QuEChERS PROCEDURE FOR MULTI-RESIDUE PESTICIDE ANALYSIS

- DisQuE Dispersive Sample Preparation

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INTRODUCTION

The term "pesticides" is commonly used to describe a very broad class of crop-protecting chemicals, including insecticides, herbicides, and fungicides. Together, these compounds have become a primary tool in modern agriculture and have contributed to a dramatic increase in crop yields in recent decades for most field, fruit, and vegetable crops. Along with the obvious benefits, concerns have also surfaced regarding the effect of these chemicals on human health. In order to address these concerns, governments have initiated monitoring programs to enforce regulatory compliance and ensure food safety.

Initially, the availability of analytical methods was a limiting factor in enforcing pesticide limits in food stuffs.[1] With focused efforts, additional techniques have been developed but have not completely addressed the logistical challenges associated with testing the vast combinations of pesticides and commodities. Since the sheer volume of work would overwhelm available resources, many areas adopted a selective monitoring approach.[2] The combinations of pesticides and foods which are tested rely on the analytical method capabilities and the potential consumer health risks.

In recent years, researchers have developed rapid multi-residue analysis methods. These techniques quickly analyze many compounds, leading to more food samples being examined for a larger number of pesticides. In turn, this leads to quicker turnaround times, increased sample sizes and a more complete screening program with which to protect public health. The benefits of multi-residue methods to address the challenges of screening a very large number of samples for a variety of chemical compounds have quickly been realized and the implementation of these types of techniques into regular testing programs has become more widespread in recent years.

Multi-Residue Pesticide Analysis

Pesticide residue analysis in food samples has evolved steadily over the last 40 years. Today, there are more than 1,000 chemicals registered as pesticides that are screened using multi-class, multi-residue protocols. In 1963, the first multi-residue method for organochlorine insecticides was developed.[3] Acetonitrile was used as the extraction solvent and partitioning with petroleum ether used for sample cleanup. As method requirements increased, the analytical range expanded to include organophosphorous and organonitrogen pesticides. Becker[4] (1971) and Luke et al.[5] (1975) realized that the non-polar solvents used in the earlier methods resulted in a partial loss of the more polar pesticides and they switched the initial extraction solvent from acetonitrile to acetone. This expanded the analytical range; however, non-polar solvents, such as methylene chloride and/or petroleum ether, were still used for partitioning. Salts, such as sodium chloride, were added during the partitioning steps to improve the recoveries of polar analytes.

Today, these methods continue to be used as detection limits become lower. Recent development and improvements in analytical instrumentation make this possible. In 1993, the original "Luke" method was further modified.[6] The method, referred to as the Luke II Method, introduced solid-phase extraction (SPE) cleanup steps in both pre- and post- partitioning steps. The novel use of SPE for cleanup with the combination of appropriate detection techniques such as GC/MS, GC/ECD, or GC/NPD, proved that quantification limits of previous methods could be reduced 5-fold to approximately 10 ppb for an incurred pesticide sample. Water was introduced as an integral part of the initial extraction to facilitate SPE loading, but it also had additional benefits. Water partitioning enables the more polar set of analytes to be determined using the new approach.
The modified Luke method has become very popular and is widely accepted by many pesticide screening laboratories because of its good performance. However, the method has many shortcomings. The sample preparation procedures are complicated and tedious, taking as long as 1.5 days. The method requires large amounts of solvent, lengthy evaporation steps and extensive use of glassware. As a consequence, there have been great efforts to streamline sample preparation procedures to reduce both time and solvent consumption.

New environmental regulations and health concerns forced the use of safer alternatives to chlorinated solvents. Ethyl acetate, a common substitute, increases the extraction of non-polar components such as lipids and waxy materials. This substitution increases the complexity of the extraction protocol and now frequently includes gel-permeation chromatography (GPC) cleanup to help isolate the pesticides from the fatty matrix components. During the 1990s, many new extraction techniques were developed, such as microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) to help "green" the extraction process. Although they have individual advantages, these techniques were not widely accepted due to various practical limitations. For example, instrument-based techniques, such as ASE, require significant numbers of preparative steps including sequential, semi-automated extraction resulting in sample throughput bottlenecks and higher maintenance costs.

