

Pesticide Analysis Using QuEChERS Extraction : A Comparison of Manual and Automated Approaches

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. The European Standard is Method EN 15662:2008. While originally developed for plant matrices, the technique has since been adapted to be used in several applications and in many additional matrices. The rise in popularity of the technique and the increase in sample testing loads have led to automation as a possible solution to increase productivity. The method, as mentioned, is a simple and fast extraction when compared to traditional semi volatile extraction techniques. However, it still requires dedicated manpower, sample prep equipment, manual spiking, manual pipetting, and a wide range of reagents and consumables. The analytical and practical challenges of automating this technique will be discussed and a comparison of extraction recoveries achieved both manually and by automation will be evaluated. Data will be presented that justifies automation as a possible solution to increase sample throughput, reduce costs, and improve the accuracy and precision of the extraction.

Introduction

With the increasing globalization of the food industry there are consequently more concerns about food 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. The European Standard Method EN 15662:2008 and the AOAC 2007.01 methods were in part, derived from these refinements¹⁻³. These methods (EN 15662:2008 and AOAC 2007.01) require several manual steps, such as addition of extraction solvent, salts/buffer addition, spiking, shaking, mixing, centrifugation, transferring to a dispersive solid phase extraction (dSPE) step, and finally measuring and transferring a portion of the extract.

The focus of this poster is on the analytical and practical challenges of automating the QuEChERS extraction technique discussed above. This poster will be broken down into five sections detailing potential advantages of automating the QuEChERS extraction.

- Solvent addition, spiking and preservation
- Salt/buffer addition
- Sample Mixing
- Pipetting
- Liquid level sensing (VialVision)

Results/Discussion

Solvent Addition, Spiking and Preservation

In addition to accuracy and precision, automating the liquid handling needs of a QuEChERS extraction requires a versatile, robust, and flexible design to meet the needs of the wide range of applications used in the laboratories today. This is accomplished with a dual syringe drive system, one equipped with a 25ml syringe to handle larger solvent volumes, and a 250ul syringe to handle smaller spiking volumes. Multiport ceramic distribution valves allow for the user to connect multiple extraction solvents and spiking solutions to the fluidic pathway without cross contamination.

The larger 25ml syringe drive and distribution valve combination can accommodate any combination of three different extraction solvents or water. These reagents can be delivered to the sample vial over a volume range of 1-25mls. The 250ul syringe drive and distribution valve combination has the option to connect up to three separate 50ml sealed amber coated vessels. These vessels can contain spiking solutions and preservation reagents to be added either to the sample or to the final extract.

In both the EN 15662:2008 and the AOAC 2007.01 methods, the main extraction solvent is acetonitrile or an acidified acetonitrile solution. Samples with water content <80% require the addition of water to hydrate the sample prior to extraction. The syringe drive and valve are chemically compatible with virtually any extraction solvent; but this study validated the accuracy and precision of delivering water and acetonitrile. (Table 1) The system was confirmed to deliver within 1% accuracy and have repeatability of less than 1%RSD.

Acetonitrile		Water	
Avg (mL)	9.93	Avg (mL)	9.95
%Rec	99.3	%Rec	99.5
%RSD	0.05	%RSD	0.18

Table 1: Accuracy and precision of the 25mL syringe drive. (n=25)

Validation of the syringe system for spiking was done both analytically by GC-MS and volumetrically. The system can be programmed to deliver spike volumes of 25ul, 50ul, 75ul, 100ul, 150ul, and 200ul. Ten replicates were delivered at each spike volume to empty tared vials and the weights were recorded. Accuracy and precision of the spike recoveries were calculated and recorded. Table 2 illustrates that the 250µL syringe drive is capable of delivering the prescribed spike volumes accurately and precisely.

Amount	25µL	50 µL	100 µL	200 µL	250 µL
Avg(µL)	24	48	97	197	248
%Rec	96.0	96.0	97.0	98.5	99.2
%RSD	3.9	0.8	0.3	0.2	0.04

Table 2: Accuracy and precision of the 250uL syringe drive. (n=10)

To test the liquid handling system as one entity, the system was programmed to add 10ml of acetonitrile and a known spike value into the QuEChERS sample prior to extraction. The samples were then extracted following the procedure outlined in EN 15662:2008. These spike results were calculated and the results were compared to an identical set of extractions that were performed by manually adding extraction solvent and spikes. (Table 3)

