An Overview of Multi-residue Pesticide Testing

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

CONTENTS

Introduction

Plant protection products, more commonly known as pesticides, are used to control pests, weeds, and diseases. They may include the following:

- **Herbicides** to control weeds before and during growth
- **Insecticides** to protect seeds and plants from damage by insects
- **Nematicides** and molluscicides to control attack on growing plants by worms and slugs
- **Rodenticides** to prevent damage and contamination by small mammals such as mice and rats during growth and storage
- **Fungicides** to prevent mold forming on plants in the field and in store

Pesticide residues resulting from the use of plant protection products on crops or food products may pose a risk factor for public health or hinder trade. This eBook aims to provide a short background on multi-residue pesticide analysis, discussing elements of sample preparation and sample analysis.

DRIVERS FOR PESTICIDE TESTING

WHY TEST FOR PESTICIDES?

Many individual governments or groups of nations monitor pesticide residues in domestic and imported agricultural produce and other foods each year.
This monitoring verifies that:

- No unexpected residues are occurring in crops in support of the national statutory approvals process for pesticides
- Residues do not exceed the statutory maximum residue limits (MRLs)
- Human dietary intakes of residues in foods are within acceptable levels

In food safety, the phrase “due diligence” refers to being able to prove that a business has taken reasonable steps to prevent food safety breaches.

Food businesses are responsible for ensuring that the food they produce, or import is compliant with current, relevant legislation, including MRLs. This may cover more pesticides at concentrations lower than legal limits, analysis of finished products as well as ingredients.
A second reason for food businesses to conduct testing is to protect their brand.

REGULATORY LIMITS

Maximum residue levels (MRLs) are established in raw food commodities and animal tissues. MRLs are set at the highest level of pesticide that the relevant regulatory body would expect to find in that crop when it has been treated in line with critical Good Agricultural Practice.

WHAT DOES AN MRL DO?

The main function of an MRL is to act as a control mechanism to ensure the product has been correctly used according to its label. The value assigned to a selected pesticide will vary depending upon the commodity for which the MRL was set.

RESIDUE DEFINITIONS

In some cases, the compound applied as the plant protection product is transformed by the time samples are taken for analysis. These changes are considered as part of the approval process when the residue definition is created. For example, in the EU, the residue definition for aldicarb is the sum of aldicarb, its sulfoxide, and its sulfone, expressed as aldicarb. Residue definitions vary between country and commodity.