The Development of the QuEChERS Method

To improve the efficiency of traditional methods a new sample preparation approach was introduced by Anastassiades et al..[7] This procedure known as "QuEChERS" is an anagram for Quick, Easy, Cheap, Effective, Rugged, and Safe. This procedure uses a single extraction in acetonitrile and requires a very small (10-15 g) sample. Similar to previous methods, a large excess of salts or buffers are added to the extract to aid in the extraction of both polar and non-polar pesticides. This simple initial step simultaneously extracts the pesticides from the sample and prepares it for the next dispersive solid-phase extraction (d-SPE) step. The salts and SPE sorbents chosen for the d-SPE step serve to remove residual water and further remove matrix interferences from the sample. The resulting acetonitrile extract is typically analyzed directly by GC/MS or by LC/MS/MS with proper dilution.

The performance of the QuEChERS extraction method was revolutionary and was further optimized to include buffers during sample extraction to improve analyte stability and extract quality. Today, there are two commonly used buffered methods. European Committee for Standardization (CEN) Method 15662[8] uses a citrate buffer for extraction. The second method uses acetic acid buffer and was adopted as the AOAC Official Method 2007.01.[9]

Method	Sample Size	Solvent	Tube Content
AOAC Method 2007.01 Acetate Buffer	15 g	15 mL 1% acetic acid in acetonitrile	6 g $MgSO_4$ 1.5 g sodium acetate
CEN Method 15662 Citrate Buffer	10 g	10 mL acetonitrile	4 g $MgSO_4$ 1 g NaCl 1.5 g sodium citrate

Table 1. Extraction tube of original and buffered QuEChERS methods.

The sample preparation protocol for AOAC Official Method 2007.01 is presented in Figure 1. This procedure was designed for commodities such as fruits and vegetables and optimized for samples that have high water content. For dry commodities, such as cereals, a similar approach can be used. However, in this case it is necessary to either first homogenize the sample in water or hydrate the commodity by soaking it in water. After the hydration step, the extraction solvent can be added and the procedure followed. This topic is covered in more detail in the "Extraction of Low Water Content Commodities" section.

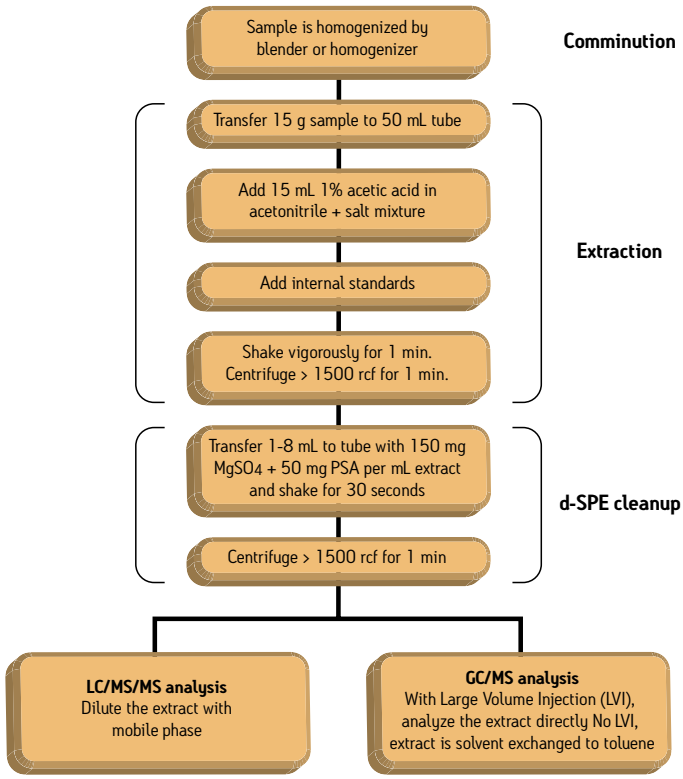

Figure 1. Sample preparation protocol of AOAC QuEChERS Method.

