Pesticide Analysis Using the AutoMate-Q40: An Automated Solution to QuEChERS Extractions

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, AOAC 2007.01 and EN15662:2008 were created and adapted for use with many additional matrices. The rise in popularity of this technique and the increase in sample testing have driven the need for automation of these extractions to increase productivity and throughput. The AutoMate-Q40 streamlines the QuEChERS method from adding Acetonitrile (ACN) and buffering salts, shaking, mixing, centrifugation the sample, sample transferring to a dispersive solid phase extraction (d-SPE) tube using an air displacement pipetter (ADP), and finally measuring and delivering the extract. The advances made by automating this technique will not only greatly enhance laboratory production, but will improve precision and accuracy as well.

The goal of this experiment is to evaluate the performance of the AutoMate-Q40 by monitoring multi-pesticide class residues in agricultural commodities. The target pesticides in the commodities will be determined and calculated by using GC/MS and LC/QQQ techniques and directly compared to manual extractions under the same chromatographic conditions.

Introduction

Sample preparation for multiresidue pesticide screening for agriculture commodities can be cumbersome and laborious for multiple sample extractions. With recent advanced in multiresidue pesticide screening, methods have been simplified by the introduction of QuEChERS. While QuEChERS is more effective than its predecessors, this extraction is still a laborious procedure and requires great detail from the analyst performing the extraction.

The use of either the AOAC Official Method 2007.01, or EN15662:2008 requires several manual steps to extract the pesticides of interest. These steps include the measurement and addition of an extraction solvent, weighing and dispensing mixed salts with buffers, measuring and adding control standards, sample shaking, and centrifugation of all samples. Once this is complete, extract clean up is performed using dispersive solid phase extraction (dSPE) in which, shaking, centrifugation, and extract measuring are repeated.

With the amounts of samples being required for pesticide residue analysis continually increasing, Teledyne Tekmar has developed the AutoMate-Q40. This revolutionary system is designed to automate the QuEChERS extraction workflow, allowing laboratories to be more efficient and timely in meeting their customer requirements for fast and reliable results. This system will help keep sample preparation and the time an analyst spends on it at a minimum, while producing highly accurate, precise, and traceable results.

The intent of this poster is to evaluate the performance of the AutoMate-Q40 by monitoring pesticide residues in apples and lettuce. The target pesticides will be analyzed using both GC/MS and LC/QQQ.

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Experimental

Sample Preparation

Apples and lettuce were purchased from a local supermarket. Per each method, the apples were chopped into small cubes. The whole apple was used aside from the seeds. Once chopped, the apple cubes were then placed into a plastic bag and frozen. Similarly, the lettuce was cut into small strips and frozen.

Extraction/Cleanup

For the extractions, a 15 g (+/-0.1 g) homogenized apple and lettuce samples was placed into a 50 mL centrifuge tube, and then placed into the AutoMate-Q40 sample trays. From this point, the AutoMate-Q40 performed all of the method steps previously performed by a laboratory professional.

The AutoMate-Q40 moved the centrifuge tubes to a de-capping station where the caps were removed. Using a dual-pump liquid handling system, the instrument was programmed to add extraction solvent and spiking solutions to the samples. 15 mL of 1%HAc in ACN and 75 µL of internal standard (20 µg/mL) were added to each sample to yield a 100 ng/g concentration. QC samples were fortified with 50 µL and 250 µL of the QC spiking solution (6 µg/mL), yielding 20 ng/g and 100 ng/g check samples. 7.5 g of AOAC extraction salts were added by the solids dispenser. The samples were automatically capped and moved to the shaker where they were shaken vigorously for 1 minute to ensure complete mixing. Once the mixing was complete, the samples were centrifuged for 5 minutes at 4000 rpm.

The centrifuged samples were transferred to the VialVision™ (patent-pending) housing. This innovative technology uses a camera to determine the positions of the layers in the vial and calculates the available supernatant that can be transferred to a dSPE cleanup vial. The samples are then transferred from the VialVison housing to the shuttle station where the necessary 15 mL dSPE cleanup tube is positioned. The centrifuge tubes were uncapped, and using the air displacement pipetter (ADP), an 8 mL aliquot was transferred from the extraction tube to the cleanup tube containing 400 mg of PSA and 1200 mg of $MgSO_4$. The 50 mL extraction tube was returned to the tray, while the cleanup tube was moved to the shaker and shaken for 1 minute. Once the mixing was complete the sample was taken to the centrifuge and spun for 5 minutes at 4000 rpm.