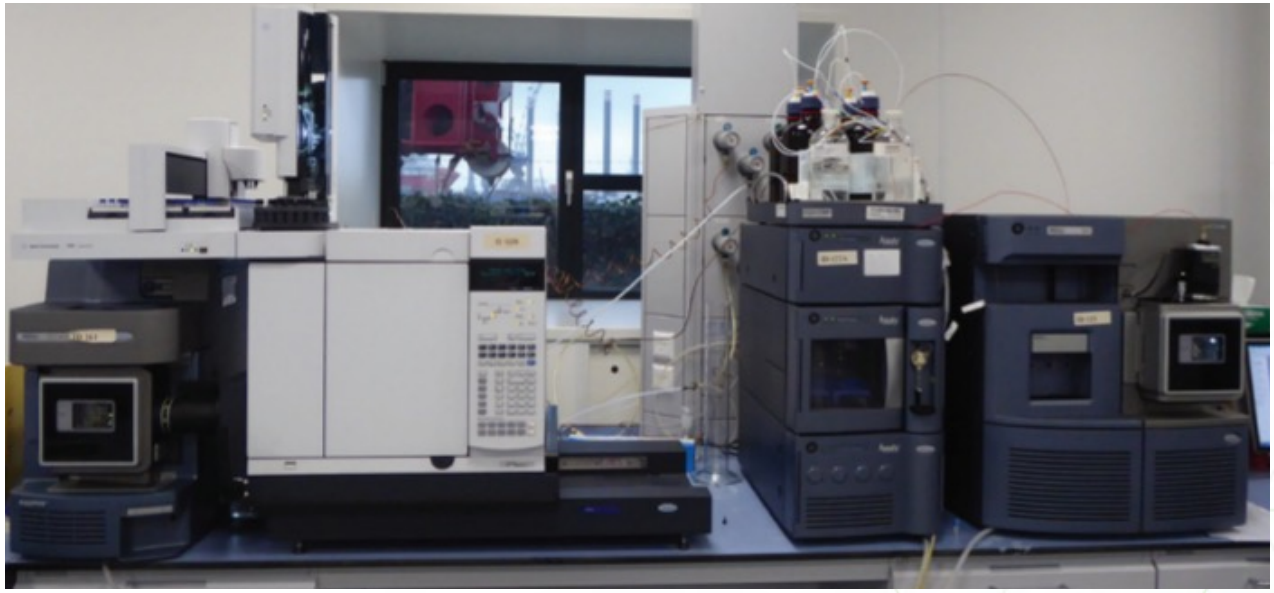
MULTI-RESIDUE PESTICIDE METHODS

WHY MULTI-RESIDUE ANALYSIS?

A primary goal for all laboratories involved with the analysis of pesticide residues in food is accurately determining as many compounds as possible or compounds in a prescribed list in the most cost-effective manner.

Laboratories must address capacity and efficiency issues constantly to meet sample throughput requirements.

By implementing multi-residue methods, many labs have significantly extended their scope of analyses and achieved effective and efficient implementation using generic extraction, matrix clean-up, and determination with gas and liquid chromatography coupled with mass spectrometry.



Waters LC-MS/MS and APGC™-MS/MS systems in the NofaLab laboratory.

Read this case study to learn how NofaLab meets the growing needs of its customers by improving method development for rapid food contaminant detection.

Sample Preparation

There are several key aspects to consider prior to sample extraction.

PREPARING SAMPLES FOR ANALYSIS

Samples received at the laboratory may need work to prepare a test sample for analysis by removal of parts (soil, stones, bones, etc.) not to be analyzed. Representative samples should be homogenized to provide uniform particle size, uniform residue distribution, and to increase the surface area available for extraction. To minimize degradation of labile compounds, samples of fruit and vegetables are comminuted (cut and homogenized) at low temperature (e.g., frozen and in the presence of "dry ice" or liquid nitrogen) to transform the sample into a fine, homogenous powder which is frozen for storage until required for analysis.

IMPROVING EXTRACTION EFFICIENCY

Test portions should be extracted frozen or while in the process of thawing (except dry samples with water content <20%). To improve the extraction efficiency of low moisture commodities (cereals, spices, and dried fruits), addition of water to the milled samples prior to extraction is recommended. When looking at the influence of various factors on the extraction yields, the use of samples with incurred pesticide residues is advised.

SAMPLE EXTRACTION

CHALLENGES WITH LEGACY METHODS

Legacy multi-residue methods relied on liquid-liquid extraction and clean-up using gel permeation chromatography (GPC) or solid phase extraction (SPE). These methods were typically labor-intensive, required specialist equipment, and involved the use of a lot of glassware and large volumes of organic solvents, including chlorinated solvents for the extraction, with associated disposal costs.

MODERN APPROACHES

There are now several generic extraction protocols in common use for multi-residue methods:

- Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) remains the most popular approach for pesticide residue analysis in fruit, vegetables, cereals and sometimes products of animal origin[1]
- Dutch mini-Luke ("NL-") method using extraction with acetone followed by partition with dichloromethane/ petroleum ether (1:1 v/v)[2]
- Swedish ethyl acetate ("SweEt") method[3].

QuEChERS EXTRACTION

WHAT IS QuEChERS?

QuEChERS is a versatile, streamlined approach using a rapid solvent-based extraction in a centrifuge tube, often followed by dispersive solid-phase extraction (dSPE) for clean-up.

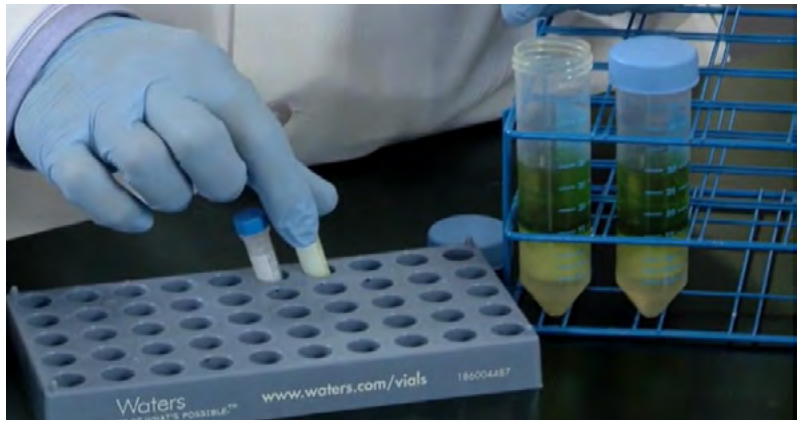
WHY QuEChERS?