The CEN QuEChERS Method is similar to the original QuEChERS procedure, but additional citrate salts are added to the tube during liquid-liquid partitioning. The sample preparation protocol is summarized in Figure 2. The d-SPE cleanup typically uses 1 mL of the acetonitrile extract with 150 anhydrous $MgSO_4$ and 25 mg PSA per gram of sample.

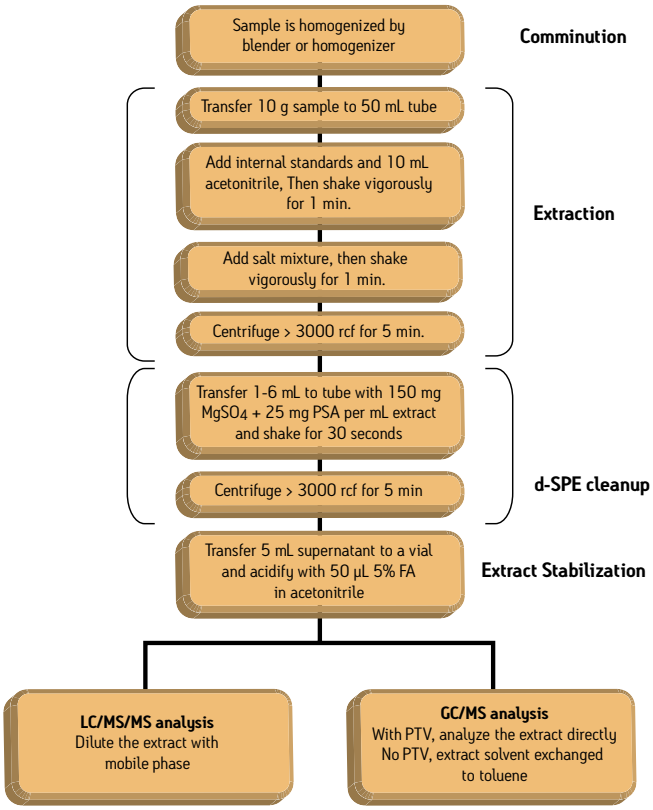

Figure 2. Sample preparation protocol of EU CEN 15662 QuEChERS Method.

Comparison of the Buffered QuEChERS Methods

Both buffered methods are suitable for screening hundreds of pesticides in fruits and vegetables. The performance of both methods is comparable. Figure 3 and Figure 4 summarize the results for a grape sample using both LC and GC analysis. Consistent recoveries are observed for most pesticides, but there are some notable exceptions based on the differences in initial extraction conditions. The pH of acetonitrile extract is a critical parameter that affects the performance of the extraction procedure. The citrate buffered extract has a nominal pH near 8, which is higher than the extract obtained from the acetate buffered method. The acetate buffered method yields extracts with a nominal pH near 5. For base sensitive pesticides the pH of the extraction conditions becomes critically important and many researchers prefer the AOAC acetate buffered extraction. To obtain consistent and reliable recovery of base sensitive pesticides (such as tolyfluanid or chlorothalonil), using the CEN method, it is essential to acidify the final extract prior to the analysis to prevent unwanted analyte degradation.

As mentioned, the AOAC Method maintains a stable pH near 5 throughout the entire extraction and d-SPE procedure. However, the advantage of the citrate buffered method (CEN Method 15662) is that it provides a more optimized pH for utilizing d-SPE sorbents and the cleanliness of the extract is improved. This applies specifically to GC analysis where the types of matrix constituents removed by the d-SPE sorbent (PSA), can create problems for the high temperature GC injection port and chromatographic column. For LC-based methods, there is little or no effect on the chromatographic performance making LC-based methods less dependent on extraction method choice.

