

Application Note

Abstract

With the rise in popularity of QuEChERS, due to its reliable multiresidue methods it has driven the need for automation of the QuEChERS extraction to increase productivity and throughput. The AutoMate-Q40 streamlines the QuEChERS method from adding Acetonitrile (ACN) and buffering salts, shaking, mixing, centrifugating the sample, transferring to a dispersive solid phase extraction (d-SPE) tube, measuring and delivering the extract.

The aim of this project is to validate the extraction performance of the AutoMate-Q40 by monitoring pesticides in oranges. The target residues will be determined by Liquid Chromatography tandem mass spectrometry.



Introduction

The continual increase of the globalization in the food industry has led to increased concerns about food safety. Global regulation on food analysis requires screening for pesticide residues on all produce. With the ever increasing amount of pesticides being developed, over 1000 pesticides must be monitored in a wide range of commodities. With the increasing amount of samples required for analysis, the preferred extraction is the QuEChERS method.¹⁻²

Even though QuEChERS is a simplified extraction technique, it still requires many manual steps including addition of solvent, extraction salts, centrifugation, shaking, decanting and performing the dSPE cleanup.¹⁻² To modernize the traditional QuEChERS extraction Teledyne Tekmar has developed the AutoMate-Q40. This automated platform will streamline the two part QuEChERS method from the liquid extraction through the cleanup.

The orange is a lovable fruit and, has a flavorful zest that is released from the peel of the orange. The orange is also known to be one of the most popular fruits in the world according to Purdue University.³ The aim of this work is to showcase the AutoMate-Q40 for the determination of pesticides in orange samples. Pesticide residues were extracted from the orange samples using the AutoMate-Q40. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. QC samples were evaluated at levels of 10.0, 20.0 and 60.0 µg/Kg to ensure precision and accuracy of the AutoMate-Q40.

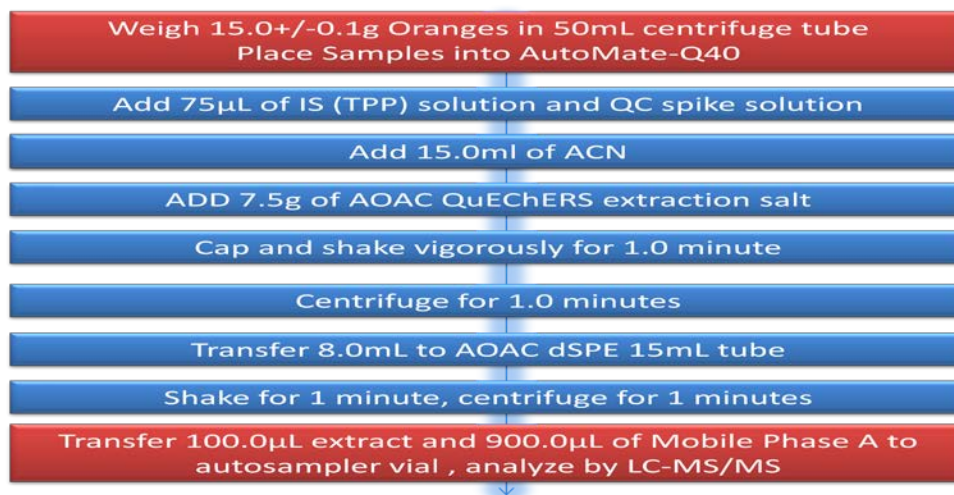


Experimental Instrument Conditions

Oranges were purchased from a local market in Ohio. The samples were prepared according to the procedure described in the "AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate".¹ The processed sample was frozen until extraction. **Figure 1** shows the sample preparation and steps followed to extract pesticide residues from oranges. For this analysis, the AutoMate-Q40 used 7.5 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 cleanup step.

