Determination of Pesticide Residue in Apple Juice Using the AutoMate-Q40

Application Note

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. While the original unbuffered method was developed for plant matrices, since 2003, two additional buffered methods were created and adapted to many additional matrices such as fruit juices. The rise in popularity of the QuEChERS technique and the increase in sample testing have driven the need for automation for this extraction technique. The AutoMate-Q40 streamlines the two part QuEChERS method from the liquid extraction to the cleanup step.

The aim of this project is to evaluate the performance and versatility of the Automate-Q40. A LC-MS/MS was used to determine pesticide residues in fruit juices, particularly in apple juice. Pesticide residues were extracted from the apple juice by 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, 50, 100 ng/g to ensure precision and accuracy of the AutoMate-Q40.

Introduction

Recent regulations on food analysis require screening for pesticides using confirmation techniques, such as LC-MS/MS. With the ever increasing amounts of pesticides being employed, 500 or more pesticides must be analyzed on a wide range of commodities.

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 in a multi-lab validation study (Teledyne Tekmar and Pacific Agricultural Lab) for the determination of pesticides in Apple Juice. Pesticide residues were extracted from the apple juice by 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, 50, 100 ng/g to ensure the precision and accuracy of the AutoMate-Q40.

Procedure

Sample Preparation/Extraction

Apple juice 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 at room temperature and then stored in the refrigerator after opening.

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 ($MgSO_4$ and NaOAc). The

AutoMate-Q40 also used the AOAC version of $MgSO_4$, and PSA for the dSPE cleanup step. The following extraction will be utilized in both analytical labs (Teledyne Tekmar and Pacific Agricultural Lab).

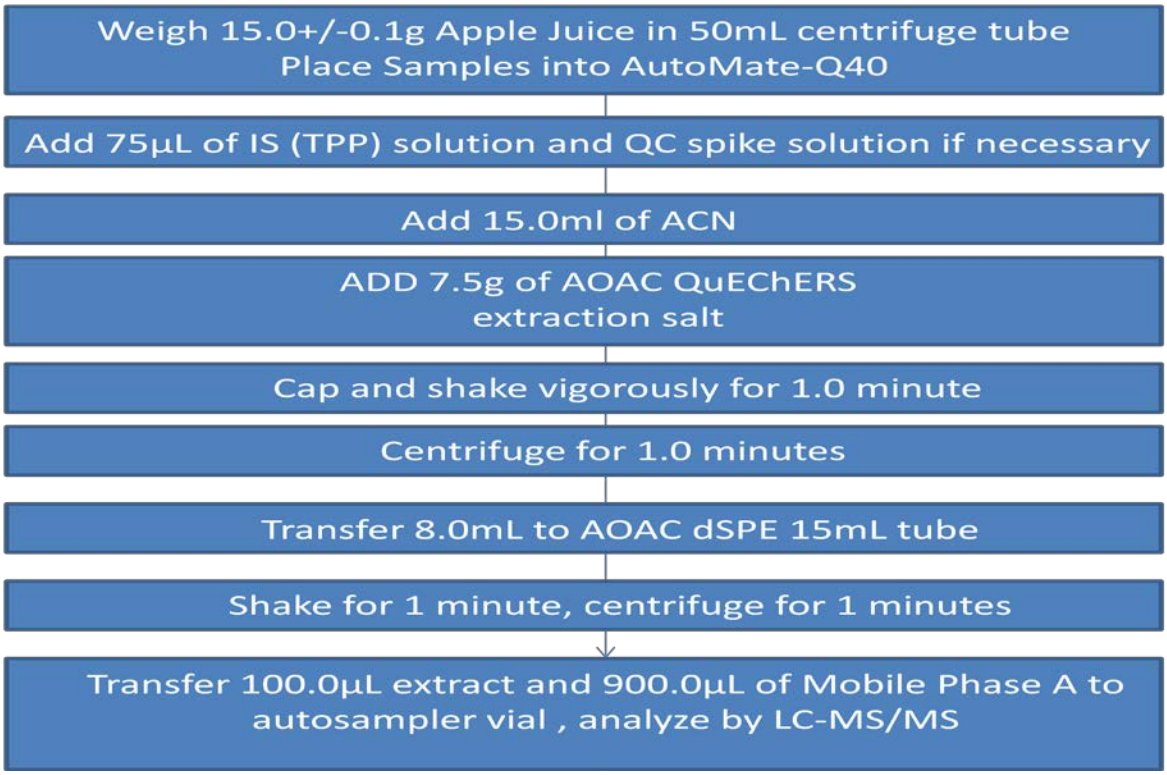

Figure 1: AutoMate-Q40 extraction parameters.

Instrumentation and Analytical Conditions

Two LC-MS/MS were run in Multiple Reactions Monitoring mode (MRM) for the initial Retention Time (RT) setup. Once the RT was established, the LC-MS/MS was switched over to Schedule Reaction Monitoring (SRM) for rest of the analysis. For confirmation of the extracted compounds, two transitions were determined for each compound. The mass transitions used are presented in **Table 1**, while the LC conditions are presented in **Table 2**.