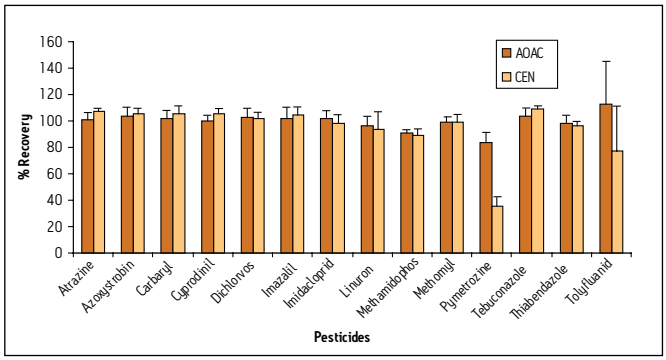

Figure 3. Pesticide recovery in grapes by GC/MS analysis. n=6

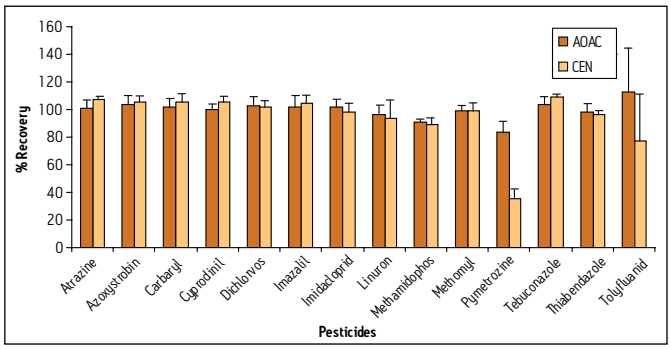

Figure 4. Pesticide recovery in grapes by LC/MS/MS analysis. n=6

The Sorbent Selection for d-SPE Cleanup

As mentioned in the previous sections, PSA is the most commonly used QuEChERS d-SPE sorbent. Its main function is to remove constituents such as fatty acids, sugars, and some ionic-lipids, making PSA sorbents very suitable for most plant-based commodities. Samples that contain large amounts of pigments, or contain very large amounts of fat may require additional cleanup specific for these matrices. Two commonly used sorbents that supplement the d-SPE cleanup step (Tube 2) are graphitized carbon black (GCB) and C_{18}.

Graphitized Carbon Black (GCB)

Some fruits and vegetables including spinach, red sweet pepper, and carrots have high content of non-polar pigments, such as carotenoids or chlorophyll. QuEChERS procedures effectively remove the most common matrix constituents, such as fatty acids and sugars; however,

the PSA sorbent is not capable of adsorbing non-polar pigments. Conversely, GCB is a very retentive chromatographic sorbent that is highly effective for removing these pigments and is often added along with the PSA sorbents and magnesium sulphate as part of the d-SPE cleanup step.

Unfortunately, using GCB sorbents in the d-SPE cleanup tube can not only retain the unwanted chlorophyll, but it can also retain certain planar aromatic analytes such as cyprodinil, thiabendazole, and hexachloro-benzene. Figure 6 shows an example where the recoveries of cyprodinil and thiabendazole are lower in grape extracts cleaned up using 7.5 mg GCB, than in those cleaned up without GCB. As this example shows, planar pesticides are the most affected with approximate losses of up to 50% of during the d-SPE cleanup. Even with the limitations of GCB, its use is especially important when preparing samples for GC instrumentation. This is because chlorophyll and other higher weight matrix components accumulate in the injection port and at the head of the GC column. As a result, the injection liners are quickly fouled requiring more frequent instrument maintenance. Figure 5 demonstrates how differing accounts of GCB affect spinach cleanup.

