

Application Note

Page | 1

Abstract

QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method that has been developed for the determination of pesticide residues in agricultural commodities. The rise in popularity of the QuEChERS technique and the increase in sample testing have driven the need for automation for this extraction technique. By using the AutoMate-Q40, it streamlines the two part QuEChERS extraction from the liquid/solid extraction to the cleanup step.

The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40. A liquid mass spectroscopy/ mass spectroscopy (LC/MS/MS) was used to determine 14 pesticides from spinach using, the AutoMate-Q40, an automated QuEChERS solution. 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 20, 40, 100 ng/g to ensure precision and accuracy of the AutoMate-Q40. Recoveries of all but one pesticide fell outside of the 70% to 120% range with sample reproducibility <8%.

Introduction

With the increasing globalization of the food industry there are consequently more concerns about our safety. As a result, the number of pesticides which must be regulated and monitored has increased. QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method developed in 2003 for the extraction of pesticide residues in agricultural commodities. Modifications to the method have expanded the scope to include many additional matrices and target analytes.

Even though the QuEChERS is a simplified extraction technique, it still requires many manual steps ranging from addition of solvent, extraction salts, centrifugation, shaking, decanting and performing the dSPE cleanup.¹⁻³ To modernize the traditional QuEChERS extraction through the use of automation, 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 goal of this work is to utilize the AutoMate-Q40 an automated QuEChERS extraction for the determination of pesticides in spinach. Pesticide residues were extracted from the spinach by using the AutoMate-Q40 following a modified AOAC 2007.01 extraction. 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 20, 40, 100 ng/g to ensure the precision and accuracy of the AutoMate-Q40.

