Pesticide analysis using QuEChERS extraction: A comparison of manual and automated approaches

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Abstract

The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) extraction method is a simple and fast procedure when compared to traditional semi-volatile extraction techniques. However, it still requires dedicated manpower, extraction equipment, manual spiking and pipetting, and a wide range of reagents and consumables. In this poster the analytical and practical challenges of automating this technique is discussed and a comparison of extraction recoveries achieved both manually and by automation is evaluated. Data is presented that justifies automation as a possible solution to increase sample throughput, reduce costs, and improve the accuracy and precision of the extraction.

Introduction

The QuEChERS method was published in 2003 for the extraction of pesticide residues in agricultural commodities.[1] Modifications to the method have expanded the scope to include many additional matrices and target analytes. After validation, the methods were adopted as AOAC Official Method 2007[1], and EN 15662:2008.[2] These methods require several manual steps, such as addition of extraction solvent and of salts/buffer, spiking, shaking, mixing, centrifugation, transferring to a dispersive solid phase extraction (dSPE) step, and finally measuring and transferring a portion of the extract.[3]

The rise in popularity of the technique and the increase in sample testing loads have lead to automation as a possible solution to increase productivity. This poster presents a system designed and optimized to automate the QuEChERS sample extraction workflow. Results from both the automated and manual QuEChERS extractions are compared using the AOAC Method 2007.[1]

Experimental

Instrument Conditions

Analyses were performed on a Thermo Scientific TRACE 1310 GC coupled to an Thermo Scientific ISQ single quadrupole mass spectrometer. Instrument conditions can be seen in Tables 1 and 2. The QuEChERS extractions were performed automaticaly by the AutoMate-Q40 (Teledyne Tekmar) and manually by an analyst.

Table 1. GC Conditions

Column	Restek 5MS-Sil 30 m x 0.25 mm x 0.25 µm
Column Constant Flow	1.0 mL/min
Oven Program	40 °C (1.5 min), 25 °C/min to 150 °C (0 min), 7 °C/min to 250 °C (0 min) 25 °C/min to 290 °C (10 min)
S/SL Temperature	250 °C
S/SL Mode	Splitless with Surge Pressure
Split Flow	50 mL/min
Transfer Line Temperature	290 °C

Table 2. GC-MS acquisition parameters

Analyte	SIM	RT (min)
Trifluralin	306.0, 264.0	10.74
Atrazine	200.0, 215.0	11.81
Chlorothalonil	266.0, 264.0	12.45
Chloropyrophos-methyl	286.0, 125.0	13.52
Cyprodinil	224.0, 225.0	15.64
Procymidone	283.0, 285.0	16.12
2,4'-DDD	235.0, 237.0	17.55
Kresoxim-methyl	116.0, 131.0	17.61
Triphenyl Phosphate	77.0, 65.0	19.96
Bifenthrin	181.0, 165.0	20.69
Lambda-Cyhalothrin	181.0, 197.0	21.62
Cis-Permethrin	183.0, 163.0	22.31
Trans-Permethrin	183.0, 163.0	22.43

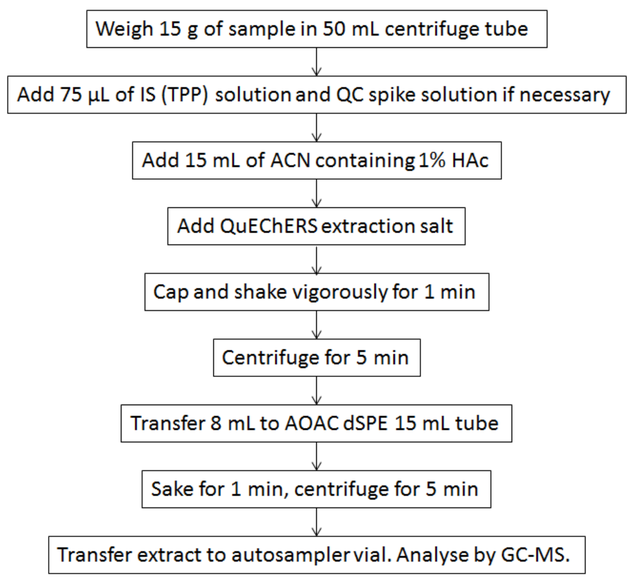
Sample Preparation

Six pounds of apples were purchased from a local grocery store in Mason, Ohio, USA. All six pounds were chopped into small cubes as prescribed in the method. The whole apple was used but the seeds were discarded. The chopped apple cubes were then placed into a plastic bag and frozen overnight. On the day of analysis the required amount of sample was removed and then carefully blended.