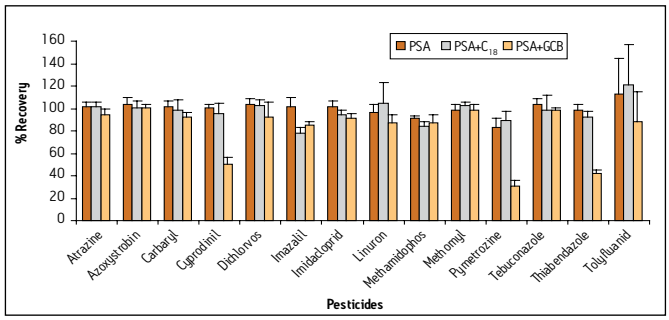

Figure 6. The pesticide recoveries in grapes with addition of various sorbents by using LC/MS/MS. n=6

Recommended Usage

GCB sorbent is only recommended if it is absolutely necessary because of the potential loss in recovery of some pesticides. AOAC Official Method 2007.01 suggests using 50 mg GCB per milliliter of extract for enhanced matrix removal when no structurally planar pesticides are present among analytes. For the CEN Method, the amount of GCB sorbent used in the d-SPE step is commodity based. Some guidelines have been proposed and follow that for carrots and romaine lettuce, use 2.5 mg GCB per 1 mL of extract. For commodities with higher pigment content such as red sweet pepper and spinach, 7.5 mg GCB is added per 1 mL of extract. It is noted that adding as high as 50 mg of GCB sorbent per milliliter of extract may be desirable for any commodity with higher pigment content, such as spinach.

Figure 5. The effect of GCB amounts on the de-coloring of spinach extracts.

The amount of GCB sorbent used should be kept as low as possible, but still meet the desired cleanup. For new or unfamiliar commodities that require additional pigment removal, the optimized amount of GCB sorbent should be determined experimentally by systematically increasing the amount added to the extract. For ease of use, bulk GCB sorbent is available and provides the flexibility to customize the d-SPE step for these commodity dependant samples.

C_{18} Sorbent (Trifunctionally-Bonded C_{18} Silica)

QuEChERS methods perform well for most pesticides in non-fatty matrices such as fruits and vegetables. For samples with a relatively high level of fat content, such as avocado or milk, recoveries of the more lipophilic pesticides may suffer. In Figure 7, the relative analyte response was calculated by normalizing the measured response of a sample treated with both PSA and C_{18} sorbents to the response for a sample treated with PSA sorbent alone. As seen in Figure 7, the relative responses for most pesticides in avocado were not affected by the addition of up to 100 mg C_{18} sorbent. In general, there is no major adverse effect of pesticide responses by adding C_{18} sorbent. When compared to adding GCB sorbents, C_{18} sorbents are more forgiving. The possible benefit of removing any matrix component that causes chromatographic interference outweighs the adverse effect found with using more retentive sorbents such as GCB. The additional C_{18} sorbent often improves analyte detection in very complex sample matrices (see Figure 8). For endosulfan sulfate, chlorothalonil, and tolyfluanid, the relative responses of these three compounds were increased by almost 100% in the samples using a cleanup tube containing PSA and 100 mg of C_{18} compared to the samples using cleanup tube with only PSA.

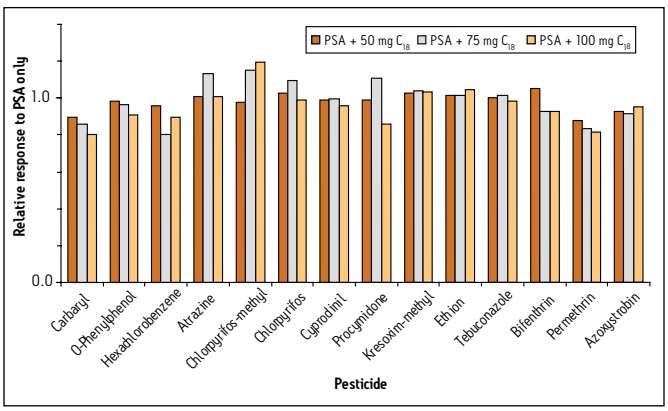

Figure 7. The effect of C_{18} amounts to the relative responses of pesticides compared to the sample cleanup by PSA only in avocado.

