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Trusted Answers

# GC/MS/MS Pesticide Residue Analysis

A reference guide

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# 1. Introduction

This reference guide provides examples of recommended, proven, and robust gas chromatography/tandem mass spectrometry (GC/MS/MS) methods for pesticide multiresidue analysis using Agilent triple quadrupole GC/MS/MS systems. The majority of pesticide laboratories have transitioned from GC/MS to GC/MS/MS techniques due to enhanced sensitivity and selectivity, especially in the presence of coeluting matrix interference. Included in this guide are practical tips and considerations for method development, optimization, modification, and routine use. This document discusses important aspects of GC and MS/MS analysis of pesticides, especially when dealing with matrix-related issues, which are largely affected by the chemical composition of the analyzed sample extract. For this reason, we also cover the basics of sample preparation in pesticide multiresidue analysis with a focus on the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach. Additional topics include the best practices for setting up and configuring triple quadrupole GC/MS system (GC/TQ) for hydrogen carrier gas, dMRM/scan acquisition mode, and advances in software tools, such as the Agilent MassHunter Optimizer for GC/TQ.

Four GC/MS/MS methods are included in Annex IV to Annex VII at the end of this guide. The first two methods (Annex IV and Annex V) are legacy methods used by the authors as a reference for method development and optimization. The second two methods (Annex VI and Annex VII) present the recommended instrument configuration, GC columns, consumables, and method parameters for the best method performance with helium and hydrogen as a carrier gas.

# 2. Pesticide multiresidue analysis

A **pesticide** is defined by the Food and Agriculture Organization (FAO) of the United Nations as *"any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport".*[1]

Based on the biological effect on target pest species, such as algae, birds, bacteria, fungi, plants, insects, mites, snails, nematodes, rodents, or viruses, pesticides can be divided into groups, including algicides, avicides, bactericides, fungicides, herbicides, insecticides, miticides/acaricides, molluscicides, nematicides, rodenticides, or virucides, respectively. Pesticide residues on food commodities, such as insecticides (and acaricides), fungicides, and herbicides are typically of major concern. **Annex I** provides the most important chemical classes of those three major groups, together with examples of representative compounds.

The number of pesticides is continuously increasing as new active substances are being developed and registered. Published in 2021, the 19th edition of The Pesticide Manual,[2] which is a comprehensive source of information about pesticides, includes over 2,000 compounds. Many of them should no longer be used, but they can still be present in a sample due to their persistency in the environment or as a result of illegal use. Pesticide application and their residue levels in foods, feed, and drinking water are strictly regulated, with maximum residue limits (MRLs) or tolerances being set by national regulatory authorities and international bodies, such as the Codex Alimentarius. For regulatory purposes, pesticide residue definitions include the parent compound and any specified derivatives, such as degradation and conversion products, metabolites, and impurities that are considered to be of toxicological significance. Consequently, there are many compounds that should be analyzed to enforce or comply with pesticide MRLs/tolerances, detect illegal residues or unexpected contamination, ensure the safety of foods and feeds, support organic product labeling, provide dietary intake data for toxicological risk assessment, and study the fate of pesticides in food chains and the environment.

For this reason, **multiresidue methods**, enabling simultaneous determination of multiple analytes, typically represent the most time- and cost-effective approach to pesticide residue analysis in routine practice.[3] Ideally, all existing pesticide residues would be analyzed by a single method; however, no current method or technology is capable of that. Even if we disregard a much smaller group of inorganic pesticides that requires a different analytical methodology, the major group of organic pesticides still represents compounds of diverse physicochemical properties, mainly in terms of polarity, solubility, volatility, and stability. This can make inclusion of some pesticides or their metabolites into multiresidue methods difficult or simply impossible. Those difficult analytes, such as highly polar, ionic compounds (for example, quaternary ammonium herbicides diquat or paraquat), must be analyzed by single-residue or single-class methods. Other cases, which typically require special methods for compliance purposes, involve pesticides with complex residue definitions such as those including metabolites with a common moiety or those including salts, esters, and conjugates, thus typically requiring a conversion, or hydrolysis, or both. Examples of the former case include US and EU definitions of the herbicide diuron (diuron and its metabolites convertible to 3,4-dichloroaniline) or the fungicide vinclozolin (vinclozolin and its metabolites containing the 3,5-dichloroaniline moiety). The latter case typically relates to certain acidic pesticides that can be applied as

salts or esters and can be present in the samples in various bio-available forms, including conjugates (for example, herbicides 2,4-D, MCPA, or haloxyfop). In practice, however, laboratories often ignore these difficult residue definitions and monitor only parent compounds or forms that can be included in multiresidue methods such as diuron, vinclozolin, or haloxyfop-methyl, thus making their analysis cost-effective for at least screening purposes if not for full compliance with the set MRL/tolerance.

Multiresidue methods consist of two important parts: sample preparation and determination of residues. Sample preparation usually involves sample homogenization (to obtain a representative sample for the analysis), extraction (isolation of residues from a representative sample), and cleanup (separation of residues from co-extracted matrix components that would interfere in the determinative step). **Chapter 3** provides information about the QuEChERS sample preparation approach that is suitable for the analysis of a wide range of pesticides and has become the method of choice in pesticide residue laboratories worldwide due to its cost, speed, and effectiveness.

For the determinative step, GC has historically been the prevailing technique used in pesticide multiresidue analysis. Traditionally, the GC detection has been conducted using a halogen-selective detector, such as electron capture or electrolytic conductivity detector (ECD or ELCD), in conjunction with phosphorus- or nitrogen-selective detectors like the nitrogen phosphorus detector (NPD) or flame photometric detector (FPD). As a result, pesticides suitable for GC-based multiresidue analysis were divided into organochlorine (OC), organophosphorus (OP), and organonitrogen (ON) based on their elemental composition and response in the different detection systems. GC combined with MS detection was historically used for confirmation of results obtained from the element-selective detectors. Over the last three decades, however, GC/MS instruments (mainly single quadrupole MS, ion traps, and triple quadrupole MS/MS) have become primary determinative tools in most pesticide laboratories, replacing GCs with conventional detectors and enabling simultaneous identification and quantification of a wider range of GC-amenable analytes, independent of their elemental composition.

Many pesticide residues are not directly amenable to GC analysis, and their continuously increasing number reflects a trend in pesticide product development, which can be seen as a transition from the use of persistent and less polar compounds to more readily degradable, more (sometimes very) polar, and less volatile active substances. Determination of these modern pesticides and their metabolites had been rather difficult until liquid chromatography/mass spectrometry (LC/MS) with electrospray ionization (ESI) became available in routine laboratories, enabling direct, selective, and sensitive multiresidue analysis. The implementation of LC/MS has also improved analysis of certain pesticides, such as more polar organophosphorus insecticides (for example, acephate, methamidophos, omethoate, dimethoate, dicrotophos, monocrotophos, malaoxon, and paraoxon). These were traditionally included in GC-based multiresidue methods because there was no other way to easily analyze them in a multiresidue fashion.

**Annex I** indicates which important pesticide classes (and their major representatives) should be analyzed by GC/MS and which by LC/MS. It also shows those which can be analyzed by both techniques equally well (providing that instrumentation of similar selectivity and sensitivity is used in both cases), or with one technique being inferior (listed in parentheses) but still suitable for the analysis if the other technique is not available, or serving as an orthogonal technique for confirmatory purposes.

# 3. Sample preparation using the QuEChERS approach

## 3.1 QuEChERS history and major modifications

The QuEChERS sample preparation approach was first introduced by Anastassiades; et al. at the European Pesticide Residue Workshop (EPRW) in Rome in 2002, then published in the Journal of AOAC Int. in 2003 as the *"quick, easy, cheap, effective, rugged, and safe"* method for the multiresidue analysis of pesticides in fruits and vegetables.[4] The method was tailored for pesticide determination using modern GC/MS and LC/MS instruments, taking advantage of their selectivity, sensitivity, and wide analytical scope, therefore enabling highly streamlined sample preparation with just enough cleanup, small volumes, no concentration steps, and analysis of both GC- and LC-amenable pesticides in basically the same final extract. The QuEChERS sample preparation approach has been adopted widely worldwide in food testing labs and has become one of the most popular methods used for food safety testing, especially for multiclass multiresidue pesticides analysis. The method shows the following features:

- Method targets to clean and remove the major matrix interferences
- Minimal impact on targets to allow a multiclass multiresidue extraction
- Easy adoption to most food matrices without major modifications
- Sample preparation to be compatible with both LC and GC analyses
- Simple, fast, and reliable for highly demanding food analysis sample preparation

A QuEChERS method includes two major parts: sample extraction using acetonitrile, followed by a salt partition to separate the acetonitrile and aqueous layers. The salts used for the partition include two types, the nonbuffered, and the buffered salts. Nonbuffered salts are used in the original method, including 4 g of anhydrous $MgSO_4$ and 1 g of NaCl for extraction of 10 g of sample. There are two types of buffered salts: the acetate buffering salts used in AOAC method 2007.01[11] and the citrate buffering salts used in EN method 15662.[12] The AOAC method uses 1% acetic acid in acetonitrile for the sample extraction, 6 g of anhydrous $MgSO_4$, and 1.5 g of sodium acetate (NaOAc) per 15 g sample extraction. The EN method also uses acetonitrile for sample extraction, but instead uses 4 g of anhydrous $MgSO_4$ + 1 g NaCl + 1 g $Na_3$Cit+ 0.5 g $Na_2$HCit·1.5H2O per 10 g sample extraction. The QuEChERS extraction step removes most of the water, solid residues, proteins, and salts from the sample matrix.

The stability of the analytes is mainly an issue for base-sensitive pesticides, such as N-trihalomethylthio-fungicides (captan, captafol, folpet, dichlofluanid, and tolylfluanid; see **Figure 1 (Page 9)** for their structures and degradation scheme), dicofol, or chlorothalonil, which can degrade at a higher pH, or can be unstable in acetonitrile itself[10]. Losses during the partition step can occur in acidic pesticides (for example, acidic imidazoline and pyridinecarboxylic or phenoxycarboxylic acid herbicides) in neutral/less acidic matrices. In the case of basic pesticides (for example, carbendazim, imazalil, pymetrozine, or thiabendazole), losses can occur at a lower pH (in acidic matrices) when a portion of the analyte molecules may be in ionic form (anion or cation, respectively) and partition into the aqueous layer. To remedy these problems for a wide-scope multiresidue method covering these analytes, it is important to: (i) acidify the final extract (to ≈0.1 % acetic or formic acid content) to improve stability of base-sensitive analytes[10], and (ii) use buffering at pH ≈5 during the extraction process to provide optimum recoveries for most pH-sensitive analytes.

Compared to the non-buffered salts used in original method, the buffered salts used in either the AOAC method or European Standard (EN) method provide a buffering extraction system during the salt partition step, and thus protect certain pH-sensitive pesticides from loss during the sample extraction. Both the acetate and citrate buffering methods improve recoveries of the problematic compounds and provide similar results in side-by-side comparisons[13,14] for the majority of pesticides. The acetate buffer in the AOAC International official method provides somewhat better results for pH-sensitive analytes, especially pymetrozine in acidic matrices.

The QuEChERS extraction procedure starts with 10 or 15 g of fresh sample. Although, for dry samples, 1 to 5 g is used with the addition of water for sample hydration. The appropriate homogeneous fresh sample or dry sample is weighed into a 50 mL centrifuge tube. The internal standard and QC spike are added along with additional water if needed. The sample is then vortexed for 1 to 2 minutes. For dry samples, the vortex mixing time is increased to 10 to 50 minutes after water addition. This is to allow the dry sample to be completely hydrated and at equilibrium. Depending on the extraction method, 10 or 15 mL of extraction solvent is added. For the original and EN methods, 10 mL of ACN is added, but for the AOAC method, 15 mL of ACN w/ 1% acetic acid is added. The sample tube is capped tightly and vortexed for 1 minute. The corresponding QuEChERS extraction salt is added. This should be Bond Elut QuEChERS extraction salt for the original method (p/n 5982-5550 or p/n 5982-5550CH), Bond Elut QuEChERS extraction salt for the EN method (p/n 5982-5650 or 5982-5650CH), or Bond Elution QuEChERS extraction salt for the AOAC method (p/n 5982-5755, or 5982-5755CH).

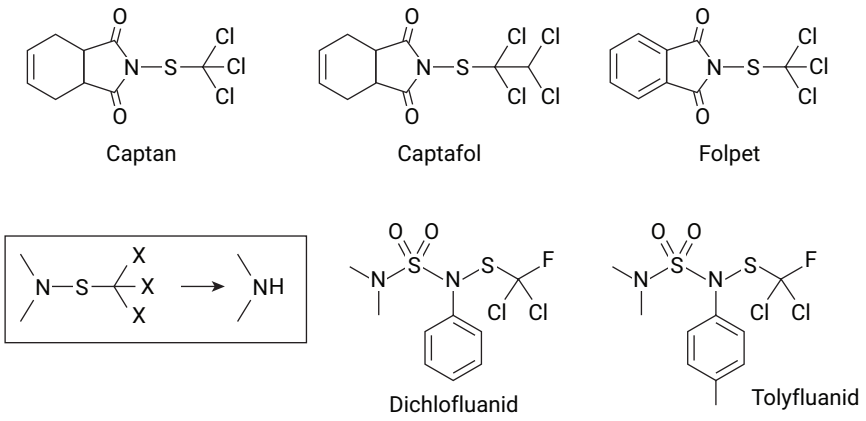



**Figure 1**. Structures of N-trihalomethylthio fungicides and the scheme of formation of their main degradation products. Reprinted with permission from.[10]

It is advisable to add 1 to 2 ceramic homogenizers (p/n 5982-9313) after the salt addition. The use of ceramic homogenizers (CHs) is highly recommended during QuEChERS salting out extractions. It assists in the consistency of sample extraction with salt, breaks up salt agglomerates, facilitates homogenization, and thus increases pesticide extraction from sample matrices. **Figure 2** shows the visual comparison of food samples after vertical shaking (left) and after centrifugation (right). In each step, two sample tubes with CHs (left) versus without CHs (right) are compared side-by-side for their sample homogeneity appearance. The comparison clearly shows that the samples using CHs generated a much more homogenous sample/salt mixture, with significantly fewer salt chunks.

The tubes are capped tightly, and samples are shaken vigorously and vertically on a mechanical shaker, such as Gino Grinder for 5 minutes. The samples are then centrifuged at 4000 to 5000 rpm for 5 minutes. After this, the supernatant sample will be ready for the next step, which is matrix cleanup treatment. The QuEChERS extraction procedure is shown in **Figure 3, step 1**.

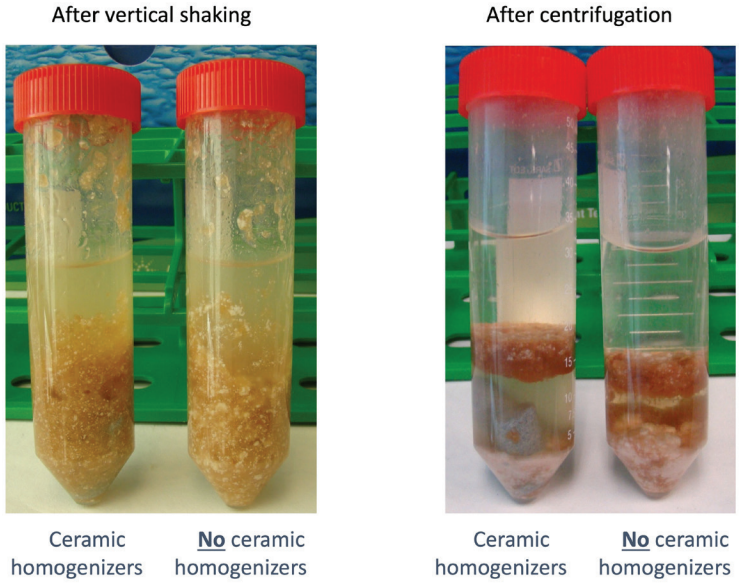



**Figure 2**.A comparison study of the use of ceramic homogenizers for QuEChERS extraction.


**Figure 3**. Typical QuEChERS extraction procedure (step 1) and dSPE matrix cleanup procedure (step 2).

The typical extraction solvent used in QuEChERS extraction is acetonitrile (ACN). It has been demonstrated to provide the acceptable extraction efficiency for a broad panel of pesticides with different physical properties, from relatively polar to nonpolar, and from relatively acidic to neutral and basic properties. Compared to other solvents, ACN also demonstrates a cleaner crude extract with fewer matrix interferences being co-extracted, such as sugars, lipids, and proteins. It is also a relatively low cost and less toxic solvent, which evaporates easily (when needed) and is GC compatible. Other solvents, such as acetone and ethyl acetate (EtOAc), can be used in QuEChERS extraction, but they may present a compromise on some pesticide extraction recoveries and extract more matrix interferences. The acidified ACN is used to assist in the extraction of some labile pesticides.

## 3.2 Cleanup options in the QuEChERS method

As discussed in **Chapter 4**, additional quality or process standards can be added at various steps of the method, such as the addition of triphenyl phosphate (TPP) to the final extract before the determinative step. Some laboratories, such as the pesticide residue laboratories involved in the Pesticide Data Program (PDP) in the U.S., prefer adding ISTDs to the final extract and checking the overall method performance using process control compounds added to the sample matrix before the extraction. An example of such a method is given in **Annex II (Page 85)**, which provides a QuEChERS protocol for the preparation of fruit and vegetable sample extracts and matrix-matched standards for GC/MS(/MS) analysis of pesticides using the acetate buffering procedure.

