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Analytical Challenges for Pesticide Residue Analysis in Food: Sample Preparation, Processing, Extraction and Cleanup

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Key Words

Cleanup, Food, GC Orbitrap, GC-MS, LC-MS, LC Orbitrap, Pesticides, Sample Comminution, Sample Preparation, Single Residue Methods, Solvent Extraction

Background

The British Crop Production Council (BCPC) Pesticides Manual[1] includes more than 1600 substances that have been used as pesticides. They can be grouped according their function (algicides, avicides, bactericides, fungicides, herbicides, insecticides, miticides, molluscicides, nematicides, rodenticides, and virucides) or based on their chemical classes (organophosphates, carbamates, pyrethroids, organochlorines, etc.). See Table 1.

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Table 1. Pesticides and the pest group they are utilized to control.

Pesticide	Pest Group
algicide	algae
avicide	birds
batericide	bacteria
fungicide	fungi
herbicide	plant
insecticide	insects
miticide	mites
mollusicide	snails
netamaticide	nematodes
rodenticide	rodents
virucide	viruses

To ensure compliance with maximum residues levels (MRLs) of pesticides in foods, analytical laboratories are expected to be able to detect, quantitate and identify hundreds of different pesticides with diverse physicochemical properties in hundreds of different sample types. For a truly comprehensive analysis of hundreds of thousands of different pesticide-commodity combinations, the analyst will typically use targeted multi-analyte methods together with a number of different single residue methods (SRMs). These so-called SRMs are specific to individual pesticides or a small group of pesticides with similar properties that do not give satisfactory results when analyzed using the multi-analyte methods. Most of the methods are typically based on generic sample extraction followed by gas chromatography (GC) and/or liquid chromatography (LC) coupled to mass spectrometers (MS).

For comprehensive analysis of 100,000's of different pesticide-commodity combinations, the analyst will typically use multi-analyte methods based on both GC-MS and LC-MS techniques together with a number of different single residue methods

The careful distribution of the target pesticide analytes into the different methods is critical in achieving the best overall performance in terms of accuracy, precision, speed and cost of analysis. Because of the diversity of physicochemical properties, not all of the pesticides in a multi-class, multi-analyte method will give results compliant with guideline validation or analytical quality control criteria (e.g., European Commission Directorate-General for Health and Food Safety or EU SANTE Guidelines)[2], but the results may still be considered adequate to demonstrate the absence of a particular residue in the sample. This is more acceptable when the pesticide in question is not present as a residue.

The challenge to the analyst is to optimize the distribution of the target pesticide analytes into the different methods to achieve the best overall performance in terms of accuracy, precision, speed and cost for the analysis

Taking representative samples at the farm, at the distribution depot, or in the supermarket for the purpose of pesticide residue analysis is an important task, but presents many challenges and is outside of the scope of this paper. Some of the challenges have been outlined elsewhere.[3-6] After sampling, transportation of the sample is equally important to avoid deterioration and contamination of the sample, and to maintain traceability, etc. Again this topic is outside the scope of this paper and guidance can be found elsewhere.[2]

After the sample is received into the laboratory, inspected and accepted to be in good condition, the analytical workflow consists of a number of defined steps.

1. Sample Preparation: defined as the procedure used, if required, to convert the laboratory sample into an analytical sample by removal of parts (soil, stones, bones, etc.) not to be analyzed[7]
2. Sample Processing or Comminution: defined as the procedure (e.g., cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to analyte distribution prior to removal of the analytical portion[7]
3. Extraction: extraction or transfer of analytes into the extracting phase (usually a solvent)
4. Cleanup (optional): procedure to remove non-specific matrix co-extractives
5. Analytical determination (separation, detection, identification and quantification of target analytes) and reporting of the results

The typical workflow from Steps 3–5, inclusive, is shown in Figure 1.

The development of an analytical strategy requires the analyst to make decisions at each step of the workflow. This will inevitably involve the need to make compromises, either because of the difficulty of analyzing pesticides with diverse chemical properties in the same method or to minimize costs.

This paper will focus on the first steps in the analytical workflow: sample comminution and the most widely used methods of solvent extraction and cleanup. Techniques such as solid phase micro-extraction (SPME), stir bar sorptive extraction (SBSE), matrix solid phase dispersion (MSPD) and single drop micro-extraction (SDME), membrane extraction, supercritical fluid extraction (SFE), etc., are outside of the scope of this paper. Information on the analysis of extracts by GC-MS and LC-MS techniques will be covered in separate papers on this topic.