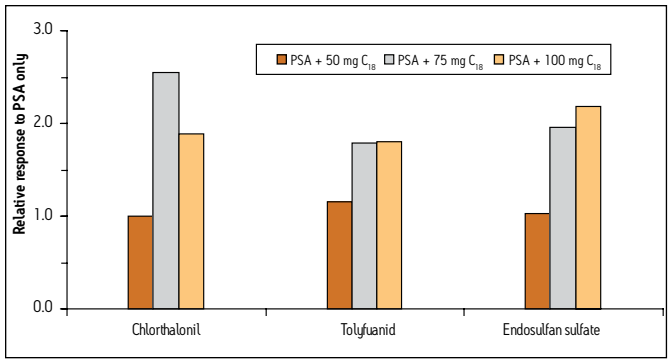

Figure 8. The effect of C_{18} amounts to the relative responses of endosulfan sulfate, cholorthalonil, and tolyfluanid compared to the sample cleanup by PSA only in avocado.

Recommended Usage

The AOAC Official Method 2007.01 recommends that 50 mg of C_{18} sorbent be used per milliliter of extract in addition to the $MgSO_4$ and PSA sorbents. The CEN Method follows a similar recommendation and suggests using 25 mg of C_{18} sorbent per milliliter of extract. Waters data indicates that the added amount of C_{18} sorbent could be as high as 100 mg per milliliter of extract without adversely affecting pesticide recovery.

EXTRACTION OF LOW WATER CONTENT COMMODITIES

QuEChERS extractions are most effective for fruits and vegetables with high water content. For commodities that have low water content, additional buffer, or water is added to optimize the extraction. The CEN Method guidelines for the addition of water are shown in Table 2.

Sample Type	Sample Weight	Water Added	Note
Fruits & Vegetables >80% water content	10 g	-	
Fruits & Vegetables 25-80% water content	10 g	X g	X = 10 g - water amount in 10 g sample
Cereals	5 g	10 g	
Dried fruit	5 g	7.5 g	Water can be added during comminution step
Honey	5 g	10 g	
Spices	2 g	10 g	

Table 2. The CEN Method guidelines of adding water into commodities with low water content.

Because there are no specific guidelines for the AOAC Official Method for analysis of commodities with low water content, it is recommended that the CEN Method guidelines be followed for those commodities. For example, 15 mL water was added into 7.5 g of rolled oats, and then 15 mL of 1% acetic acid was transferred to the tube for extraction. The results are displayed in Figures 9 and 10.