QuEChERS requires a small sample size (10 – 15 g) and low solvent volume (10–15 mL) so waste disposal costs are minimized (no chlorinated solvents). There is no requirement for glassware so no need for washing or storage. It speeds up sample throughput and simultaneously generates extracts for both GC-MS(/MS) and LC-MS/MS. Ready-to-use extraction and clean-up tubes are commercially available, which contain pre-weighed salts and sorbents.

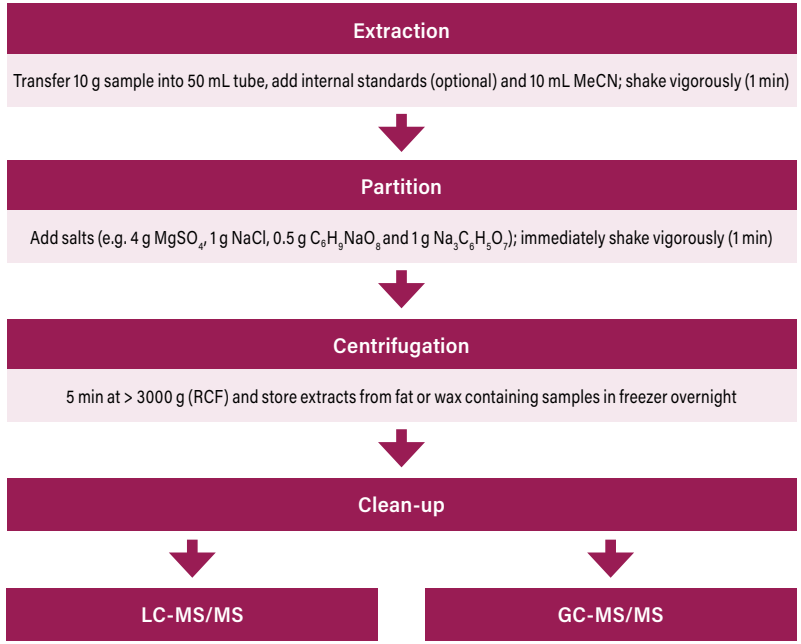
The use of acetonitrile (MeCN) provides extraction of a broad range of the compounds, from polar to nonpolar. The addition of buffering salts into a mixture of water and MeCN causes the formation of a two-phase system and improves recoveries of pH-dependent analytes.

>> Click here to learn more about our commercially available products.

Watch this video on Simplifying your QuEChERS Extractions using DisQuE™ Sample Preparation Products.



Analyst transferring QuEChERS extract to dSPE tube.



QuEChERS CEN method workflow.

DISPERSIVE SOLID PHASE EXTRACTION (dSPE)

WHAT IS dSPE?

Dispersive solid phase extraction (dSPE) is a rapid, simple, and straightforward technique suitable for the clean-up of extracts from a wide variety of food and agricultural commodities.

An aliquot of acetonitrile supernatant from a QuEChERS extraction is added to a disposable tube containing sorbent(s). The tube is shaken, centrifuged and the supernatant is collected for further analysis. Unlike in conventional SPE, it is the co-extractives that are retained on the sorbent whilst the analytes remain in the solution. Varying amounts of different sorbents are added depending on the commodity and pesticides targeted.

The most popular commercially available kits were developed in accordance with the official standardized version of QuEChERS and typically include magnesium sulfate to remove any remaining water and one or more of three sorbents for the dSPE step:

- Graphitized carbon black (GCB; for pigments)
- Primary-secondary amine (PSA; for sugars, fatty acids, organic acids)
- Octadecyl-bonded silica (C_{18}; for long chain hydrocarbons, lipids, waxes)

