Determination of Pesticide Residues in Oats by Automated QuEChERS and LC/QQQ

Application Note

Abstract

The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method was developed for the determination of pesticide residues in agricultural commodities. Since development, QuEChERS has been modified to accommodate other matrices such as cereal grains. The rise in popularity and the ease of QuEChERS has driven the need for automation for this manual extraction technique. By using the AutoMate-Q40, it has streamlined 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. Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (LC/QQQ) was employed for the analysis of the LC amenable pesticides in oats. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. By using the AutoMate-Q40 to streamline the QuEChERS method to cereal grains, this provides us with suitable analytical results falling in the method guidelines (range of 70-120% and RSD <20%) for the majority of the target compounds.

Introduction

Increased globalization of the food industry has led to additional concerns about food safety. With recent advancements in multiresidue pesticide screening, the methods have been simplified from the Luke extraction which uses large volumes of dichloromethane which generates a lot of waste per sample The Luke extraction has been simplified by the introduction of the QuEChERS method in 2003[1].

With the ever increasing amount of samples being required for pesticide residue analysis, the QuEChERS extraction is the go to extraction of choice since its installment. Even though 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[2-3].

The aim of this work was to modernize the traditional QuEChERS extraction through the use of automation. Teledyne Tekmar has developed the AutoMate-Q40 for this purpose. This automated platform will streamline the two part QuEChERS method from the liquid extraction through the cleanup. Utilizing the AutoMate-Q40, the QuEChERS method was modified to accommodate various grain matrices such as oats and provides us with good analytical results falling in the method guidelines (range of 70-120% and RSD <20%) for the majority of the target compounds.

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Procedure

1.) Sample Preparation/Extraction

An oat sample was purchased from a local grocery store in Mason, Ohio. The sample was thoroughly homogenized using a mill to create flour like consistency. 3.5 g of oats were placed into a 50 mL centrifuge tube along with 10 mL of water and allowed to swell for 20 minutes. Once the sample swelled, the oat samples were placed into the AutoMate-Q40.

Once placed in to AutoMate-Q40 the system automated the entire QuEChERS extraction method. **Figure 1** shows the steps that are taken by the AutoMate-Q40 to extract the pesticides from the oats. For this analysis, the AutoMate-Q40 used unbuffered QuEChERS extraction salts ($MgSO_4$ and NaCl). The AutoMate-Q40 used the AOAC version of $MgSO_4$, PSA and C18 for the cleanup step. This cleanup step removed fatty acids from the oat matrix[4].