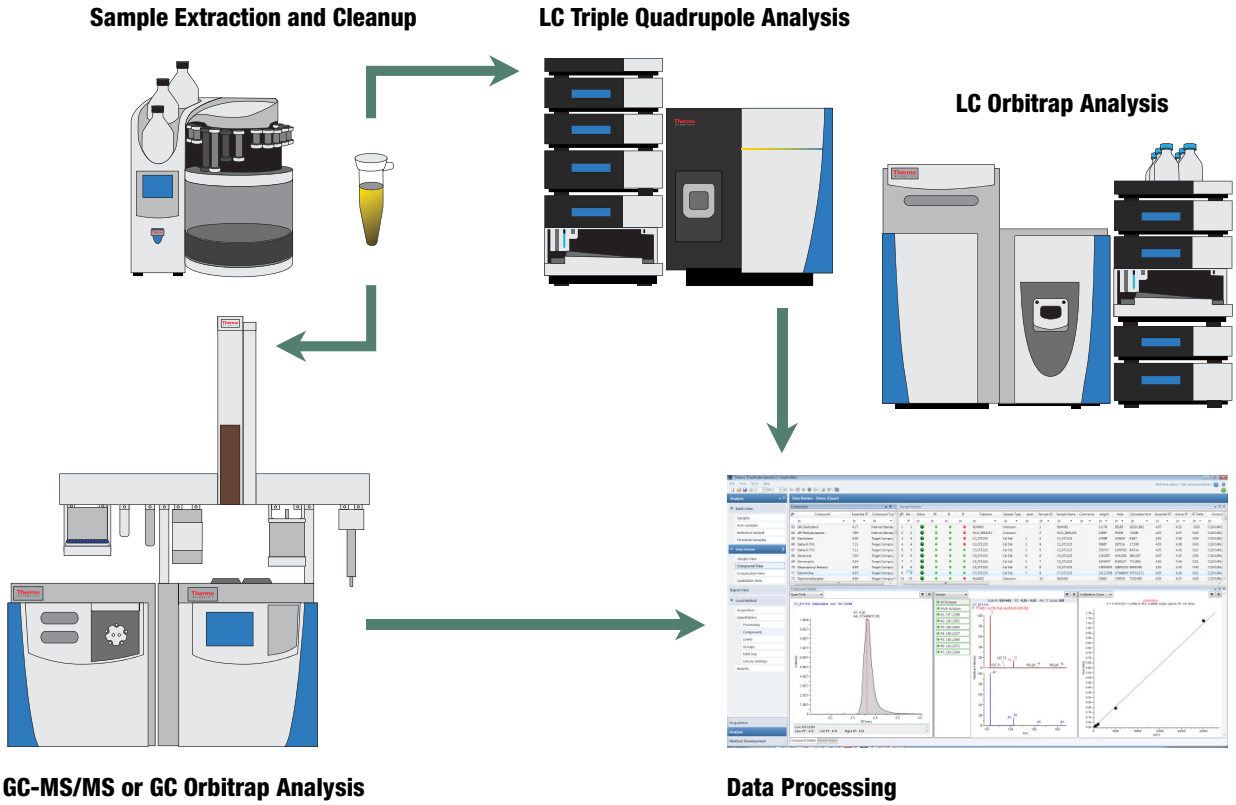


Figure 1. Overview of a typical workflow for pesticides.

Sample Processing

After receiving a sample in the laboratory, the first step is to ensure that it is acceptable for analysis. The sample must not show visible signs of deterioration and must be compliant with the appropriate requirements (minimum number of units and weight, etc.). For official control samples in Europe, Commission Directive 2002/63/EC (based on Codex CAC/GL 50-2004) applies. The next step is to remove parts not to be analyzed (soil, stones, bones, stems, etc.) in accordance with Commission Regulation EC No. 178/2006 which is based on Codex CAC/GL 41-1993. The final procedure for converting the laboratory sample into the analytical sample is the cutting, grinding, or mixing necessary to make the analytical sample sufficiently homogenous – with respect to the analyte distribution – prior to removal of a representative analytical portion.

Field trial samples collected for pre-registration analysis are typically frozen and then homogenized or comminuted cryogenically using liquid nitrogen or liquid CO_2 (dry ice). This is done to prevent degradation or losses of field-incurred residues, and to convert the sample to a homogenous flowable powder made up of small-sized particles. This allows small-scale methods (e.g., 100 mg/1 mL solvent) to be used, facilitating very high sample throughput (hundreds per day). Since this data is used to determine MRLs, it might be expected that the analysis of post-registration (monitoring) control samples would be treated in the same or similar way, but this is not always the case.

Field trial samples are typically frozen and then comminuted cryogenically using liquid nitrogen or liquid CO_2 (dry ice) to prevent degradation or losses of field-incurred residues

Most laboratories comminute samples of fruit and vegetables at room temperature, forming a chemically active liquid/solid 'soup' (see Figure 2). Losses of pesticides can occur through hydrolysis by free water, oxidation, enzymatic degradation following the release of enzymes when cells are ruptured, degradation due to pH, or the formation of insoluble complexes by interaction with matrix components. Each of these factors, individually or in combination, can lead to an underestimation of the true residue concentration.[8,9] Another issue with this approach is that the homogeneity of the samples can be dependent on the equipment used and the commodity type.[9]

Loss of pesticides can occur through hydrolysis by free water, air oxidation, enzymatic action after release of enzymes when cells are ruptured, degradation due to pH, or the formation of insoluble complexes by interaction with matrix components

Figure 2. Sample processing at room temperature. Photograph courtesy of Fera, UK.

Due to their elasticity, the skins of commodities such as grapes, plums, and tomatoes may not be finely chopped; this can be a source of heterogeneity especially in the case of contact pesticides which are more concentrated on the skins. Sufficient homogeneity is also dependent on thorough mixing during the withdrawal of sub-samples (test portions). Otherwise the different fractions (solid, liquid, skins) can form layers causing further heterogeneity.