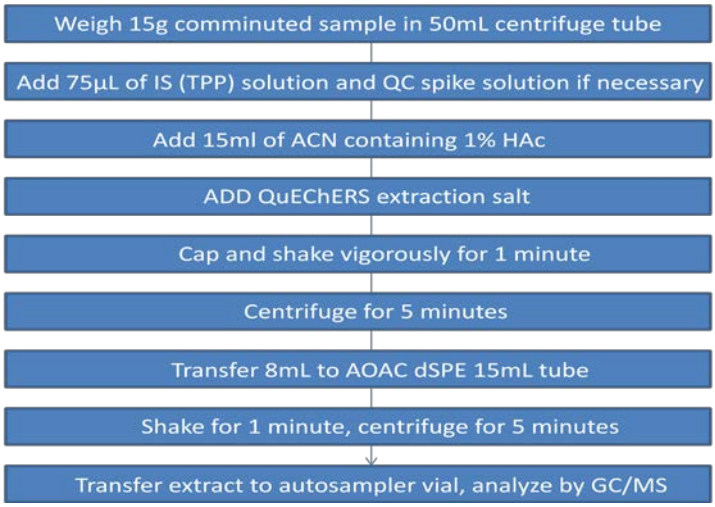

Figure 1. Flow chart of AutoMate™Q40 AOAC QuEChERS extraction

Once centrifuging was complete, the 15 mL dSPE cleanup tube was moved to the VialVision™ station to determine the amount of extract available to be transferred to the final extract tube. The cleanup tube was taken from the VialVison housing to the shuttle station where the corresponding 15 mL final extract tube is positioned. The cleanup and final extract tubes were uncapped and, using the ADP 5mL of the final extract was transferred to the final tube. Finally, aliquots from the final extract tube are manually placed into autosampler vials, and analyzed by LC/QQQ and GC/MS.

Figure 1 shows the flow chart for the AOAC QuEChERS extraction procedure for both the AutoMate-Q40 and the manual hand extraction.

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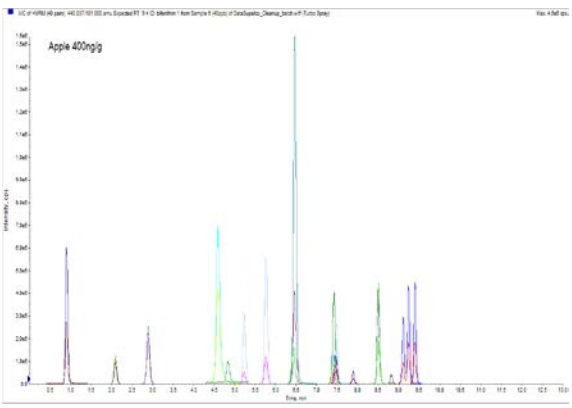
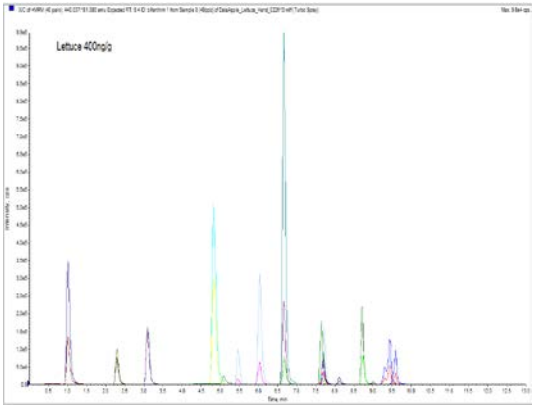
Instrument Conditions

LC/QQQ analysis was performed using a Synergi 4u Fusion-RP 80A 50 x 2.00 mm HPLC column on a Shimadzu Nexear LC system, interfaced with an AB SCIEX Q-Trap 4500 triple quadrupole mass spectrometer, and analyzed by ESI in positive ion mode. LC/QQQ parameters and transitions can be found in Table 1. Figure 2 show MRM chromatograms for both apples and lettuce. An Agilent 7890/5975 GCMS was utilized for the GC analysis. The parameters for the GC/MS can be found in Table 2. Figure 3 shows SIM chromatograms for both apples and lettuce.

