The Applications of QuEChERS Beyond Fruits and Vegetables

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As analytical instrumentation becomes faster, more selective, and more sensitive, the preparation of samples for analysis has emerged as a common slow step for laboratory workflows. Additionally, routine analysis of samples can include the detection and quantification of hundreds of chemically diverse compounds at low concentrations in different types of matrices.

One solution to this analytical challenge is the implementation of a sample preparation technique known as Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), which was developed by Steven Lehotay and Michelangelo Anastassiades in 2003 for the analysis of pesticide residues in fruits and vegetables [1]. The QuEChERS method presents many advantages over traditionally used techniques by decreasing the time spent preparing samples, providing fewer procedural steps, reducing the amount of sample and chemicals needed, and delivering method transferability for a number of matrices.

Unlike many other sample preparation techniques that are tailored for the selective extraction of target analytes, the QuEChERS approach is targeted towards the removal of the matrix, which can be grouped into different categories relating to the pigmentation and the amount of fattiness in the sample type. The procedure implements three overall steps: 1) extraction with a solvent, typically acetonitrile, and partitioning salts, 2) cleanup with dispersive solid phase extraction (dSPE) using adsorbent materials (sorbents) that remove unwanted matrix components, and 3) detection, typically using gas chromatography-mass spectrometry (GC/MS) and/or liquid chromatography-mass spectrometry (LC/MS) (Figure 1). Over the past 10 years, the QuEChERS approach evolved to include buffering salts and additional sorbents and was adopted as the official Association of Analytical Communities (AOAC) and European methods for pesticide residue analysis [2,3].

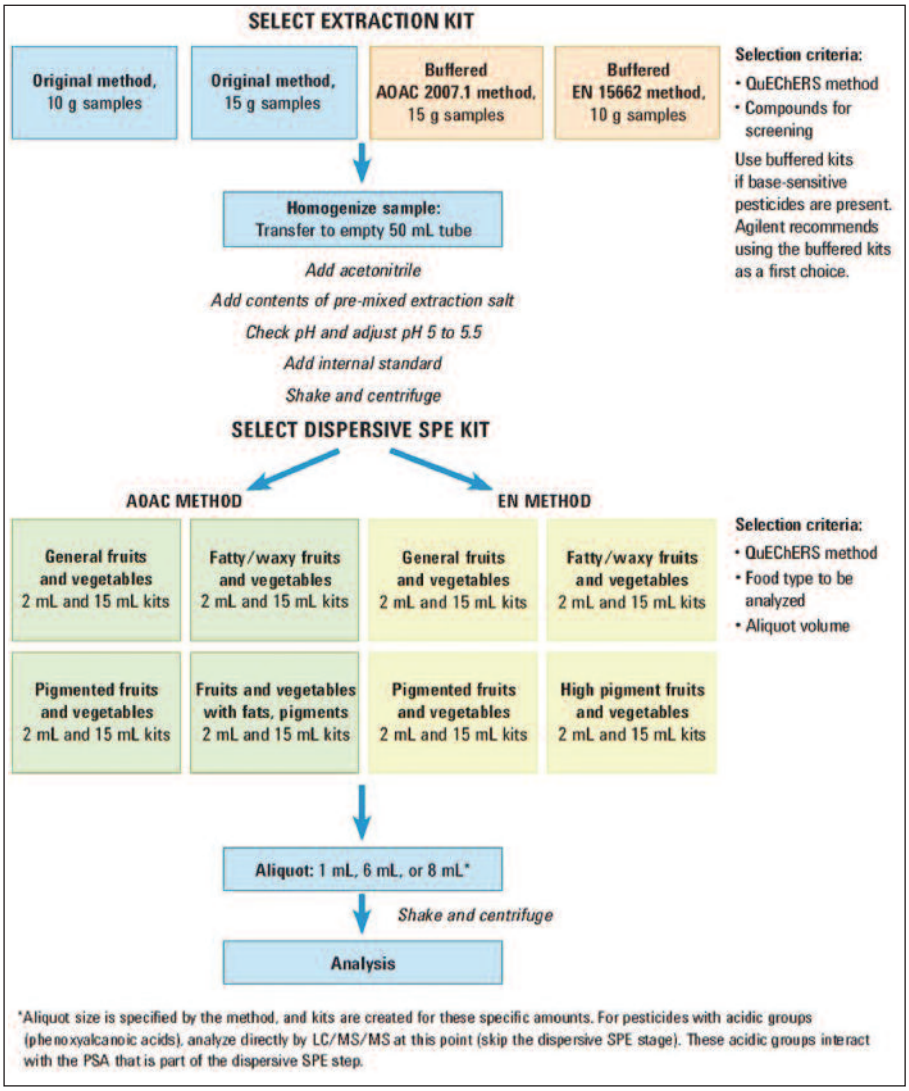

Figure 1: QuEChERS sample preparation workflow highlighting the selection of an extraction salt kit and later the matrix dependent dSPE cleanup kit.