Figure 3. Left: Cryogenic sample processing using dry ice. Right: Flowable powder produced after cryogenic processing. Photographs provided by Fera, UK.

Comminution or homogenization of high water content samples such as fruits and vegetables at low temperatures, using liquid nitrogen or dry ice, will inactivate enzymes and slow down chemical reactions during comminution of the samples.

Cryogenic processing (see Figure 3) will usually provide sufficient homogeneity to facilitate implementation of the smaller-scale, more environmentally-friendly methods that have gained in popularity during recent years. Cryogenic processing is less popular than processing at room temperature because it requires robust equipment, is more costly, requires additional health and safety procedures, and if using dry ice it requires additional time for pre-freezing of the sample and sublimation of CO_2 after comminution. This additional time is a restraint for laboratories who need to produce results within 24–48 hrs to be able to stop the product in transit or to remove the product from the market.

The pressure of turnaround deadlines means that laboratories sometimes compromise results for specific pesticides so the substantial errors that can occur at the sample comminution stage may not be taken into account. Ironically the same analysts often strive hard to improve the precision of the determination step by a few % relative standard deviation (RSD) and calculate uncertainty values that rarely include the contribution of sample homogenization, an integral and unavoidable step in the workflow. Pesticide residue analysts should at least evaluate the homogenization procedure used in their own laboratories to determine the minimum size of test portion required to be representative of the analytical sample and thus be able to justify the implementation of the small-scale extraction methods described below.

Low water content samples such as grain (wheat, rye, oats, etc.) tend to be physically disintegrated in a mill or grinder. The samples need to be disintegrated into small particles to provide homogeneous sub-samples (dependent on the size of sub-samples required) without causing separation of the endosperm and husk. Since some pesticides may be present at high concentration on the outer husks, any disproportionate separation will cause irreproducible and inaccurate results. Additionally, grinding the samples into smaller particles increases heat generation, and this may be sufficient to degrade some pesticides. Dry ice can be used to cool the mill (before grinding) or can be added to the sample during grinding to prevent overheating. The compromise recommended in the SANTE guidelines[2] is to mill the samples to a particle size of preferably less than 1 mm.

Tissue samples (meat, fish, etc.) can be minced, but again homogenization in the presence of dry ice in a food processor produces samples with improved homogeneity and with less likelihood of degradation of the pesticides.

The next step after sample comminution or sample processing is solvent extraction and cleanup.

Solvent Extraction and Cleanup

Prior to the publication of the 'generic' small-scale QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method in 2003[10], pesticides methods usually required 30–100 g of sample and 60–200 mL of solvent just for extraction. The most commonly used extraction solvents were acetonitrile[11], ethyl acetate[12], and acetone.[13-15] These methods – especially the ones based on acetone extraction with liquid/liquid partition – often required a collection of expensive glassware (e.g., separating funnels) and equipment (homogenizers), and generated large volumes of solvent waste (often chlorinated) for disposal. Those methods using liquid/liquid partition gave lower recoveries for polar compounds such as acephate and methamidophos.[13-15] Often time-consuming cleanup techniques such as gel permeation chromatography (GPC) or cartridge solid phase extraction (SPE) provided relatively clean extracts for determination by GC with electron capture detector (GC-ECD), GC with Flame Photometric Detector (GC-FPD), GC with Nitrogen Phosphorus Detector (GC-NPD), and GC-MS. At that time LC-MS was not widely available. Although these methods were capable of consistently producing accurate and reproducible results for a large number of pesticides, they were time-consuming and relatively costly.

Prior to the publication of the QuEChERS method in 2003, pesticides extraction required 30–100 g of sample and 60–200 mL of solvent

Extraction and Cleanup of High Moisture Samples

To perform an extraction:

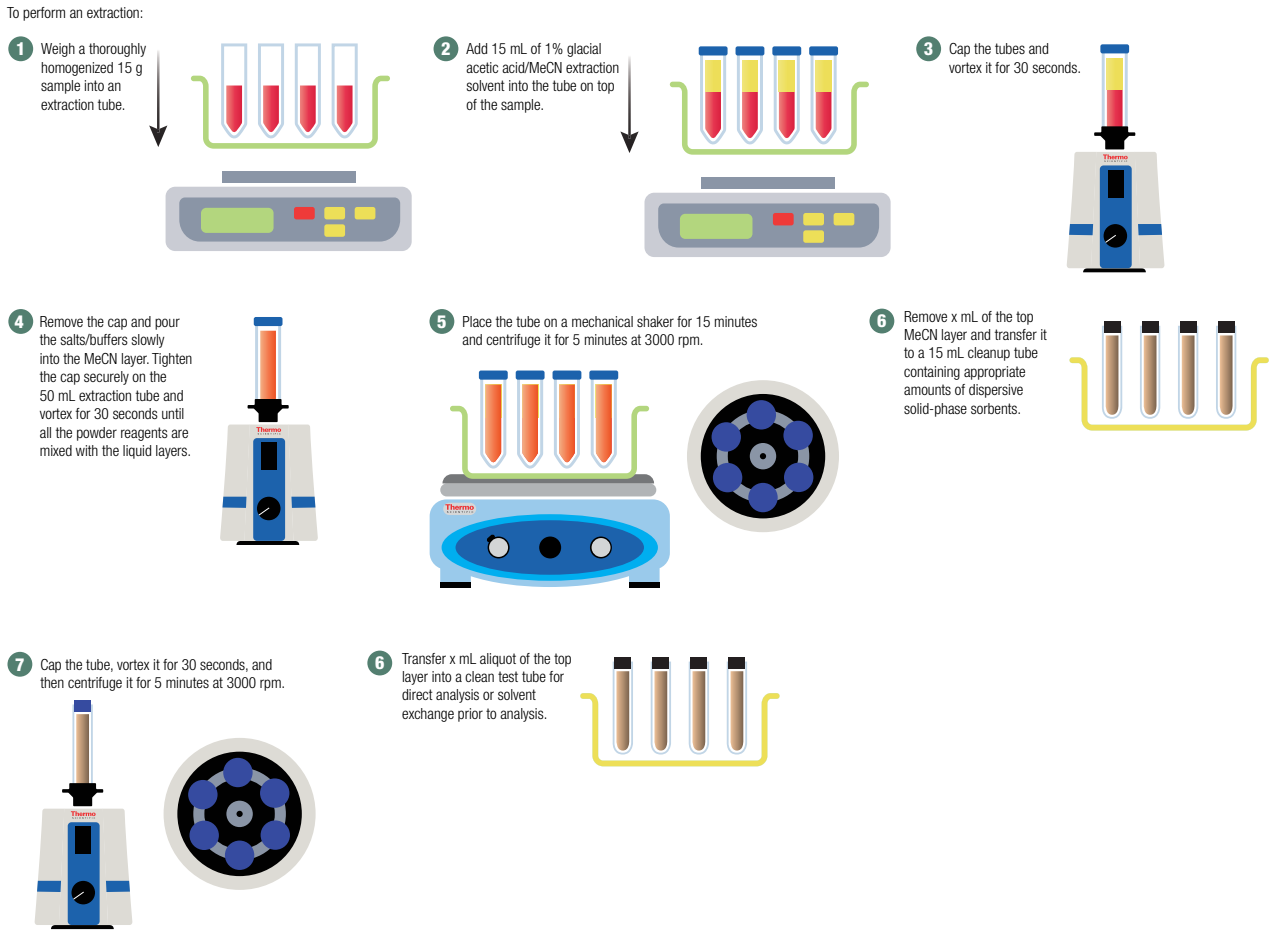
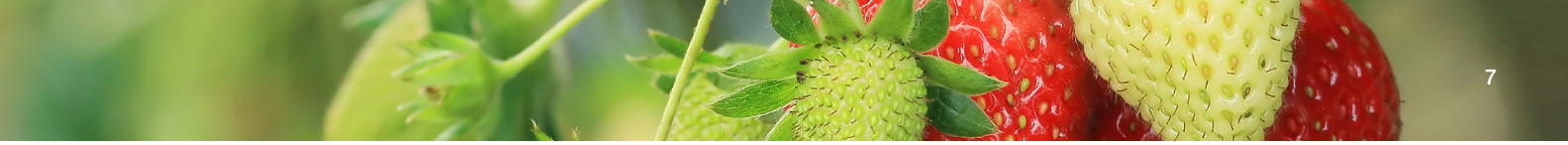


Figure 4. Extraction and cleanup process steps when using the QuEChERS method of sample preparation.



In more recent times the simplicity, low cost, speed, and wide analyte scope of QuEChERS acetonitrile extraction combined with 'just enough' dispersive SPE (dSPE) cleanup, has completely changed the pesticide residues analyst's approach to multi-residue extraction procedures. This concept of a reduced-scale generic extraction in a tube requiring a small sample size (10–15 g), low solvent volume (10–15 mL, typically 1 mL/g of sample), less waste, no need for homogenization (shaking is sufficient), and requiring minimal equipment, glassware, reagents, and bench space has proved very successful (see Figure 4).

The original QuEChERS method uses magnesium sulphate and sodium chloride to partition the aqueous and organic phases and to salt out analytes into the acetonitrile. The mixture is centrifuged, and an aliquot of the supernatant is taken for further analysis. The method includes an optional cleanup step using various solid-phase sorbents in the dispersive mode (dSPE) to remove co-extractives from an aliquot of the acetonitrile supernatant. Sorbents used, either individually or in combination, include primary secondary amine sorbent (PSA) to remove acidic co-extractives, carbon to remove pigments, C18 or zirconium coated silica to remove lipids, amino sorbents to remove sugars, and calcium chloride to remove specific co-extractives from tea.[16]

The original publication that introduced the QuEChERS technique[10] was based on GC-MS, which is rather ironic since acetonitrile is not an ideal solvent for GC separations. The development of more sensitive LC-MS/MS instrumentation during the last 5–10 years allows dilution (typically by a factor of 10) of the extracts prior to injection of low volumes (1–3 µL) of sample extract. The net result of the 'dilute and shoot' approach is that matrix co-extractives are diluted and matrix suppression of ionization in LC-MS is minimized. Today QuEChERS is probably the most widely used multi-residue approach in laboratories worldwide with many modified versions published in the literature. Variations include the development of buffered versions of the method using acetic acid[17] or citrate salts[18] to improve the stability of base-sensitive pesticides such as captan, captafol, chlorothalonil, dicofol, and others. A comparison of different versions of QuEChERS has been published in other literature.[19]

Buffered versions of the QuEChERS method using acetic acid or citrate salts have been developed to improve the stability of base-sensitive pesticides

The QuEChERS approach is not without problems, especially when dealing with certain matrices. The dSPE sorbents can decrease the recovery of pesticides, and may not clean up the extract to a great extent, increasing the possibility of faster contamination of the instrumentation.