For the extraction, a 15 g (+/-0.1 g) homogenized apple sample was placed into a 50 mL centrifuge tube. The AutoMate-Q40 then 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 are then transferred from the VialVison station – a liquid level sensor - to a pipetting station where 15 mL dSPE cleanup tube waits. The centrifuge tubes are uncapped, and using the air displacement pipetter (ADP) an 8 mL aliquot is transferred from the extraction tube to the 15 mL dSPE tube containing 400 mg of PSA and 1200 mg of $MgSO_4$. The 15 mL dSPE tube is moved to the shaker and shaken for 1 min. Once the mixing is complete the sample is moved to the centrifuge and spun for 5 min at 4000 rpm.

Once centrifuging is complete, the 15 mL dSPE cleanup tube is moved to the VialVision station to determine the amount of extract available to be transferred to the final extract tube. The 15 mL dSPE cleanup tube is moved from the VialVison station to a shuttle where the corresponding 15 mL final extract tube waits. The cleanup and final extract tubes are uncapped and, using the ADP 5 mL of the final extract is transferred to the final tube. Then an aliquot from the final extract tube is manually placed into an autosampler vial, and analyzed by GC-MS. The flow chart below shows the AOAC QuEChERS extraction procedure for both the AutoMate-Q40 and the manual hand extraction.



AOAC QuEChERS steps for automatic and manual procedures

Results

By automating the liquid handling, addition of salts/buffers, sample mixing, centrifugation, pipetting and liquid level sensing (VialVision), the QuEChERS extraction process is fast, and easy. The AutoMate-Q40 offers time and labor savings, while improving consistency and repeatability when compared to manual QuEChERS. Two sets of data were analyzed to illustrate the differences between the AutoMate-Q40 and manual QuEChERS extraction.

Linearity

AOAC Method 2007 specifies preparation of a matrix matched calibration to compensate for potential interferences. Six matrix blanks were extracted with internal standard and surrogates and were spiked with a prescribed amount of a 2 µg/mL calibration stock standard. The calibration ranges for all compounds were performed at 5-400 ng/g. The calibrations were extracted both by hand and by automation (Table 3). Each calibration curve was prepared at levels of 5, 10, 20, 50, 100, and 400 ng/g. The results for both sets of extractions are shown in Tab 4.

Table 3. Calibration framework for both hand and automated QuEChERS extractions

Standard (µL)[a]	Extract (µL)[b]	Concentration (ng/g)[c]
5.0	995	5.0
10.0	990	10.0
20.0	980	20.0
50.0	950	50.0
100.0	800	100
400.0	600	400

[a]Amount of 2 µg/mL AOAC Pesticide Stock Std
[b]Amount of Final Extract Used
[c]Final Concentration in 2 mL sample

Table 4. Linearity study for both the AutoMate-Q40 and manual extractions

	Linearity (R^2)	
Component in Apple Matrix	**AutoMate-Q40**	**Hand Extraction**
Trifluralin	0.9976	0.9930
Atrazine	0.9992	0.9969
Chlorothalonil	0.9990	0.9926
Chloropyrophos-methyl	0.9980	0.9955
Cyprodinil	0.9970	0.9986
Procymidone	0.9987	0.9961
2,4'-DDD	0.9981	0.9970
Kresoxim-methyl	0.9991	0.9986
Triphenyl Phosphate (TPP)		
Bifenthrin	0.9988	0.9987
Lambda-Cyhalothrin	0.9974	0.9993
Cis-Permethrin	0.9995	0.9994
Trans-Permethrin	0.9998	0.9992
Average	0.9971	0.9962

Recovery and Reproducibility

A precision and accuracy study was performed using both manual and automated QuEChERS extraction techniques. A 6 µg/mL stock AOAC QC pesticide standard was used to fortify the homogenized apple samples. Using the AutoMate-Q40, the system spiked the samples with 50 and 250 µL of the AOAC QC check standard yielding 20 and 100 ng/g check samples. The same process was done for the manual extractions using a micro syringe. These QC samples were quantitated against their corresponding matrix-spike calibration curve. The analyses were performed in replicates of five (n=5).

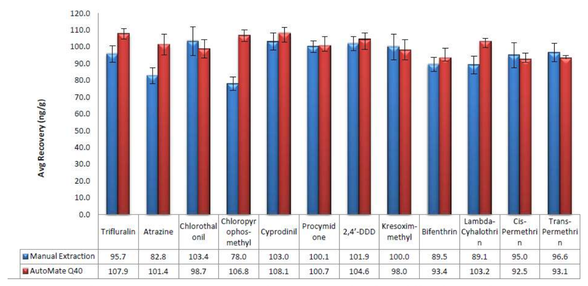


Figure 1. Recovery for 20 ng/g fortified apple.