Commodity Type	Cleanup Provided	Size	AOAC Method 2007.01	CEN Method 15662
General Fruits and Vegetables (Celery, Head Lettuce, Melon)	Removes polar organic acids, some sugars and lipids	2 mL Tubes 15 mL Tubes	50 mg PSA, 150 mg $MgSO_4$ Part #186004572 400 mg PSA, 1200 mg $MgSO_4$ Part #186008072	25 mg PSA, 150 mg $MgSO_4$ Part #186004831 150 mg PSA, 900 mg $MgSO_4$ Part #186004833
Fruits and Vegetables with Fats and Waxes (Cereals, Nuts, Dairy, Avocado)	Removes polar organic acids, some sugars, more lipids and sterols	2 mL Tubes 15 mL Tubes	50 mg PSA, 50 mg C_{18}, 150 mg $MgSO_4$ Part #186004830 400 mg PSA, 400 mg C_{18}, 1200 mg $MgSO_4$ Part # 186008073	25 mg PSA, 25 mg C_{18}, 150 mg $MgSO_4$ Part #186004832 150 mg PSA, 150 mg C_{18}, 900 mg $MgSO_4$ Part #186004834

dSPE sorbent suggestions for different commodities.

Oasis™ PRiME HLB Plus Cartridge, Pass-Through SPE

SOLID PHASE EXTRACTION (SPE)

LIMITATIONS OF dSPE

The use of dSPE is not without problems, especially when dealing with certain commodities. The dSPE sorbents can decrease the recovery of some compounds and may not provide sufficient clean-up of the extract, increasing the possibility of faster contamination of the instrumentation.

ALTERNATIVES TO dSPE

Laboratories may turn to conventional trap and elute SPE, which can be more effective for removal of matrix co-extractives but increases the risk of poor recovery of certain pesticides.

Using SPE in pass-through mode, the extract is passed straight through the cartridge and the matrix co-extractives remain on the sorbent and analytes are collected in the eluant.

One advantage over conventional SPE is the omission of the extra load, rinse and subsequent elution steps typically involved with SPE. SPE using Oasis™ PRiME HLB can be used as an alternative to dSPE, especially where removal of lipids is important or to avoid use of PSA or GCB sorbents in dSPE.

Discover the benefits a simple pass through offers for the removal of chlorophyll from highly pigmented samples in this **application brief**.

GAINING EFFICIENCIES IN LIQUID HANDLING

LIQUID HANDLING: FROM PAINS TO GAINS

A common and significant step in multi-residue pesticide testing workflows is the preparation of matrix matched standards. The accuracy, repeatability and traceability of this step are essential for the reliable quantitation of pesticides.

Automation of routine liquid handling steps such as this can provide several benefits, including:

- Minimized pipetting errors
- Reduced waste
- Increased traceability
- Reduced risks of repetitive injury
- Freeing analysts to be deployed for other high value tasks

Read this application note: Automating Preparation of Matrix-Matched Standards for Pesticide Residue Analysis Using the Andrew+™ Pipetting Robot.

Sample Analysis

KEY TECHNOLOGIES FOR THE DETERMINATION OF MULTI-RESIDUES

Mass spectrometry coupled with both gas chromatography (GC) and liquid chromatography (LC) is needed to provide comprehensive analysis of the wide range of pesticide residues. Tandem quadrupole mass spectrometry (MS/MS) has become the most widely adopted technique due to its high selectivity in multiple reaction monitoring (MRM) mode and sensitivity to meet global MRL regulations.

Like the evolution of sample preparation techniques, MS/MS has continued to develop to meet users' growing needs, where:

- Sensitivity to exceed MRL requirements has allowed for sample preparation to be streamlined and reduce matrix loaded
- Acquisition speeds yield sufficient data points to reliably quantify even the most well-focused and overlapping peaks
- The reduced instrument footprint offers laboratories improved performance per square meter of coveted bench space

Read this application note on the determination of multi-residue pesticides in fruits and vegetables by UPLC and APGC coupled with the Xevo™ TQ-S micro.

GAS CHROMATOGRAPHY TANDEM QUADRUPOLE MASS SPECTROMETRY (GC-MS/MS)

Originally gas chromatography coupled to mass spectrometry (GC-MS) methods were mostly based on selected ion monitoring (SIM) or full scan modes, using either single quadrupole or ion trap mass analyzers. However, the determination of GC-amenable pesticides in food by using MS/MS has grown considerably over the last decade as this provides higher selectivity and sensitivity that minimizes most chromatographic interferences.