If dSPE does not provide sufficient cleanup, laboratories may resort to SPE cartridge cleanup which can be more effective but increases the risk of recovery losses of certain pesticides. However, for specific pesticide-sample combinations, SPE in the cartridge format can be a good option.

The use of PSA in either format will remove acidic matrix components and cause an increase in the pH of the extract. The citrate version of QuEChERS includes the addition of formic acid after PSA dSPE to lower the pH, which improves the stability of base-sensitive pesticides such as captan, dicofol, and others. During the extraction step, the buffering capacity of the citrate version is less than the acetate version, so pH adjustment of certain sample types prior to extraction may be required to prevent degradation of base-sensitive or acid-sensitive pesticides. Most laboratories typically prefer to employ a single method at a slightly acidic pH, accepting the inevitable compromises.

Pesticides such as captan, dicofol, and captafol are particularly challenging and will degrade during sample processing, extraction, and determination by GC analysis. Although there are some issues with the compatibility of acetonitrile as a GC solvent, a published article on the evaluation of common organic solvents for GC concluded that acetonitrile was the best solvent for the stability of a number of pesticides.[20] Still, if these pesticides are those degraded during sample processing, then the results will simply be underestimated. With care, good results can be obtained by direct GC analysis of QuEChERS acetonitrile extracts, but a solvent exchange to a more GC-compatible solvent will permit injection of larger volumes. A solvent exchange can also reduce the concentration of coextractives, which may in turn help to reduce analyte degradation, reduce system contamination, and increase column longevity. Steiniger reported that solvent exchange from acetonitrile to hexane:acetone (9:1) removed high concentrations of polyphenols from green tea.[21,22]

The extraction efficiency of rapid methods such as QueChERS for certain incurred pesticides from low moisture content samples has also been questioned. This is partly due to the fact that there is no homogenization of the sample in the solvent. In the case of low moisture commodities such as cereals, rice, tea, and spices, water should be added to the sample and the re-hydrated sample left to stand for 10–20 minutes prior to addition of acetonitrile. The timing is critical especially in the case of cereals; if the time is too short the sample may not be sufficiently hydrated while if it is too long the added water can activate carboxylesterase enzymes that degrade certain pesticides, including malathion.[23] Freezing of sample extracts for at least a few hours at -20 °C or through use of an acetone-dry-ice bath after centrifugation to precipitate lipids – which have a relatively low solubility in acetonitrile – is also recommended for extracts of milled cereals. QuEChERS is applicable for oils and fats in liquid form as it is relatively easy to separate lipids from acetonitrile, but it is not the most appropriate option for the extraction of pesticides from fatty tissues.

There is no doubting the popularity of the QuEChERS acetonitrile extraction approach worldwide. It has been successfully validated for hundreds of pesticides in a large variety of sample matrices in many different laboratories. The method has also been commercialized with manufacturers providing kits containing the various reagents pre-weighed for convenience. For example, Thermo Scientific kits use high-quality reagents (salts and sorbents) to ensure excellent results with all QueChERS method variants. It is important to recognize that variation in the quality of reagents over time can impact the analytical results. More information on the reagents, SPE cleanup options, and the applications of the various methods can be found at www.thermofisher.com/QueChERS.

Because of its limitations and compromises, QuEChERS acetonitrile extraction is not the preferred multi-residue method of choice for all laboratories. A number of other ‘reduced-scale’ methods, based on the QuEChERS approach but using different extraction solvents including acetone[24] or ethyl acetate[25], are also used in many laboratories. The accelerated solvent extraction technique is also used as it can provide benefits of automation or more effective extraction of pesticides from difficult matrices. These options are discussed in more detail in the following text.