The QuEChERS method enables extraction of a wide polarity range of pesticides, which also means that a wide range of matrix co-extractives can be present in the extract. Although the use of ACN for extraction can limit the co-extraction of matrix interferences, the crude sample extract is still too complex for direct injection onto an instrument for analysis. The complex sample matrix co-extractives may significantly impact the accuracy, reproducibility, and reliability, as well as the instrument method sensitivity and selectivity, long-term robustness, and needs for routine maintenance. Therefore, usually, the sample crude extract still needs to be further cleaned up after the extraction step. All multiresidue pesticide methods have to balance between the degree of matrix cleanup and the analytical scope or recoveries of certain analytes. The following sample matrix cleanup may or may not result in lower recoveries of certain analytes, depending on their structure, amount of sorbent, format (dispersive versus packed in a cartridge), and matrix type. The balance between matrix cleanup and pesticide recoveries thus becomes a critical point to consider.

The traditional sample matrix cleanup method after QuEChERS extraction is dispersive solid phase extraction (dSPE). Two formats of dSPE tubes are commercially available, the 2 mL dSPE kit for 1 mL sample crude extract cleanup, and the 15 mL dSPE kit for 6 or 8 mL sample crude extract cleanup. Depending on different food matrices and methods, there are many dSPE products available, including various blended sorbents using different ingredients, formulas, and sorbent mass. The typically used sorbents in dSPE kits include $MgSO_4$, PSA, C18, and GCB. Other rarely used sorbents include Fluorosil, alumina, silica, polystyrene, $Na_2SO_4$ and more.

## Anhydrous $MgSO_4$ salt

Anhydrous $MgSO_4$ (150 mg per 1 mL extract) is added to the raw extract for drying purposes to significantly reduce the amount of water in the acetonitrile extract. This could otherwise affect the SPE cleanup and the GC analysis. determination. Additionally, $MgSO_4$ is added to the sample extract after the clean up step as discussed in section 3.3.2.

### 3.2.1 Sorbents used for dSPE cleanup

#### PSA sorbent

PSA is a sorbent with the following primary secondary amine structure:

It is mainly used to remove compounds with carboxylic groups such as fatty acids or other organic acids present in the sample matrix., but It can also remove compounds with carbonyl groups, such as sugars. However, the use of **PSA sorbent may impact the analysis of acidic pesticides, especially in the dSPE step**. Larger amounts of PSA (>50 mg per 1 mL extract) can lead to lower recoveries of certain pesticides with a carbonyl group, such as acephate, chinomethionat, clethodim, hexythiazox, or sethoxydim. This is especially the case if used for matrices with a lower content of acidic matrix co-extractives, which would normally compete with them for the active sites on the PSA sorbent. Similarly, **larger amounts of PSA (and longer contact with it) can cause degradation of base-sensitive analytes due to removal of acids from the extract**. These larger amounts of PSA, such as 150 mg per 1 mL extract, can be justified for cleanup of extracts with a higher amount of fatty acids, for example cereal grains[15].

Alternatively, a cartridge format can be used to further increase the cleanup efficiency. **Prolonged contact of extracts with PSA should be avoided**. After centrifugation, the supernatant aliquot should immediately be placed in a vial and acidified (see **Annex II (p. 85)**).

#### EC-C18 sorbent

Endcapped C18 (EC-C18) sorbent is added to the dSPE step at 50 mg per 1 mL of extract to remove highly lipophilic matrix components, such as sterols or waxes. However, its cleaning efficiency on lipids and fats is limited for fatty food matrices. The hydrophobic interaction-based mechanism is not selective, so it may cause hydrophobic pesticide loss when more C18 sorbent is used. Alternatively, freezing out can be performed to remove (solidify) fats and waxes before dSPE with PSA (it can also help remove some additional co-extractives with limited solubility in acetonitrile, such as sugars). However, **freezing out (typically conducted overnight) adds to the analysis time, and can be less effective than the easier addition of $C_{18}$.**[13]

## Bond Elut EMR-Lipid

Agilent Bond Elut Enhanced Matrix Removal - Lipid (EMR-Lipid) is a novel sorbent material developed by Agilent that can be used in dSPE format. The sorbent interacts with lipid molecules, based on a combination of size exclusion and hydrophobic interactions. It is used in dSPE cleanup after QuEChERS extraction for highly efficient and selective removal of lipids from food matrices. This sorbent and dSPE kit are especially helpful on high-fat food matrices, such as avocado, edible oils, and animal origin food matrices. **Figure 4** shows the GC/MS full scan chromatograms using QuEChERS extraction with dSPE cleanup for avocado. The significantly improved sample matrix cleanliness also improves the reliability of sensitive pesticides, such as captan, permethrin, and deltamethrin, as shown in **Figure 5**.

Bond Elut EMR-Lipid dSPE kit replaces the traditional dSPE cleanup after QuEChERS extraction for fatty food matrices. Using this kit requires pre-activation with water at a ratio of 1:1 water/sample crude extract. This is to allow the EMR-Lipid sorbent full functionality for lipid removal.

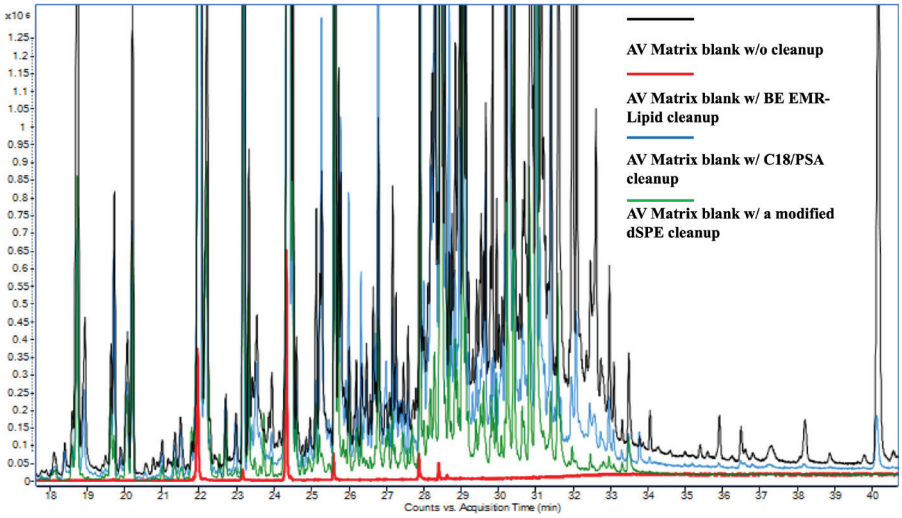



**Figure 4**.An avocado extract GC/MS full scan chromatogram after QuEChERS extraction (black), and traditional dSPE (blue), a modified dSPE (green), and Bond Elut EMR-Lipid dSPE cleanup (red).

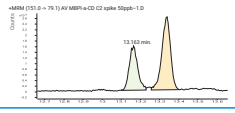
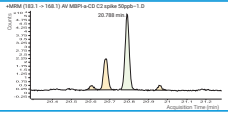
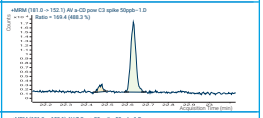
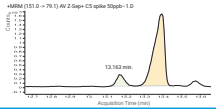
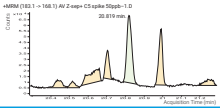
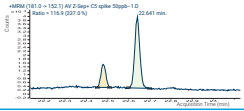
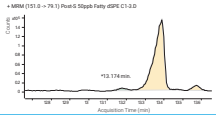
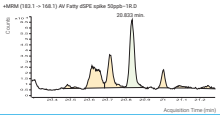
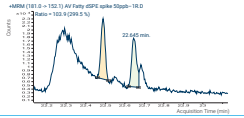
| Pesticide | Captan | Permethrin | Deltamethrin |
|---|---|---|---|
| BE EMR-Lipid cleanup |  |  |  |
| Modified dSPE cleanup |  |  |  |
| C18/PSA cleanup |  |  |  |

**Figure 5**. Chromatogram comparison for sensitive pesticides and the affect of matrix on peak response, peak quality, and interferences in the MRM window. Blank samples were treated with either Bond EMR-Lipid dSPE, a modified dSPE, or traditional C18/PSA dSPE cleanup.

## GCB sorbent

Graphitized carbon black (GCB) adsorbs compounds with a planar structure and can be added to the dSPE step used for removal of certain pigments (carotenoids and chlorophyll), sterols, and other planar matrix co-extractives potentially present in the sample extract. The problem is that certain pesticides, such as hexachlorobenzene, thiabendazole, coumaphos, cyprodinil, chlorothalonil, pentachlorothioanisole (MPCPS), or pentachloroaniline, also have planar structures, and are retained by GCB. Therefore, larger amounts of GCB are not recommended if those analytes are included in the method. A compromise between cleanup efficiency and recoveries of these analytes is necessary in practice, such as the use of only 7.5 mg GCB per 1 mL extract of highly pigmented samples in the European standard method EN 15662[12]. It still leaves some chlorophyll and other pigments in the extract but provides acceptable recoveries (>70%) of planar pesticides. Toluene can recover planar compounds from GCB partially. Toluene is miscible with acetonitrile and can be added at the dSPE step to improve recoveries of planar pesticides when larger amounts of GCB are used, such as 50 mg. Unfortunately, toluene also elutes matrix pigments, so the overall cleanup effect of this procedure is typically diminished.[17]

## Carbon S sorbent

Agilent Carbon S sorbent is an advanced hybrid carbon material with optimized carbon content and pore structure. The improved sorbent provides equivalent or better pigment removal from plant-origin sample matrices than GCB sorbent, but significantly improves sensitive analyte recoveries, including planar pesticides. As a result, Carbon S sorbent delivers a better balance between analyte recovery and matrix pigment removal efficiency than traditional GCB sorbent.

The use of Carbon S sorbent for dSPE kits is just the direct replacement with GCB using an identical or very similar formula. **Figure 6** shows the spinach extract color appearance after QuEChERS extraction using the AOAC pigmented matrix dSPE kit with GCB versus with Carbon S. The final sample extract color and LC/UV chromatograms both confirmed the equivalence of pigment removal for Carbon S and GCB sorbents. However, the planar pesticide recovery is doubled or more, as shown in **Figure 7**.

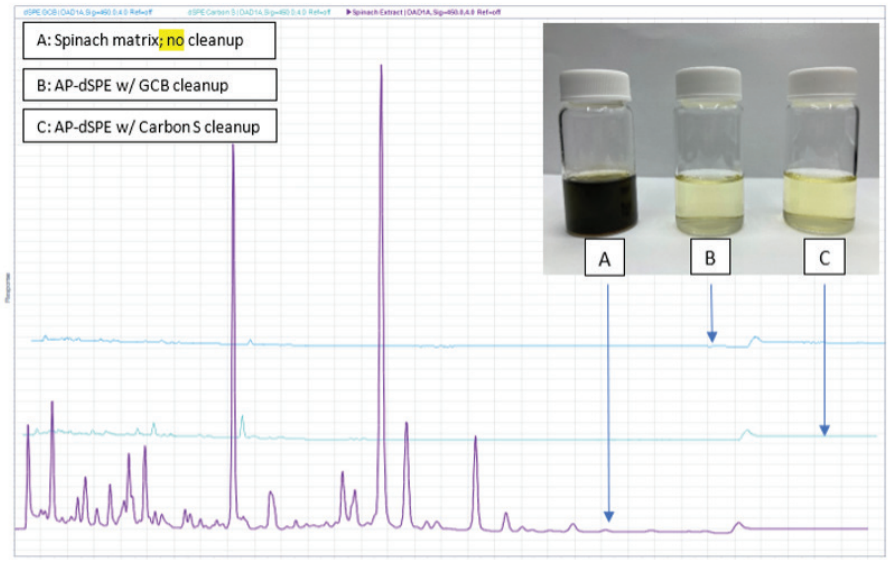



**Figure 6**. Comparison of spinach crude matrix pigment removal using the dSPE kit with Carbon S (C) vs. GCB (B) vs no dSPE cleanup (A). Chromatograms were collected by LC-UV at 450 nm.

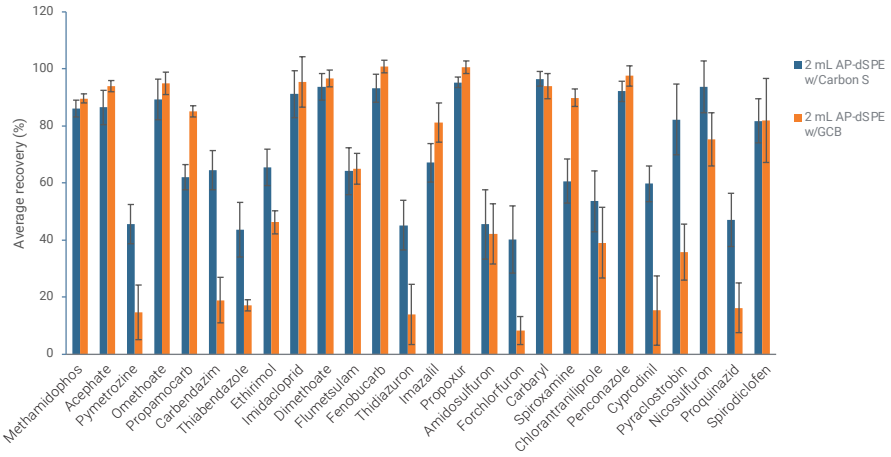



**Figure 7**. Comparison of Agilent Bond Elut AP-dSPE with Carbon S versus AP-dSPE with GCB for 26 representative pesticides analysis in spinach.

### 3.2.2 dSPE cleanup procedure and selection

**Figure 3 step 2** shows the typical procedure of using a dSPE kit for sample cleanup after QuEChERS extraction. Depending on the different kit and format, the appropriate volume of crude sample extract is transferred into a dSPE tube. The tube is capped and vortexed for 3 to 5 minutes, followed by centrifuging for 5 minutes. The supernatant is then ready for analysis.

For the use of the Bond Elut EMR-Lipid dSPE kit (p/n 5982-01010), the procedure is different. The 5 mL of water needs to be added first, then 5 mL of QuEChERS crude extract. After sample vortexing and centrifuging, the supernatant is then transferred to a polishing tube (p/n 5982-0101), containing 2 g of anhydrous $MgSO_4$/NaCl (4:1) for water removal. However, since the original extract contains about 50% water, one step of drying cannot completely remove the water residue,

and a second drying step is necessary. After vortexing and centrifuging polishing tube, a 1 mL aliquot of supernatant is transferred to a 2 mL tube, followed by the addition of about 300 mg anhydrous $MgSO_4$ (from the drying pouch, p/n 5982-0102), vortexing, and centrifugation. The supernatant is then ready for analysis via GC/MS/MS.

The selection of dSPE cleanup is complicated and depends on the sample matrices (such as general fruit and vegetables, pigmented and highly pigmented samples, fatty samples, fatty and pigmented samples), the sample volume to be cleaned (for example, 1 mL versus 6 or 8 mL), and extraction method (AOAC methods versus EN methods and other local methods). For all dSPE kits containing GCB, Agilent also provides the equivalent counterpart with Carbon S instead. To simplify the dSPE kit selection, Agilent has developed the Agilent Bond Elut QuEChERS Universal dSPE kit. This includes kits with Carbon S for the 2 mL (p/n 5610-2058) and 15 mL format (p/n 5610-2060), and kits with GCB for the 2 mL (p/n 5982-0028) and 15 mL format (p/n 5982-0029). Universal dSPE provides a relatively generic dSPE solution for various food matrices, which makes the selection of a dSPE kit simpler. Bond Elut EMR-Lipid dSPE provides an excellent solution for fatty food matrices, providing superior matrix cleanup without significant negative impact on pesticide recovery.

### 3.2.3 Limits of dSPE cleanup

The dSPE cleanup has been recommended for post-QuEChERS extraction since the method was developed to provide 'good enough' sample cleanup before instrument analysis. It is a relatively simplified, fast, and easy procedure that uses less apparatus. The cost is relatively low, without generating additional solvent waste. The methods provide acceptable recoveries for many pesticides, but also result in moderate to significant matrix effects caused by matrix co-extractives. In addition, the use of GCB and PSA could cause the loss of some sensitive pesticides, such as planar and acidic compounds. The use of an endcapping C18 (EC-C18) sorbent does not provide efficient fatty matrix component cleanup.

The poor matrix cleaning efficiency makes the method not suitable for complex sample matrices and causes the significant failure during certain pesticide analyses. Dirty samples also impact the instrument detection reliability and robustness. Although the QuEChERS method can potentially support both LC/MS/MS and GC/MS/MS analysis, it can be difficult to align for complex sample matrices, such as herbal supplement material, spices, tea, and essential oils. More complex and separate sample preparation methods must be used to achieve acceptable testing results.

The large variety of different dSPE kits may cause confusion and complicate selection, making the dSPE cleanup difficult in terms of method alignment. The dSPE cleanup procedure still involves many time-consuming and labor-intensive steps, such as multiple transfers and uncapping and capping dSPE tubes. The sample volume recovery for dSPE cleanup is only around 50%. The impact on low volume recovery makes the transferring supernatant step difficult, as salt can easily get into transfer pipette tips, and limits some post-treatment, such as drying and reconstitution for sample concentration.

## 3.3 EMR cartridge passthrough cleanup

The Captiva EMR passthrough cleanup methodology was first introduced with the Captiva EMR–Lipid products. The method offers high selectivity and efficiency on comprehensive matrix removal, making it a convenient, rapid, and reliable sample matrix cleanup technique. This sample cleanup methodology is especially suitable for multiclass, multiresidue analysis, as the matrix cleaning is based on selective retention of unwanted matrix interferences, and therefore provides minimal impact on target recoveries.