SOFTER IONIZATION FOR ENHANCED SENSITIVITY AND SELECTIVITY

GC-MS/MS typically uses EI at 70 eV, extensive fragmentation is often observed so the selection of the precursor ion for MS/MS is often a compromise between sensitivity and selectivity. APGC™ is a soft ionization technique which generates higher abundance of molecular ions, with less fragmentation than conventional EI. As the molecular ion (or protonated molecular ion) is highly abundant under the soft ionization occurring in APCI, selectivity and sensitivity are notably enhanced when it is used as the precursor ion for MRM methods. Moreover, APGC operates at atmospheric pressure which removes the restriction imposed by pumps, allowing a much wider range of flow rates for GC separations and use of alternative gases to helium, such as nitrogen.

Waters APGC System, the Xevo TQ-XS.

Read this white paper on Atmospheric Pressure Gas Chromatography (APGC) to learn more.

ACQUITY™ UPLC Premier and Xevo TQ Absolute system

LIQUID CHROMATOGRAPHY TANDEM QUADRUPOLE MASS SPECTROMETRY (LC-MS/MS)

With the introduction of UltraPerformance Liquid Chromatography™ (UPLC™), laboratories reported significant method efficiency benefits over traditional LC. The true benefits of this technology was further realized with the advancement of MS/MS technology almost a decade later, offering:

- Improved system sensitivity, allowing for simple dilution of crude QuEChERS extracts, avoiding timely and labor-intensive extract cleanup
- Increased MS/MS scanning capabilities enabling the analytical scope in a single injection to be extended, without impacting data quality
- Reduced analytical runtimes, allowing twice the samples to be analyzed by UPLC-MS/MS vs traditional LC-MS/MS
- Less solvent usage and associated disposal costs

Read this application note: Multi-residue Method for the Quantification of Pesticides in Fruits, Vegetables, Cereals and Black Tea using UPLC-MS/MS.

RETENTION

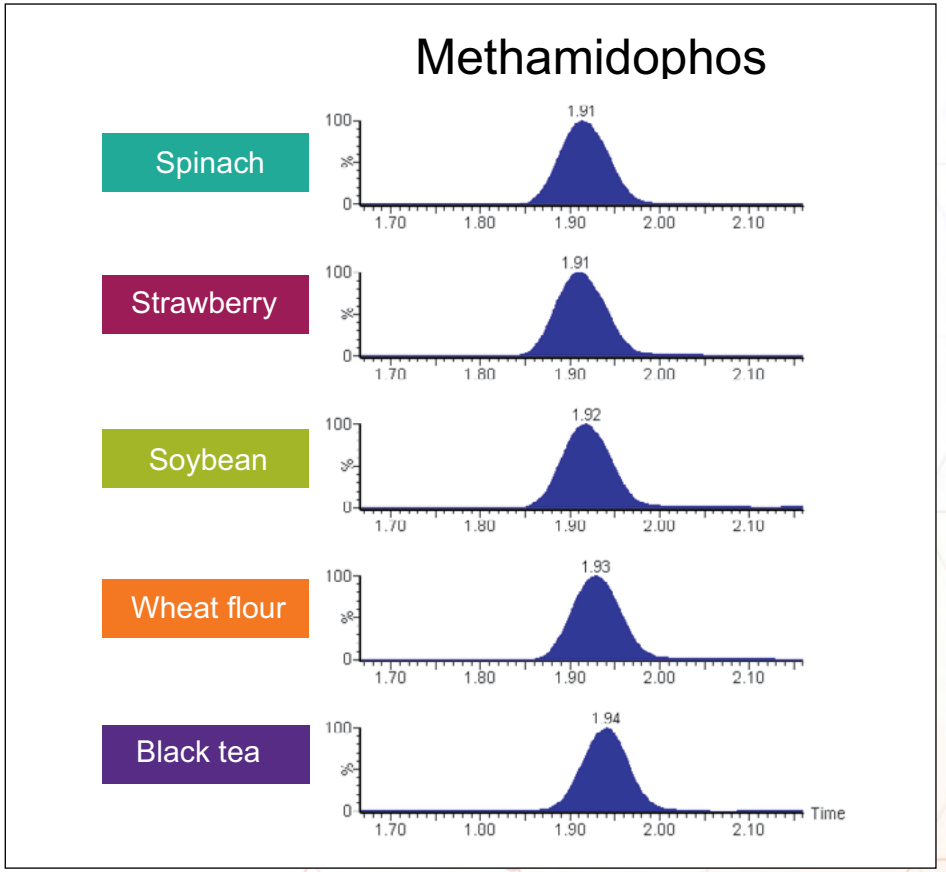
WHAT IS RETENTION TIME?