QuEChERS Extraction

This step includes the addition of organic solvent and salts to extract the analytes of interest and induce phase separation. A homogenised sample is added to centrifuge tubes along with any water, internal standard, and/or QC spikes. A suitable solvent is then added and mixed thoroughly with the sample to extract the analytes of interest. While the original, unbuffered QuEChERS procedure uses magnesium sulphate and sodium chloride as extraction/partitioning salts it was later revealed that some pesticides are more stable at lower pH and were extracted in higher recovery by buffering the solution at pH-5 using sodium acetate (AOAC method) or citrate salts (EN method) [4,5]. This is followed by shaking and centrifuge allowing for convenient phase separation for the subsequent steps.

QuEChERS dSPE Cleanup

Following the extraction step, the upper organic layer is typically added to a centrifuge tube containing sorbent materials that remove unwanted matrix components which result in analytical interferences or instrument contamination. Sorbents for dSPE are selected based on their ability to retain matrix compounds known to be in the sample. For example, primary secondary amine (PSA) is offered in the majority of the kits listed in Figure 1 and is effective at removing organic acids and sugars. If the sample is also known to contain fat and pigments such as chlorophyll, C18 and graphitised carbon black (GCB) can be included in the dSPE mixture to remove the respective compounds. These sorbents must be used with discretion as they can also retain analytes of interest. For instance, PSA will retain acidic analytes and GCB will retain analytes with planar geometry. The complexity and diversity across matrices requires an arsenal of sample cleanup options and for this reason, dSPE sorbents exist in a variety of sorbent mixtures and ratios as shown in Figure 1. After mixing the sample extract with a dSPE kit, the mixture is centrifuged and the supernatant can be added to vials for either dilution or evaporation and reconstitution prior to analysis.

In recent years the popularity of QuEChERS continues to grow as fit-for-purpose modifications to the procedure accommodate modern analytical challenges. Furthermore, the utility of the method has expanded outside of the traditional pesticide residue analysis for fruits and vegetables. The following sections will highlight several examples which deviate from traditional applications and explore new target analytes and new matrices using the QuEChERS technique.

Sample Preparation of Non-Traditional Analytes

The successful utility of QuEChERS for multi-analyte, multi-class pesticide residue analysis provided evidence for its transferability to other analytes of interest. Pharmaceuticals are another group of compounds which exhibit chemical diversity and are desirable for multi-analyte, multi-class analysis. The preparation of forensic and clinical types of samples was demonstrated in whole blood samples using a miniaturised QuEChERS technique, followed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) (Figure 2) [6]. Several classes of pharmaceuticals were spiked into the blood, including anesthetics, analgesics, antidepressants, and opioid receptor antagonists. The procedure was miniaturised to account for small sample volumes typical for blood samples. Recoveries were determined to be between