### 3.3.1 Captiva EMR-Lipid passthrough cleanup

The Captiva EMR-Lipid sorbent was the second generation of EMR-Lipid sorbent. It still uses similar chemistry for the size exclusion and hydrophobic interaction combination mechanism. Only lipid-like molecules containing unbranched hydrocarbon chains can enter the EMR-lipid sorbent pores and be retained by hydrophobic interactions. Target analytes that do not have a lipid-like structure are unable to enter the sorbent pores and remain in solution for subsequent analysis. As a result, the EMR-Lipid sorbent can differentiate lipids from other target analytes, and deliver high-analyte recovery and lipid removal efficiency. Captiva EMR-Lipid is an Si-based sorbent where the function groups are bonded on an Si base, which improves sorbent strength and allows the sorbent to be packed in cartridge format. Compared to the Bond Elut EMR-Lipid sorbent, Captiva EMR-Lipid allows the use of less water (down to 20% in sample mixture) while still providing equivalent lipid removal efficiency. The reduced water premixing improves the recovery of more hydrophobic pesticides and other hydrophobic contaminants, such as PAHs. Less water in the sample eluent also makes water removal easier and complete water residue removal is possible with one-step drying.

Pass-through cleanup using Captiva EMR–Lipid products has been used for pesticides analysis in fatty matrices by GC/MS/MS. The procedure in **Figure 8** shows a representative example for olive oil sample preparation. For a highly oily matrix, QuEChERS extraction was not used due to the loss of some pesticides during extraction. Instead, liquid-liquid extraction (LLE) was used with 80:20 ACN/EtOAc. The addition of 20% EtOAc improves solvent strength for pesticide extraction from fatty matrices. To ensure sample mixture homogeneity and prevent phase separation after the water premixing step, using over 20% EtOAc or more hydrophobic solvents is not recommended.

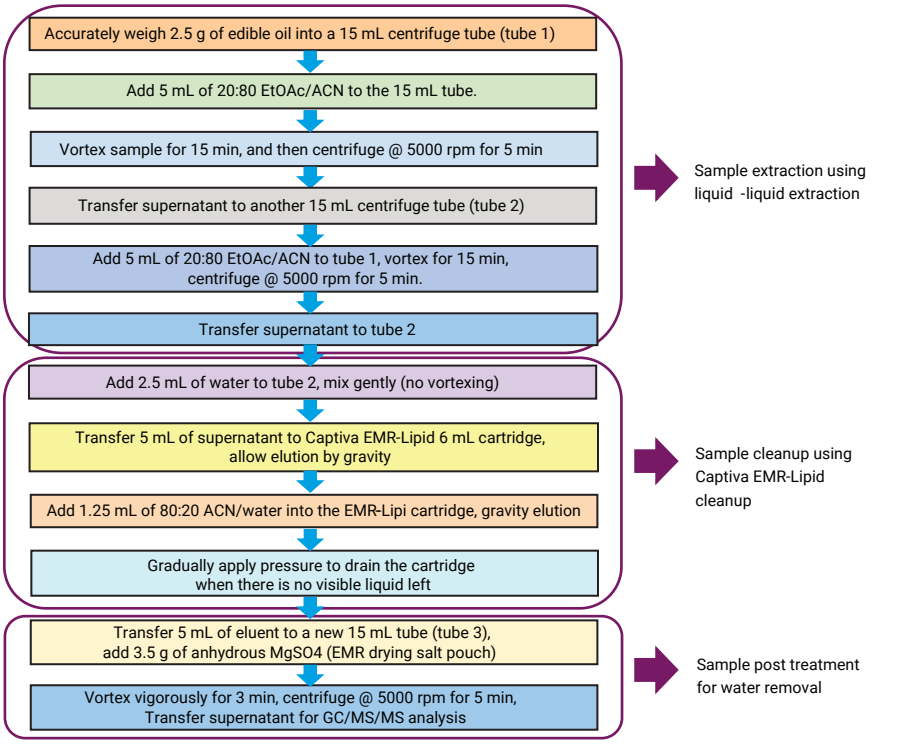



**Figure 8**. Olive oil sample preparation for pesticides analysis using liquid-liquid extraction followed by Captiva EMR-Lipid passthrough cleanup.

### 3.3.2 Captiva EMR with Carbon S passthrough cleanup

Captiva EMR with Carbon S cartridges include a series of mixed-mode EMR cartridges using the optimized blended sorbents like Carbon S, Captiva EMR-Lipid, PSA, and EC-C18 sorbents. These cartridges provide comprehensive cleanup for matrix co-extractives, including organic acids, sugar, lipids and fats, pigments, sterols, and other hydrophobic interferences.

Anhydrous $MgSO_4$ is commonly included in dSPE kits for simultaneous water residue removal during sample cleanup. However, simultaneous water removal can cause significant loss for sensitive pesticides, especially acidic compounds. Considering the exceptional improvement of sensitive pesticide recoveries without $MgSO_4$ during matrix cleanup, the $MgSO_4$ was not included in any EMR cartridges for passthrough cleanup.

Five new Captiva EMR cartridges were developed with optimized formulas for various complicated plantation sample matrices. These are Captiva EMR-HCF 1 (p/n 5610-2088) and 2 (p/n 5610-2089), Captiva EMR-GPF (p/n 5610-2090), Captiva EMR-GPD (p/n 5610-2091), and Captiva EMR-LPD (p/n 5610-2092). **Table 1** shows the detailed description of all Captiva EMR cartridges and their recommendations.

**Table 1.** Agilent Captiva EMR cartridges and their recommendations for different plant-origin matrices.

| Product name | Sorbents | Sample loading volume | Recommendations based on sample matrices | Examples of applicable sample matrix |
|---|---|---|---|---|
| Captiva EMR-Lipid | Carbon EMR-Lipid | 2.5 – 3 mL for 3 mL cartridges; 5-6 mL for 6 mL cartridges | High fatty oily matrices | Edible oils |
| Captiva EMR-HCF1 | Carbon S/NH2 | 3 mL | High chlorophyll fresh leafy vegetables | spinach, parsley, alfalfa |
| Captiva EMR-HCF2 | Carbon S/PSA | 3 mL | High chlorophyll fresh leafy vegetables | spinach, parsley, alfalfa |
| Captiva EMR-GPF | Carbon S/PSA/ EC-C18 | 3 mL | General pigmented fresh plant-origin matrix | berries, peppers, broccoli, grapes |
| Captiva EMR-GPD | Captiva EMR-Lipid /PSA/EC-C18/ Carbon S | 2.5 – 3 mL | General pigmented dry plant-origin matrix | Spices, tea, coffee |
| Captiva EMR-LPD | Captiva EMR-Lipid /PSA/EC-C18/ Carbon S | 2.5 – 3 mL | Low/none pigmented dry plant-origin matrix | Nuts, light pigmented spices, tobacco |

Captiva EMR passthrough cleanup is a simple and easy procedure. The crude sample extract from a previous sample extraction is transferred onto appropriate Captiva EMR cartridges, either through direct transfer or with 10% premixed water. Sample elution usually uses gravity or low-level external forces, such as positive pressure or vacuum. The eluent is collected following a drying step using anhydrous $MgSO_4$ treatment to remove the water residue. The addition of $MgSO_4$ can be as simple as a small spatula of the powder (~200 to 300 mg) from the Agilent Bond Elut QuEChERS EMR–Lipid polish pouch (p/n 5982-0102). The added amount does not have to be exact, and the complete water residue removal can be confirmed by two indicators. First, a “milky” white homogenous sample mixture should be visible during vortexing. Second, the salts should settle down as powder, rather than coagulated chunks, at the bottom. **Figure 9** shows the pictured steps for sample drying after Captiva EMR cleanup, but before GC/MS/MS analysis.

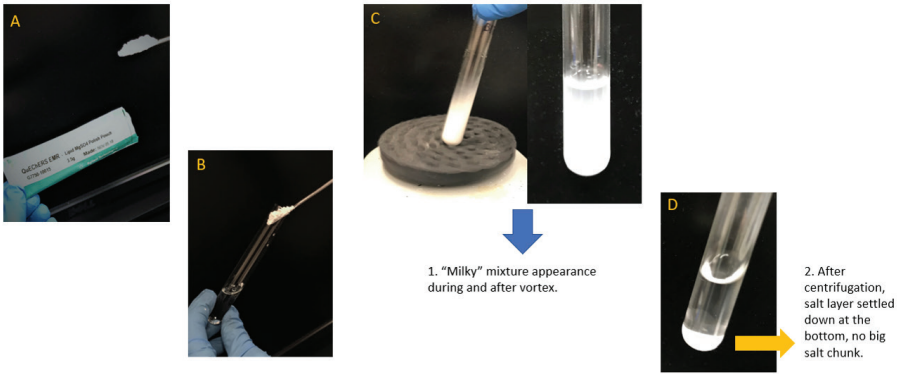



**Figure 9**. Sample drying after Agilent Captiva EMR-GPF cleanup for GC/MS/MS analysis. A) Take out a spatula of $MgSO_4$ anhydrous powder for the Agilent Bond Elut QuEChERS EMR-Lipid polish pouch. B) Add the $MgSO_4$ powder to the collection tube containing the sample eluent after cleanup. C) Vortex the sample for 2 to 3 minutes. D) Centrifuge the sample for 3 minutes.
Note that 1 and 2 are critical indicators of complete water residue removal.

Compared to traditional dSPE cleanup, the passthrough cleanup on EMR cartridges provides simplified workflow steps, such as the elimination of uncapping and capping the tubes, vortexing, centrifuging. The crude sample extract can be loaded onto EMR cartridges for passthrough cleanup. For LC-type detection, the sample eluent can be diluted with water for injection. For GC-type detection, the sample eluent needs a further drying step for water removal. In addition, the passthrough cleanup on EMR cartridges improves the recovery of certain sensitive pesticides. Both EMR-GPD and EMR-LPD cartridges offer decent matrix removal for complex plant origin dry matrices; EMR-GPD cartridges are more suitable for heavy pigmented dry matrices, and EMR-LPD cartridges are more applicable for light pigmented dry matrices. EMR-GPF and EMR-HCF cartridges demonstrate sufficient matrix removal for fresh plant origin matrices. EMR-GPF cartridges are applicable for all fresh matrices except high chlorophyll leafy matrices, and EMR-HCF cartridges provide intensive pigment removal for high chlorophyll leafy matrices.

Captiva EMR passthrough demonstrates significantly higher efficiency for complex matrix removal. **Figure 10** shows the GC/MS full scan chromatograms of cayenne pepper prepared by QuEChERS extraction, followed by Captiva EMR-GPD cleanup, versus two types of dSPE cleanup. The pictures on the right show the dry residue of cayenne pepper extracts. Both the chromatographic background and the final sample extract dry residue comparison demonstrate the superior matrix removal efficiency provided by Captiva EMR-GPD passthrough cleanup.

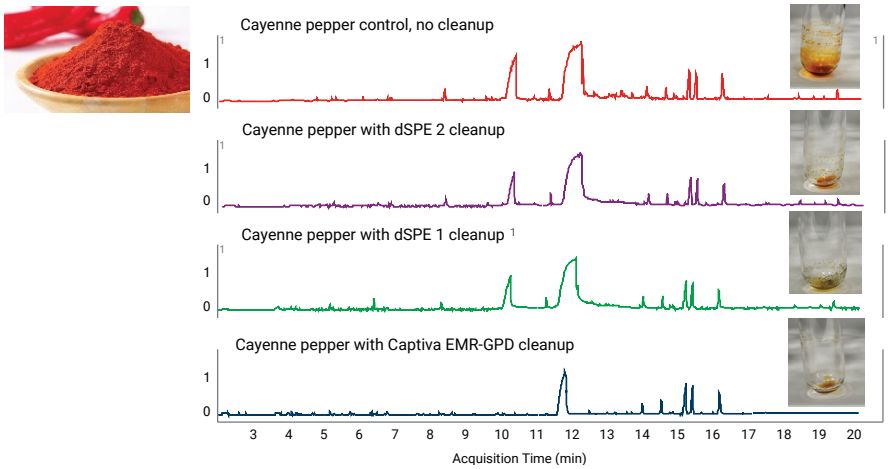



**Figure 10**. Cayenne pepper matrix blank chromatograms in GC/MS full scan data acquisition mode.

The cleaner sample provides a better chromatographic background even with the highly selective target MRM acquisition method. **Figure 11** shows the GC/MS/MS MRM chromatograms for the bell pepper extracts postspiked with pesticide standard at 10 ng/mL. The expanded images in the ovals show the MRM chromatograms for the compound molinate at a 1.5 minute acquisition window. The result confirms that more reliable and consistent target integration can be obtained in cleaner sample extract, prepared using Captiva EMR-GPF cleanup.

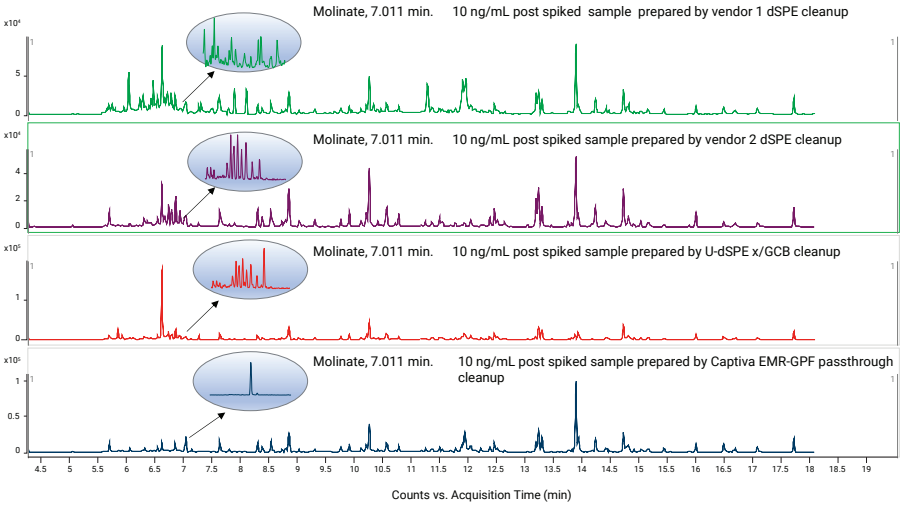



**Figure 11**. GC/MS/MS MRM chromatograms for the bell pepper extracted samples postspiked at 10 ng/mL. The expanded view in the ovals shows the MRM chromatograms for the compound molinate, with a 1.5 minute acquisition window.

The use of Captiva EMR passthrough cleanup also improves sensitive pesticide recovery and reduces matrix effects. **Figure 12** shows four typical GC-amenable sensitive pesticides, chlorothalonil, dichlofluanid, tolyfluanid, and fenhexamid, analyzed in blackberry, using Captiva EMR-GPF cleanup versus a typical dSPE cleanup. The dSPE cleanup not only caused the significant loss of these sensitive pesticides, but also resulted in low responses of targets on GC/MS/MS detection. Because of these improvements, the use of Captiva EMR passthrough cleanup reduced the overall failure rate for large-panel pesticide analysis, by providing higher recovery and better reproducibility. The improved sample preparation method also improves the GC/MS/MS method robustness over multiple sample injections, demonstrated in **Figure 13** for deltamethrin response consistency in cayenne pepper extract (prepared by Captiva EMR-GPD cleanup).

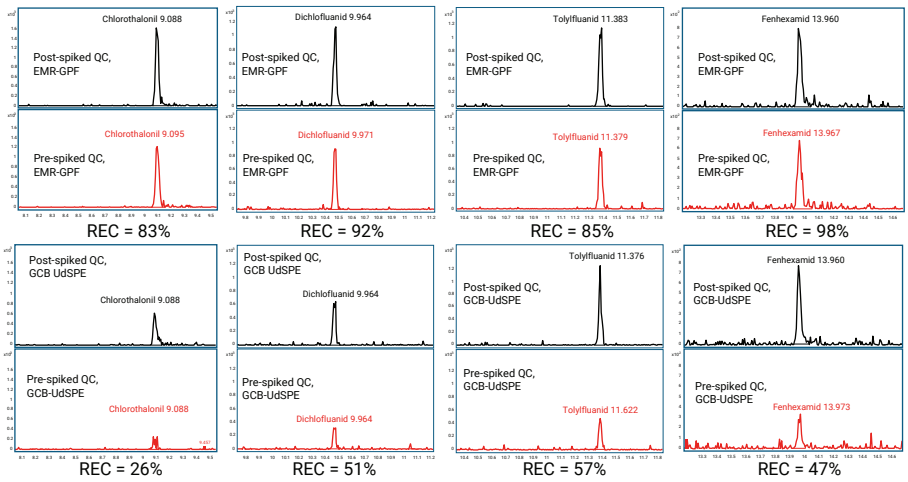
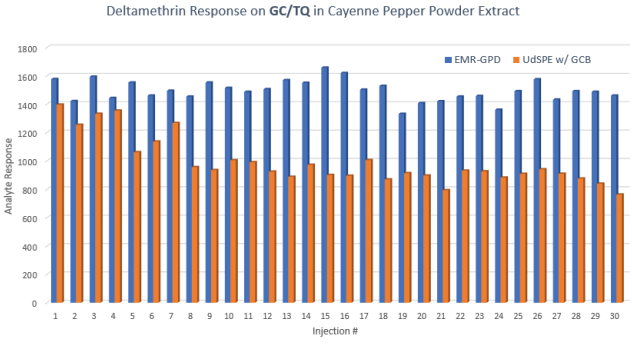



**Figure 12**. Sensitive targets chromatographic comparison for samples prepared using different cleanup methods.




| Ave. RSD% for 28 pesticides | |
|---|---|
| Sample cleanup | Ave. RSD% (n = 30) |
| Captiva EMR-GPD | 7.3 |
| UdSPE w/ GCB | 13.1 |

**Figure 13**. Deltamethrin response on GC/MS/MS for 30 injections of cayenne pepper extract by Captiva EMR-GPD cleanup vs typical dSPE cleanup.