Table 1.LC/QQQ MRM Transition and Parameter					
Curtain Gas (CUR)					30
Ion Spray Voltage (IS)					5500
Temperature (TEM)					400
Collision Gas (CAD)					Medium
Analyte Transitions					
Analyte	**RT**	**Q1/Q2**	**DP(V)**	**CE(V)**	**CXP(V)**
Atrazine	5.8	216/173	11	25	14
Azoxystrobin	6.5	404/372	71	19	10
Bifenthrin	9.4	440/181	6	19	16
Carbaryl	5.2	202/127	41	39	12
Chlorpyrifos	8.6	352/96	51	43	10
Chlorpryifos methyl	7.9	322/125	51	28	10
Cyprodinil	7.4	226/93	81	53	8
Dichlorvos	4.8	221/109	51	23	10
Ethion	8.5	385/143	56	21	12
Imazalil	7.4	297/189	1	29	14
Imidacloprid	2.9	256/209	46	21	6
Kersoxim-methyl	7.4	314/116	51	19	10
Lambda-Cyhalotrhin	8.8	467/141	1	55	12
Linuron	6.5	249/160	56	25	12
Methamidophos	0.9	142/94	1	19	8
Methomyl	2.1	163/163	41	13	8
Cis-Permethrin	9.1	408/183	26	25	14
Trans-Permethrin	9.1	408/183	26	25	14
Tebuconazole	7.4	308/70	26	51	8
Thiabendazole	4.6	202/131	46	42	10
Tolyfluanid	7.5	347/238	56	15	18

Table 1. LC/MS/MS Transition and Parameters





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LC Condition	Shimadzu Nexera	
Column	Synergi 4u Fusion-RP 80A	
Dimensions	50.0 x 2.00mm	
Mobile Phase	A:5 mM Ammonium Formate in H_2O	
	B:5 mM Ammonium Formate in MeOH	
Gradient	Time (min)	%B
	0.1	20
	9	100
	10	100
	10.1	20
	13.0	20
	13.1	Stop
Flow Rate (mL/min)	0.3	
Column Temperature (°C)	30	

Figure 2. MRM chromatogram of Apple (Top) and Lettuce (Bottom) extract spiked at 40 ng/g

SIM Transitions.			
Compound	Base Peak	Ms Transition	RT
Atrazine	200	215	7.4
Azoxystrobin	344	372	20
Bifenthrin	181	115	13.9
Carbaryl	144	115	5.3
Chlorpyrifos	97	197	9.6
Chlorpryifos methyl	125	286	8.7
Chlorothanlonil	266	264	8
Cyprodinil	224	225	10.3
Dichlorvos	109	185	3.1
Ethion	97	157	12.3
Endosulfan Sulfate	272	274	13
Imazalil	41	215	11.3
Kersoxim-methyl	116	131	11.6
Lambda-Cyhalotrhin	181	208	14.8
Linuron	61	46	9.4
Procymidone	96	67	10.6
Cis-Permethrin	183	163	15.7
Trans-Permethrin	183	163	15.8
Tebuconazole	250	163	13.3
Thiabendazole	201	174	10.6
Tolyfluanid	137	181	10.4
Trifuralin	306	264	6.6
Triphenyl Phospahte (TTP)	326	325	13.5
2,4'-DDD	235	237	11.7