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Lab A (Tekmar) AB Sciex 4500 QTrap				Lab B (Pacific Agricultural Lab) Waters Xevo TQ-MS			
Compounds	Precursor Ion (m/z)	Quantization Product Ion (m/z)	Confirmation Product Ion (m/z)	Compounds	Precursor Ion (m/z)	Quantization Product Ion (m/z)	confirmation Product Ion (m/z)
Acetamiprid	222.93	125.90	98.90	Acetamiprid	223.00	56.10	126.00
Azoxystrobin	403.92	328.90	307.00	Azoxystrobin	404.10	329.00	372.00
Boscalid	344.80	307.00	139.00	Boscalid	342.90	139.90	307.00
Carbaryl	201.97	144.90	126.90	Carbaryl	202.00	127.00	145.00
Carbendazim	191.91	159.90	131.90	Carbendazim	192.00	160.00	132.00
Clofentezine	304.83	137.90	101.90	Clofentezine	303.00	102.00	138.00
Cyprodinil	225.93	93.00	108.10	Cyprodinil	226.00	93.00	108.00
Difenoconazole	405.79	250.80	187.90	Difenoconazole	406.00	251.10	111.10
Hexythiazox	352.90	227.70	167.90	Hexythiazox	353.00	168.00	228.00
Metconazole	320.01	124.90	98.90	Metconazole	320.10	70.00	125.00
Methoxyfenozide	369.02	148.80	91.00	Methoxyfenozide	369.10	149.10	313.20
Oxamyl	237.00	72.00	90.00	Oxamyl	237.00	72.00	90.00
Propiconazole	341.89	158.80	122.80	Propiconazole	342.00	159.00	69.00
Pyraclostrobin	387.89	193.90	162.80	Pyraclostrobin	388.10	163.00	193.90
Pyrimethanil	200.72	106.90	169.00	Pyrimethanil	200.00	82.00	107.00
Tebufenozide	353.03	132.90	296.90	Tebufenozide	353.10	133.00	297.10
Thiabendazole	201.86	174.90	131.00	Thiabendazole	202.00	131.00	175.00
Thiophanate Methyl	342.89	150.70	93.10	Thiophanate Methyl	343.00	93.00	151.00

Table 1: SRM transitions for LC-MS/MS parameters.

Lab A (Teledyne Tekmar)			Lab B (Pacific Agricultural Lab)		
Shimadzu Nexera LC Parameters			Waters LC Parameters		
Column	Restek Ultra AQ C18		Column	Waters BEH C18	
Dimensions	100 mm x 2.1 mm, 3 µm		Dimensions	2.1 mm x 100 mm, 1.7 µm	
Mobile Phase	A 5mM Ammonium Formate in H_2O		Mobile Phase	A:20mM Ammonium 0.2% acetic Acid in H2O	
	B 5mM Ammonium Formate in MeOH			B: 20mM Ammonium 0.2% acetic Acid in MeOH	
Gradient	Time (min)	%B	Gradient	Time (min)	%B
	0.10	10		Initial	30
	1.50	10		2.00	30
	4.00	60		8.00	50
	8.00	70		14.00	90
	11.00	100		17.00	90
	12.00	100		18.00	30
	12.01	10			
	15.00	10			
Injection Vol (µL)	10.0		Injection Vol (µL)	10.0	
Flow Rate (mL/min)	0.31		Flow Rate (mL/min)	0.45	
Column Temperature (C˚)	30.0		Column Temperature (C˚)	60.0	

Table 2: LC conditions parameters.

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Experimental Results

By automating the QuEChERS extraction, it enables a fast, easy, reliable and more reproducible extraction. The AutoMate-Q40 offer significant labor savings, while it improves the repeatability and consistency between the samples.

A precision and accuracy study was performed in both labs using the AutoMate-Q40. A 6 µg/mL stock pesticide solution was used to fortify the apple juice samples. Using the AutoMate-Q40, the system is able to automatically spike the samples with 25.0, 125.0 and 250.0 µL of the pesticide standard. These volumes produce a 10.0, 50.0 and 100 µg/L check samples respectively. Also, the AutoMate-Q40 spiked in 75.0 µL of the internal standard TPP in each sample to produce a concentration of 100.0 µg/L of. These QC samples were quantitated against their corresponding matrix matched calibration curve.

The analysis was performed in two parts. The AutoMate-Q40, can extract the apple juice samples with and without a dSPE cleanup. Two sets of data will be presented in this precision and accuracy study. **Table 3** shows data using the AutoMate-Q40 to extract unclean apple juice samples. While **Table 4** shows data that was sent through the dSPE cleanup option using the AutoMate-Q40.