Retention time (RT) is the time between the start of an injection to the emergence of the peak maximum of the analyte(s).

WHY IS RT IMPORTANT?

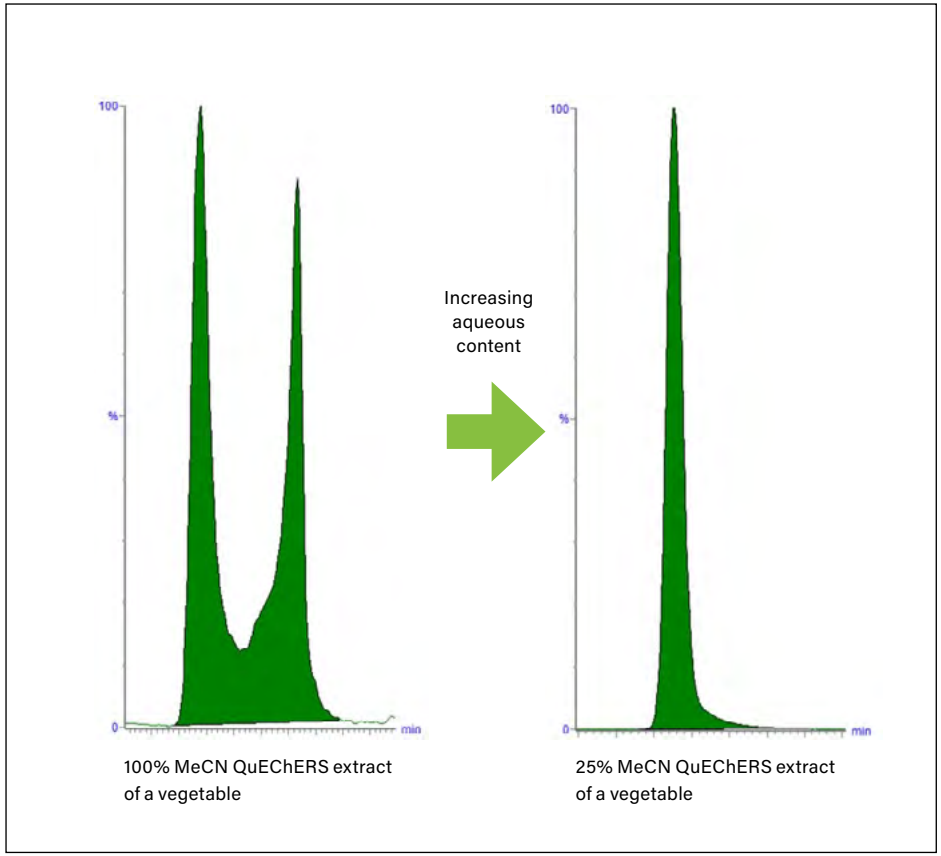
If this interaction is too short, then little to no chromatography has taken place, separations will be less stable, and there will be a high chance of ion suppression when LC-MS is used. The column void volume (v) is a measure of the internal volume inside the column packed with the stationary phase particles and can be estimated from a column's length (L) and internal diameter (ID).

Analytical quality control, performance and method validation guidelines such as SANTE/11312/2021 state "the minimum acceptable retention time for the analyte(s) should be at least twice the retention time corresponding to the void volume of the column."[4]



Methamidophos retention time in different samples.

Visit the Column Coach to Assist in Column Selection for your Analysis.



Methamidophos in a QuEChERS extract with different acetontrile and water ratios.

SAMPLE INJECTION

Pesticides, such as methamidophos and acephate, are polar and elute early in the chromatogram. Injecting samples containing moderate amounts of organic solvent (≥25%) on a reversed-phase column often results in fronting and/or split peaks for the early eluting compounds. Reducing the organic content used in the sample diluent prior to transfer onto the column may help to improve the peak shape of early eluting analytes. However, maintaining a portion of organic in the extract is essential for analyte stability.

ALTERNATIVES TO MANUAL DILUTION

Post injector mixing can allow the injection of typical QuEChERS extracts into high aqueous mobile phase without compromising peak shape. An extension loop is placed in between the injector port and column. Before the injection is made, the extension loop is filled with the high aqueous mobile phase, which provides more volume to aid dispersion of the sample into the aqueous solvent prior to transfer onto the column.