Table 2. GC/MS SIM Transitions

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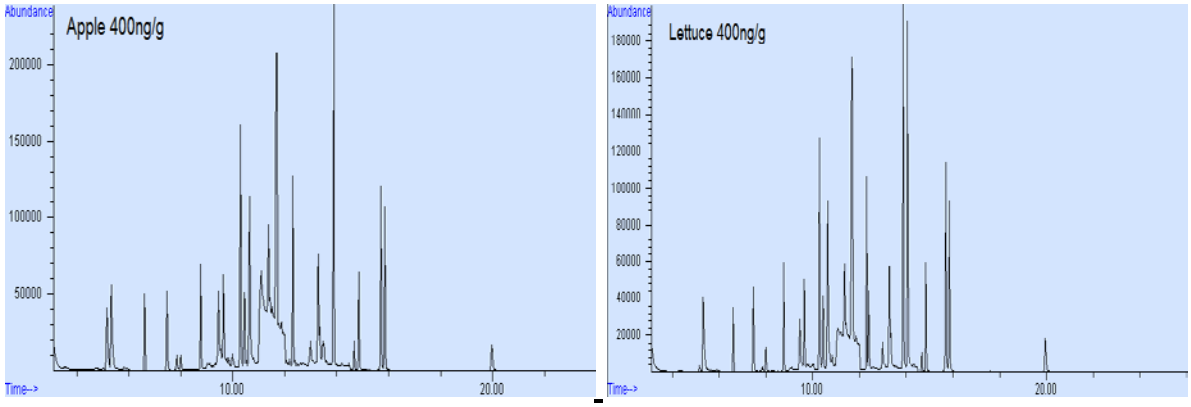

Figure 3. SIM chromatogram of Apple (Left) and Lettuce (Right) extract spiked at 400 ng/g

GC/MS Parameters	
GC Conditions	**Agilent 6890 GC 5975 MSD**
Column	Restek 5MS-Sil 30.0m x 0.25mm x 0.25µm
Column Constant Flow	1.0mL/min
Oven Program	100°C (0.0 min), 25°C/min to 150°C (0.0min), 10°C/min to 280°C (10.0min), 25°C/min to 325°C (5.0min)
S/SL Temperature	250°C
S/SL Mode	Splitless
Transfer Line Temperature	290°C

LC/QQQ and GC/MS Sample Preparation

LC samples were prepared by adding 100 µL of extract into 900 µL of HPLC grade water. The GC samples were prepared by transferring 1.0 mL of extract into a 2.0 mL vial.

Results and Discussion

Automating QuEChERS enables fast, easy, reliable and, more reproducible extractions. The AutoMate-Q40 offers significant labor savings, while improving