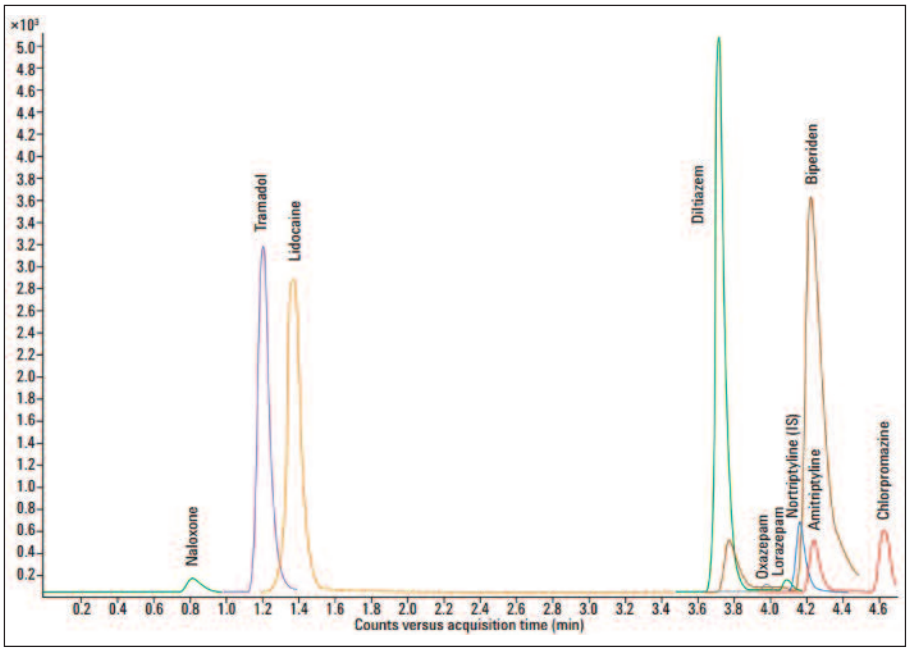

Figure 2. LC-MS/MS chromatograms of a 10 ng/mL spiked whole blood sample after mini-QuEChERS extraction (AOAC extraction and d-SPE with PSA). Separation accomplished using a Poroshell 120 EC-C18, 2.7µm,2.1 x 100mm column (p/n 695775-902). Mobile phase A: 5mM Ammonium acetate, pH 5, 20:80 MeOH:Water; B: 5mM Ammonium acetate in ACN.

77% and 105% for 50ng/mL and 100ng/mL spiked samples demonstrating the effectiveness of the sample preparation.

Preservatives are a common additive used to promote longer shelf life in food. While they accomplish this quite successfully, the long term consumption of compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), and ethoxyquin have been related to health issues including liver disease and cancer. An application explored these three preservatives in pet food using QuEChERS and LC-MS/MS and provided evidence for ethoxyquin in non-organic pet foods, as shown in Figure 3 [7]. The method gave recoveries between 55% and 64% for ethoxyquin and recoveries between 100% and 130% for BHA and BHT with all relative standard deviations (RSD) below 8%.

Fried foods are very popular; however the high temperatures can produce acrylamide in carbohydrate-rich foods. Acrylamide has been associated with neurotoxicity and cancer in animals and is a suspected carcinogen in humans. A modified QuEChERS procedure was used in the preparation of acrylamide in fried potatoes to improve upon a previous method, which involved time-consuming liquid-liquid extraction. Detection and quantification using LC-MS/MS following the sample preparation gave recoveries between 97% and 116% and RSD 5% or below,

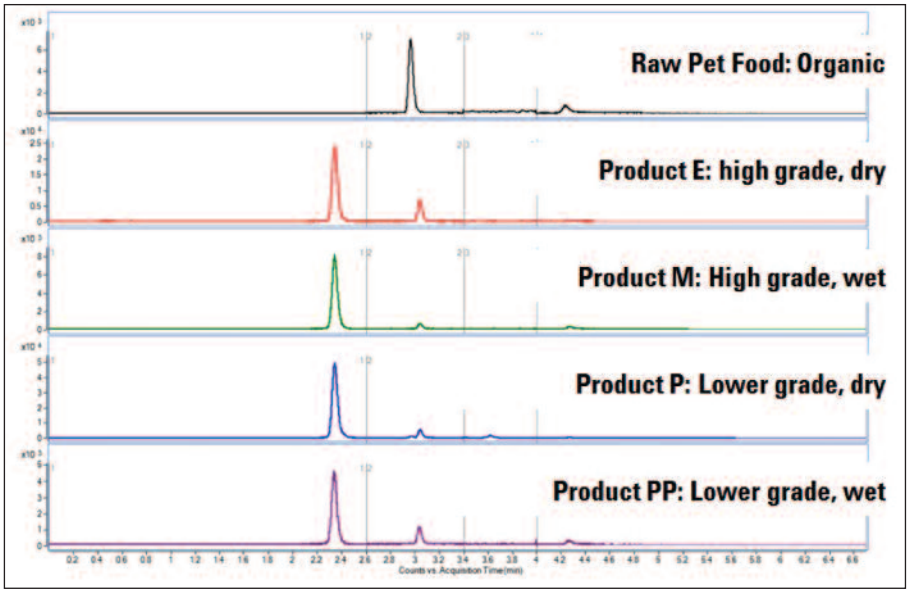

Figure 3. LC-MS/MS chromatograms for incurred ethoxyquin in dry pellets and canned, wet pet foods. Grade indicates the quality of pet food. Separation accomplished using a Poroshell 120 SB-C18, 2.7µm,2.1 x 50 mm column (p/n 689775-902). Mobile phase A: Water and 0.1% formic acid (FA); B: Acetonitrile and 0.1% FA.

demonstrating that the QuEChERS approach is an appropriate option for acrylamide analysis [8].