Pesticide	Mid-QC 100ng/ml				Low -QC 20ng/ml			
	Automated Spike Recovery	Automated %RSD	Hand Spike Recovery	Hand Spike %RSD	Automated Spike Recovery	Automated %RSD	Hand Spike Recovery	Hand Spike %RSD
2,2',5'-Trichlorobiphenyl (PCB-18) surrogate	99.1	5.4	99.2	9.0	97.6	3.5	97.6	4.1
2,4,4'-Trichlorobiphenyl surrogate	106.4	3.7	104.7	8.0	112.4	3.2	107.8	3.3
2,2',5,5'-Tetrachlorobiphenyl (PCB-52) surrogate	81.3	3.1	79.1	7.9	86.3	3.0	82.8	3.9
Triphenylphosphate (TPP) IS	106.8	1.4	106.8	9.4	106.7	1.9	106.0	2.4
Dichlorvos (DDVP)	94.1	7.7	98.6	8.4	83.3	5.7	91.9	5.6
Trifluralin	95.5	5.6	94.7	9.5	116.5	4.5	124.6	3.1
Atrazine	96.9	3.1	99.0	6.3	104.4	5.5	99.2	4.2
Chlopyrifos Methyl	107.3	3.2	104.5	9.8	116.0	4.1	119.2	3.2
Linuron	105.5	6.1	109.9	10.6	93.3	7.8	103.2	2.8
Chlorpyrifos (Dursban)	99.0	4.9	108.1	15.0	99.2	13.4	101.6	7.1
Cyprodinil	99.3	2.3	100.7	8.7	108.3	4.4	107.8	3.9
Thibendazole	100.9	6.4	93.4	13.7	130.0	5.4	115.1	5.2
Procymidone	102.0	1.5	99.8	5.3	112.0	3.9	110.1	3.3
2,4'-DDD	99.7	2.3	100.0	8.9	103.9	3.4	106.4	1.9
Kesoxim Methyl	97.9	2.8	101.7	8.7	95.4	4.0	100.0	3.2
Ethion	95.7	2.9	99.3	12.2	119.7	2.3	129.1	6.8
Endosulfan Sulfate	110.2	2.2	106.8	9.3	113.4	4.5	108.1	6.4
Bifenthrin	99.4	2.7	103.0	10.1	98.9	2.9	103.2	3.1
Lambda-Cyhalothrin	90.7	4.6	94.6	10.7	112.5	4.8	121.3	4.0
cis-Permethrin	99.4	3.6	99.3	9.2	106.9	4.2	102.5	3.6
trans-Permethrin	100.8	2.1	100.2	8.0	106.8	4.4	107.3	1.9
Average	99.4	3.7	100.2	9.5	105.9	4.6	106.9	4.0

Table 3: Recoveries and reproducibility of pesticides spiked in Apple using a manual and automated technique. n=8

The experiment was performed in sample batches of 8 replicates so the automated and manual spiking precision could be evaluated. As expected, the automated spiking proves to have equal or if not better precision over the manual spiking technique, with the average recoveries ranging from 99.4-105.9%.

Salt/Buffer Addition

Several suppliers offer QuEChERS packets or tubes that contain pre-weighed salts and buffers for the EN15662, AOAC 2007.01, and the original unbuffered methods. This has simplified the extraction process and eliminated the tedious task of individually weighing out the prescribed amounts needed for each sample. Automating this process has the potential to further simplify the process and reduce consumable costs associated with the extraction. While automating the salt delivery would prove to be a less expensive and more efficient process, there are some challenges to overcome in order to ensure a robust, reliable, accurate and precise system.

The salt delivery system must be flexible enough to accommodate different types of extraction salts to comply with the numerous variations of QuEChERS methods in use today. In addition, the system must be free of contamination, and have the ability to keep the extraction salts in an anhydrous state to work effectively. Lastly, the delivery system must be accurate and precise enough to meet the demands of the methods. With these prerequisites in mind, Tekmar has developed a salt delivery system to overcome these challenges. (Figure 1) The salt delivery dispenser is equipped with a “hopper” capable of holding over 2Kg of premixed QuEChERS salts. This volume of salts is enough to extract between 250-400 samples depending on the extraction method. To keep the salts free of contamination and moisture, the container is sealed and kept under a blanket of dry gas during use. To achieve the necessary accuracy, precision and reliability of the system a “grit” form of magnesium sulfate was used. This enables a proper flow of materials in the system to avoid clogging, and clumping. Using the “grit” form of magnesium sulfate has also been proven to increase recoveries of some pesticide residues⁴. The salts are volumetrically added into the centrifuge tube utilizing a gravity fed hopper and indexing rotor system. The volume is method dependent and can be adjusted to accommodate a delivery of between 4-10g of salts.



Figure 1: Salt dispenser equipped with gravity fed hopper.

Tests were performed to confirm the precision and accuracy of the salt dispenser. The feed hopper was loaded with 500.0g of the bulk extraction salts endorsed in AOAC2007:01 (a ratio of 6.0 g $MgSO_4$: 1.5 g NaOAc). The volume of the rotor was set to dispense 7.5g of the salts and the dispenser was programmed to deliver 25 doses. The weights of the doses were recorded and evaluated for accuracy and precision of the delivery. The same test was performed using the extraction salts endorsed in EN15662:2008 (a ratio of 4.0 g $MgSO_4$: 1.0 g NaCl: 1.0 g TSCD: 0.5 g DHS). The rotor volume was adjusted to deliver 6.5g of the extraction salts and again the weights were recorded and evaluated for precision and accuracy of the delivery. (Table 4)

	EN 15662:200 Extraction Salts (6.5g)	AOAC 2007.01 Extraction Salts (7.5g)
Avg (g)	6.53	7.48
Stdv	0.05	0.08
%RSD	0.77	1.12

Table 4: Precision and accuracy of the solids dispenser for both the EN and AOAC extraction salts. (n=20)

As can be seen from the data, the delivery of the extraction salts is very accurate and repeatable. The ability to adjust the dosage volume also allows the user to customize their sample preparation to meet their specific needs. Additionally, the range of the delivery volumes meets the requirements of EN15662:2008, AOAC2007:01, and the original unbuffered QuEChERS methods. The salt dispenser eliminates the task of weighing out individual doses for each sample, and the use of secondary tubes to transfer the salts, and virtually ends spillage and waste.