Figure 1 AutoMate-Q40 Extraction Parameters (Blue Automated, Red Manual)

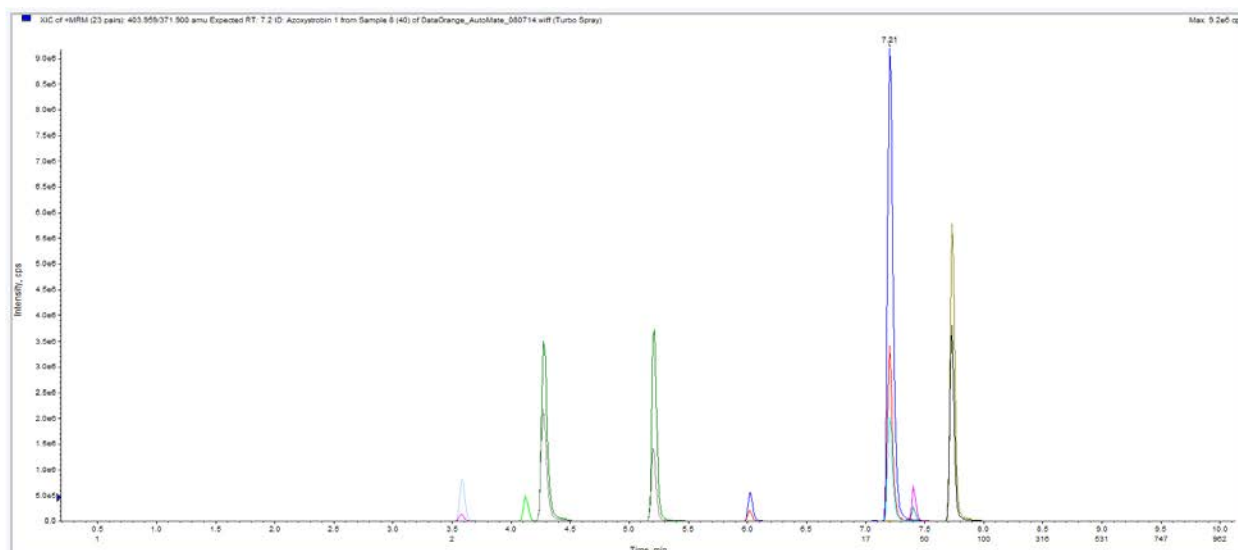


Sample analysis was conducted using a Shimadzu Nexera HPLC system coupled to an AB SCIEX 4500 QTrap tandem mass spectrometer (MS/MS) via electrospray ionization (ESI). For separation of the compounds of interest, a Phenomenex Kinetex 2.6µm Biphenyl (50 x 2.1mm) column was used. The unique biphenyl stationary phase combined with the high performance Kinetex core-shell platform provided exemplary chromatographic separation of the pesticides by focusing on hydrophobic, aromatic, and polar-basic differences. [Table I](#) and [Table II](#) contain 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 µL/L.

Table I LC-MS/MS SRM Transitions and Parameters for AB SCIEX 4500 QTrap						
Curtain Gas (CUR)				30		
Ion Spray Voltage (IS)				5500		
Temperature (TEM)				500		
Collision Gas (CAD)				Medium		
Analyte Transitions						
Compounds	RT (min)	Precursor Ion (m/z)	Quantization product Ion (m/z)	DP(V)	CE(V)	CXP(V)
Azoxystrobin	7.21	403.95	371.90	56	19	28
Carbaryl	5.21	201.97	144.90	36	13	10
Carbendazim	3.57	191.96	159.90	51	25	12
Imazalil	7.21	296.90	158.80	66	31	16
Imidacloprid	4.11	255.90	174.90	51	27	14
Pyraclostrobin	7.73	387.94	193.90	41	17	14
Pyrimethanil	6.00	200.07	107.00	56	31	10
Thiabendazole	4.26	201.95	174.90	81	33	14
TPP	7.39	326.91	214.80	86	35	15