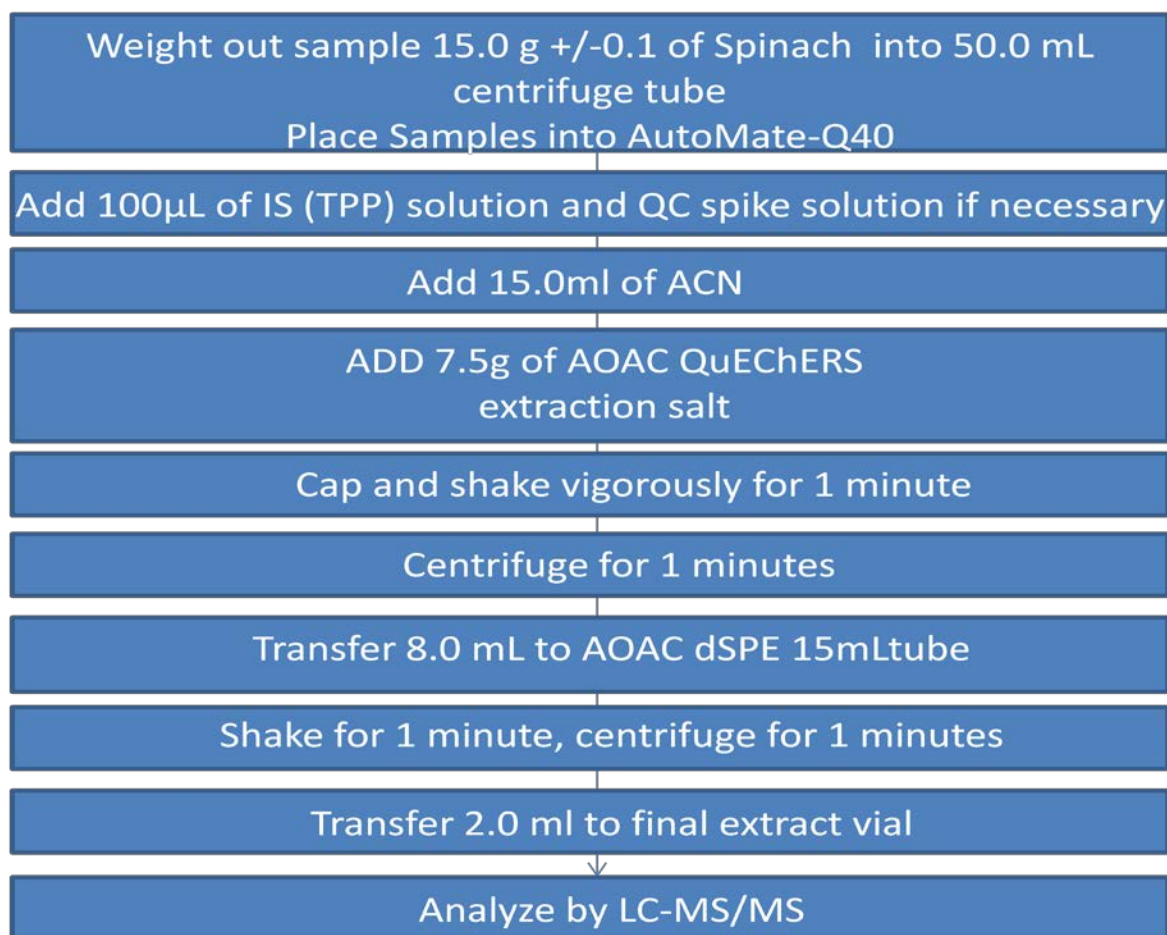
Experiment - Instrument Conditions

Sample Preparation/Extraction

Spinach 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 samples were stored in the freezer until sample analysis.

Figure 1 shows the sample preparation and extraction steps that are needed to extract the pesticide residues from apple juice. For this analysis, the AutoMate-Q40 used AOAC QuEChERS extraction salts (MgSO₄ and NaOAc). The AutoMate-Q40, also, used a version of MgSO₄, PSA, and GCB (Graphitized Carbon Black) for the dSPE cleanup step.

Figure 1 AutoMate-Q40 Extraction Parameters.



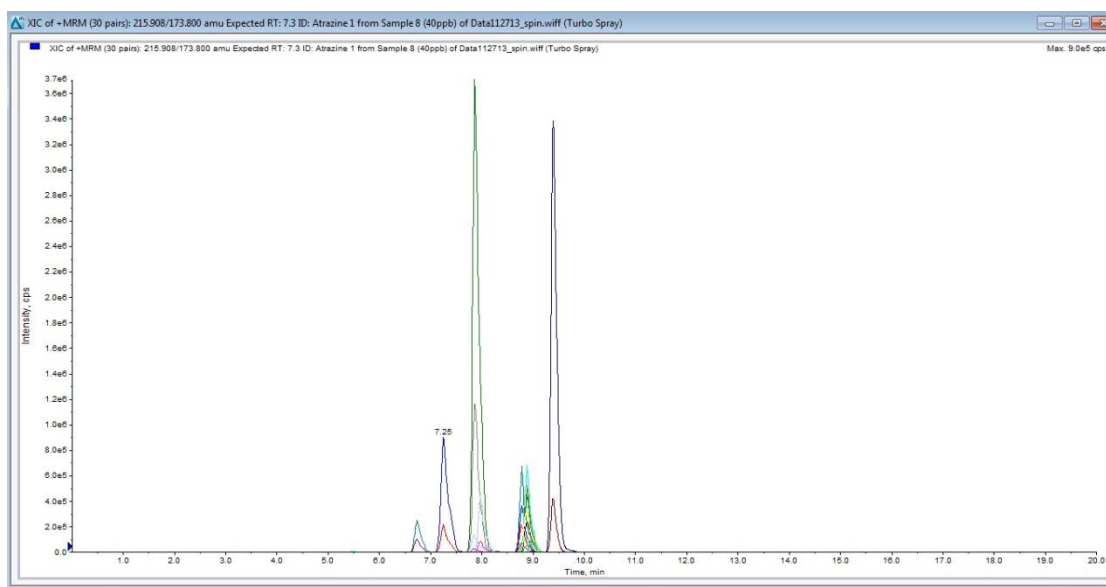
Instrumentation and Analytical Conditions

The analysis was conducted on the Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap triple-quadrupole mass spectrometer (LC-MS/MS). For separation of the compounds of interest, a Phenomenex Synergi 4u Fusion-RP (50 x 2.0 mm, and 80Å pore size) 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 µL/L. LC samples were prepared by adding 100.0 µL of final extract into 900.0 µL of HPLC grade water.

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)						400			
Collision Gas (CAD)						Medium			
Analyte Transitions									
Compounds	Precursor Ion (m/z)	Quantization product ion (m/z)	DP(V)	CE(V)	CXP(V)	Confirmation product ion (m/z)	DP(V)	CE(V)	CXP(V)
Atrazine	215.9	173.8	36.0	23.0	14.0	103.8	36.0	37.0	10.0
Azoxystrobin	403.9	372.0	41.0	21.0	10.0	343.9	41.0	35.0	26.0
Boscalid	342.8	306.7	81.0	27.0	24.0	139.7	81.0	25.0	14.0
Carbaryl	202.0	144.9	11.0	13.0	22.0	126.9	11.0	39.0	10.0
Carbendazim	191.9	159.9	91.0	27.0	16.0	131.9	91.0	41.0	12.0
Cyprodinil	225.9	92.9	111.0	47.0	8.0	76.9	111.0	65.0	8.0
Imazalil	296.9	158.8	36.0	35.0	12.0	254.6	36.0	25.0	20.0
Kresoxim-methly	313.9	205.7	10.0	9.0	16.0	115.9	10.0	19.0	10.0
Propiconazole	341.9	158.8	81.0	35.0	12.0	122.8	81.0	81.0	20.0
Pyrimetanil	200.0	107.0	86.0	31.0	10.0	77.0	86.0	59.0	10.0
Tebuconazole	308.0	69.9	71.0	61.0	10.0	124.8	71.0	55.0	12.0
Thiabendazole	296.8	158.7	56.0	31.0	16.0	200.8	56.0	25.0	16.0
TPP	326.9	214.7	91.0	35.0	16.0	167.7	91.0	47.0	14.0
Triflumazole	345.9	277.6	1.0	15.0	24.0	72.8	1.0	21.0	6.0

