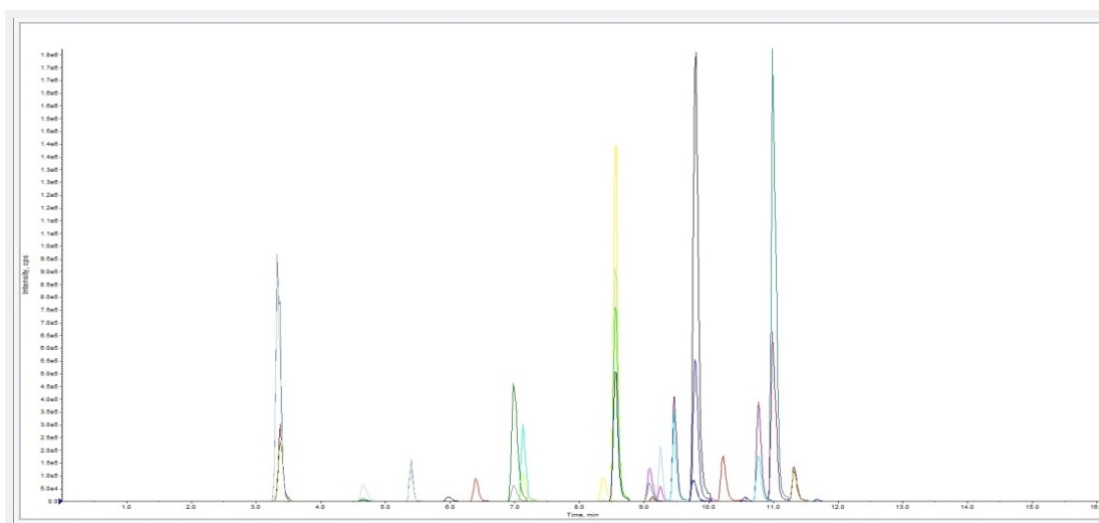
Figure 1 AutoMate-Q40 Extraction Parameter


Table I UHPLC-MS/MS Parameters	
Shimadzu Nexera	
Column	Kinetix 2.6u Biphenyl 100Å
Column Temp	40 °C
Column Flow	0.450 mL/min
Mobile Phase	A: 5 mM Ammonium Acetate in Water B: 5 mM Ammonium Acetate in Methanol
Injection Volume	10 uL
AB Sciex 4500 QTrap	
Curtain Gas	30
Ion Spray (V)	4500
Temperature (°C)	500
GS1	50
GS2	60

Table II LC-QqQ Transitions		
Compound	Q1 Mass	Q3 Mass
Azoxystrobin	404.0	371.9
Atrazine	216.0	173.9
Buprofezin	306.0	201.0
Carbaryl	202.0	144.9
Carbendazim	192.0	159.9
Chlorpyrifos	349.8	197.8
Cyprodinil	226.0	93.1
Imazalil	296.9	158.9
Iprodione	329.9	244.8
Malathion	330.9	126.9
Metalaxyl	280.0	220.0
Methiocarb	225.9	168.9
Methomyl	163.0	87.9
Metolachlor	284.0	252.0
Metribuzin	214.9	187.0
Monocrotophos	223.9	192.8
Myclobutanil	288.9	192.8
Pyrimethanil	200.0	107.0
Simazine	202.0	131.9
Thiodicarb	355.8	107.9

Figure 2 Grapes Spiked at 400 ng/mL



Results

A precision and accuracy study was performed using the AutoMate-Q40. A 2.0 µg/mL stock pesticide solution was used to fortify the grape samples. Using the AutoMate-Q40, the system is able to spike samples with 50.0, 100.0 and 200 µL of the pesticide standard that yielded 10.0, 20.0 and 40.0 ng/mL QC samples. These QC samples were quantitated against their corresponding matrix-matched calibration curve.

Figure 3 displays the AutoMate-Q40's ability to extract pesticide residues from grapes with all recoveries falling within 70-120%. These spike recoveries fall well within the recommended values in Sanco/12495/2011 which states that mean recoveries must fall within 70.0% to 120.0% with a RSD <20%.⁵ Figure 4 demonstrates the AutoMate-Q40's exceptional precision with an average of 5.5% RSD for the spiked QC samples.