Table II Shimadzu Nexera LC Parameters		
Column	Kinetex 2.6um Biphenyl	
Dimensions	50 X 2.1 mm	
Mobile Phase	A:5mM Ammonium Formate in Water	
	B: 5mM Ammonium Formate in Methanol	
Gradient	Time	%B
	0.10	5
	8.00	90
	10.00	90
	10.10	5
	12.1	Stop
Flow Rate (mL/min)	0.5	
Column Temperature (°C)	40	

Figure 2 400.0 µg/Kg Spiked Orange Sample



Experimental Results

Automating the QuEChERS extraction enables a fast, easy, reliable and more reproducible extraction. The AutoMate-Q40 offers significant labor savings, while improving the reproducibility and consistency between samples.

A precision and accuracy study was performed using the AutoMate-Q40. A 6.0 µg/mL stock pesticide solution was used to fortify the orange samples. QC samples were fortified at 10.0 20.0 and 60.0 µg/Kg using the AutoMate-Q40's ability to make standard additions. The AutoMate-Q40 was also used to spike 75.0 µL of the internal standard, Triphenylphosphate, (TPP) in each sample. These QC samples were quantitated against their corresponding matrix matched calibration curve.

Figure 3 Average %Recovery for the Orange Validation using the AutoMate-40

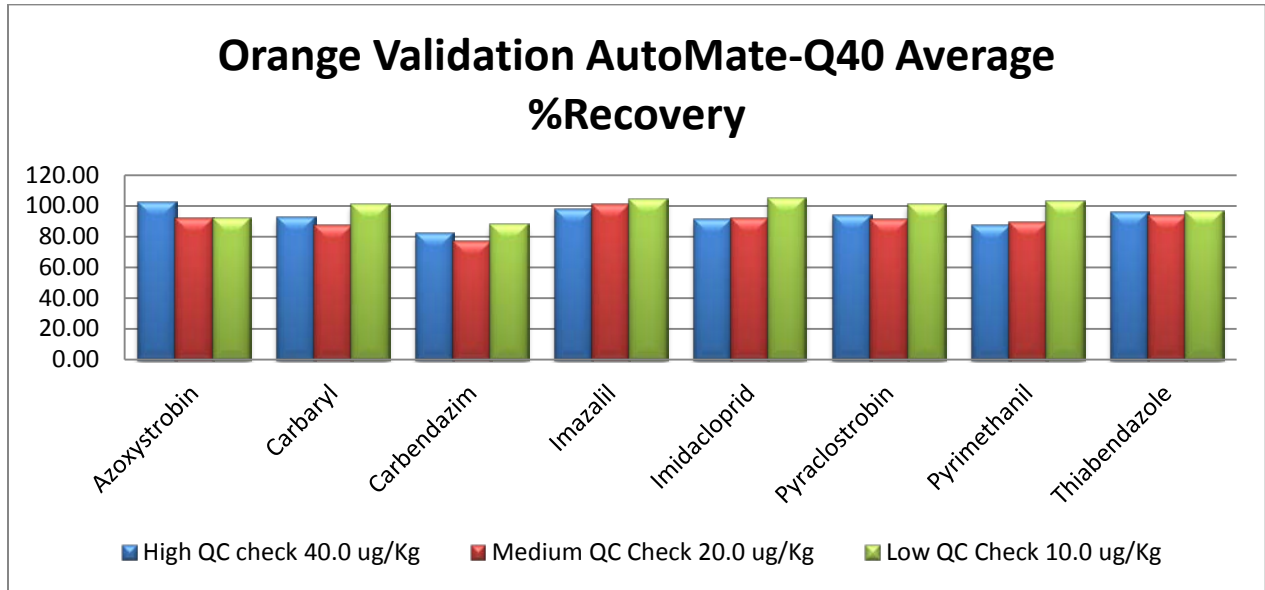


Figure 4 %RSD for the Orange Validation using the AutoMate-40

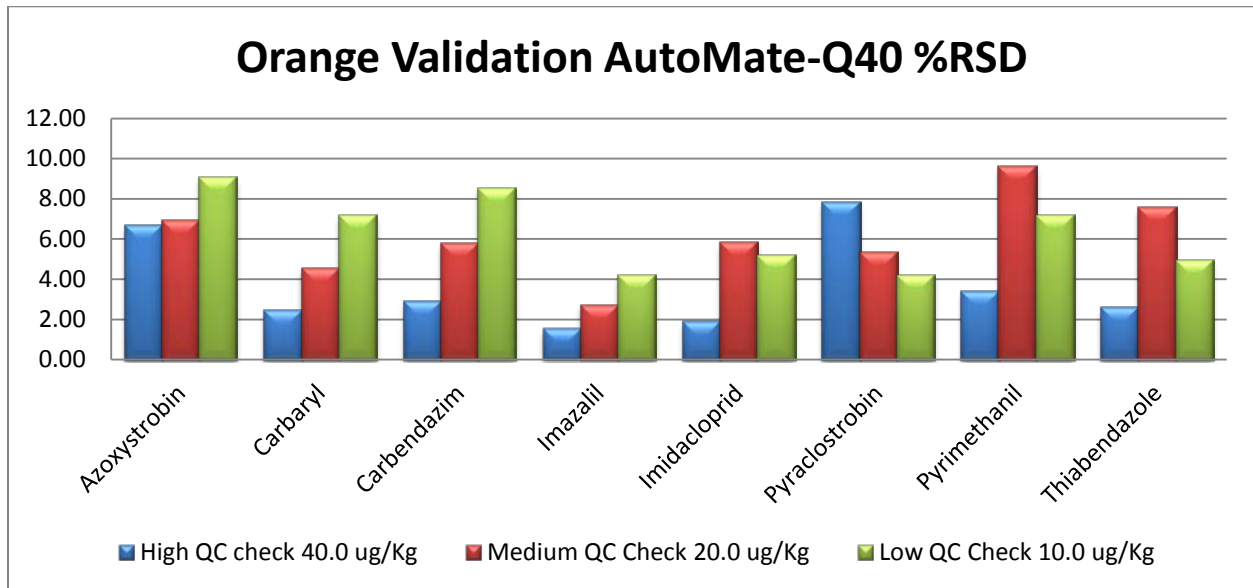