consistency and repeatability when compared to the manual QuEChERS extraction. Two sets of commodities - apples and lettuce - were analyzed by both LC/QQQ and GC/MS to illustrate analytical differences between using the AutoMate-Q40 and manual QuEChERS extraction.

A precision and accuracy study was performed using both manual and automated QuEChERS extraction for both commodities. A 6 µg/mL stock pesticide standard was used to fortify the samples. Using the AutoMate-Q40, the system spiked the samples with 50 and 250 µL of the pesticide standard yielding 20 ng/g and 100 ng/g check samples. The same process was done for the manual extraction using a micro-syringe. These QC samples were quantitated against their corresponding matrix-matched calibration curve. The analysis was performed in replicates of seven (n=7).

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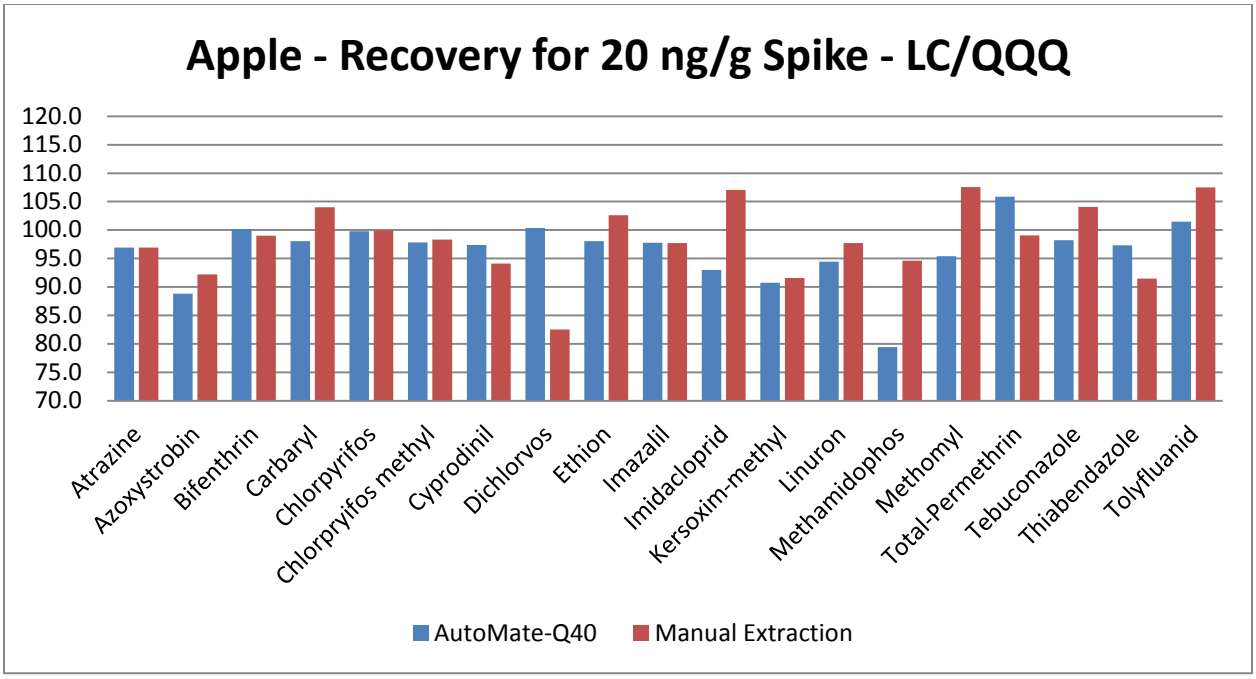