Reduced-Scale Alternatives to QuEChERS

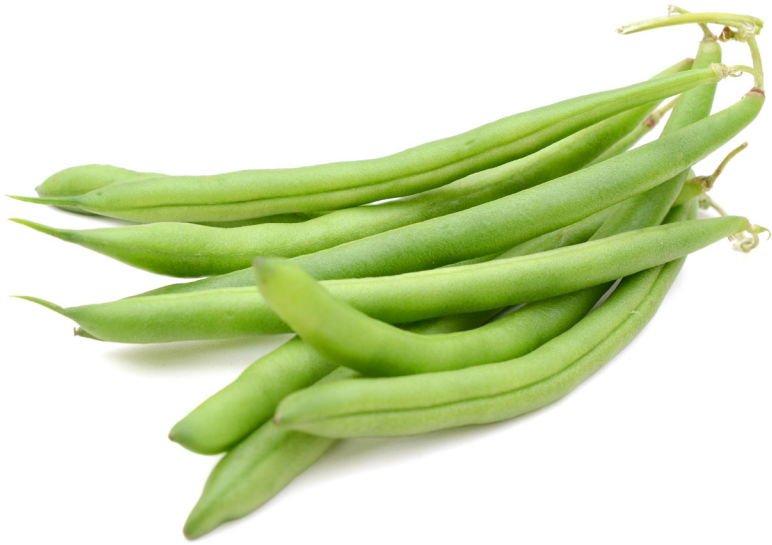
Reduced-Scale Acetone-Based Extraction

The so called 'Dutch mini Luke' method[25], which uses a combination of acetone/petroleum ether/dichloromethane (v/v 1/1/1), has been successfully validated for a wide range of LC and GC amenable pesticides. It is preferred by some laboratories because liquid/liquid partitioning provides relatively 'clean' extracts without the need for additional cleanup. The lower concentration of co-extractives compared to acetonitrile and ethyl acetate results in less contamination of the instrument systems. Wider adoption of the method has possibly been hindered by the need for slightly higher volumes of solvent including dichloromethane. However, experiments to further reduce the volume of solvents required (dichloromethane reduced to 10 mL) and validate the improved 'NL method' were recently reported.[26]

Reduced-Scale Ethyl Acetate-Based Extraction

The routine analysis of 341 pesticides, metabolites, and degradation products in more than 100 different matrices using a semi-miniaturized method based on ethyl acetate with a phosphate buffer and dSPE has been reported by Mol et al. For a comprehensive and informative discussion, refer to the published article.[25]

Ethyl acetate in combination with QuEChERS salts in a tube can be used successfully for a wide range of pesticides, but experience shows the recovery of polar pesticides such as acephate, methamidophos, and aldicarb sulphoxide are lower than with acetonitrile. The recovery of polar pesticides is more influenced by the temperature of extraction when using ethyl acetate, which is probably due to the efficiency of the removal of water, especially when using sodium sulphate. Another issue encountered with ethyl acetate is the formation of emulsions for crops with a high starch/sugar content. This is easily overcome by increasing the solvent volume:sample weight ratio (typically from 2:1 to 4:1), or by placing the extract in a refrigerator to reduce the temperature of the final extract.



The National Food Administration in Sweden has developed the so-called 'SweEt' (Swedish Ethyl Acetate) method[27] which is a modification of an ethyl acetate method previously published by the same group.[28] The SweEt method uses a 10 g sample for high water content samples and a 5 g sample hydrated with 10 mL water for low water content samples such as cereals. It uses ethyl acetate extraction with sodium sulphate to bind water and sodium hydrogen carbonate to adjust the pH. The extracts only require filtration before direct injection in GC-MS/MS or LC-MS/MS systems. A low injection volume, around 1 µL, is recommended for LC-MS to avoid peak splitting for early eluting compounds. Because of the higher solubility of fats in ethyl acetate, a modified version of the method is also applicable to animal tissues such as meat and offal. For low fat samples, the sample (5 g) is extracted with ethyl acetate:cyclohexane (1:1), 10 mL, and the extracts are cleaned up with GPC. Evaporation of the solvent and reconstitution in ethyl acetate is performed before GC-MS/MS or LC-MS/MS.

The European Union Reference Laboratory (EURL) for animal products has combined elements of the SweEt method and the citrate-buffered QuEChERS method to form a new method for the analysis of 'less polar' pesticides in products of animal origin.[29] (See Figure 5) For complex samples such as liver, water, and citrate, buffer salts are added followed by ethyl acetate/ cyclohexane (1+1 v/v), sodium sulphate, and PSA before centrifugation. An aliquot of the supernatant is cleaned up by GPC and then sequential dSPE with zirconium oxide and silica gel before solvent exchange into a more suitable solvent such as toluene prior to GC-MS/MS analysis. There are various steps (shaking, concentration, and solvent exchanges) between the stages mentioned. The method can be simplified for less complex matrices. For example the silica gel cleanup can be excluded for the analysis of eggs, and both the zirconium oxide and silica gel – and perhaps the GPC step – may be eliminated for the analysis of honey (under investigation). The method may seem complex, but it is much more efficient than older methods using greater volumes of solvent.

An excellent method to check the effectiveness of different cleanup steps during method development for the analysis of pesticides in animal products is to use Thin Layer Chromatography.[30]

Ralph Lippold, head of the EURL for pesticides in products of animal origin, recently presented use of dual axis centrifugation for speeding up QuEChERS.[31] The samples rotate while being centrifuged, resulting in more efficient mixing. This allows the consecutive adding of reagents, mixing, and shaking to be combined into a single process.

Generic Non-Partitioning Reduced-Scale Methods for Extraction

A simple generic extraction and dilution approach with no liquid/liquid partition or cleanup has been reported for the multi-class, multi-analyte screening of samples for pesticides, mycotoxins, plant toxins, and veterinary medicines in a single analysis.[32] Various solvent options were evaluated. The best recoveries were obtained using acidified acetone, but with respect to the amount of matrix co-extractives, acetonitrile was best and methanol the worst.