Figure 3 and Figure 4 documents the AutoMate-Q40 ability to extract pesticide residues from orange samples with recoveries ranging from 77.4% to 102.1%. These spike recoveries fall well within the recommended mean values in **Document N° Sanco/12495/2011**⁴ which states the mean recoveries must fall within 70% to 120% and a RSD <20%. The AutoMate-Q40, also, demonstrated great precision ranging from 1.8% to 9.6%RSD for the spiked QC samples

Conclusion

The Automate-Q40 successfully processes orange samples for pesticide residue utilizing the QuEChERS method. By automating the liquid handling, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using patent pending VialVision™. Today's scientists are freed from labor-intensive extraction methods and exposure to unhealthy chemicals.

This creates an extraction process that is faster, more reliable, and easier than the traditional manual based procedure. As seen above in [Figure 3](#) and [Figure 4](#), excellent precision and accuracy can be achieved without consuming valuable analysts' time.

Additionally, the combined pesticide spike recoveries of 95.0%, at an average RSD of 5.3%, exceed the requirement outlined in **The Document N° Sanco/12495/2011**⁴, validating the performance of the AutoMate-Q40 to perform the QuEChERS pesticide extraction method for fruits.

References

1. AOAC 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
2. 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 cleanup by dispersive SPE QuEChERS-method.
3. Orange Citrus sinensis <http://www.hort.purdue.edu/newcrop/morton/orange.html>
4. Method Validation and Quality Control Procedure for Pesticide Residues Analysis in Food and Feed (Document N° SANCO/12495/2011)