Mechanical Mixer

Extraction efficiency and reproducibility are affected by many variables throughout the QuEChERS extraction. One of the keys is sample mixing. The pesticide residues are most effectively extracted from the sample matrix and into the extraction solvent when the sample is properly buffered (if needed), and when there is an adequate surface interaction between the extraction solvent and the sample matrix. Doing this consistently in a manual process can be challenging. Equally important to the sample preparation process is the removal of matrix interferences that negatively affect the quantitation of the pesticide residues. The removal of these interferences is done by way of dispersive solid phase extraction (dSPE). This technique has proven successful, provided the proper solid phase media is selected, and the extract is sufficiently mixed with the cleanup media. There are several advantages to automating the mixing step, as well as the dSPE cleanup portion of the extraction. Besides the obvious labor saving, employing a mechanical mixer for the QuEChERS extraction eliminates the variation of the shaking techniques of different analysts, and improves the sample interaction with the extraction solvents due to a more rapid and vigorous mix.

Teledyne Tekmar has developed a mechanical shaker that is placed within the AutomMate-Q40 platform which is capable of mixing the samples approximately twice as fast as an analyst shaking the samples manually. The increased speed and force of the mechanical shaker could potentially reduce the shaking time needed for the extraction. The shaker mechanism is capable of mixing up to 2-50mL centrifuge tubes and 2-15mL centrifuge tubes at one time to allow for increased through-put in the automated platform. (Figure 2)

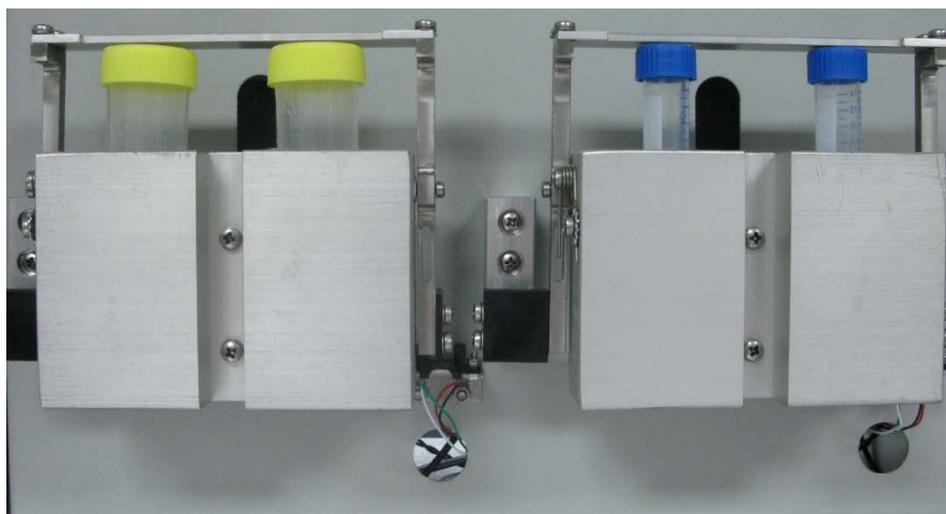


Figure 2: Mechanical shaker.

The shaker design has a mechanical stroke of 2.00" and reaches speed of 7 strokes/sec. to completely mix any sample matrix. Figure 3 is a comparison of several sample matrices that were mechanically shaken and manually shaken. All matrices that were mechanically mixed appeared to be mixed equal to or better than samples that were manually shaken. Confirmation of mixing efficiency was also proven with a spike recovery study.