Figure 4. Apple recovery for 20 ng/g QC sample on the LC/QQQ

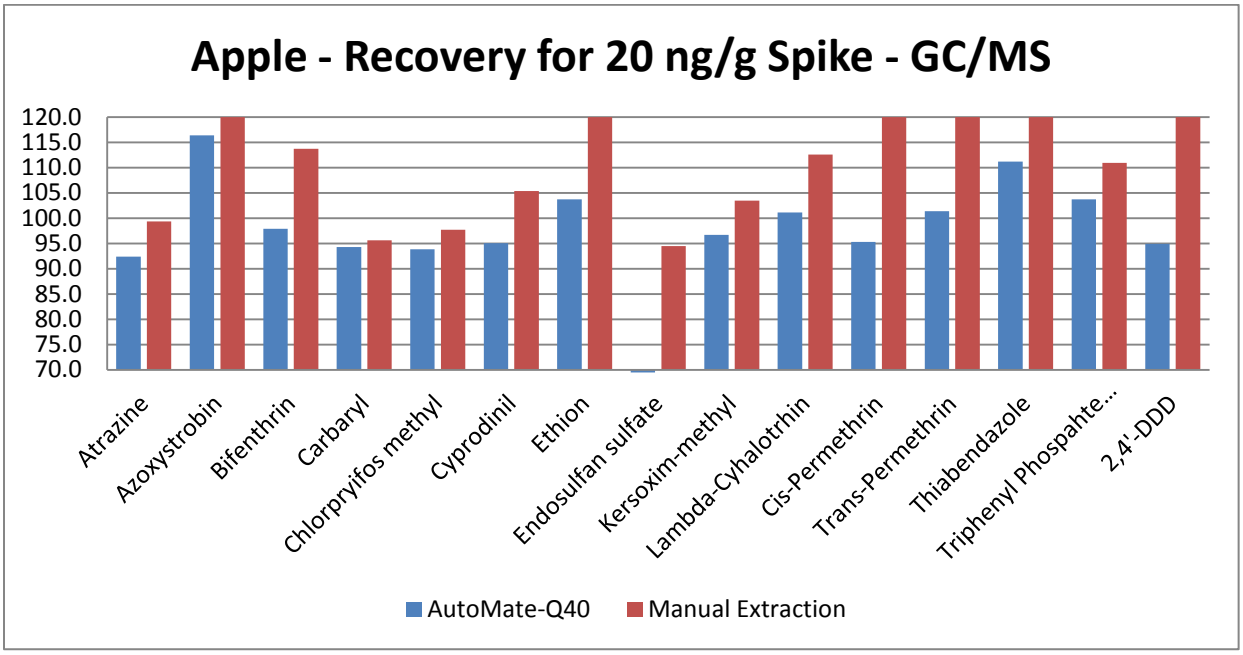

Figure 5. Apple recovery for 20 ng/g QC sample on the GC/MS

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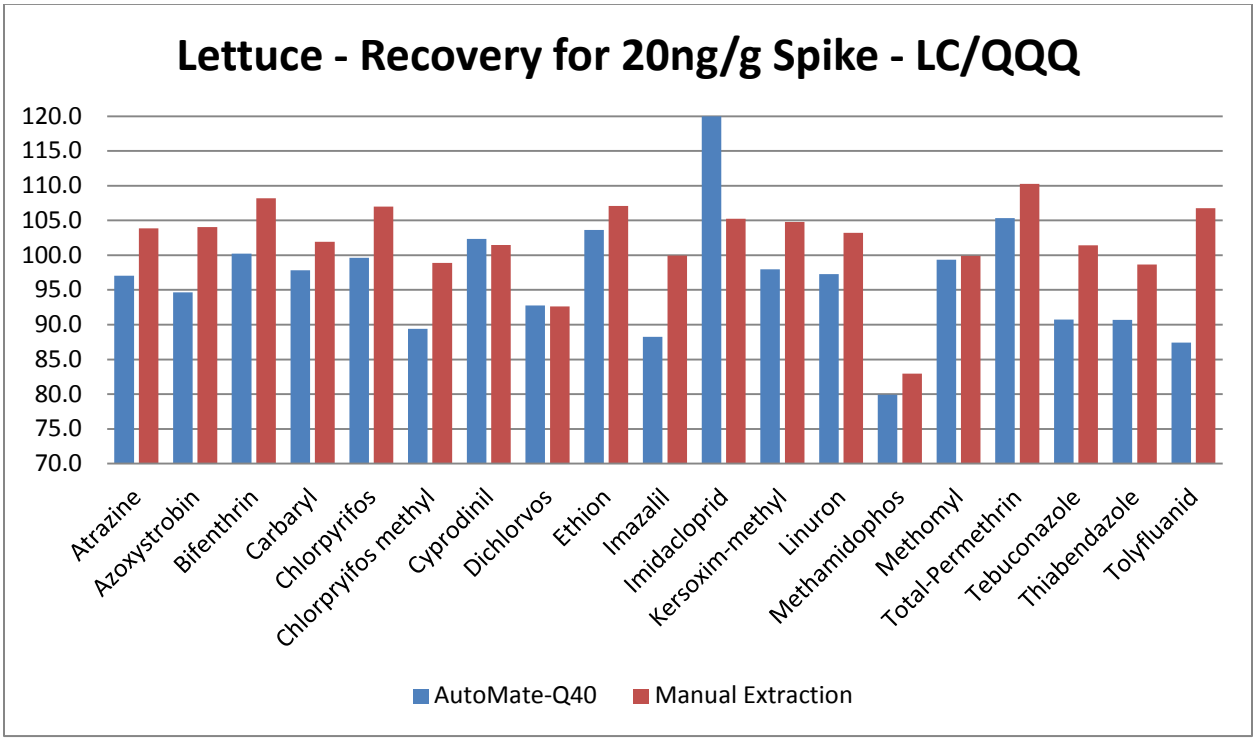