# 4. Use of internal and quality/process control standards

The use of suitable **internal standards (ISTDs)** is a good analytical practice to improve precision and trueness. In the QuEChERS method, ISTDs are typically added to the sample right after the extraction solvent to volumetrically control the entire analytical process. This approach is recommended because **signal normalization to ISTDs can correct volumetric errors and fluctuations during addition of the extraction solvent to the sample, with the injected volume in the determinative step** (due to potential injector variability or bubbles in the syringe), **or from losses due to extract evaporation**. Also, the volume of the acetonitrile layer formed during the partition step could potentially fluctuate due to the different sugar or water content, or variable room temperature. A suitable ISTD should have good stability and recoveries independent of matrix pH, fat content, or cleanup options (for example, the use of GCB). It should not be present as an incurred residue in any samples, should be available as neat material at a reasonable price, and should also represent the analytes well in the given chromatographic system. For example, $d_{10}$-parathion is recommended as an ISTD for GC-amenable pesticides to control the entire analytical process.

Additional compounds can be added as **quality control (QC) standards** together with the ISTDs at the beginning of the QuEChERS procedure as their backup (for example, in cases where matrix interferences occur for the ISTD signal), or to check recoveries of certain problematic groups of analytes. For example, planar polycyclic aromatic hydrocarbons such as $d_{10}$-anthracene or $d_{10}$-phenanthrene, can be used to check for potential losses of planar pesticides when GCB is used in the dSPE cleanup. For samples with a higher lipid content, losses of highly lipophilic pesticides (due to partition between the acetonitrile and fat/oil layer) can be assessed by checking recoveries of PCB congeners 138 or 153.[12] To isolate potential issues in sample preparation from instrument problems, other QC standards can be added just before the determinative step for troubleshooting purposes. For example, TPP is a suitable compound for this purpose, especially if both GC/MS and LC/MS are used for the extract analysis, because it is inexpensive and amenable to both techniques. (Note: TPP can be retained by GCB, and is therefore not suitable for addition before dSPE when this sorbent is used). It is convenient to do a postextraction QC addition, such as adding a TPP solution in acetonitrile containing 1% acetic acid to achieve approximately 0.1% acetic acid in the final extract, for acidification and stabilization.

Some laboratories, such as those involved in the Pesticide Data Program (PDP), use **process control** compounds that are spiked into each sample and are intended to ensure the integrity of individual samples within an analytical system. Based on PDP procedures,[19] each sample, except reagent and matrix blanks, should be spiked with a process control at approximately five times the limit of quantification (LOQ) before the extraction step (prior to the addition of the extraction solvent).

For process control criteria, PDP laboratories can use either the absolute or statistically calculated range criteria:

- **Absolute range criteria**: Each process control recovery should fall between 50 to 150% for all detection systems used to calculate sample data.
- **Statistically calculated range criteria**: Each process control recovery should fall within its acceptance recovery range, which is the process control mean recovery (calculated for a given sample set) plus and minus three standard deviations.

Control charting or other appropriate statistical tools should be used to evaluate recoveries on a set-to-set basis and to monitor trends over time. Chlorpyrifos-methyl and propoxur have been used as process control compounds for PDP sample analysis using GC/MS and LC/MS, respectively.

Some laboratories prefer not to add ISTDs at the beginning of the entire analytical procedure, but instead add them to the final extract before instrumental analysis, therefore controlling only the determinative step and mainly correcting for potential injection volume fluctuations. Specific issues, such as compound losses or signal variability due to degradation in the GC inlet or column, can be addressed when a suitable, compound-specific ISTD is used for signal normalization, as demonstrated in **Figure 14 (Page 25)**. This shows calibration curves for *p,p'*-DDT and *p,p'*-methoxychlor obtained with and without normalization to different ISTDs added postextraction in plum matrix-matched standards.[20] These two pesticides have similar structures (see **Figure 15**, **Page 25**) and are known to degrade in the GC inlet.

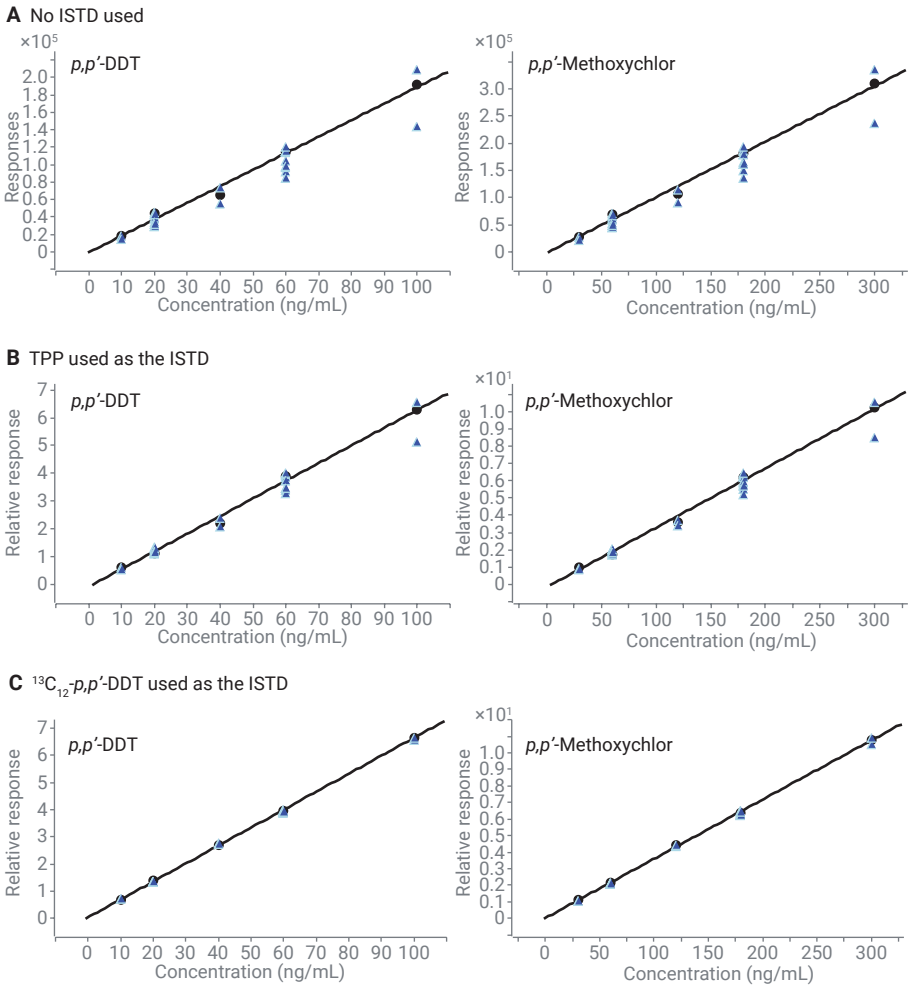



**Figure 14**. Calibration curves and QC results obtained for *p,p′*-DDT and *p,p′*-methoxychlor in plum matrix: (A) without the use of any ISTD, (B) using TPP as a generic ISTD for pesticide residue analysis, and (C) using $^{13}C_{12}$- *p,p′*-DDT as the ISTD for both *p,p′*-DDT and *p,p′*-methoxychlor.

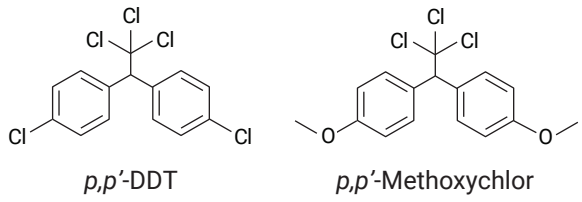



**Figure 15**. The structures of *p,p′*-DDT and *p,p′*-methoxychlor.

**Figure 14** compares calibration curves and QC results obtained for *p,p'*-DDT and *p,p'*-methoxychlor in plum matrix: (A) without the use of any ISTD, (B) using TPP as a generic ISTD for pesticide residue analysis, and (C) using labeled $^{13}C_{12}$-p,p'-DDT as the ISTD for both *p,p'*-DDT and *p,p'*-methoxychlor. The calibration curves (calibration points shown as black dots) were constructed using a matrix-matched standard set injected in the middle of the sequence. The QC results (depicted as blue triangles in the charts) are absolute or relative responses obtained from the QC samples injected throughout the sequence and in the calibration standards analyzed at the beginning and end of the sequence. **Table 2** gives the mean accuracies (relative ratios of calculated versus theoretical/expected concentration) obtained for *p,p'*-DDT and *p,p'*-methoxychlor in the QC samples and all calibration standards (n = 31) using the ISTD options and calibration curves provided in **Figure 14**.

**Table 2.** Comparison of mean accuracies and their RSDs obtained for *p,p'*-DDT and *p,p'*-methoxychlor in the QC samples and calibration standards (n = 31) in plum matrix: A) without the use of any ISTD, B) using TPP as a generic ISTD for pesticide residue analysis, and C) using $^{13}C_{12}$- *p,p'*-DDT as the ISTD for both *p,p'*-DDT and p,p'-methoxychlor.[20]

| | ***p,p'*-DDT** | | ***p,p'*-Methoxychlor** | |
|---|---|---|---|---|
| **ISTD** | Mean accuracy (%) | RSD (%) | Mean accuracy (%) | RSD (%) |
| A) None | 95.5 | 14 | 94.3 | 13 |
| B) TPP | 100 | 7.8 | 98.0 | 6.9 |
| C) 13C12- p,p'-DDT | 100 | 1.5 | 98.3 | 2.0 |

The results in **Figure 14** and **Table 2** show that the use of a generic ISTD, such as TPP, improves calibration curve fits and overall precision compared to not using an ISTD. This is demonstrated in **Table 2** by the almost 50% reduction in the RSD values when TPP was used as the ISTD. An even more dramatic reduction, and therefore an improvement in precision, was obtained when using $^{13}C_{12}$-*p,p'*-DDT as the ISTD for both *p,p'*-DDT and *p,p'*-methoxychlor. The general use of labeled ISTDs in pesticide multiresidue methods is problematic because of their availability and cost. In specific cases, such as *p,p'*-DDT, for which a labeled standard is commercially available and the issues are mainly GC-related, the postextraction addition represents a more cost-effective use of the ISTD than if it were added before the extraction. For example, if the final extract volume is 0.25 mL, and the initial acetonitrile extract volume is 10 mL, then only 1/40 of the ISTD is required when it is added post- versus preextraction. Moreover, compounds with similar properties can share the same ISTD, addressing similar behavior, as demonstrated by the use of $^{13}C_{12}$-*p,p'*-DDT as the ISTD for *p,p'*-methoxychlor.

# 5. GC analysis of pesticides

A conventional approach to GC multiresidue analysis of pesticides uses capillary columns with low-bleed stationary phases, mostly consisting of dimethylpolysiloxane with 5% phenyl (other possible methyl substituents include cyanopropyl, cyanopropylphenyl, or increased phenyl content up to 50%), and typical column dimensions of 0.25 mm inner diameter (id), 0.25 µm stationary film thickness, and 30 m column length. When using GC/MS(/MS), shorter columns, such as 20 m, can be used for the separation of pesticides. This results in shorter GC runs (typically around 10 to 20 minutes) with minimal sacrifices in analyte separation, and maintains similar method ruggedness.[15,20,21]

The shorter run times can be achieved with some sacrifices in column capacity or separation efficiency when using fast GC/MS approaches. Some techniques used to achieve this include employing short, MicroBore columns (<0.2 mm id), fast temperature programming with the Agilent Intuvo GC or other fast GC hardware, or low-pressure GC/MS.[22–25,72] An example of a fast method can be found in the Agilent application note, "A Fast and Robust GC/MS/MS Analysis of 203 Pesticides in 10 minutes in Spinach" (publication number 5994-4967EN).[67]

## 5.1 Matrix effects

In real-world pesticide residue analysis, co-extracted matrix components play an important role, affecting all steps in the GC analysis (injection, separation, and detection). They can result in inaccurate quantitation, decreased method ruggedness, high detection limits, or false positive or negative results. Therefore, appropriate handling of various matrix effects is essential for obtaining reliable, sensitive, and rugged results in routine GC and GC/MS(/MS) analysis. This chapter provides practical tips for dealing with adverse matrix effects in GC, including use of various injection techniques, column backflushing, analyte protectants, and suitable calibration approaches.

In general, matrix effects are adverse phenomena caused by the presence of matrix components in the analyzed sample.[26] In chromatographic separations, the most obvious matrix effects are coelutions of matrix components with analytes that affect analyte detection. Those effects can be overcome by improved selectivity of the detection (for example, using MS/MS, as discussed in **Chapter 6**), chromatographic separation, or sample preparation. There are, however, matrix effects that are more difficult to manage because the culprit cannot easily be eliminated. These effects include mainly matrix-induced response enhancement in GC and signal suppression/enhancement in LC/MS, with atmospheric pressure ionization (API) techniques (the latter is outside of the scope of this document).

**Matrix-induced response enhancement**, first described by Erney; *et al*.,[27] is a matrix effect impacting quantitation accuracy of certain susceptible analytes that is well known in GC analysis of pesticide residues in foods. When a real sample is injected, the matrix components tend to block active sites (mainly free silanol groups) in the GC inlet and column, reducing losses of susceptible analytes caused by adsorption or degradation on these active sites. This phenomenon results in higher analyte signals in matrix-containing versus matrix-free solutions, thus precluding the use of calibration standards in solvent only, which would lead to overestimation of the calculated concentrations in the analyzed samples.

The extent of the matrix-induced enhancement effect is primarily related to the following factors.[28]

- Number and type of active sites in the GC system (mainly in the GC inlet and column)
- Chemical structure (hydrogen-bonding character and thermolability) of the analytes (for example, organophosphorus pesticides containing P=O bonds, such as methamidophos, acephate, omethoate, or dimethoate, are particularly prone to this matrix effect)
- Analyte concentration (more pronounced at lower analyte concentrations)
- Matrix type and concentration
- Interaction time (a function of flow rate, pressure, injection volume, solvent expansion volume, liner diameter and design, column diameter and length, and retention time)
- Injection temperature

In theory, elimination of active sites or matrix components would overcome the matrix-induced enhancement effect; however, **absolute and permanent GC system deactivation or thorough sample cleanup are virtually impossible in practice**. Careful optimization of injection and separation parameters (such as the injection technique, temperature and volume, liner size and design, solvent expansion volume, column flow rate, or column dimensions) can lower the number of active sites (due to a decreased surface area) or shorten the analyte interactions with them. This results in a reduction—but rarely complete elimination—of the effect. For example, application of temperature programming or a pressure pulse during the injection (to reduce residence time or thermal degradation in the injection port) may serve as examples of this effort (see **Section 5.2**).

Since effective elimination of the sources of the matrix-induced response enhancement is not likely in practice, laboratories should compensate for the effect by using alternative calibration methods[29] (see **Section 5.3**). The current compensation approaches include the use of matrix-matched standards, standard addition method, and isotopically labeled internal standards (not generally feasible in multiresidue analysis due to their unavailability or prohibitive price). All of these techniques require extra labor and costs; moreover, they may lead to quantitation inaccuracies because the extent of the effect depends on analyte concentration and matrix composition.[30]

In 2003, the concept of analyte protectants was introduced[28,31] to add suitable compounds to sample extracts as well as matrix-free (solvent) standards to induce an even response enhancement in both instances, resulting in equalization of the matrix-induced response enhancement effect (see **Section 5.4**). In addition to the potential compensation for **matrix-induced response enhancement**, the application of analyte protectants can also significantly reduce another matrix effect called matrix-induced response diminishment.[32,33] This effect is caused by gradual accumulation of less volatile or nonvolatile matrix components in the GC system, resulting in the formation of new active sites and a gradual decrease in analyte responses. The use of analyte protectants provides GC system deactivation in each injection, resulting in improved ruggedness, that is, long-term repeatability of analyte peak intensities, shapes, and retention times.[31] Another way to minimize problems with less- volatile matrix components, improve ruggedness, and greatly reduce the need for frequent GC and MS system maintenance is to use column backflushing (see **Section 5.5**).

## 5.2 GC injection techniques

Injection (sample introduction) usually represents the most crucial part (and often the weakest link) of the GC analysis. In pesticide residue analysis and other trace-level applications, splitless injection techniques are typically used for transfer of analytes from the inlet to the column.

**Hot splitless injection** involves rapid volatilization of the injected sample in the injection port and transfer of the entire sample vapor to the column using the column flow. Despite some noticeable imperfections, it is a popular technique in pesticide residue analysis, mainly because of its relatively easy operation and legacy methods. Disadvantages of this technique include thermal degradation and adsorption of susceptible analytes in the inlet, leading to strong matrix-induced response enhancement, small injection volumes, and potential discrimination of volatile analytes due to the liner overflow. As opposed to **on-column injection**, which is another classic injection technique, hot splitless injection configuration provides some protection of the analytical column against the deposition of nonvolatile matrix components by retaining them in the inlet liner. This provides better ruggedness for routine analysis than on-column injections.[34]

A significant improvement on the hot splitless technique can be achieved using a carrier gas pressure pulse during injection. This modification is called **pulsed splitless injection**. The application of a pressure pulse leads to a higher carrier gas flow rate through the inlet, and thus faster transport of sample vapors onto the GC column. Under these conditions, the residence time of the analytes in the injection port is much shorter compared to the classic hot splitless injection.

It results in a significant decrease of analyte discrimination, adsorption, or degradation in the injection port, and therefore a reduced matrix-induced response enhancement effect[34–37]. In addition, due to the increased pressure (resulting in reduced solvent vapor volumes), larger volumes of sample can be injected without the risk of liner overflow; consequently, lower detection limits can be achieved.