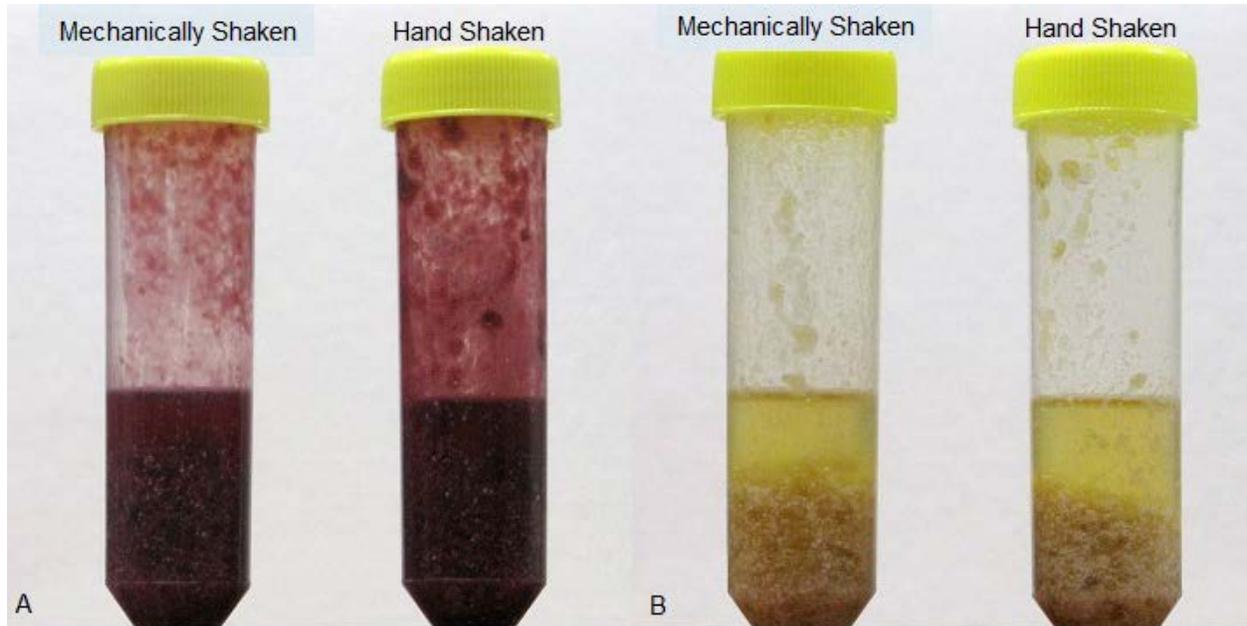
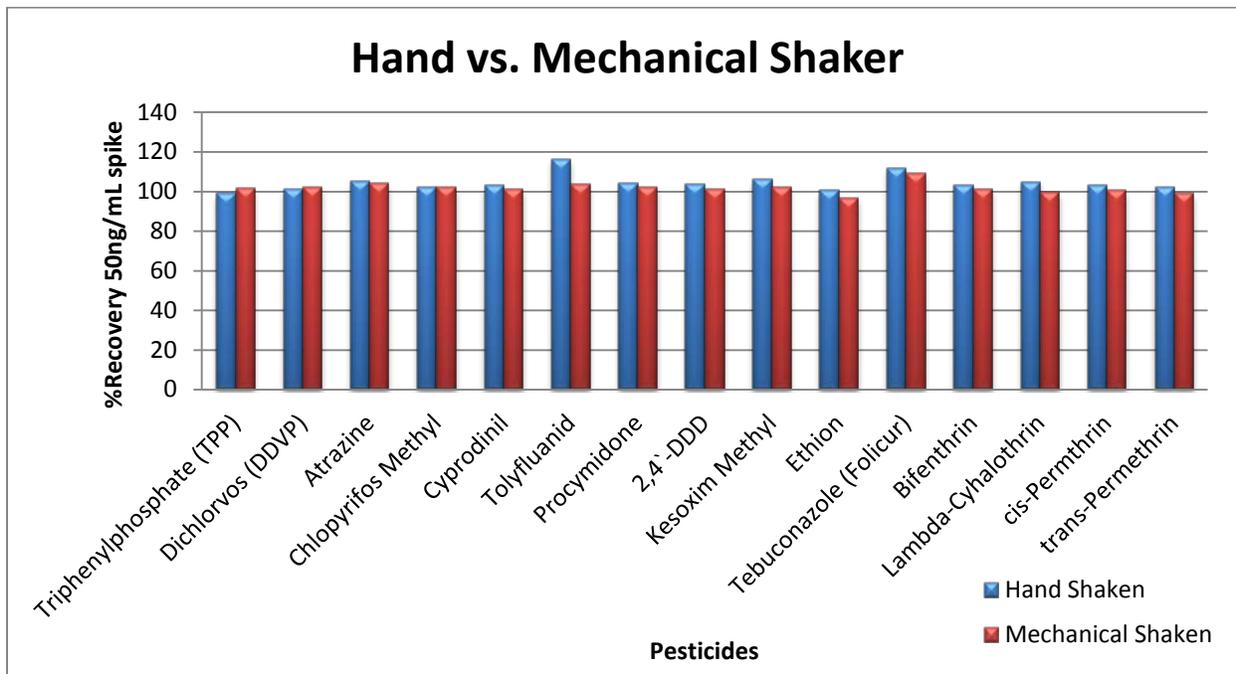


Figure 3: Visual representation of both mechanical and hand shaken techniques.

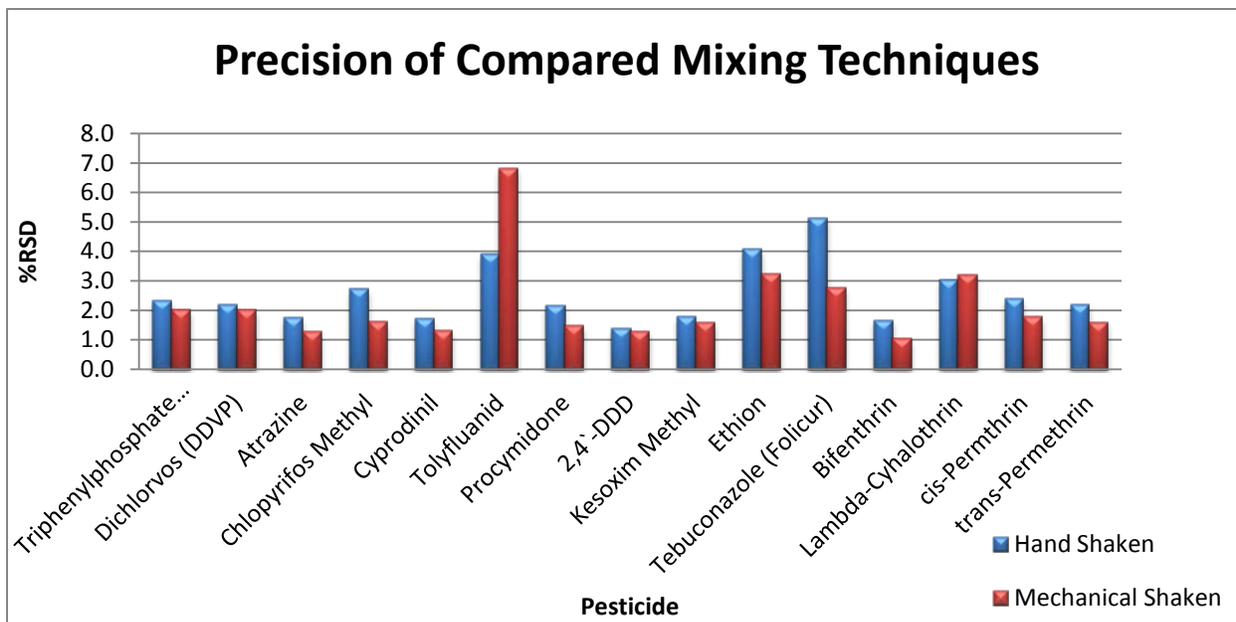
A: Blackberry B: Apple

Several experiments were performed to confirm the reproducibility and efficiency of the mixer. In the first experiment, an apple matrix was manually spiked with a 50ppb pesticide spiking solution. The samples were then extracted following the EN 15662:2008 extraction method parameters. The extractions were performed using both a manual shaking technique and the mechanical shaker for the mixing steps of the method. The sample extracts were analyzed and the spike recoveries were compared. (*Graph 1*) All spike recoveries were within +/-10% of the expected value regardless of the shaking technique employed.