Table II Shimadzu Nexera LC Parameters		
Column	Synergi 4u Fusion RP 80Å	
Dimensions	50.0 X 2.00 mm	
Mobile Phase	A: 5 mM Ammonium Formate in H ₂ O	
	B: 5 mM Ammonium Formate in MeOH	
Gradient	Time (min)	%B
	0.1	5.0
	9.0	90.0
	12.0	100.0
	15.0	100.0
	17.0	Stop
Flow Rate (mL/min)	0.3	
Column Temperature (°C)	30.0	

Figure 2 400.0 ng/g Spike of Compounds in Spinach



Experimental Results

Automation of the QuEChERS extraction allows for a fast, easy reliable and more reproducible extraction. By using the AutoMate-Q40, it offers significant labor savings, while improving the repeatability and consistency between the samples.

A precision and accuracy study was performed on all three commodities using the AutoMate-Q40. A 6.0 µg/mL stock pesticide solution was used to fortify the spinach samples. Using the AutoMate-Q40, the system is able to spike the following samples with 50.0, 100.0 and 250.0 µL of the pesticide standard that yielded a 20.0, 40.0, and 100.0 ng/g check samples. These QC samples were quantitated against a corresponding matrix matched calibration.

Table III Data Table For Spinach							
Compounds	R ²	20.0 ng/g Spike		40.0 ng/g Spike		100.0 ng/g Spike	
		% Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Atrazine	0.9992	102.30	1.52	95.67	2.62	88.45	1.94
Azoxystrobin	0.9997	98.34	1.61	97.64	2.76	93.32	2.18
Boscalid	0.9983	109.95	8.37	105.28	4.84	92.31	2.74
Carbaryl	0.9988	100.94	2.06	95.23	2.94	90.14	1.91
Carbendazim	0.9655	n/d	n/d	n/d	n/d	n/d	n/d
Cyprodinil	0.9987	94.93	2.73	87.98	1.97	83.46	3.93
Imazalil	0.9995	87.93	1.89	85.35	2.48	80.85	1.43
Kresoxim-methly	0.9995	103.63	3.17	96.32	3.25	93.32	2.58
Propiconazole	0.9991	101.72	1.17	96.67	2.98	91.14	2.50
Pyrimethanil	0.9987	97.81	1.72	91.91	3.64	85.73	1.19
Tebuconazole	0.9993	100.65	1.64	95.68	2.46	88.81	2.04
Thiabendazole	0.99946	90.85	2.94	86.46	1.89	81.09	2.69
Triflumazole	0.9991	92.58	2.11	82.34	1.82	77.58	2.78

Table III shows that when using the AutoMate-Q40 to extract pesticide residues from spinach samples, it exhibits recoveries ranging from 77.58% to 109.95%. Since GCB was used in the cleanup plainer compounds such as carbendazim were absorbed into the loose media. These spiking recoveries fell within the recommend values for the Document N° Sanco/12495/2011.⁴ This document also states that the %RSD must be below 20%. By using the AutoMate-Q40 for the QuEChERS extraction it showed great precision ranging from 1.17% to 8.37%RSD for the spiked QC samples.

Conclusion

This study demonstrates the feasibility of automating the QuEChERS extraction method using the AutoMate-Q40. By automating the liquid handing, addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision™. The extraction process is faster, more reliable, and easier. This enables time and labor savings, while improving consistency and repeatability of the extraction. As shown above in **Table III** combined pesticide spikes recovered at 90.99% with an average RSD of 2.57%RSD. These numbers indicate superb precision and accuracy thus validating the performance of the AutoMate-Q40 and its use as an excellent analytical tool.

References

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 cleanup by dispersive SPE QuEChERS-method.
2. 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
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 N° SANCO/12495/2011)