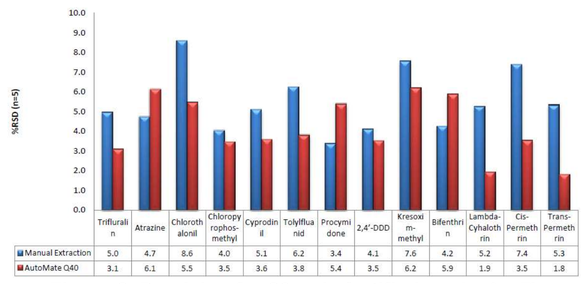


Figure 2. Precision for 20 ng/g fortified apple. (n=5)

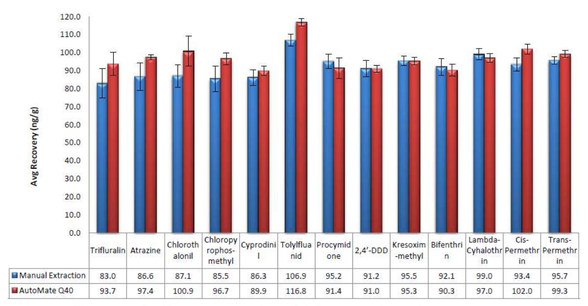


Figure 3. Recovery for 100 ng/g fortified apple.

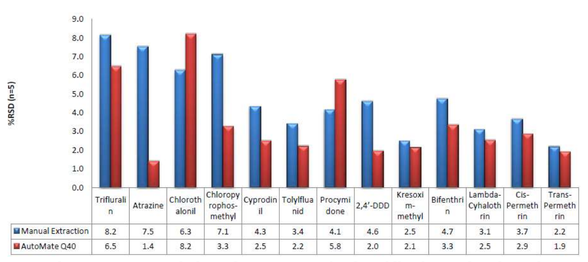


Figure 4. Precision for 100 ng/g fortified apple. (n=5)

Figures 1 to 4 show the recoveries and precision (%RSD) for both the manual and automated QuEChERS extractions. It can be seen from the results that when using the AutoMate-Q40 that all of the pesticides exhibited excellent recovery (average of 100.8% for 20 ng/g spike and 97.1% for 100 ng/g spike) and precision (average of 4.1%RSD for the low spike and 3.4%RSD for the high spike). In comparison, the results of the manual QuEChERS exhibit slightly lower recoveries (average of 87.3% for 20 ng/g spike and 92.1% for 100 ng/g spike) and comparable precision (RSD 4-5% on average for both spikes). Figure 5 shows a chromatographic comparison of a 100 ng/g fortified apple extract extracted by both the AutoMate-Q40 and manual QuEChERS.

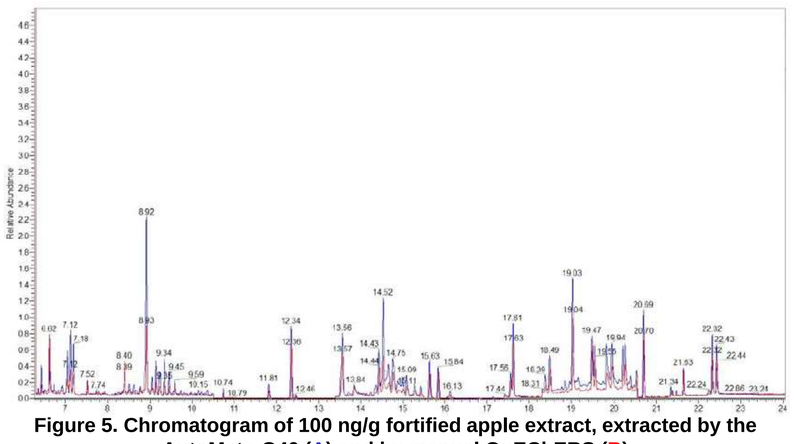


Figure 5. Chromatogram of 100 ng/g fortified apple extract, extracted by the AutoMate-Q40 (A) and by manual QuEChERS (B)

Conclusion

By automating the liquid handling, the addition of salt/buffers, sample mixing, pipetting, and liquid level sensing using the patent pending VialVision, the extraction process is fast, and easy. The AutoMate-Q40 not only offers time and labor savings, but also improves extraction precision and accuracy. As shown in the data above, all of the pesticides give excellent spike recoveries on average 100.8% for the 20 ng/g spike and 97.1% for the 100 ng/g spike and excellent precision on average of 3-4%RSD.

References

1. AOAC Official Method 2007.07 Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.
2. 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. (http://quechers.cvua-stuttgart.de/pdf/reality.pdf . Accessed on June 25, 2015.)

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