Graph 1: Pesticides recoveries for 50ppb spiked apple matrix.

The experiment was performed in sample batches of 8 replicates so the repeatability of the mixing could be evaluated. (Graph 2) As expected, the mechanical shaking technique shows improved consistency (%RSD below 7%) from sample to sample exhibiting slightly better precision of the analyte recoveries.



Graph 2: Precision of the mechanical shaker.

Current QuEChERS methods call for a shake time of one minute. In most cases, a one minute extraction is sufficient to extract the pesticide residues from the sample matrix over to the extraction solvent. As the QuEChERS technique has evolved, many modifications have been attempted in an effort to improve the recoveries, increase sample throughput, and expand the technique into other markets. These modifications often include alternate

mixing times than those published in the validated methods. The use of an automated mechanical shaker allows the analyst to devise unattended mixing time studies to validate potential improvements.

In addition to the precision and accuracy experiments, a mixing time study was performed. Again, the apple matrix was spiked with a 50ppb pesticide spiking solution. The samples were extracted using the EN 15662:2008 extraction method. The mixing times of the extractions were varied by programming the mechanical shaker to shake sets of samples for 15, 30, 45, and 60 seconds. (Figure 4) The average area recovery of the extraction sets was calculated and then plotted for comparison. (Graph 3) For the samples analyzed in this study, the mechanical shaker proved capable of effectively extracting the pesticide residues from the sample matrix in as little as 15sec. with all recoveries greater than 85%.

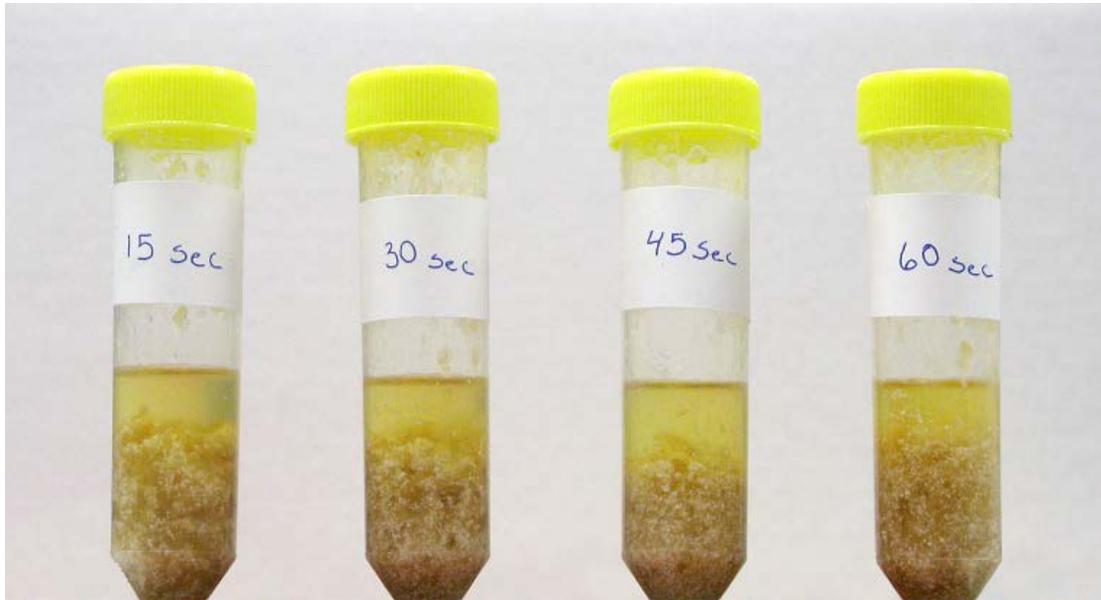
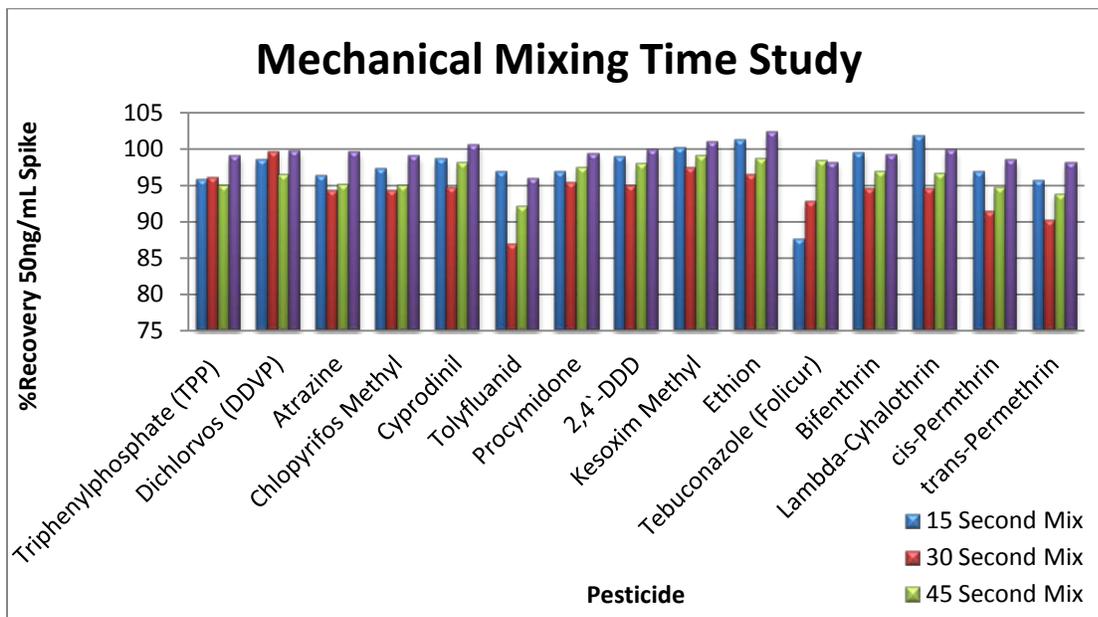