A generic method based on methanol extraction for targeted analytes has been reported by Klein and Alder.[33] The method became a European Committee for Standardization (CEN) Standard Method EN15637: http://www.cen.eu, but is not widely used, most likely because the method involved a solid phase partition with ChemElut followed by elution of analytes with dichloromethane. Hanot et al.[34] recently published a method that involved extraction of a 10 g sample with 40 mL ammonium acetate buffered methanol. The method was based on the method published by Granby et al.[35] but an important modification was homogenization of the sample in the solvent using a high speed IKA Ultra Turrax™, instead of ultrasonication. The improved extraction efficiency resulted in a 40% increase in the measured concentration of certain incurred residues. Methanolic extracts were diluted with water to improve the peak shapes for early eluting pesticides. A disadvantage of using methanol is non-compatability with GC-MS.

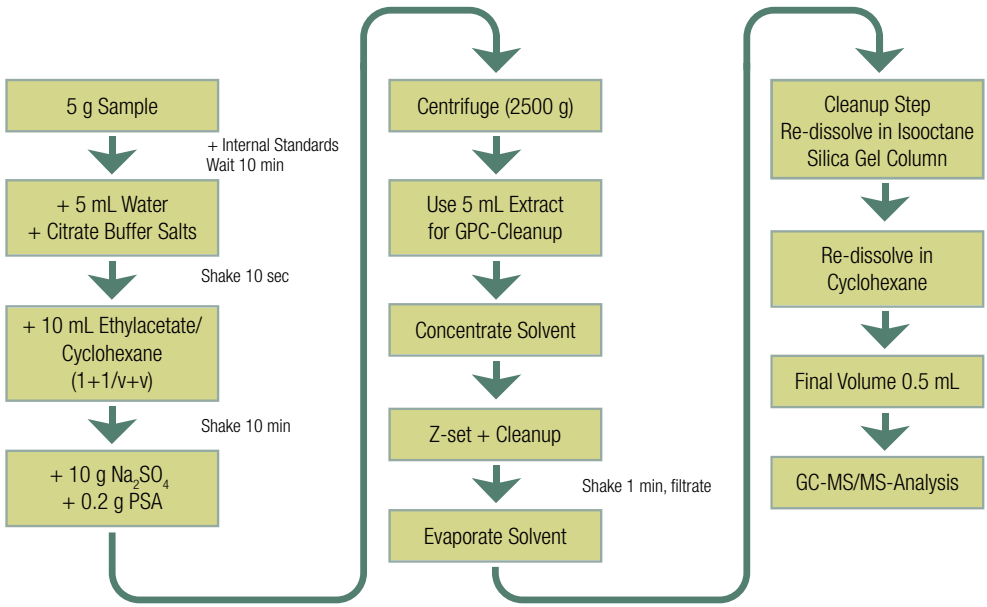
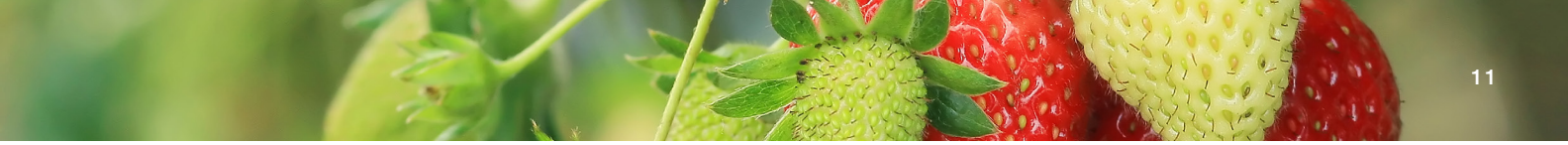


Figure 5. Schemetic courtesy of Dr. Ralf Lippold, Head of the EURL for pesticides in products of animal origin, CVUA, Freiburg, Germany.



Single Residue Methods

In addition to multi-residue methods, laboratories have a requirement to use SRMs to analyze those pesticides which do not fit into the multi-residue methods. This has led to the development of the quick polar pesticides extraction (QuPPe) method[36] for the analysis of LC amenable polar and ionic compounds that remain in the aqueous phase. The QuPPe method uses acidic methanol so there is no phase separation and no cleanup. The main advantage is that a number of pesticides that were once analyzed by single methods can be combined into a single extraction, but the extracts contain high concentrations of co-extractives requiring increased column and instrument maintenance.

Aliquots from a single extraction are analyzed using a number of different LC columns types including ion exchange, hydrophilic interaction liquid chromatography (HILIC), and Thermo Scientific™ Hypercarb™. The addition of labelled procedural internal standards before sample extraction is often necessary to improve accuracy and precision. The overall outcome is that the cost per pesticide has been reduced enabling these pesticides to be included in monitoring programs. This is important because some of these pesticides, including ethephon, fosetyl, glyphosate, etc., are frequently used, and indeed residues are now being detected and reported. However, there is also a need for SRMs to monitor pesticides that require specialized extraction and detection methods. These include:

- Paraquat and diquat which require extraction with relatively strong acids[37]
- Fumigants requiring headspace analysis (e.g., phosphine)[38] or hexane extraction for the simultaneous multi-analysis of fumigant compounds[39]
- Phenoxy acid herbicides requiring some form of hydrolysis to include esters and conjugates[40]
- Dithiocarbamates[41,42] determined as carbon disulphide evolved after acid digestion and determined by GC-MS

For the analysis of the low moisture commodities (cereal, tea, herbs, spices) and fatty tissues, pressurized liquid extraction is also an option for both single and multi-residue analysis.