Sample Preparation for Non-Traditional Matrices

Although QuEChERS is traditionally applied to fruits and vegetables, other commodities containing pesticide residues have come under increased scrutiny. The following examples examine applications for pesticide residue analysis in non-traditional matrices.

Pesticides are chemical compounds prevalent in the modern world of agriculture. Through numerous pathways, these pesticides can enter the food we eat, justifying the importance of monitoring our food to ensure contaminants are at or below regulatory tolerance levels. The analysis of pesticides in chicken was performed using gas chromatography and tandem mass spectrometry (GC-MS/MS). QuEChERS was employed as the sample preparation method in this instance, and 54 pesticides were monitored in the chicken meat [9]. Figure 4 shows the improvement in solution clarity (right) through the removal of matrix components by including PSA and graphitised carbon black (GCB) during the dispersive cleanup step. Despite the increased complexity of the matrix, relative to fruits and vegetables, the method delivers recoveries between 73% and 113% and RSD below 16% for all pesticides, demonstrating the robustness of QuEChERS for pesticide residue analysis.

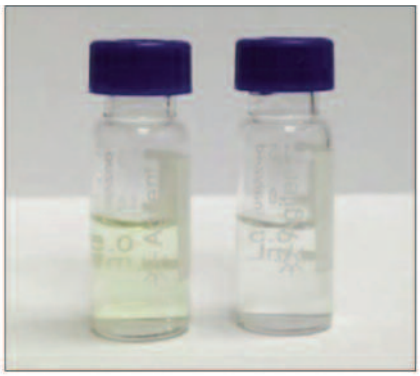

Figure 4: Sample solutions of chicken muscle after cleanup using dispersive-SPE kit for other food methods (left) and after dispersive-SPE kit for all food types (right).

	10 ng/g	Spike	100 ng/g	Spike	500 ng/g	Spike	
	%	%	%	%	%	%	R^2
	Recovery	RSD	Recovery	RSD	Recovery	RSD	
Nicotine	113.7	6.4	97.7	1.6	90.0	2.1	0.9996
Anabasine	91.8	6.2	86.4	1.6	77.8	2.5	0.9993
Cotinine	127.9	5.4	117.0	2.2	93.4	2.1	0.9978

Figure 5: Recovery and precision data for nicotine and its metabolites anabasine and cotinine. Sample preparation used QuEChERS AOAC extraction kit (p/n 5982-5755CH) and dispersive SPE kit (p/n 5982-5022). LC separation was performed on a Agilent Poroshell 120 HILIC, 2.1 x 100mm, 2.7µm column (p/n 695775-901).

Although nicotine and its metabolites are generally associated with tobacco products, these compounds can also be applied as an insecticide in agriculture. These compounds can eventually enter the water and the aquaculture that inhabits it. A QuEChERS approach was employed for the extraction of nicotine and its metabolites cotinine and anabasine in cod samples for LC-MS/MS analysis [10]. The QuEChERS method developed for this extraction delivered adequate cleanup of the complex, fatty matrix and resulted in a 1ng/g and 5ng/g limit of detection (LOD) and limit of quantitation (LOQ), respectively, as well as excellent recovery and reproducibility (Figure 5).

Summary and Conclusion

Although the QuEChERS approach was developed as a time-saving and rugged technique for pesticide residue analysis in fruits and vegetables, its versatility is continuously demonstrated as scientists explore its use for new matrices and new analytes. As laboratories demand faster, more rugged, and high-throughput sample preparation methods compatible with modern instrumentation, QuEChERS applications continue to grow. The easily customisable steps of QuEChERS provide an essential path towards future applications, where adequate sample cleanup is accomplished for diverse classes of compounds, in the minimal amount of time to satisfy the needs of busy analytical laboratories.

References

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