Unclean Apple Juice Samples											
LAB A (Teledyne Tekmar)							LAB B (Pacific Agricultural Lab)				
Compounds	Low Spike		Medium Spike		High Spike		Compounds	Medium Spike		High Spike	
	% Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD		%Recovery	%RSD	%Recovery	%RSD
Acetamiprid	96.99	9.8	97.70	2.9	95.55	3.0	Acetamiprid	92.75	13.2	100.70	6.6
Azoxystrobin	104.09	2.9	99.90	3.4	101.13	1.7	Azoxystrobin	99.07	15.3	106.94	8.3
Boscalid	118.73	21.6	107.00	4.8	104.46	3.6	Boscalid	93.11	10.2	101.06	8.1
Carbaryl	106.86	3.5	103.95	2.8	103.33	3.4	Carbaryl	92.72	10.3	97.73	8.9
Carbendazim	98.02	2.7	100.63	2.4	96.60	6.5	Carbendazim	93.79	13.5	102.93	9.8
Clofentezine	104.73	8.3	101.37	3.2	102.24	4.0	Clofentezine	83.97	13.1	91.77	6.1
cyprodinil	102.93	6.2	101.13	2.2	102.27	5.6	Cyprodinil	91.43	11.3	95.43	4.9
Difenoconazole	105.14	2.6	95.22	2.0	98.83	3.5	Difenoconazole	95.43	10.0	104.46	2.6
Hexythiazox	113.82	9.8	97.63	2.6	97.79	4.9	Hexythiazox	90.18	10.4	93.66	3.2
Oxamyl	99.50	5.4	100.36	1.9	103.33	3.3	Oxamyl	86.36	15.9	93.87	7.4
Pyraclostrobin	90.13	7.9	98.77	3.1	98.82	5.3	Pyraclostrobin	81.32	13.5	88.31	8.0
Pyrimethanil	103.24	8.8	101.97	6.9	104.44	3.3	Pyrimethanil	92.39	11.5	102.44	4.4

Table 3: Unclean apple juice sample extracted using AutoMate-Q40

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Cleaned Apple Juice Samples											
LAB A (Teledyne Tekmar)							LAB B (Pacific Agricultural Lab)				
Compounds	Low Spike		Medium Spike		High Spike		Compounds	Medium Spike		High Spike	
	% Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD		%Recovery	%RSD	%Recovery	%RSD
Acetamiprid	83.68	9.9	104.41	2.5	101.31	4.0	Acetamiprid	103.14	11.6	103.89	3.1
Azoxystrobin	101.53	7.2	104.66	2.7	102.21	4.5	Azoxystrobin	98.64	13.5	104.86	12.3
Boscalid	106.22	6.9	105.67	4.5	104.30	1.8	Boscalid	103.97	9.8	102.70	7.4
Carbaryl	104.09	7.4	105.57	2.0	104.51	2.9	Carbaryl	104.11	12.2	101.61	8.8
Carbendazim	93.74	11.8	101.89	3.5	93.36	6.2	Carbendazim	96.25	12.6	107.27	12.1
Clofentezine	98.06	6.6	102.26	2.8	99.69	3.2	Clofentezine	90.68	9.5	93.06	4.9
cyprodinil	104.61	5.2	106.10	3.9	104.03	3.2	Cyprodinil	97.61	11.1	101.16	4.3
Difenoconazole	102.79	9.0	99.73	3.1	96.79	5.0	Difenoconazole	103.50	9.6	108.14	2.5
Hexythiazox	102.65	7.6	100.49	3.2	99.06	4.9	Hexythiazox	87.25	8.8	91.44	4.4
Metconazole	98.22	8.8	102.58	4.0	98.96	5.0	Metconazole	98.22	12.9	105.61	4.2
Oxamyl	98.37	6.7	100.07	2.7	99.69	3.8	Oxamyl	93.82	17.0	87.59	7.5
Pyraclostrobin	88.02	16.5	102.36	6.8	98.51	6.1	Pyraclostrobin	87.54	8.5	99.23	4.1
Pyrimethanil	105.54	7.1	107.11	5.1	104.44	4.2	Pyrimethanil	95.68	6.2	106.67	8.3

Table 4: dSPE clean apple juice sample extracted using AutoMate-Q40

Table 3 and 4 shows that when using the AutoMate-Q40 to extract pesticide residues from apple juice, it exhibits recoveries ranging from 83.97% to 113.82% for the unclean samples, while the dSPE cleaned samples exhibits excellent recoveries ranging from 87.54% to 107.27%. These spike recoveries fall within the recommended mean values for the Document N˚ Sanco/12495/2011[4]. This document states that the mean recoveries must fall within 70% to 120% and a RSD <20%. The AutoMate-Q40, also, demonstrated great precision ranging from 1.7% to 21.6 %RSD for the unclean samples and while the samples that used a dSPE cleanup showed precision ranging from 2.0% to 17.0 %RSD.

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 3 and 4** all pesticides gave excellent spike recoveries, ranging from 83.97% to 113.82% and excellent precision ranging from 1.7% to 21.6%.

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Reference

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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

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