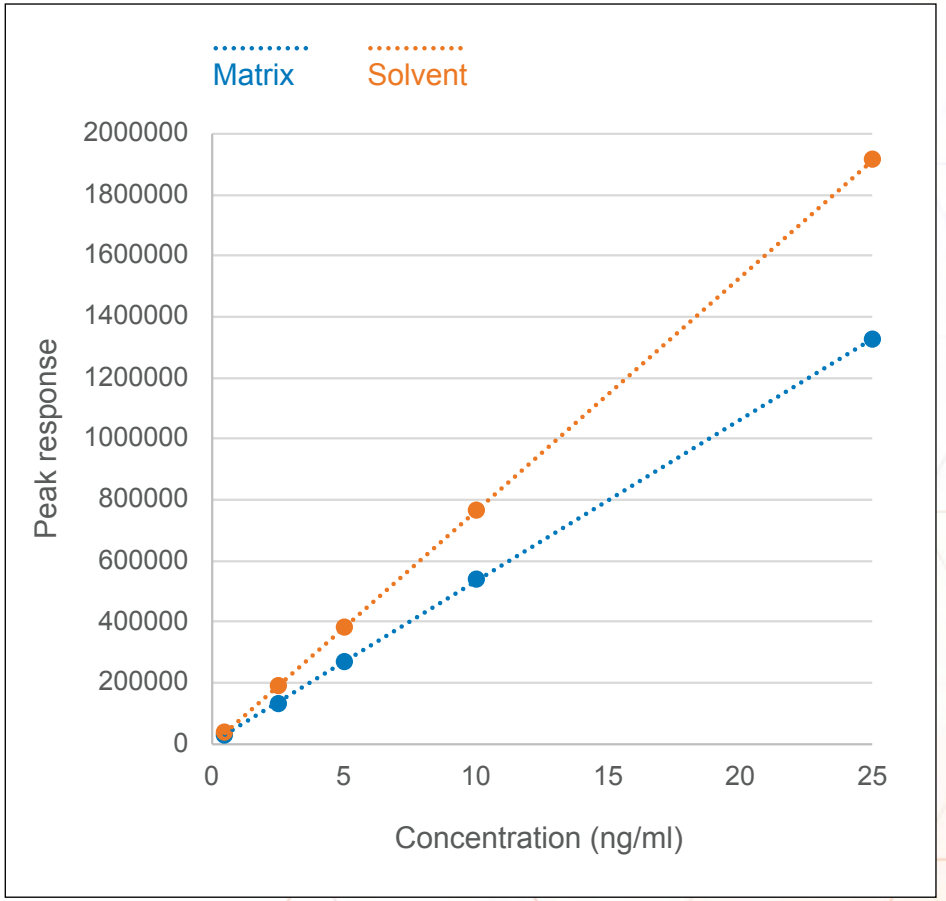
Read this application note: Multi-residue Method for the Quantification of Pesticides in Fruits, Vegetables, Cereals and Black Tea using UPLC-MS/MS.

MATRIX EFFECTS

The hyphenation of chromatographic and mass spectrometry technologies has revolutionized food contaminant testing laboratories. However, one major drawback is the potential for the phenomenon of matrix effects. The influence of matrix on the reliability of your method should be determined when implementing new methodologies, commodities or analytes into your laboratory's analytical scope.