The injection volumes in pulsed hot splitless injections are limited by the liner size (internal volume) and solvent expansion volume at the given injection pressure and temperature. **Table 3** compares maximum injection volumes obtained at different column head pressures for six solvents that have been used in pesticide residue analysis as GC injection solvents: acetonitrile, acetone, ethyl acetate, hexane, toluene, and isooctane.[10]

**Table 3**. Properties of six different GC injection solvents,[10] including maximum safe injection volumes at different column head pressures (injection temperature = 250 °C). The injection volumes in a pulsed hot splitless injection are limited by the liner size (internal volume) and solvent expansion volume at the given injection pressure and temperature.

| Solvent | $M_r$ (g/mol) | ρ (g/mL) | b.p. (°C) | P' | Pv (kPa) | $V_{vapor}$ (µL) | $V_{inj}$ max (µL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 10 psi | 20 psi | 40 psi | 60 psi |
| Acetonitrile | 41 | 0.78 | 82 | 6.2 | 9.6 | 486 | 1.2 | 1.7 | 2.7 | 3.7 |
| Acetone | 58 | 0.79 | 56 | 5.4 | 24.6 | 348 | 1.7 | 2.4 | 3.8 | 5.2 |
| Ethyl acetate | 88 | 0.90 | 77 | 4.3 | 9.7 | 261 | 2.3 | 3.2 | 5.1 | 6.9 |
| Hexane | 86 | 0.66 | 69 | 0.0 | 16.3 | 196 | 3.1 | 4.3 | 6.8 | 9.3 |
| Toluene | 92 | 0.87 | 111 | 2.3 | 2.9 | 242 | 2.5 | 3.5 | 5.5 | 7.5 |
| Isooctane | 114 | 0.69 | 99 | -0.4 | 5.1 | 155 | 3.9 | 5.5 | 8.6 | 11.7 |

Mr — Molecular mass

ρ — Solvent (liquid) density (at 20 °C, $P_{atm}$)

b.p. — Boiling point (at $P_{atm}$)

P' — Polarity index

$P_v$ — Vapor pressure (at 20 °C)

$V_{vapor}$ — Vapor volume generated by 1 µL injection ($V_{inj}$ = 1 µL) of the given solvent at 10 psi (a pressure close to head pressure in typical GC/MS pesticide analysis without a pressure pulse) and injection temperature $T_{inj}$ = 250 °C; calculated using the following equation:

$$\mathbf{V_{vapor} = 22.4 \times 10^3 (\rho/M_r) [(t_{inj} + 273)/273] [P_{atm}/(P_i + P_a)] V_{inj}}$$

**where $P_{atm}$ = 14.7 psi (101 kPa) and $P_a$ is ambient pressure, usually taken as $P_{atm}$**

$V_{inj}$ max — Maximum safe injection volume for the 800 µL liner used at different column head pressures (10, 20, 40, and 60 psi) and an inlet temperature of 250 °C, that is, an injection volume that generates 600 µL of vapors (75% of the liner volume)

A potential drawback of the pulsed hot splitless injection technique involves the potential to force nonvolatile matrix components farther into the column with the increased flow during the pressure pulse. In this respect, **programmable temperature vaporizer (PTV) injection** provides better column protection, and better ruggedness, than both classical and pulsed hot splitless injection[34]. The PTV technique has been shown to significantly reduce immediate and long-term matrix effects by decreasing thermal degradation and enabling effective analyte transfer to the column through rapid temperature and flow programming.[34]

A PTV injection can be performed using the Agilent multimode inlet (MMI). The MMI can work in a manner similar to a standard Agilent split/splitless inlet or it can function with the capabilities of a PTV inlet without hardware modification. It can perform large volume injections for trace analysis, cool injections for improved signal response, and also facilitates pulsed injections.

A PTV injection can be conducted in two basic modes: **cold splitless** and **solvent vent**. In both cases, the sample is injected at a temperature below the boiling point of the injection solvent and retained in a liquid form in the inlet. This prevents thermal shock and immediate volatilization of the entire sample, which can lead to column contamination by less volatile or even nonvolatile matrix components that can be dispersed in the gas phase and carried to the column during a hot splitless injection. **In the solvent vent mode, most of the injection solvent is eliminated through the split vent at a low temperature, enabling the introduction of larger injection volumes,[38] and allowing improved peak shapes of early eluting analytes when injecting as little as 2 µL of acetonitrile**. In both solvent vent and cold splitless, the analytes are transferred to the column by a rapid heating of the inlet to a temperature needed for an effective transfer of the least volatile analyte, while less volatile and nonvolatile matrix components can be retained in the inlet liner. Careful optimization of the PTV conditions, including selection of an appropriate liner, is necessary for successful PTV injection and overall long-term system performance[38,39] (see **Section 7.2**).

## 5.3 Calibration approaches

The use of calibration standards prepared in neat solvents represents the easiest, cheapest, and most straightforward way for external calibration. Unfortunately, because those techniques are prone to the discussed matrix effects, they are not reliable enough to provide accurate quantification in pesticide residue analysis in foods and other complex matrices using GC(/MS) and LC/MS. The European guidelines SANTE/11312/2021 v2 for Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed[29] state that matrix-matched calibration is commonly used to compensate for matrix effects. Extracts of blank matrix, preferably of the same type as the sample, should be used for calibration.

The SANTE guidelines suggest the use of suitable analyte protectants as a practical, alternative approach to minimize matrix effects in GC analysis by adding them to both the sample extracts and the calibration solutions (in pure solvent or in matrix) to produce equivalent matrix effects (see **Section 5.4**) for more details about analyte protectants). Based on the SANTE guidelines and general analytical practice, the most effective approaches to compensate for matrix effects are calibrations by standard addition and by isotope dilutions, with isotopically labeled internal standards being added at any stage of the analytical procedure prior to the determinative step. Those two approaches are also used for enforcement purposes in the U.S. because the U.S. federal regulatory agencies do not allow the use of matrix-matched standards for determination of compliance of detected pesticide residues with the established tolerances in food and feed.

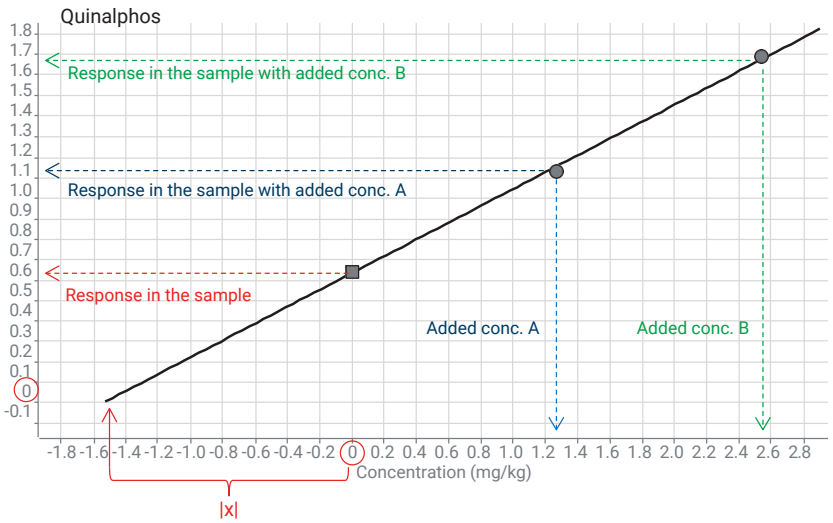
As discussed in **Chapter 4 (Page 23)**, the general use of **isotopically labeled internal standards** is not feasible in pesticide multiresidue analysis because they are not commercially available for all analytes (this approach would basically double the number of compounds to be included in the method and standard solutions), and if they are available, their cost is typically prohibitive for routine analysis. Some, such as $d_{10}$-parathion, can be obtained as neat (solid) materials at a reasonable cost, and can be recommended as generic ISTDs for analyte signal normalization. In certain cases, such as the example of *p,p'*-DDT and *p,p'*-methoxychlor in **Chapter 4**, postextraction addition of a labeled ISTD ($^{13}C_{12}$-*p,p'*-DDT) can be a cost-effective way to counter degradation and other potential GC-related issues, especially if compounds with similar properties can share the same ISTD, addressing similar behavior.[20]

**Matrix-matched standards** are calibration standards prepared in blank (pesticide-free) matrix extracts (as opposed to neat solvents) to achieve the same extent of matrix-induced enhancement as in the sample extracts. **Annex II (Page 85)** provides an example of a procedure for the preparation of matrix-matched standards when using a QuEChERS-based sample preparation method. A matrix-matched calibration procedure is the most widely used compensation calibration approach, despite certain practical problems, including the rather time-consuming and laborious preparation of matrix-matched standards, the need for an appropriate blank material (ideally the same as the analyzed samples), and a greater amount of overall matrix injected onto the GC column during the analytical sequence. One potential problem is that, due to the different compositions and concentrations of various matrix co-extractives, different samples, even of the same commodity, may exhibit different magnitudes of matrix effects. This becomes especially problematic when different commodity types are analyzed in one batch of samples, which is often the case in routine pesticide residue analysis. The SANTE guidelines[29] suggest using a representative matrix calibration, with a single representative matrix or a mixture of matrices, which can calibrate a batch of samples containing different commodities. It is recommended that the relative matrix effects are assessed and the approach is modified accordingly.

**Standard addition** is a procedure in which the test sample is divided into three (or more) test portions. One portion is analyzed in its current state, and known amounts of the analyte standard are added to the other test portions immediately prior to extraction[29]. It is also possible to add known analyte amounts to aliquots of final sample extract prior to the injection to compensate for matrix effects in the determinative step. The standard addition prior to the extraction is more laborious, but recommended in practice because it also inherently takes into account analyte recovery. This can be beneficial, especially in instances of unknown sample types or if lower (but consistent) recoveries are suspected or expected, such as if lipophilic pesticides are quantitated in samples with a higher fat content using the QuEChERS method (see **Annex III (Page 91)**).

Using the standard addition procedure, the analyte concentration in the sample is derived by extrapolation from a linear regression curve (see **Figure 16**).

A linear response over the appropriate concentration range is therefore essential for achieving accurate results. The amount of analyte standard added should be between one and five times the estimated amount of the analyte in the sample; thus knowledge of the approximate residue level is required.[29] This approach is important for minimizing the potential differences in the matrix-induced response enhancement effect obtained at different analyte concentrations.




Linear regression equation: y = ax + b; where: a = slope, b = Y-axis intercept of the regression curve Extrapolated concentration in the sample (y = 0): |x| = -b/a

**Figure 16**. Extrapolation of an unknown analyte concentration in the sample using the standard addition procedure in the MassHunter Quantitative Analysis software. The sample (with zero added concentration) is depicted as a square on the calibration curve. The concentration in the sample is calculated as an absolute value of the Y-axis intercept, divided by the slope of the calibration curve, thus 0.627748/0.411234 = 1.53 mg/kg in this example.

## 5.4 Analyte protectants

Analyte protectants are compounds that strongly interact with the active sites (mainly free silanol groups and active sites created by nonvolatile matrix deposits) in the GC system, decreasing degradation or adsorption of susceptible co-injected analytes.[28,31] They protect analytes against losses in the GC system in a similar fashion to matrix components in the matrix-induced enhancement effect. This approach takes advantage of the response enhancement rather than trying to eliminate it.

The concept is to add suitable analyte protectants to sample extracts, as well as matrix-free (solvent) standards, to induce an even response enhancement in both instances, resulting in effective equalization of the matrix-induced response enhancement effect. In general, the hydrogen bonding capability and volatility (retention time coverage) of analyte protectants were found to be the most important factors in the enhancement and protection effect. In a study evaluating 93 different prospective analyte protectants,[28] dramatic peak enhancements were achieved using compounds containing multiple hydroxy groups, such as sugars and sugar derivatives, with L-gulonic acid γ-lactone (gulonolactone) providing the highest overall enhancements. This can be explained by the formation of several broad peaks of gulonolactone and its degradation products covering a wide pesticide elution range. For this reason, gulonolactone can serve as an effective single compound additive to improve responses (peak shapes and intensities) of analytes—mainly those eluting in the middle region of the chromatogram. However, to effectively compensate for matrix-induced response enhancement in GC multiresidue pesticide analysis, the chosen analyte protectants should induce strong response enhancement throughout the entire range of analytes. Therefore, a suitable combination of several analyte protectants, covering the volatility range of GC-amenable pesticides, is typically needed.

A mixture of 3-ethoxy 1,2-propanediol, gulonolactone, and sorbitol was found to be the most effective for the volatility range of GC-amenable pesticides.[31] **Figure 17** compares peak shapes and intensities of three selected pesticides, obtained (using a 1 µL hot splitless injection) in solvent standards and matrix extracts without and with the addition of the above mixture of analyte protectants.

**Figure 17** clearly demonstrates the beneficial effect of the analyte protectant addition, resulting in similar analyte responses in solvent and matrix solutions and reduced analyte tailing. The three pesticides were selected to represent different analyte susceptibility to matrix-induced response enhancement: lindane (usually not susceptible), phosalone (moderately susceptible), and o-phenylphenol (very susceptible).

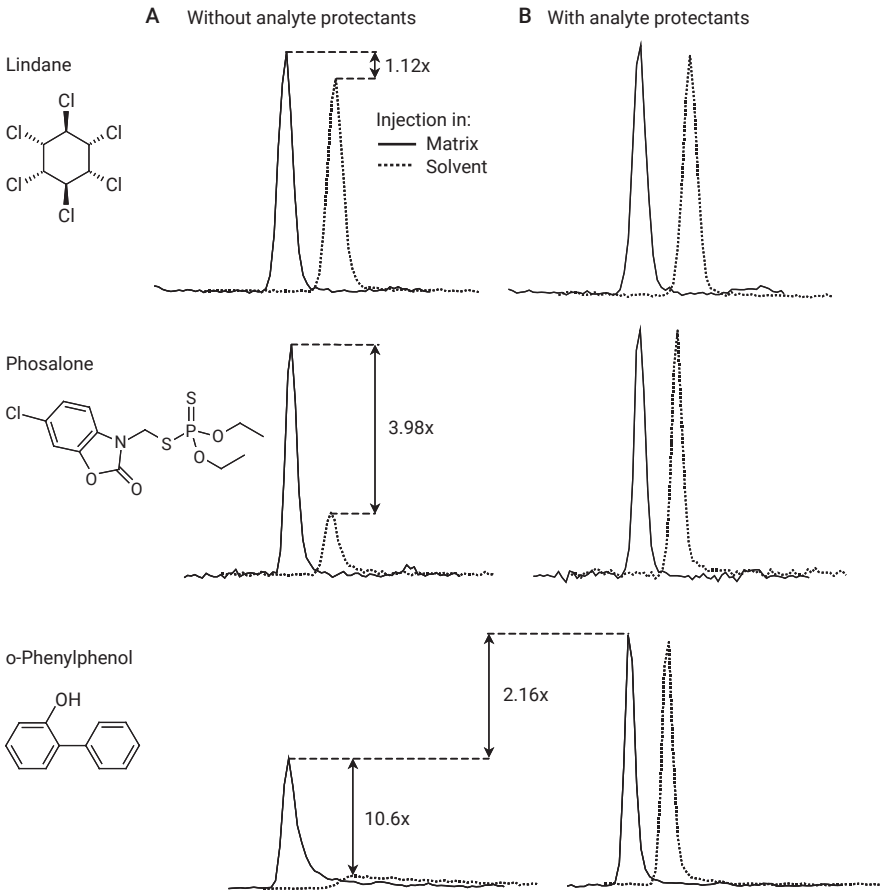



**Figure 17**. Comparison of peak shapes and intensities of three selected pesticides (with different susceptibility to the matrix-induced enhancement effect) obtained by injection in matrix (fruit extract) and solvent (acetonitrile) solutions (A) without and (B) with the addition of analyte protectants (a mixture of ethylglycerol, gulonolactone, and sorbitol). The numbers demonstrate signal (peak height) enhancement factors (signal in matrix versus solvent) obtained without the use of analyte protectants and improvement in o-phenylphenol signal intensity in matrix with the use of analyte protectants. Reprinted with permission.[31]

In addition to the compensation for matrix-induced response enhancement, the application of analyte protectants can also significantly reduce the matrix-induced response diminishment effect, which is caused by gradual accumulation of less volatile and nonvolatile matrix components in the GC system. This results in the formation of new active sites and a gradual decrease in analyte responses. **The use of analyte protectants provides GC system deactivation in each injection**. This results in improved ruggedness and a less frequent need for GC system maintenance, as demonstrated in **Figure 18**, which shows the overlaying peaks of the three above-mentioned pesticides obtained throughout a long injection sequence of mixed fruit and vegetable QuEChERS extracts.[31] Without the addition of analyte protectants, the signals for phosalone and (especially) o-phenylphenol significantly deteriorated with an increasing number of injected samples. Whereas the side-by-side injections of the same pesticide solution containing analyte protectants resulted in superior long-term signal repeatabilities, as documented by the RSDs obtained for peak areas, heights, height-to-area ratios, and retention times.