Using the Accelerated Solvent Extraction Technique

The Thermo Scientific™ Dionex™ ASE™ 150/350 Accelerated Solvent Extractor is an automated system which extracts target compounds using elevated temperature and pressure. This technique is especially suitable for more challenging matrices applicable to high lipid content such as oyster tissue[43] and low moisture content samples (herbal products[44], tea, cereal[45], and animal feeds[46]). The accelerated solvent extraction technique is also applicable to fruit and vegetables[47,48] and can reduce sample preparation time, especially when used in combination with a novel new polymer designed to absorb water from wet samples[49] and/or the Thermo Scientific™ Rocket™ Evaporator System for solvent evaporation and concentration of the extract.[50]

The accelerated solvent extraction technique is especially suitable for more challenging matrices including high lipid content (oyster tissue) and low moisture content samples (herbal products tea, cereal, and animal feeds)

The accelerated solvent extraction technique is one of the fastest, safest, easiest, and most reproducible techniques available for extracting analytes out of solid and semi-solid samples. Traditional methods such as Soxhlet and sonication are time-consuming, difficult, labor-intensive, and require large amounts of solvent which can be expensive. The accelerated solvent extraction technique is automated, requires minimal solvent, and can accommodate in-cell or in-line selective removal of interferences.[51,52]

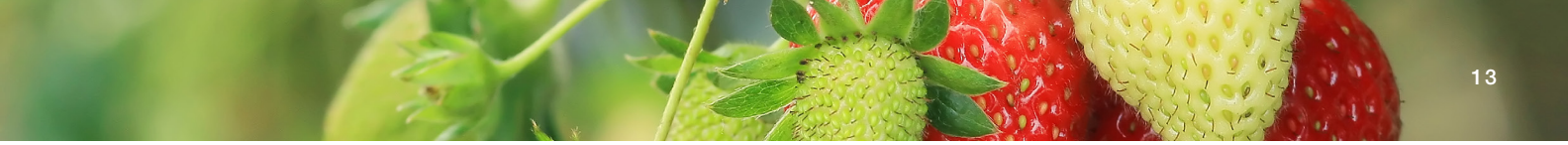
The concept is simple: The accelerated solvent extraction technique uses elevated temperature to increase the extraction efficiency of analytes of interest from their matrix. Elevated pressure is used to keep the solvents in a liquid state as the temperature is increased above their boiling points. By using increased temperature and pressure, solubility of analytes is increased, viscosity of solvent is reduced, and hence analyte diffusion into the solvent is improved, thereby improving extraction efficiency and reducing extraction time. An overview of the optimization of the use of the accelerated solvent extraction technique was published by Sun et al.[53]

While other methods take up to 8 hours and use hundreds of milliliters or more of solvent, the accelerated solvent extraction technique takes 15–30 minutes and requires minimal solvent, typically 10–30 mL depending on the application. Under these conditions the solvent penetrates the matrix more effectively so it is possible that certain types of incurred residues are extracted more effectively and thus more accurate results may be obtained. In pesticide residues analysis, the recovery is typically checked by spiking pesticides onto the surface of the sample or even into the extract. As there is minimal, if any, binding of the analyte to the matrix, this approach can only measure losses of analytes during the extraction process. Internal standards are sometimes required to correct for losses of spiked pesticides, which suggests that in these cases the recovery of incurred residues is likely to be poor. More results are required to be able to draw definitive conclusions.

Summary

All multi-residue methods will have limitations requiring inevitable comprises. There is no universal best method for all laboratories. The best method for individual laboratories will depend on many factors including the pesticide-matrix combinations to be tested, customer requirements, guidelines or regulations to be complied with, any requirements from accreditation bodies, instrumentation and facilities available, etc. The likelihood is that laboratories will need to implement a number of different extraction and cleanup methods in combination with detection technologies. Hopefully this paper will be helpful in understanding some of the critical stages, advantages, and limitations of the most widely used methods.

For more information on methods for pesticides and many other classes of residues and contaminant analyses, please visit the Thermo Scientific Food and Beverage Community online at www.thermofisher.com/foodandbeverage.



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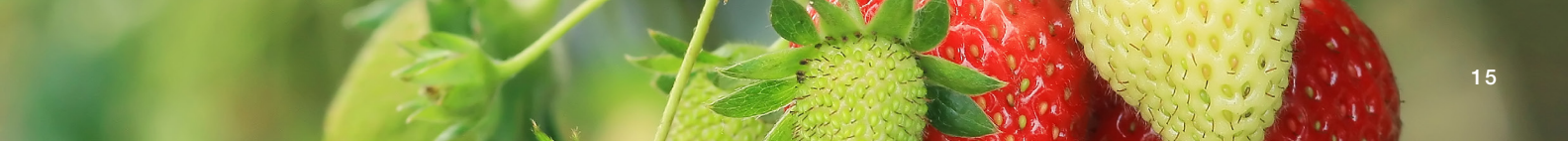

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