Figure 4: Mechanical shaken samples implementing different mix times.



Graph 3: 50ng/mL spike using different extraction times.

Automated Pipetting

One of the more labor intensive steps in the QuEChERS extraction process is pipetting. Accurate and precise pipetting is necessary to the sample extraction process to ensure accurate results. Pipetting occurs multiple times during the QuEChERS extraction, increasing the potential for human error, which can negatively affect the data. Many liquid handling instruments on the market today offer automated pipetting solutions to increase precision and accuracy by eliminating the human error factor. Tekmar has chosen an Air Displacement Pipetting (ADP) technique for the automation of the pipetting process in the new AutoMate-Q40. The ADP offers several advantages, the biggest of which is a simplistic design. Displacing volumes of air to aspirate and dispense liquids eliminates the need for sample line priming, rinsing and sweeping, and minimizing the risk of cross contamination.

Experiments were conducted to evaluate the accuracy and precision of robotically pipetting acetonitrile from a 50mL centrifuge tube containing a QuEChERS extract and dispensing it into a 15mL centrifuge tube containing dSPE cleanup media. Additionally, aliquots were pipetted from the dSPE cleanup vials and dispensed into a final screw cap storage vial to simulate the two pipetting processes involved in the QuEChERS extraction.

Automating the pipetting process for QuEChERS samples is challenging due to the multi-layered matrix which could plug the pipette tips. To overcome this issue, a three prong approach was taken to ensure accuracy, repeatability and reliability in the pipetting process. First, through the use of our patent pending VialVision system we are able to accurately determine the volume of liquid that is available for aliquoting. Secondly, through the use of a conductive pipette tip we are able to accurately detect the top layer of the extract. This allows us to determine the exact depth at which pipetting can begin. Lastly, by programming the robotic ADP probe to follow the liquid down at a controlled speed during the pipetting process we can reliably take the aliquot without fear of disturbing the underlying layer of the sample matrix. (Figure 5)

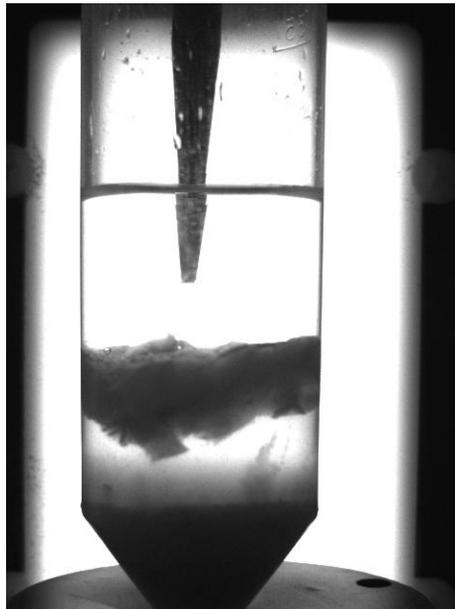
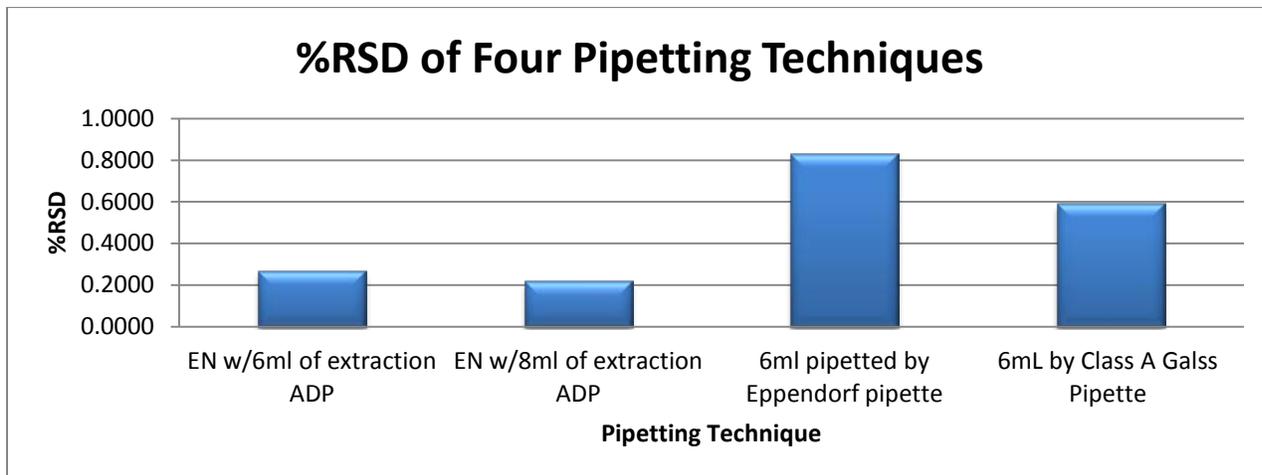