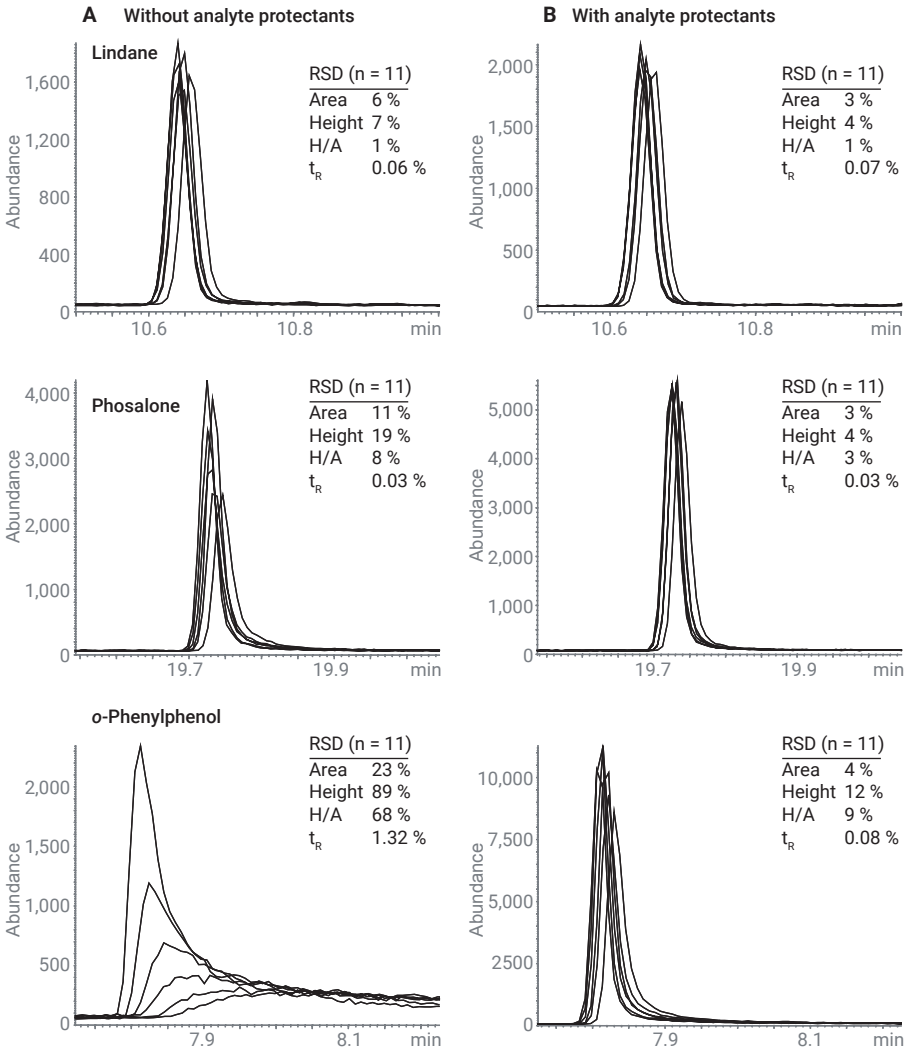



**Figure 18**. Overlay of lindane, phosalone, and o-phenylphenol GC/SIM-MS chromatograms obtained (A) without and (B) with the addition of analyte protectants (a mixture of ethylglycerol, gulonolactone, and sorbitol) at the beginning and throughout a long sample sequence (after 30, 60, 90, 120, and 150 GC injections). RSDs of peak areas, heights, height-to-area ratios (H/A), and retention times ($t_R$) are provided for all 500 ng/mL acetonitrile standards with analyte protectants and test solutions (without analyte protectants) injected immediately before them throughout the sequence (n = 11). Reprinted with permission.[31]

**Figure 19** compares calibration curves of the three selected pesticides obtained in acetonitrile and mixed fruit and vegetable extracts without and with the ethylglycerol, gulonolactone, and sorbitol mixture in the same study[31]. Without the analyte protectants, the injections of the susceptible pesticides in acetonitrile resulted in nonlinear calibration curves, with lower slopes and intercepts, compared to the situation in the matrix extracts. This is a typical manifestation of the matrix-induced response enhancement effect, which would lead to significantly overestimated results in the analyzed samples if solvent standards were used for calibration. **In this case, the addition of analyte protectants nearly eliminated the differences between calibrations obtained in matrix versus matrix-free solutions (solvent standards). In practice, however, this may not always be the case, especially if more complex and concentrated matrix extracts that can induce stronger response enhancement than the added analyte protectants are analyzed.**

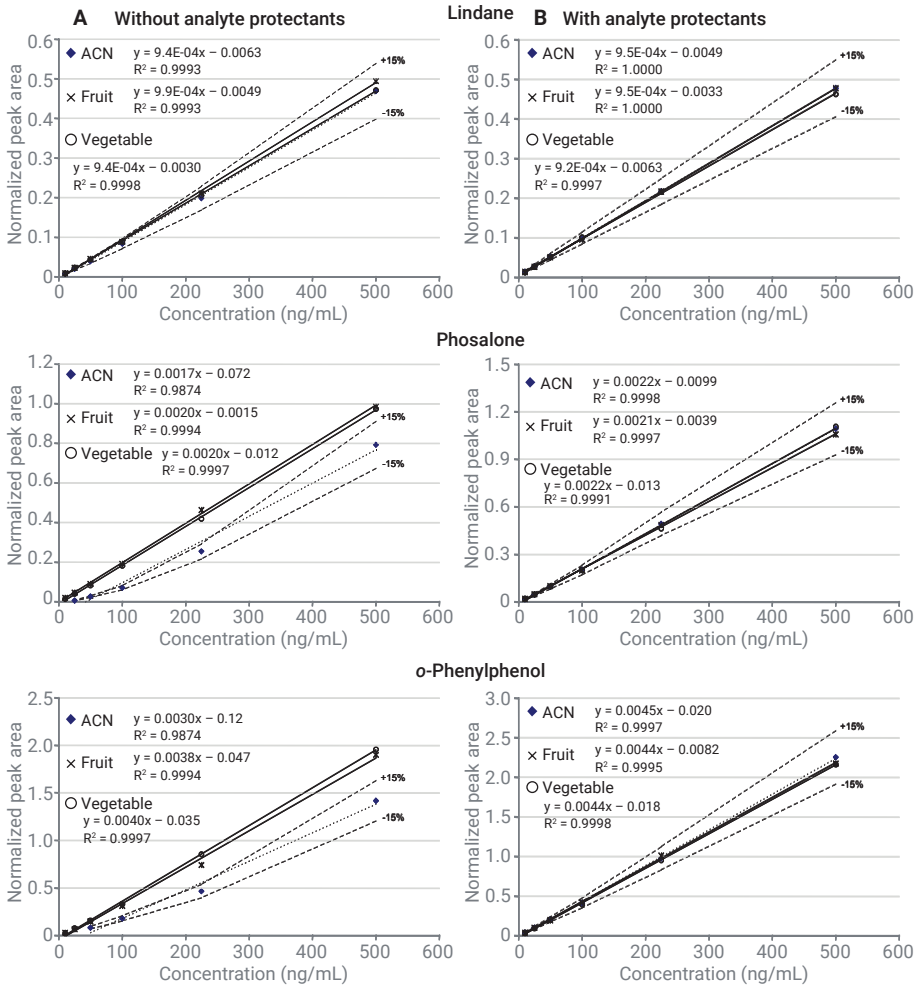



**Figure 19**. Comparison of calibration curves (based on peak areas normalized to ISTD heptachlor) of lindane, phosalone, and o-phenylphenol, obtained by injection of acetonitrile (ACN) standards and spiked fruit and vegetable extracts (A) without and (B) with the addition of analyte protectants (a mixture of ethylglycerol, gulonolactone, and sorbitol). Dashed lines denote ±15% peak area tolerance for values obtained in MeCN standards. Reprinted with permission from K. Mastovska; *et al.* Anal. Chem. 2005, 77, 8129-8137.[31]

Even if the analyte protectants may not fully compensate for matrix effects, their addition is generally beneficial because they provide system deactivation and increase analyte responses, especially in weaker matrices. As suggested in the SANTE guidelines[29], analyte protectants can be added to matrix-matched standards, helping improve method ruggedness.[20] This is done in the QuEChERS example protocol in **Annex II (Page 85)**, which uses a mixture of gulonolactone and sorbitol. Ethylglycerol was omitted from this mixture because this analyte protectant is effective only for more volatile and relatively polar analytes, such as methamidophos, acephate, or omethoate. These analytes generally do not perform well in GC(/MS) and should be moved to LC/MS, as discussed in **Chapter 2** and indicated in **Annex III**. Moreover, a relatively large amount of ethylglycerol must be used to be effective, which can interfere with the focusing of early eluting analytes.

Some studies show that oils, such as **olive or corn** oil, can also serve as promising analyte protectants[40]. The protection mechanism of oils most likely involves only the physical masking of those sites, as opposed to gulonolactone and sorbitol, which strongly interact with the active sites. The potential problem with using oils and other natural products/extracts as analyte protectants is the risk of GC system contamination. They need to be checked for pesticide residues and composition, therefore, their effectiveness may vary from lot-to-lot. In comparison, neat chemicals, such as gulonolactone and sorbitol, are inexpensive and nontoxic, and have been proven to be safe for routine use in GC/MS systems.

## 5.5 Column backflushing

Column backflushing is a technique that can eliminate less volatile matrix components from the GC column. It does this by reversing the column flow at a pressure junction point, which is also called a pressure-controlled tee (PCT) configuration.[41–43] To facilitate this configuration, Agilent Capillary Flow Technology (CFT) devices such as the Purged Ultimate Union (PUU) provide a deactivated junction with a low thermal mass and a small dead volume.

Without the use of column backflushing, less volatile, late-eluting matrix components must be baked out at a high temperature after analyte elution. This common GC practice increases the analysis time, reduces column lifetime, and leads to contamination of the MS ion source. If the column flow is reversed before the late-eluting compounds start to move or get too far down the column, it will take less time and a lower oven temperature to remove them from the system through a split vent in the inlet. Also, they will not reach the MS source, greatly reducing its maintenance needs.[20, 21]

The column flow can be reversed after (postrun) or during the analytical run (concurrently).[44] **Postrun backflushing** begins after the last analyte has been detected.[45–47] One example is a postcolumn backflush configuration, which uses short restriction capillary tubing installed after the analytical column between the purged CFT device and the MS. This also enables easy column maintenance or replacement without venting the MS system. In this mode, backflushing can start only after the last analyte of interest elutes from the entire column and reaches the MS. This increases the risk of less volatile matrix components getting further

into the column and potentially contaminating the MS source during subsequent runs. To ensure effective elimination of less volatile matrix components from the entire column, the backflushing time must be optimized carefully (see **Chapter 7.3 (Page 61)**). Another example of a commonly used type of postrun backflushing is a midcolumn backflushing configuration. In this configuration, a CFT device is installed between two columns. During backflushing, the make up flow from the Pneumatic Switching Device (PSD) is raised to a much higher value, sweeping high boilers backward out of the first column while simultaneously providing forward flow in the second column.

**Concurrent backflushing** is a more time-effective approach than postrun backflushing. It uses a purged CFT device installed between two columns with the reverse column flow starting after the last analyte elutes from the first column to the second column. Different physical column configurations are possible, including a midcolumn backflush configuration, using, for example, two 15 m columns[48,49] or a precolumn backflush configuration using a short coated[20, 21] or uncoated capillary (a retention gap)[50,51] as the first column. The uncoated capillary, however, does not provide effective retention of less volatile matrix components.[25,33]

The GC/MS/MS methods provided in **Annexes IV (Page 93)** and **V (Page 96)** use concurrent backflushing with a 5 m first column and a 15 m second column of the same column diameter, stationary phase type, and film thickness.[20,21] In this setup, the backflushing starts as soon as the last analyte elutes safely from the short column, preventing the less volatile matrix components from reaching the longer analytical column and also reducing the analysis time.

As opposed to postrun backflushing, concurrent backflushing is somewhat more difficult to optimize (see **Chapter 7.3 (Page 61**)), but, provides even more time-effective elimination of less volatile compounds and protection of the MS source and second column against contamination. As a result, the need for MS source maintenance is greatly reduced and its cleaning should typically be performed only as part of preventive maintenance (approximately every six months) if fruit and vegetable extracts are analyzed on a routine basis.[20]

## 5.6 Using hydrogen as a carrier gas

Due to recurring helium shortages and increased prices experienced in the recent years, there is an intensified demand for adapting the GC/MS analysis to hydrogen carrier gas. While helium is the optimal carrier gas for GC/MS, hydrogen has emerged as a viable alternative. Hydrogen brings chromatographic benefits to the analysis if proper measures are taken to translate the method. Additionally, hydrogen emerges as a renewable and costeffective alternative for sustainable laboratory practices. However, unlike helium, hydrogen is not chemically inert. This lack of inertness raises concerns as hydrogen can potentially react with target analytes, matrix components, or solvents. Such reactions can lead to compound degradation, chromatographic issues like peak tailing, distorted ion ratios in the mass spectrum, compromised library matching, and decreased sensitivity. Therefore, the transition from helium to hydrogen carrier gas requires due diligence.[69]

The **EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion Guide**[70] provides detailed instructions for method conversion from helium to hydrogen. The user guide outlines the considerations and procedures for hydrogen safety necessary to make the transition to hydrogen carrier gas successful.

Some important considerations when using hydrogen as a carrier gas include the following:

- **Analyte response sensitivity will generally be reduced** when using hydrogen carrier gas. A two- to ten-fold decrease in sensitivity is expected. Reduced sensitivity can be due to a combination of a decreased signal and increased noise. This decrease in sensitivity is anticipated even for compounds that do not interact with hydrogen in the GC inlet or the EI source.
- **Hydrogen is not inert.** Hydrogen can react with compounds susceptible to hydrogen reduction in the GC inlet and in the EI source. If an EI source that does not reduce source-induced reactivity is used, chemical transformations with undesired and uncontrolled reactions will take place, leading to spectral changes. This will have a negative impact on quantitation accuracy and precision, as well as calibration linearity. In the method referenced in **Annex VII**, 15 of the 203 pesticides analyzed demonstrated that they could be susceptible to hydrogenation if the EI source is not optimized for hydrogen carrier gas. These undesirable reactions can be prevented or at least significantly reduced using techniques outlined in the Agilent application note: "Hydrogen Carrier Gas for Analyzing Pesticides in Pigmented Foods with GC/MS/MS" (5994-6505EN).[69] Due to the potential for reactivity with hydrogen, there is a need to **carefully evaluate every analyte** for spectral changes after converting from helium to hydrogen.
- **Using an EI source that reduces source reactivity** is recommended. Agilent **HydroInert** EI technology is specifically designed for this purpose and reduces or eliminates in-source reactions while using hydrogen as a carrier gas.
- Due to decreased carrier gas viscosity when using hydrogen (compared to helium), **it is often necessary to reduce capillary column dimensions** to facilitate analysis with hydrogen carrier gas. For example, it is common for a method that previously used a 30 m, 0.25 mm id, 0.25 µm column with helium carrier gas to be converted to a 20 m, 0.18 mm id, 0.18 µm column when using hydrogen carrier. A conventional midcolumn backflushing configuration comprised of two 15 m, 0.25 x 0.25 mm columns can be converted to two 20 m, 0.18 x 0.18 mm columns when using a hydrogen carrier gas that allows for the same GC column phase ratio and achieves the same retention times with hydrogen and helium.

# 6. MS/MS detection considerations

The use of tandem MS (MS/MS) generally improves method selectivity, providing that suitable precursor-to-product ion MS/MS transitions are selected for the analyte detection.[52] For analyte identification, at least two MS/MS transitions are required in pesticide residue and other contaminant analysis.[29,53] More than two MS/MS transitions may be needed as a backup for compounds with less selective transitions that are prone to potential matrix interferences, such as dieldrin, endrin, or endosulfans. Also, if needed, additional transitions may provide increased confidence in positive analyte identification, or they may serve for confirmatory purposes.

**Table 4** gives tolerances for relative ion intensities (ion ratios for less versus more abundant MS/MS transitions), which are, according to the European Commission (EC) decision 2002/657/EC,[53] permitted in the analysis of certain substances and residues in animal-derived matrices. By contrast, the updated European Commission (EC) decision 2021/808/EC[71] contains simplified criteria, giving a tolerance of +/-40% relative deviation for relative ion intensities. The SANTE guidelines[29] recommend that ion ratios be within +/-30% (relative) of the average ion ratios obtained in calibration standards in the same sequence. but they also suggest conducting actual, experimental measurements of the ion ratios (during the method validation or over time) to obtain performance-based criteria for individual analytes, rather than applying the generic criterion.

**Table 4.** Maximum permitted tolerances for relative ion intensities (less versus more abundant MS/MS transition) in GC/MS/MS and LC/MS/MS analysis of certain substances and residues in animal-derived matrices, according to the European Commission (EC) decision 2002/657/EC.[53]

| Ion relative intensity | Relative tolerance |
|---|---|
| >50 % | ±20 % |
| >20–50 % | ±25 % |
| >10–20 % | ±30 % |
| ≤10 % | ±50 % |

**Chapter 7.1.3** provides a detailed description of the MS/MS optimization, and highlights the importance of testing the most promising MS/MS transitions in various matrix extracts because selectivity is often more than (or as important as) sensitivity in the trace analysis of complex matrices. As opposed to LC-API-MS/MS, which typically has only the pseudo-molecular ion as an option for MS/MS precursor selection, electron ionization (EI) spectra usually offer multiple possibilities. In general, a higher-mass precursor ion should provide better selectivity in EI-MS/MS (see procymidone example in **Figure 20**) because the number of compounds (thus potential interferences) decreases exponentially with increasing *m/z* present in the EI full scan MS spectra,[52] as demonstrated in **Figure 21**. However, certain MS/MS transitions may not be as selective as others, especially when it comes to losses of commonly occurring structures and groups. This can be seen in the loss of methyl (*m/z* 15) in the atrazine example in **Figure 22** that compares the selectivity of MS/MS transitions *m/z* 215 & 200 and 215 & 58.

The coeluting or closely eluting matrix components may produce the same MS/MS transition that are shown in **Figure 23**. This demonstrates that a higher-mass precursor ion (*m/z* 173 versus 158 in the case of malathion GC/MS/MS analysis in dietary supplements) may not guarantee better selectivity because the selectivity is given by the actual transition (*m/z* 173 & 117 compared to m/z 158 & 47), and not just the precursor ion itself.