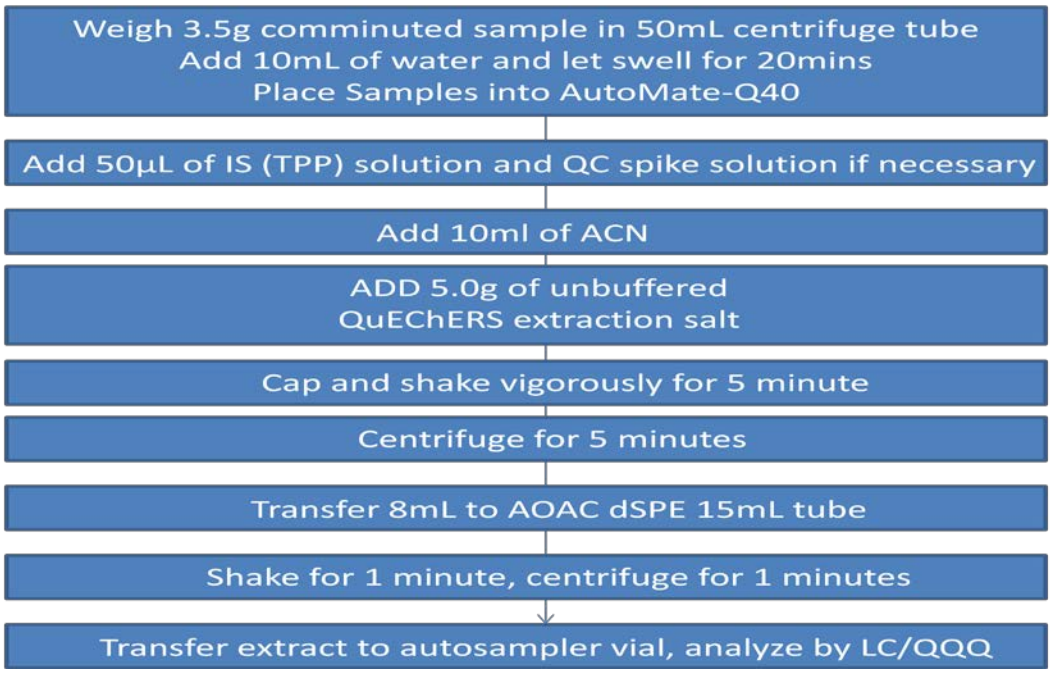

Figure 1: Extraction parameters for the AutoMate-Q40

2.) LC/QQQ Analysis

The analysis was conducted on the Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap triple-quad mass spectrometer (LC/QQQ). 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 1 and 2** give the optimized LC/QQQ analysis parameters for both the chromatographic separation and optimal analyte transitions. **Figure 2** shows the schedule MRM chromatogram for the oats samples spiked at 400 ng/mL. LC samples were prepared by adding 100 µL of final extract into 900 µL of HPLC grade water.

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LC/QQQ 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	t_R(min)	Precursor Ion (m/z)	Quantization Product Ion (m/z)	Confirmation Product Ion (m/z)	DP(V)	CE(V)	CXP(V)
Trifluralin	7.03	334.9	288.7	224.6	1	9	20
Profenofos	8.28	374.8	304.7	346.5	66.0	27.	22.0
Deltamethrin	9.04	524.9	282.7	242.0	31.0	21.0	26.0
Carbaryl	5.39	202.0	144.9	127.0	36.0	13.0	12.0
Bitertanol	7.79	338.0	268.7	99.0	11.0	13.0	26.0
Azoxystrobin	6.61	403.7	371.9	343.8	56.0	21.0	10.0
Methiocarb	6.62	225.9	168.9	120.8	46.0	13.0	14.0
Resmethrin	9.23	339.0	171.1	142.9	56.0	21.0	14.0
Pirimiphos-methyl	8.00	305.9	163.8	108.0	31.0	31.0	14.0
Methoxychlor	6.48	346.9	278.6	N/A	16.0	15.0	26.0
Methoprene	9.35	311.2	278.9	191.0	16.0	9.0	22.0
Malathion	7.01	330.8	127	98.9	56.0	17.0	14.0
Imazalil	7.63	296.9	158.8	254.6	36.0	35.0	12.0
Difenconazole	7.98	407.8	253	151.9	11.0	33.0	6.0
Dichlorvos	4.98	220.9	108.8	127.0	11.0	23.0	8.0
Chlorpyrifos-methyl	8.05	323.8	124.8	291.5	41.0	21.0	36.0
Carbofuran	5.11	222.0	164.9	122.8	56.0	17.0	16.0
Atrazine	5.9	217.0	173.9	103.9	11.0	25.0	64.0

Table 1: LC/QQQ transitions and parameters

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Shimadzu Nexera LC Parameters		
Column	Synergi 4u Fusion RP 80Å	
Dimensions	50.0 X 2.00mm	
Mobile Phase	A 5mM Ammonium Formate in H_2O	
	B 5mM Ammonium Formate in MeOH	
Gradient	Time (min)	%B
	0.1	20.0
	9.0	100.0
	10.0	100.0
	10.1	20.0
	13.0	20.0
	13.1	Stop
Flow Rate (mL/min)	0.3	
Column Temperature (C˚)	30.0	

Table 2: Shimadzu Nexera LC parameters.