Figure 6. Lettuce recovery for 20 ng/g QC sample on the LC/QQQ

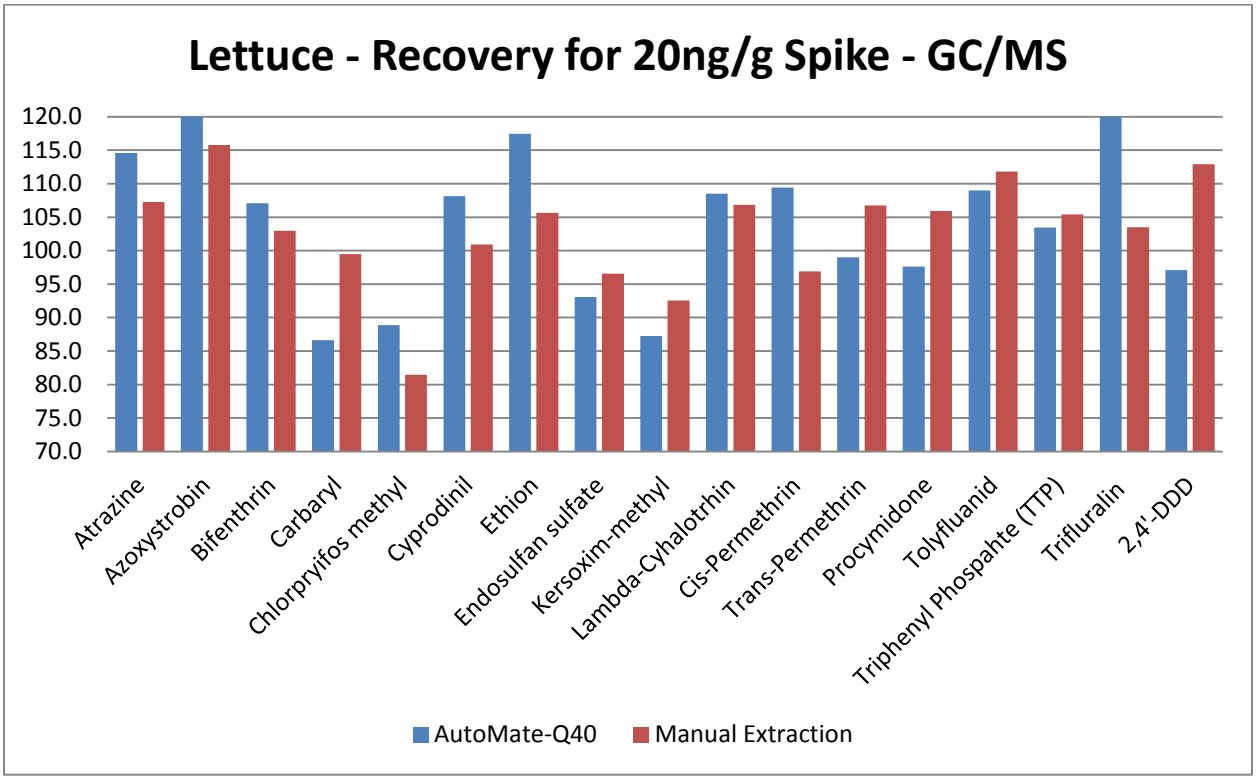

Figure 7. Lettuce recovery for 20 ng/g QC sample on the GC/MS

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Figures 4, 5, 6, and 7 show the recoveries for both the manual and automated QuEChERS extractions that were analyzed by LC/QQQ and GC/MS. The results show that when using the AutoMate-Q40, all pesticides fell within the 70 to 120% recovery range.

		LC/QQQ Average Table			
		AutoMate-Q40		Manual Extraction	
	Concentration	Avg Recovery	Avg %RSD	Avg Recovery	Avg %RSD
Apple	20 ng/g	96.4	3.8	98.3	5.2
	100 ng/g	98.7	2.8	96.6	2.8
Lettuce	20 ng/g	97.6	6.6	102.0	4.4
	100 ng/g	96.6	6.0	94.3	9.5

Table 3. Average LC/QQQ values for both AutoMate-Q40 and Manual QuEChERS Extraction. (n=7)

		GC/MS Average Table			
		AutoMate-Q40		Manual Extraction	
	Concentration	Avg Recovery	Avg %RSD	Avg Recovery	Avg %RSD
Apple	20 ng/g	97.6	4.9	114.8	3.5
	100 ng/g	96.9	3.6	101.5	4.4
Lettuce	20 ng/g	105.7	4.4	103.1	4.5
	100 ng/g	97.7	4.5	88.0	6.2

Table 4. Average GC/MS values for both AutoMate-Q40 and Manual QuEChERS Extraction (n=7)

Tables 3 and 4 show the excellent recoveries achieved when using the AutoMate-Q40, ranging from 96.4% to 105%, while the manual QuEChERS extraction had a wider range from 88.0% to 114.8%. The AutoMate-Q40 also demonstrated greater precision ranging from 2.8% to 6.6% RSD while the manual QuEChERS extraction ranged from 2.8 to 9.5% RSD.

Conclusion

This study demonstrates the feasibility of automating the QuEChERS extraction using the AutoMate-Q40. By automating the liquid handling, 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 Tables 3 and 4, all pesticides gave excellent spike recoveries, ranging from 96.4% to 105%, and excellent precision, ranging from 2.8% to 6.6%. Lastly, by employing an intuitive user interface, data audit trails can be recalled at the touch of a button.

Reference

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. M. Anastassiades: QuEChERS a mini-multiresidue method for the analysis of pesticide residues in low-fat products

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