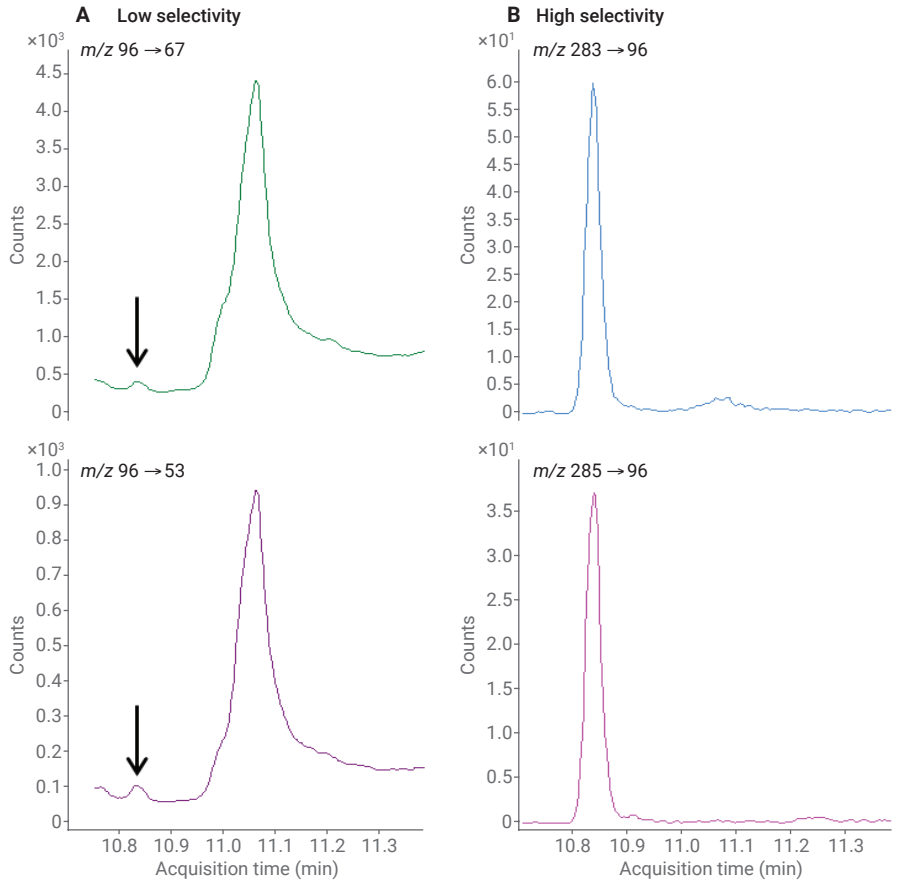



**Figure 20**. Analysis of procymidone in spinach (at 5 ng/g) using MS/MS transitions with (A) low and (B) high selectivity.

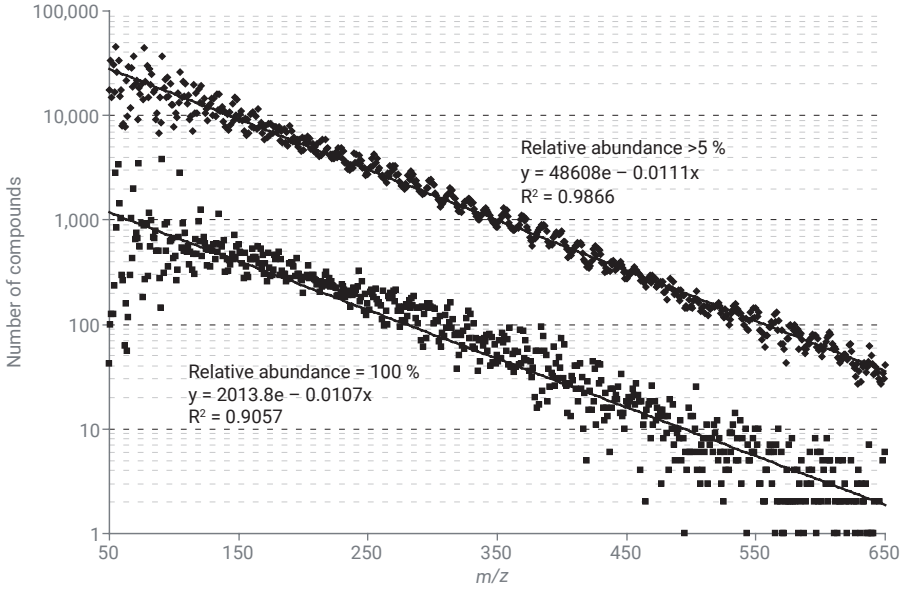



**Figure 21**. Number of spectra in the NIST'98 mass spectral library, plotted versus the m/z of the base peak (relative abundance = 100%) and the m/z of peaks with a relative abundance of >5%. Reprinted with permission.[52]

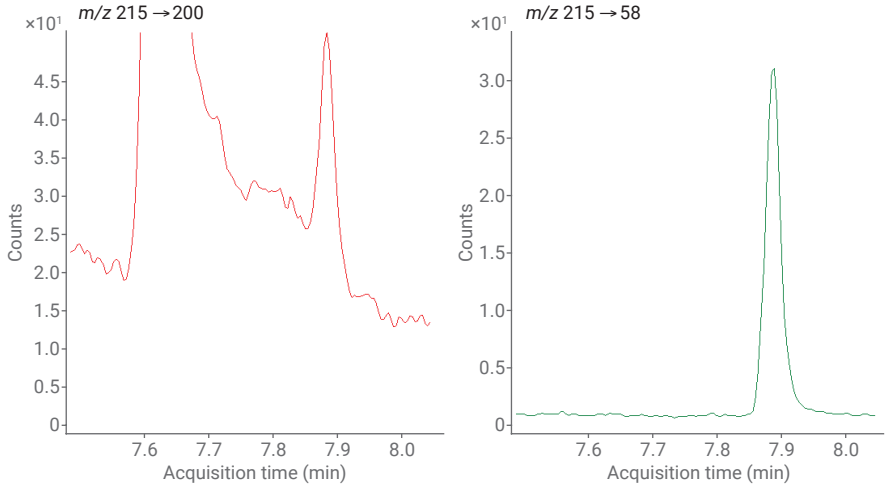



**Figure 22**. Analysis of atrazine (RT = 7.9 minutes) in flour (at 5 ng/g) using MS/MS transitions.

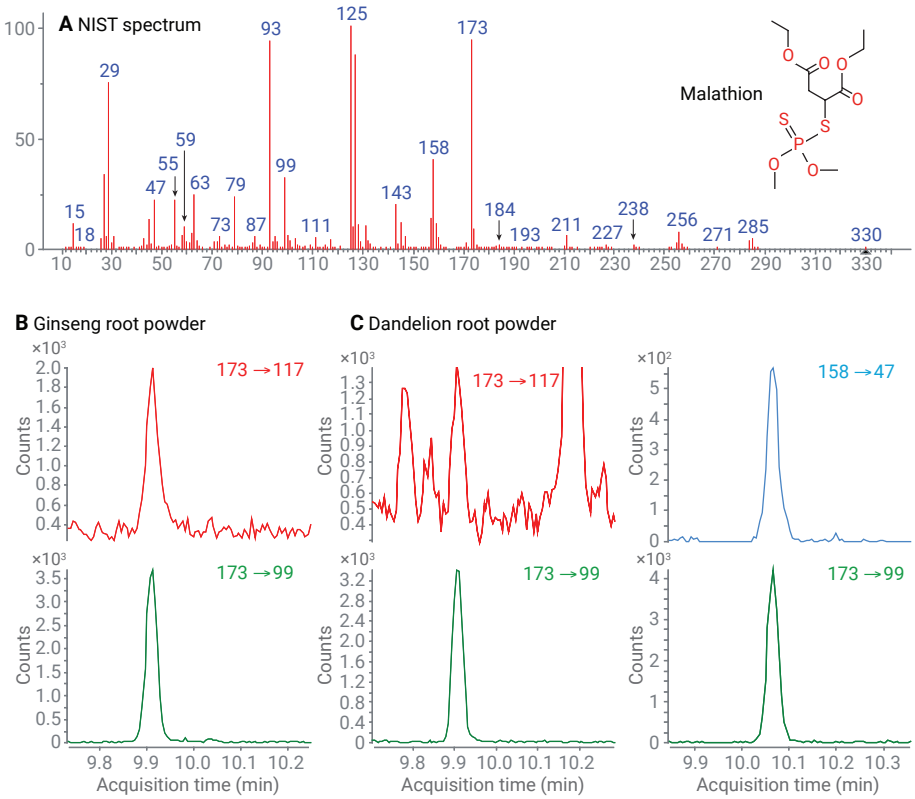



**Figure 23**. Selection of optimum MS/MS transitions for analysis of malathion in matrix extracts: (A) NIST library MS spectrum of malathion and examples of MS/MS extracted ion chromatograms of 5 ng/mL malathion in (B) ginseng root powder extract and (C) dandelion root powder extract. The MS/MS transitions *m/z* 173 & 99 and 158 & 47 were selected as optimal for the analysis of malathion in dandelion root powder and other botanical extracts. Reprinted with permission.[21]

## 6.1 JetClean

Currently, simple sample preparation methods, such as QuEChERS are routinely used for the analysis of food and feed samples, often leaving a significant amount of matrix in the extracts. Analytical laboratories are challenged by these matrix residues, which can negatively affect the responses of the analyzed pesticides, and eventually require source cleaning. The use of the Agilent JetClean self-cleaning ion source (JetClean) reduces the time between manual source cleanings while still enabling the analysis of complex samples, without losing sensitivity and reproducibility. The JetClean self-cleaning ion source introduces a precisely measured hydrogen gas ($H_2$) flow into the MS source, controlled by the MassHunter Acquisition for GC/MS software. The appropriate $H_2$ flow (µL/min) generates conditions that clean the surfaces of the source, the lenses, and other components. These actions aid in maintaining a stable detection environment and provide for response stability of the pesticides in difficult matrices.[74]

The JetClean self-cleaning ion source is equipped with two operational modes:

- Acquire and Clean (also known as Online) mode: $H_2$ is running continuously during the analysis
- Clean only (also known as Offline) mode: $H_2$ is introduced only postrun or postsequence

The JetClean system can greatly increase the number of samples that can be processed before manual cleaning of the ion source must be done. During the JetClean process, a small amount of hydrogen is introduced into the ion source's ion volume while the filament is emitting electrons, causing reactive hydrogen species to be created. Each time this process runs, contamination is removed from the ion source, depending on the conditions and the nature of the contamination.

An example of a method using JetClean can be found in the Agilent application note, "Maintaining Sensitivity and Reproducibility with the Agilent JetClean Self-Cleaning Ion Source for Pesticides in Food and Feed"(5991-7342EN).

**Figure 24** demonstrates another example of productivity enhancement when using JetClean. The blue bars correspond to the number of injections between ion source cleanings for 7010 Series GC/TQ when using JetClean in the Clean only mode. The red line represents the number of injections of the same challenging matrix that a laboratory might expect to perform between maintenance intervals when not using JetClean. In this case, the number of samples analyzed before the EI source needed cleaning, was increased 2-4-fold.

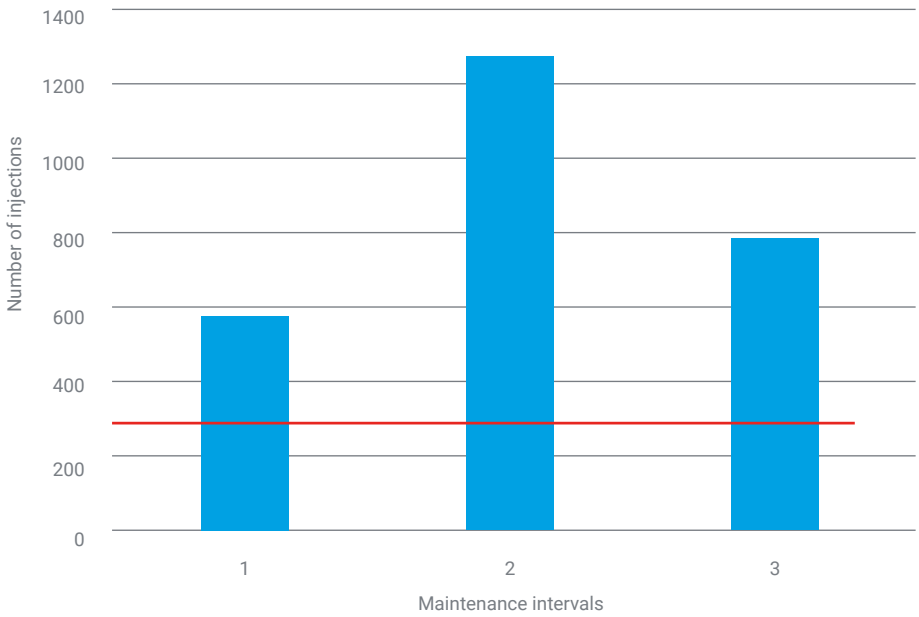



**Figure 24**. Increase in instrument uptime recognized by one laboratory performing pesticide analysis using JetClean.[75]

# 7. GC/MS/MS method development and optimization examples

## 7.1 Optimization of MS conditions

**Annex IV (Page 93)** provides an example of a GC/MS/MS method for pesticide multiresidue analysis using an Agilent 7000 series triple quadrupole, multimode inlet (MMI) in solvent vent mode and concurrent backflushing. This method was recommended in the previous edition of this reference guide.

**Annex V (Page 96)** provides another example of a pesticide multiresidue method using an Agilent 7010 series instrument with almost identical GC conditions, except for the decreased injection volume introduced using cold splitless mode. This decreased injection volume is enabled by increased detection sensitivity of the 7010 series instruments with a high-efficiency ion source (HES). Furthermore, the list of analytes has significantly been expanded in this new example method, and the run time has been extended by one minute to include some less volatile analytes. The following sections explain the most important parameters in a GC/MS/MS method and give practical tips for their optimization.

### 7.1.1 MS source temperature

To provide a compromise between good responses of late eluting analytes (mainly pyrethroids) and an acceptable degree of fragmentation, the EI MS source temperature was set at 280 °C. Higher source temperatures may lead to more extensive fragmentation, and therefore lower and more variable abundances of higher m/z ions, which are typically preferred as precursor ions for higher selectivity reasons discussed in **Chapter 6 (Page 41)**.

### 7.1.2 Gain factor

The electron multiplier voltage (EMV) is adjusted routinely using Aautotune to compensate for the detector aging or lower ion generation/transmission due to potential source contamination. To increase sensitivity, gain normalization can conveniently be used with Agilent 7000/7010 series instruments, which (as opposed to the simple EMV offset, for example, +200 V) allows for repeatable long-term method sensitivity and better agreement in analyte signal intensities between different instruments.[54] A gain setting of 10 to 20 is recommended for routine analysis to increase sensitivity for trace-level methods while having acceptable linearity and EM lifetime. A gain factor of 10 is typically sufficient for pesticide residue analysis in fruits, vegetables, and other matrices.[20, 21] The MassHunter data acquisition allows for updating the gain curve within a sequence by using the keyword “UpdateGainCurve”. The gain curve update enables users to update EM gain coefficients that result in consistent response maintenance throughout instrument use. This is also while maintaining the relative ion ratios, which are essential for maintaining calibration data validity. For more information see **9.2.1 Updating the gain curve**.

### 7.1.3 MS/MS optimization

The MS/MS conditions were optimized for each analyte, typically by selecting 2 to 4 precursor ions in the full scan spectrum and running product ion scans at multiple collision energies (CEs). This was then followed by selecting the most promising MS/MS transitions, analyzing them at different CEs (0 to 60 V range with a step of at least 5 V), testing them for sensitivity and selectivity in various target or representative matrix extracts, and finally selecting the two or three most suitable MS/MS transitions (multiple reaction monitoring, MRM) per analyte. More than three transitions may be beneficial for analytes prone to matrix interferences, such as dieldrin, endrin, or endosulfans. Automated MRM development and optimization with the Optimizer for GC/TQ software is discussed in **7.1.4 MassHunter Optimizer software**. The steps involved in manual MRM development are discussed below.

**Figure 25** illustrates the GC-EI-MS/MS optimization of MRMs for the fungicide etridiazole, starting with the evaluation of its **full scan** MS spectrum and selection of promising precursor ions. In this case, *m/z* 211 (loss of chlorine, *m/z* 35, during the EI fragmentation) looks like a good candidate due to its relative abundance in the spectrum (it is a base peak) and also its relatively high m/z value. Other ions highlighted in **Figure 25** (*m/z* 183, 185, 213, 246, and 248) could also be considered as precursors for MS/MS optimization, given their abundances or *m/z* values.

For the **product ion spectra**, Agilent MassHunter acquisition software currently enables acquisition of four different product ion events in one time segment; therefore, more methods and runs would be needed to obtain more than four product ion spectra for one analyte (unless the peak is split into two time segments).

For compounds with unknown collision-dissociation behavior, it is advisable to obtain product ion spectra for at least three different CEs (for example, 5, 10, and 20 V). But more CEs, covering the entire range of 0 to 60 V (such as 0, 2, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 60) would provide a better picture of the promising product ions. For the easiest evaluation of the product ion spectra, it is recommended to acquire and overlay them in the profile mode. The overlaid spectrum, shown in **Figure 25B**, compares the maximum abundances of the individual product ions (*m/z* 183, 140, 108, and 79 for the case of the *m/z* 211 precursor) independent of the CEs.