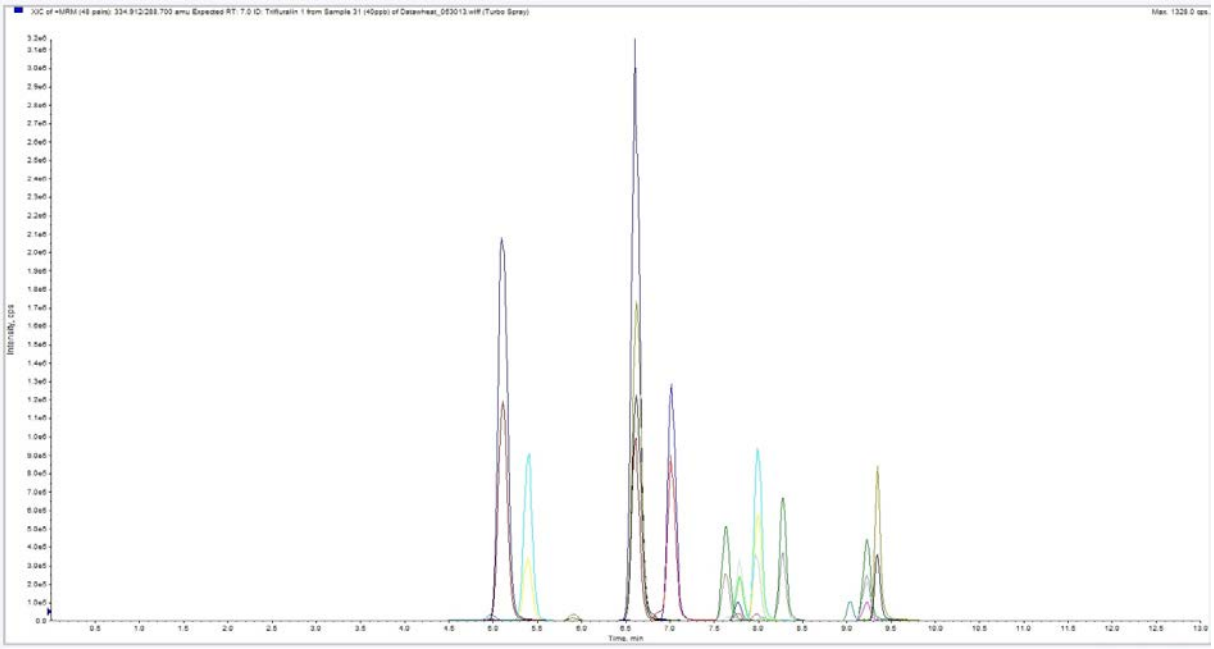

Figure 2: MRM chromatogram for the oats samples spiked at 400 ng/mL

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Experimental-Results

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

A precision and accuracy study was performed using the AutoMate-Q40. A 4 µg/mL stock pesticide solution was used to fortify the oat samples. Using the AutoMate-Q40, it was able to spike the sample with 50 and 250 µL of the pesticide standard that yielded 20 and 100 ng/g check samples. Also, 75 µL of TPP was added to each sample that yielded 100 ng/g of TPP in each sample. These QC samples were quantitated against their corresponding matrix-matched calibration curve. The analysis was performed in replicates of seven (n=7).

		Low Spike		High Spike	
Compounds	R^2	% Recovery	%RSD	% Recovery	%RSD
Profenofos	0.9992	90.50	1.44	90.63	2.27
Deltamethrin	0.9994	99.67	8.74	87.77	2.36
Carbaryl	0.9996	95.16	4.90	100.70	4.17
Bitertanol	0.9992	97.63	3.13	96.72	2.50
Azoxystrobin	0.9943	98.72	2.30	104.16	1.72
Methiocarb	0.9991	93.89	2.68	97.20	3.11
Resmethrin	0.9991	99.50	14.32	89.41	2.65
Pirimiphos-methyl	0.9993	95.64	2.80	94.09	1.89
Methoprene	0.9999	84.34	8.81	84.59	2.14
Malathion	0.9980	96.43	1.59	99.98	1.40
Imazalil	0.9989	85.18	2.11	87.76	2.07
Difenconazole	0.9996	79.43	2.27	95.09	3.73
Dichlorvos	0.9999	100.31	15.45	110.18	10.44
Chlorpyrifos-methyl	0.9997	93.70	4.87	93.43	3.80
Carbofuran	0.9991	98.61	1.48	102.55	3.37
Atrazine	0.9997	94.64	4.47	96.97	2.39

Table 3: LC/QQQ values for the check samples using the AutoMate-Q40.

Table 3 shows when using the AutoMate-Q40 to extract pesticide residues from oat samples, it exhibits excellent recoveries ranging from 79.4% to 100.3% for the low spike and 84.6% to 110.2% for the high spike. These recoveries fall within the methods guidelines of 70-120% recovery. The AutoMate-Q40 also demonstrates great precision ranging from 1.4% to 14.3%RSD for the low spike and 1. 9% to 10.4% RSD for the high spike which falls within the method guide lines of RSD <20%.

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Conclusions

The QuEChERS sample extraction method was developed for the determination of pesticide residues in agricultural commodities. Since its development, QuEChERS has been modified to accommodate other matrices such as cereal grains. This study demonstrates the feasibility of automating the QuEChERS extraction of oats using the AutoMate-Q40.

Liquid Chromatography coupled to a triple-quadrupole mass spectrometry was employed for the analysis of the LC-amenable pesticides. Quantification of check samples were based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy.

As shown above in **Table 3**, all pesticides gave outstanding spike recoveries ranging from 79.4 to 110.2% and also showing excellent precision, ranging from 1.4% to 14.3%. Utilizing the AutoMate-Q40, the QuEChERS method was modified to accommodate grain matrices such as oats that provided us with excellent analytical results falling within the method guidelines (range of 70-120% and RSD <20%) for the majority of the target compounds. This application demonstrates that using the AutoMate-Q40 enables a fast, reliable and more reproducible extraction, while offering significant time and labor savings.

References

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