Figure 3 Grape Method Recovery Validation

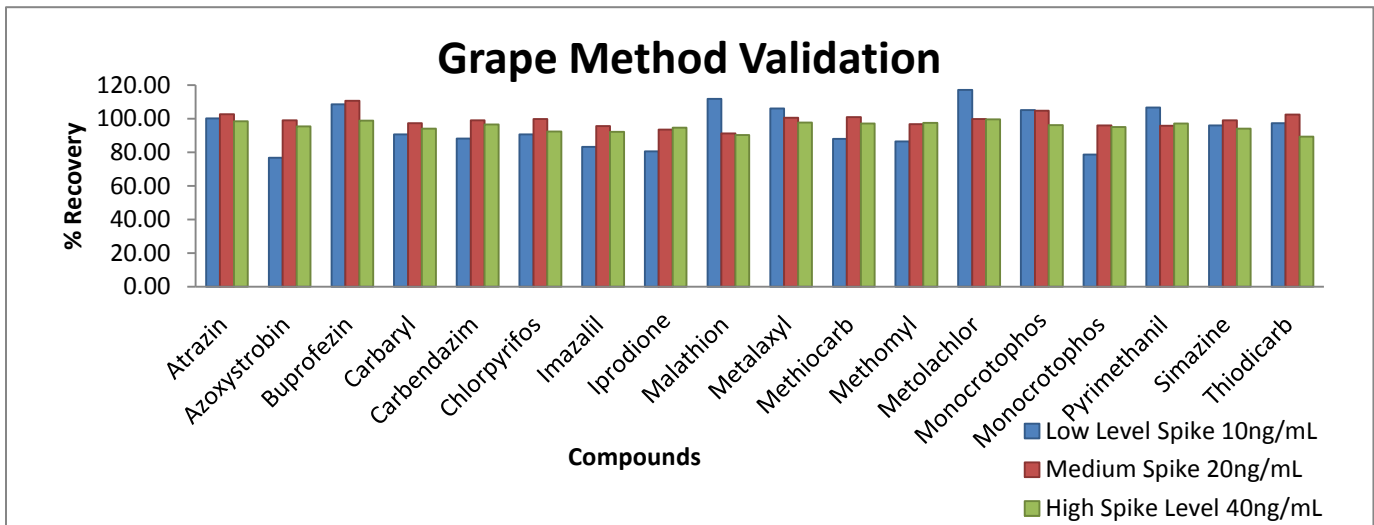
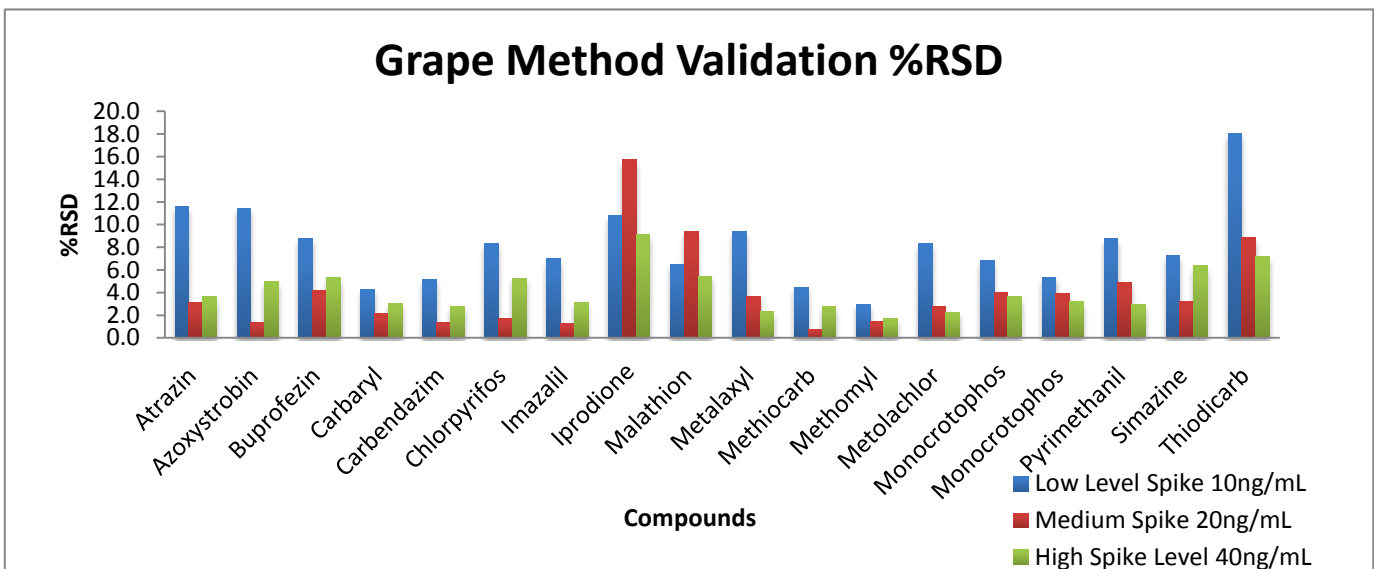


Figure 4 Grape Method %RSD Validation



Conclusion

This application note demonstrates the Automate-Q40's ability to successfully process grape samples for pesticide residue by the QuEChERS extraction method. By automating the liquid handling, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™, the AutoMate-Q40 alleviates the scientist from a labor-intensive extraction method, as well as exposure to unhealthy chemicals.

The automation of the extraction process enables an easy, consistent, reliable and ultimately more reproducible extraction with significant time and labor savings. [Figure 2](#) and [Figure 3](#) demonstrate that combined pesticide spike recoveries for all levels had a 96.7% recovery, with an average RSD of 5.5% exceeding the requirements outlined in Sanco/12495/2011. These numbers indicate superb precision and accuracy, validating the AutoMate-Q40's ability to adequately perform the QuEChERS pesticide extraction method for grapes.

Reference

1. European Committee for Standardization/Technical Committee CEN/TC275 (2008), Foods of plant origin: Determination of pesticide residues using GC-MS and/or LC-MS/MS following Acetonitrile extraction/ partitioning and clean-up by dispersive SPE QuEChERS-method.
2. AOCA Official Method 2007.07 Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry, First Action 2007.
3. M. Anastassiades: QuEChERS a mini-multiresidue method for the analysis of pesticide residues in low-fat products.
4. Method Validation and Quality Control Procedure for Pesticide Residues Analysis in Food and Feed (Document Number: SANCO/12495/2011).