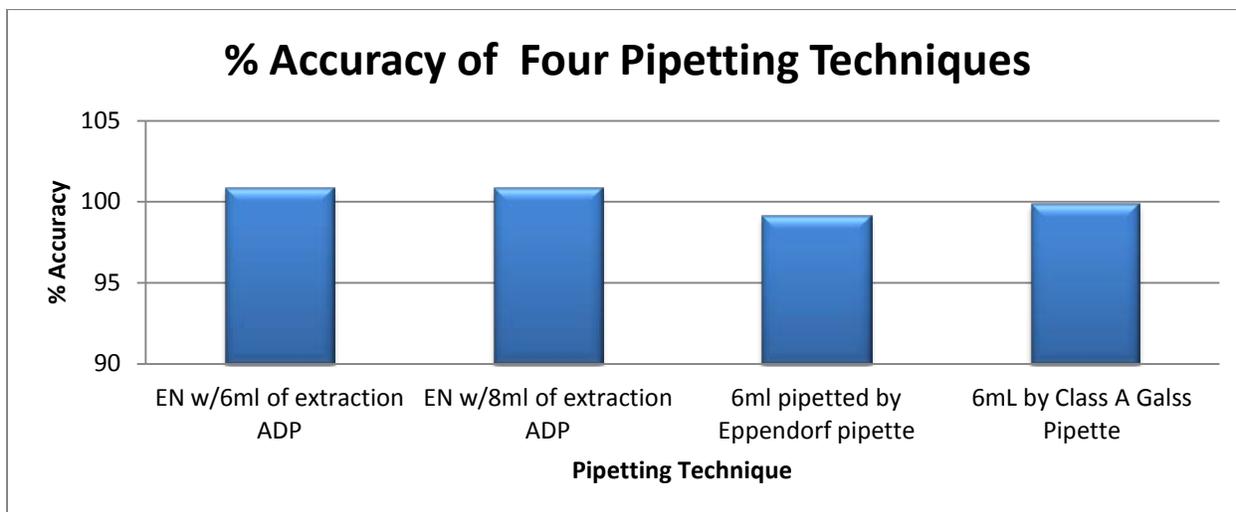
Figure 5: Visual representation of the ADP during the pipetting process.

A precision and accuracy study consisting of four sets of samples (n=10) was conducted using the ADP and manual techniques for pipetting. The sample sets were prepared according to both the EN 15662:2008 and AOAC 2007.01 methods. The appropriate amounts of sample, acetonitrile, and extraction salts were added to the 50mL centrifuge tubes. They were then shaken and spun down in a centrifuge. 6mL (EN 15662:2008) and 8mL (AOAC 2007.01) aliquots were taken from the 50mL extraction tubes and were transferred into empty extraction vials. The volumes in the vials were then calculated gravimetrically. Graphs 4 and 5 illustrate the precision of the pipetting process using both the ADP to pipette the aliquots and by pipetting manually from the extraction tubes. The results proved to be extremely reproducible having a 0.27%RSD for the 6mL aliquot (EN 15662:2008) and 0.21%RSD for the 8mL aliquot (AOAC 2007.01). The manual pipetting process, while still very precise, was less consistent from sample to sample.



Graph 4: Pipetting precision of manual and automated pipetting techniques.

The accuracy for transferring both the 6.0mL aliquots and the 8ml aliquots using the ADP was within 1% accuracy. The automated ADP technique was then compared to two different styles of manual pipetting. The first technique involved transferring the aliquots using a class A rated glass pipette and bulb. The second technique used a manual pipette and disposable pipette tips. (Graph 5)



Graph 5: Pipetting accuracy using manual and automated pipetting techniques.

The last experiment was designed to evaluate the ADP performance when taking the aliquot from the dSPE cleanup vial and transferring the aliquot to a final extract vial. Our research has indicated that these final volumes vary from lab to lab and from application to application depending on the analytical instrument chosen for the final analysis. Common practice found that this final extract volume ranged from 4-5mL. The final extract then could be distributed to multiple GC vials for analysis on several different instruments. A 4mL aliquot was pipetted using the ADP and transferred to the final extract storage vial. As expected the ADP was both extremely accurate and precise when performing this task. (Table 5)

ADP Final Extract Transfer	
Final 4mL Aliquot from d-SPE Cleanup (mL)	
% Accuracy	99.5
%RSD	0.02

Table 5: Accuracy and Precision of petting final extract.

Using the ADP, allows for complete automation of the pipetting process for the QuEChERS extraction. The ADP eliminates tedious sample line priming, rinsing, and sweeping and reduces the risk for cross contamination by using a simplistic design. Using conductive pipette tips allow for detection of the aqueous layer, which helps determine the exact depth at which the pipetting can be performed. The data above illustrates that using the ADP is equal to or better in both accuracy and precision than to manual pipetting techniques.

Liquid Level Sensing

As mentioned, pipetting is a vital part of the QuEChERS extraction. Automating this process can be challenging due to the need to pipette a limited amount of extraction solvent from a vial without disturbing a secondary layer within. When performed manually, the analyst has the option to tilt the vial in an effort to take a “clean” and full aliquot. Automating the pipetting step, the art of vial tilting cannot be performed without introducing major complexity to the mechanical design. Tekmar has overcome this obstacle through the use of VialVision, a system that can determine the position of the layers in the vial and calculate the available volume for the extraction. By converting the pixels in the camera image to millimeters, and determining the volume of the vial, we can calculate the volume of the solvent layer can be determined to within 5% accuracy. Based on these measurements, the system then warns the user if the aliquot to be taken from the vial exceeds the volume of solvent available.