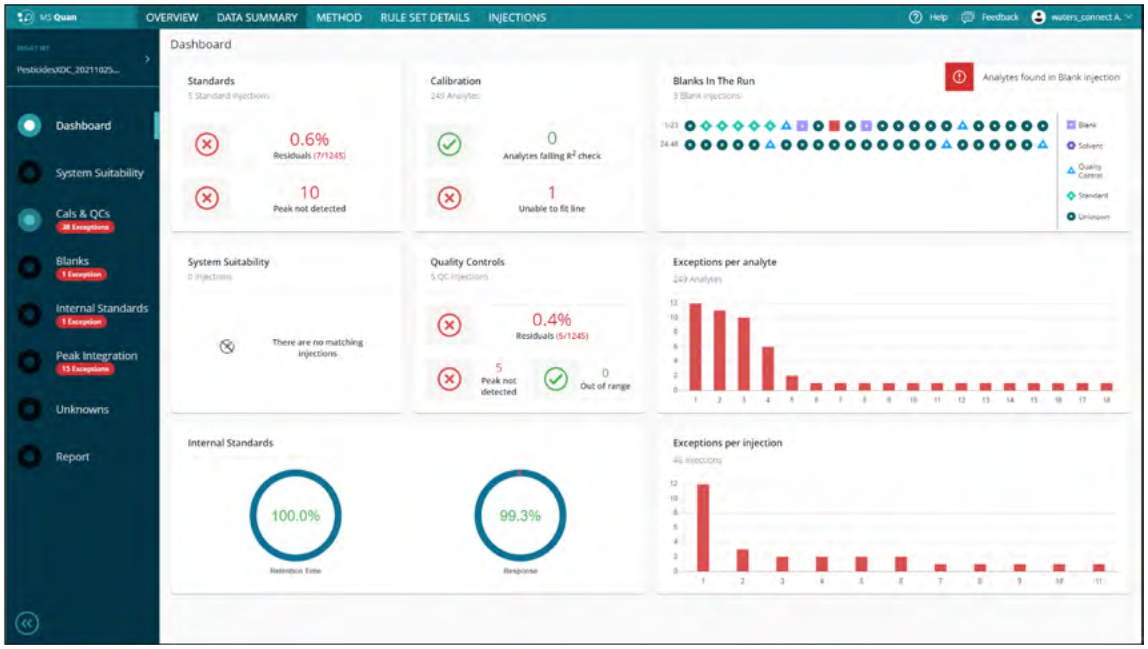
COMPENSATING FOR MATRIX EFFECTS

Matrix effects are known to occur frequently in LC-MS/MS methods and should be assessed during method validation. Ionization efficiency in the source is impacted, resulting in ion suppression or enhancement. These effects are caused by the co-elution of matrix co-extractives with analytes. Significant variations in the magnitude of matrix effects have been observed between different commodities. The use of matrix-matched calibration is recommended to mitigate for matrix effects.



Matrix effects causing ion suppression of a matrix matched calibration curve.

Watch this bite-size webinar on compensating for matrix effects in complex samples to learn more.



The MS Quan Software Dashboard makes it easy to visualize your data.

Read this white paper on the benefits of waters_connect™ MRM Processing Application, MS Quan.

DATA PROCESSING

Whilst quantitation itself is a routine experiment, the analysis of hundreds of pesticides, each with two MRM transitions creates a significant amount of data for review. Along with peak integration, retention time, ion ratios, signal to noise, calibration curve data and quality control standard performance must be assessed. As the scope of analytes continues to increase, the data review can create bottlenecks for routine testing laboratories.

Data processing software with Exception Focused Review (XFR) functionality allows customized rule-sets based on the laboratory's standard operating procedures (SOPs) to be created and enables analysts to:

- Review data more efficiently and consistently
- Quickly identify injections that fall outside the laboratory's analytical quality system
- Visually and quickly identify samples with suspected incurred residues
- Submit the sample batch for approval with confidence

Why Waters?

ENABLING TECHNOLOGIES AND SERVICES FROM WATERS

The use of plant protection products helps to secure global food supplies but can leave pesticide residues in our food chain. With an extensive regulatory framework in place across the globe and varying physicochemical characteristics of pesticide classes, food manufacturers and testing services need flexible, fast and reliable testing solutions. Whether meeting food safety regulations, quality control stipulations or undertaking metabolite discovery, efficiency is paramount to keep pace with demands.

With an extensive portfolio of instruments, services and support we provide quality, knowledge and confidence for optimum productivity in your laboratory. We partner with you to ensure a successful purchase outcome, employing our global team of application experts to assist in instrument setup and user training.

Resources

1. Anastassiades M *et al.* (2003). Fast and easy multi-residue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *J AOAC Int.* 86(2):412-31

2. Lozano A *et al.* (2016). Miniaturisation and optimisation of the Dutch mini-Luke extraction method for implementation in the routine multi-residue analysis of pesticides in fruits and vegetables. *Food Chem.* 192: 668–681

3. Pihlström *et al.* (2007). Analysis of pesticide residues in fruit and vegetables with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric detection. Anal. Bioanal. Chem. 389(6):1773-89

4. SANTE 11312/2021. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.

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