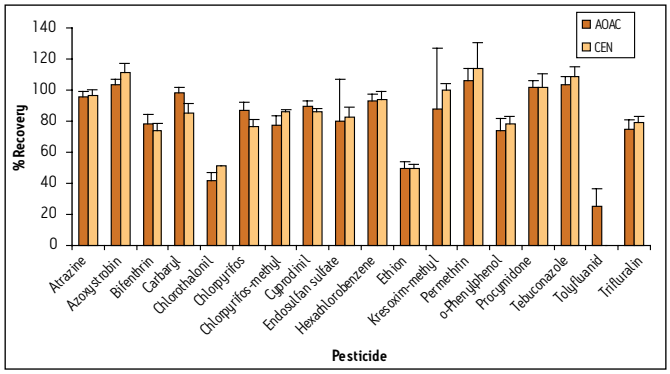

Figure 9. Pesticide recovery in rolled oats by GC/MS analysis. n=6

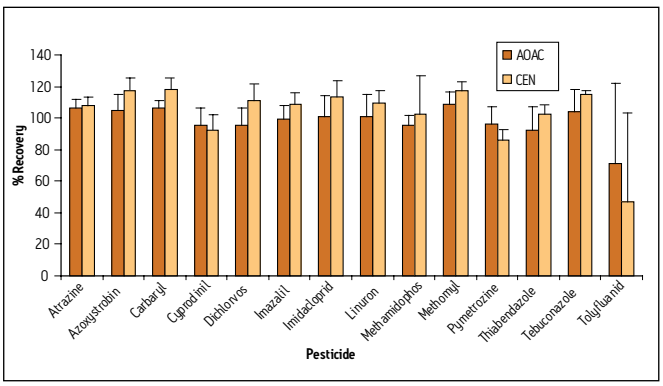

Figure 10. Pesticide recovery in rolled oats by UPLC/MS/MS analysis. n=6

EXPERIMENTAL

The choice of analytical instrumentation for QuEChERS method extracts is dependent upon the properties of the analyte being tested. For LC amenable compounds, LC/MS (tandem MS/MS or Q-TOF) is typically used. For the results presented in the study, LC/MS/MS was used with at least two Multiple Reaction Monitoring (MRM) transitions being quantitatively measured. For more non-polar compounds, GC/MS (SIR or tandem MS/MS) is the main analytical technique. In this study, GC/MS tn SIR mode was used with three Single Ion Recording (SIR) transitions being monitored for each analyte. A single QuEChERS extraction was used to generate both LC and GC data to encompass a diverse list of pesticides. The instrumental parameters used for this study are as follows:

UPLC Conditions

LC System:	Waters ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C_{18} column, 2.1 x 100 mm, 1.7 µm
Column Temp:	40 °C
Sample Temp:	4 °C
Flow Rate:	0.3 mL/min
Mobile Phase A:	Water + 0.1% formic acid
Mobile Phase B:	Methanol + 0.1 % formic acid

Gradient:

Time	Flow Rate (mL/min)	A%	B%
0.00	0.3	75	25
0.25	0.3	75	25
7.7	0.3	5	100
8.50	0.3	0	100
8.51	0.5	75	25
10.50	0.5	75	25
11.0	0.3	75	25

Injection Volume:	15 µL

MS Conditions

MS System:	Waters TQ Detector Mass Spectrometer
Ionization Mode:	ESI Positive
Capillary Voltage:	3 kV
Cone Voltage:	Specific for each analyte
Desolvation Temp:	400 °C
Desolvation Gas:	800 L/Hr
Source Temp:	150 °C
Collision Energies:	Specific for each analyte
Acquisition:	Multiple Reaction Monitoring (MRM) Mode

GC Conditions

GC System:	Agilent 6890N Gas Chromatographic system
Column:	RTX-5MS, 30 mm x 0.25 mm (0.25 µm film)
Carrier Gas:	Helium at 1 mL/min
Temp. Program:	Initial 100° C, hold 1 min, then 10° C /minute to 320° C, hold for 7 minute
Injection:	2 µL splitless

MS Conditions

MS System:	Waters Quattro micro™ GC Mass Spectrometer
Ionization Mode:	Electron Impact (70 eV)
Acquisition:	Single Ion Recording (SIR) Mode

CONCLUSION

The QuEChERS sample preparation method has become the most popular technique to prepare fruits, vegetables, and other commodity samples for multi-residue pesticide analysis. Recent approaches using QuEChERS extraction include buffers during the sample extraction. Today, the two most common methods are European Committee for Standardization (CEN) Method 15662.5 and AOAC Official Method 2007.01. These methodologies allow users to quickly and easily extract their compounds of interest from a sample and prepare them for further analysis. The benefits of these methods lead to increased numbers of tested samples and greater confidence in the safety of the food supply.

To simplify the process even further, prepared extraction tubes are now available for the standardized methods. DisQuE dispersive sample preparation kits are available for both the CEN and AOAC Official Methods, bring additional savings in terms of time and cost by providing ready to use tubes. The DisQuE products are consistent and of the highest quality (including no leaking) with a cost which is comparable to creating tubes by hand.

QuEChERS extractions carried out using DisQuE kits are the result of 40 years of evolution in multi-residue pesticide analysis. Allowing more samples to be screened for a multitude of compounds in a shorter period of time, this technique is rapidly gaining acceptance across the globe.

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