Below are images captured by the VialVision system of QuEChERS extracts after centrifugation. (Figure 6) Both samples are in 50mL conical centrifuge tubes, one sample is an olive oil matrix and the other is an apple matrix.

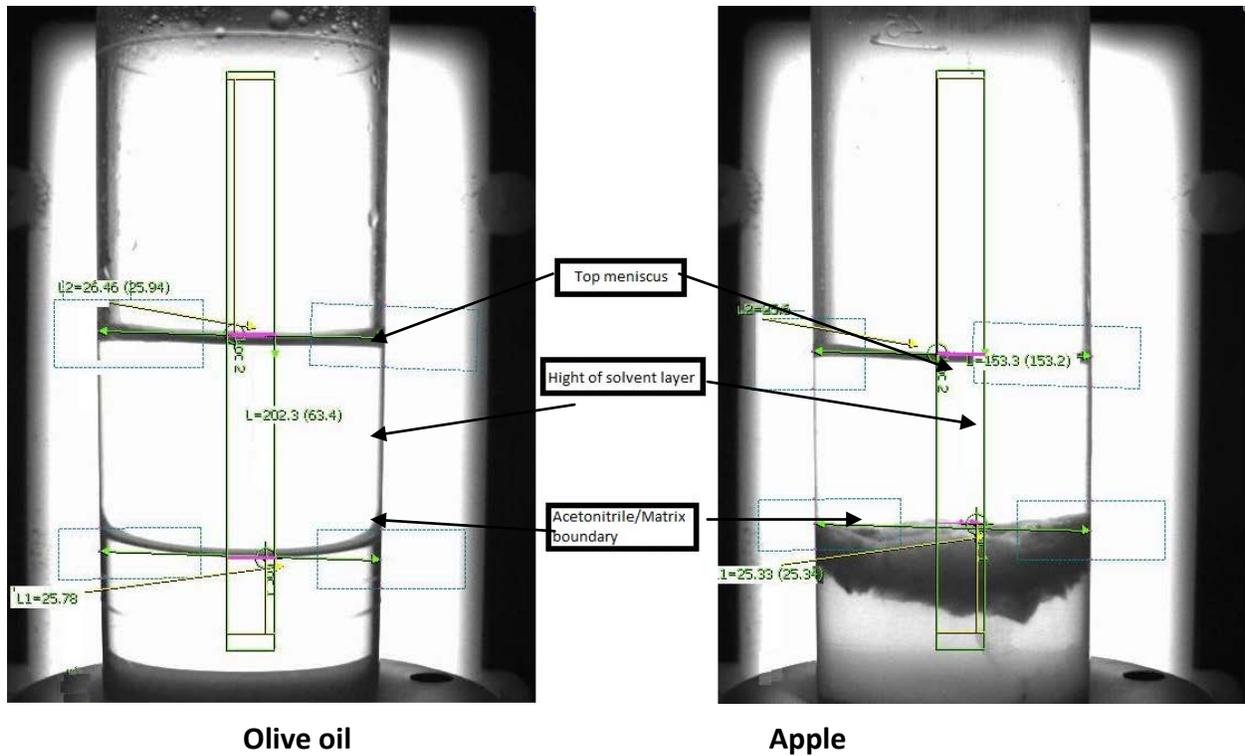


Figure 6. Olive Oil and apple samples after solvent extraction and centrifugation

The second challenge is to pipette an aliquot from the 15ml dSPE vial to a final extract vial. (Figure 7) This process is handled in the same way as the sample extract in that the top and bottom layer of the solvent are determined and used to calculate the available volume for pipetting. This allows the system to safely pipette an aliquot from the vial without disturbing the solid phase media layer below.

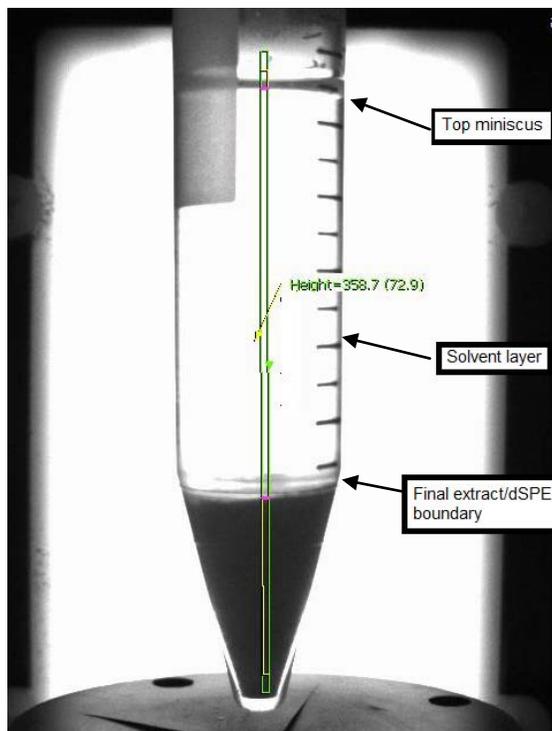


Figure 2. dSPE cleanup vial containing 6ml acetonitrile extract

Conclusions

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 fast, easy and offers time and labor savings, while improving consistency and repeatability of the extraction.

References

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