The optimum CE for each MRM can be obtained directly from the preceding product ion experiments (especially if a wide range of CEs with fine steps are evaluated), or separate MRM experiments can be conducted using methods with varying CEs.

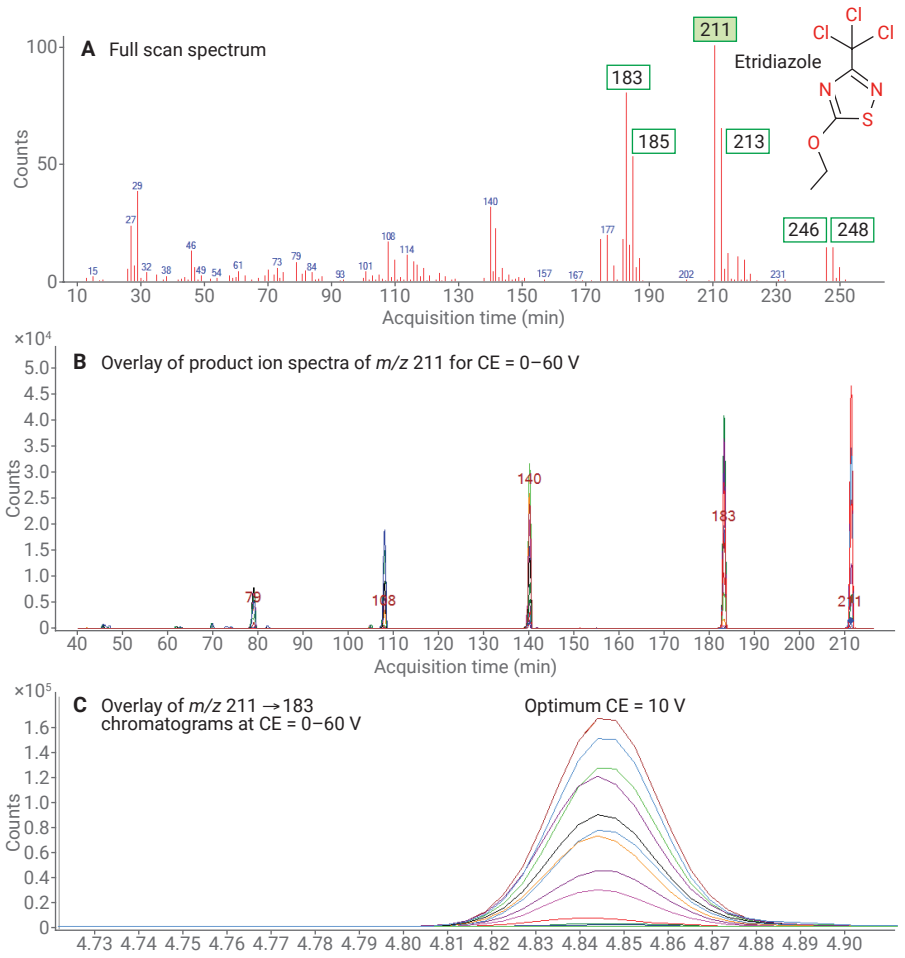



**Figure 25**. An example of MS/MS manual optimization steps for etridiazole. (A) A selection of promising precursor ions from a full scan spectrum. (B) Generation of product ion spectra at various collision energies (CEs) for each precursor ion (overlay of product ion spectra for the precursor ion *m/z* 211 is shown as an example). (C) Optimization of CEs for each MS/MS transition (overlay of chromatograms obtained at various CEs for *m/z* 211 & 183 are provided as an example).

### 7.1.4 Agilent MassHunter Optimizer for GC/TQ software

Instead of determining the precursor ions, product ions and collision energies manually, the **Agilent MassHunter Optimizer** software can be used to automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a triple quadrupole mass spectrometer instrument for each individual compound analyzed. Specifically, it automates the selection of the best precursor ions, the optimization of the … voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify.[65]

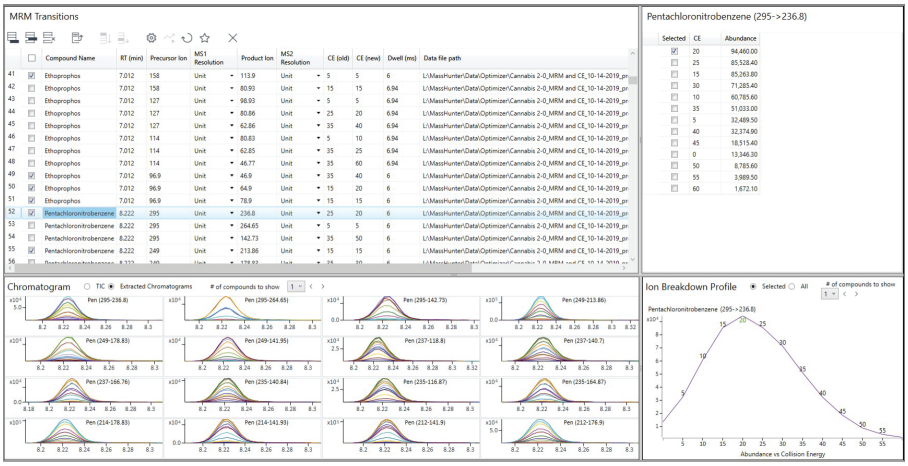
Agilent application notes are available as guides for using the MassHunter Optimizer software for the optimization of transitions and collision energies: "Automated MRM Method Development for Pesticides in Cannabis Using the Agilent MassHunter Optimizer for GC/TQ" (5994-2087EN)[64] and "Automated MRM Method Development for US EPA Method 8270 with the Agilent MassHunter Optimizer for GC/TQ" (5994-2086EN).[73]

Automated collision energy optimization step is shown in **Figure 26**. Collision energy optimization can be performed around the value chosen in the previous step or over a defined range. In this example, collision energies were optimized for 375 MRM transitions over a range of 0–60 eV with a step size of 5 eV (**Figure 26B**) by performing six injections. This step would require only three injections instead of six if no coelution occurred or if coeluting compounds were ignored. Collision energy optimization results are shown in **Figure 26A**, with the 295→236.8 transition for pentachloronitrobenzene highlighted in the MRM transitions table.

The window includes:

- An MRM transitions table, in which each line corresponds to one MRM transition
- TIC or extracted ion chromatograms for each of the transitions acquired at all the tested collision energy values
- An ion breakdown profile, which demonstrates a plot of the MRM transition abundance versus collision energy
- Collision energies with corresponding abundances for the highlighted MRM transition

A)




B)

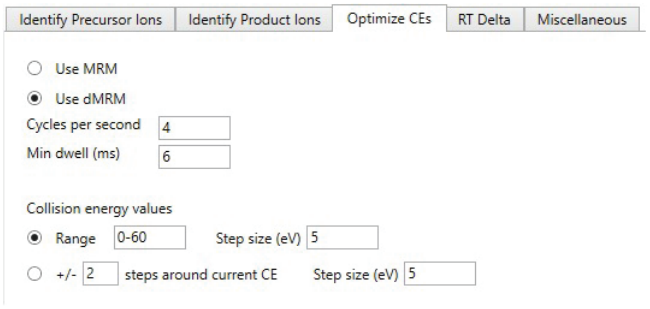
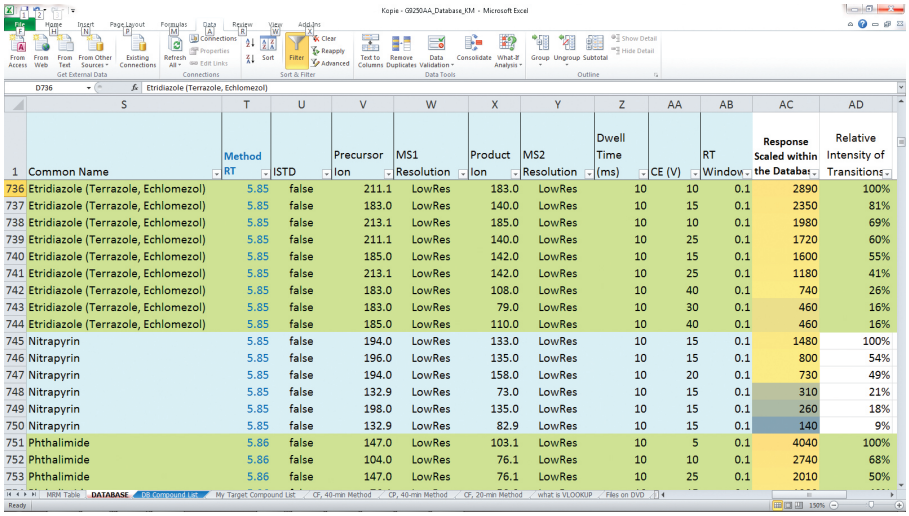



**Figure 26**. Collision energy optimization with MassHunter Optimizer for GC/TQ.

## 7.1.5 Pesticides and environmental pollutants MRM database

**Method development time can greatly be reduced by using the Agilent MassHunter Pesticide and Environmental Pollutants MRM Database (P&EP 4.0) (G9250AA)**.[76] The database contains MS/MS conditions (on average eight MRMs per analyte) and retention time information (provided for five different GC conditions, including the GC conditions for **Annex IV**–**VII** methods) for over 1,100 compounds and 7,500 matrix-optimized MRM transitions in an accessible format.[55] The database simplifies the otherwise time-consuming and costly process of manually developing MRM or dMRM methods, especially when dealing with many compounds and matrices. It also alleviates the need to purchase and analyze the expensive standards typically required in method development. **Figure 27** shows a selected view of the MRM database, listing MS/MS conditions for our etridiazole example. The database also gives relative intensities of the provided MRMs, which can help in their selection; however, the promising transitions should be tested in representative matrices to evaluate their selectivity (not just sensitivity) as discussed previously.



| | S | T | U | V | W | X | Y | Z | AA | AB | AC | AD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Common Name | Method RT | ISTD | Precursor Ion | MS1 Resolution | Product Ion | MS2 Resolution | Dwell Time (ms) | CE (V) | RT Window | Response Scaled within the Databa | Relative Intensity of Transitions |
| 736 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 211.1 | LowRes | 183.0 | LowRes | 10 | 10 | 0.1 | 2890 | 100% |
| 737 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 140.0 | LowRes | 10 | 15 | 0.1 | 2350 | 81% |
| 738 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 213.1 | LowRes | 185.0 | LowRes | 10 | 10 | 0.1 | 1980 | 69% |
| 739 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 211.1 | LowRes | 140.0 | LowRes | 10 | 25 | 0.1 | 1720 | 60% |
| 740 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 185.0 | LowRes | 142.0 | LowRes | 10 | 15 | 0.1 | 1600 | 55% |
| 741 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 213.1 | LowRes | 142.0 | LowRes | 10 | 25 | 0.1 | 1180 | 41% |
| 742 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 108.0 | LowRes | 10 | 40 | 0.1 | 740 | 26% |
| 743 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 79.0 | LowRes | 10 | 30 | 0.1 | 460 | 16% |
| 744 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 185.0 | LowRes | 110.0 | LowRes | 10 | 40 | 0.1 | 460 | 16% |
| 745 | Nitrapyrin | 5.85 | false | 194.0 | LowRes | 133.0 | LowRes | 10 | 15 | 0.1 | 1480 | 100% |
| 746 | Nitrapyrin | 5.85 | false | 196.0 | LowRes | 135.0 | LowRes | 10 | 15 | 0.1 | 800 | 54% |
| 747 | Nitrapyrin | 5.85 | false | 194.0 | LowRes | 158.0 | LowRes | 10 | 20 | 0.1 | 730 | 49% |
| 748 | Nitrapyrin | 5.85 | false | 132.9 | LowRes | 73.0 | LowRes | 10 | 15 | 0.1 | 310 | 21% |
| 749 | Nitrapyrin | 5.85 | false | 198.0 | LowRes | 135.0 | LowRes | 10 | 15 | 0.1 | 260 | 18% |
| 750 | Nitrapyrin | 5.85 | false | 132.9 | LowRes | 82.9 | LowRes | 10 | 15 | 0.1 | 140 | 9% |
| 751 | Phthalimide | 5.86 | false | 147.0 | LowRes | 103.1 | LowRes | 10 | 5 | 0.1 | 4040 | 100% |
| 752 | Phthalimide | 5.86 | false | 104.0 | LowRes | 76.1 | LowRes | 10 | 10 | 0.1 | 2740 | 68% |
| 753 | Phthalimide | 5.86 | false | 147.0 | LowRes | 76.1 | LowRes | 10 | 25 | 0.1 | 2010 | 50% |

**Figure 27**. A selected view of the Pesticides and Environmental Pollutants MRM Database (P&EP 4.0) (G9250AA),[76] showing MS/MS conditions for etridiazole.

**Figure 28** shows an example of the **evaluation of MRMs in representative matrices** for etridiazole at 10 ng/g in broccoli and tangerines. Based on this experiment (note: only five MRMs are shown in **Figure 28**, but all optimized transitions were tested), MRMs *m/z* 211 & 140 and 211 & 108 were selected for the acquisition method as the two main transitions. The MRM *m/z* 246 & 211 was also added to the methods in **Annex IV** and **V** for increased confidence in the identification of etridiazole because the *m/z* 246 precursor ion comes from the molecular ion cluster.[52.] The most abundant transition, *m/z* 211 & 183 (the first one listed in the MRM database), could also be used for the analysis, but its loss of *m/z* 28 ($C_2H_4$) is less selective than for the MRMs *m/z* 211 & 140 and 211 & 108, which results in a lower signal-to-noise ratio (S/N) despite the higher signal (1.3- and 2.4-fold larger peak areas, respectively). **Figure 29** compares signals, root mean square (RMS) noise, and S/N for etridiazole in broccoli (at 10 ng/g) for *m/z* 211 & 183, and the three MRMs included in the method.

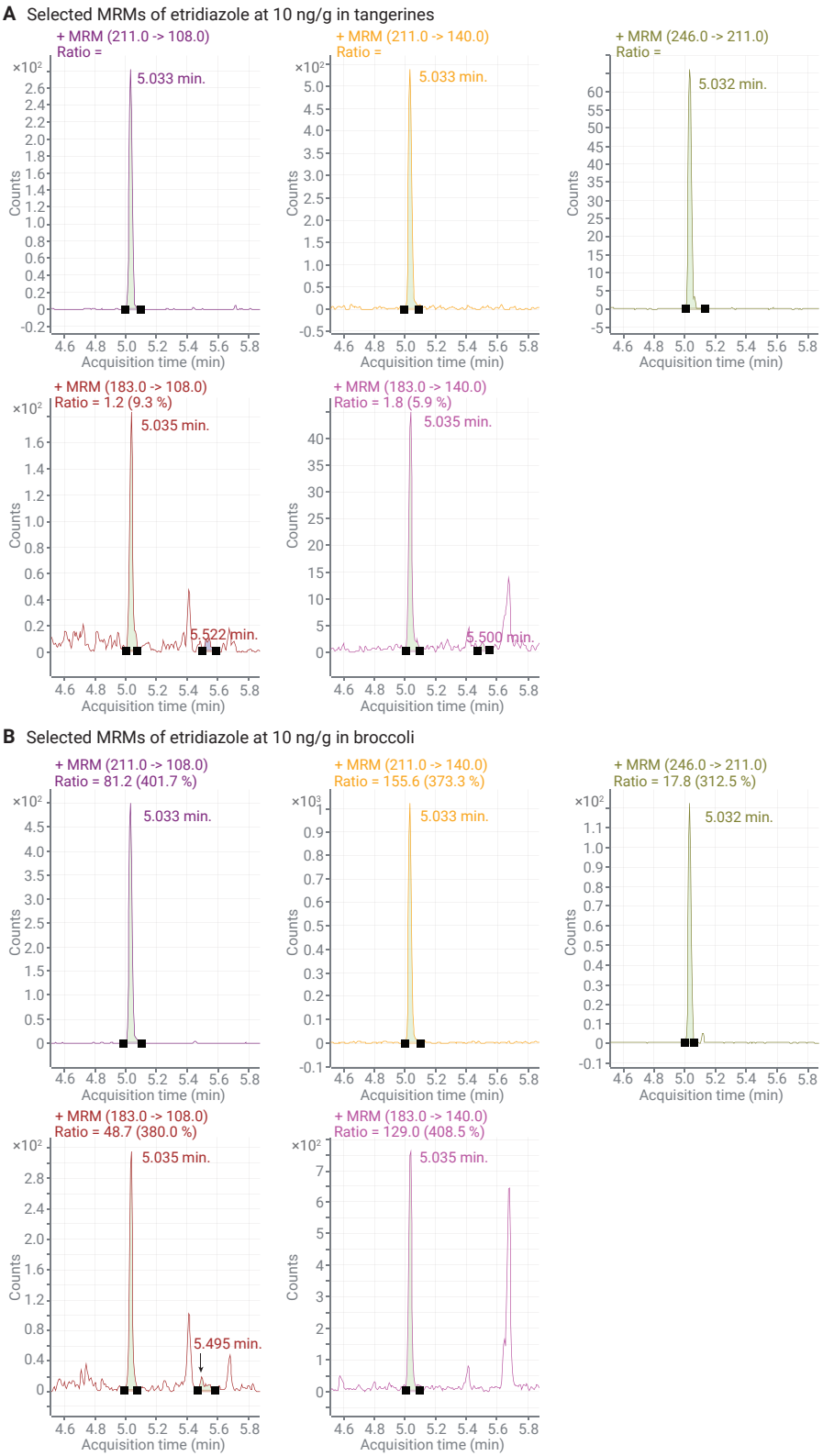



**Figure 28**. Evaluation of selected etridiazole MRMs (*m/z* 211 & 108, 211 & 140, 246 & 211, 183 & 108, and 183 & 140) at 10 ng/g in tangerines and broccoli.

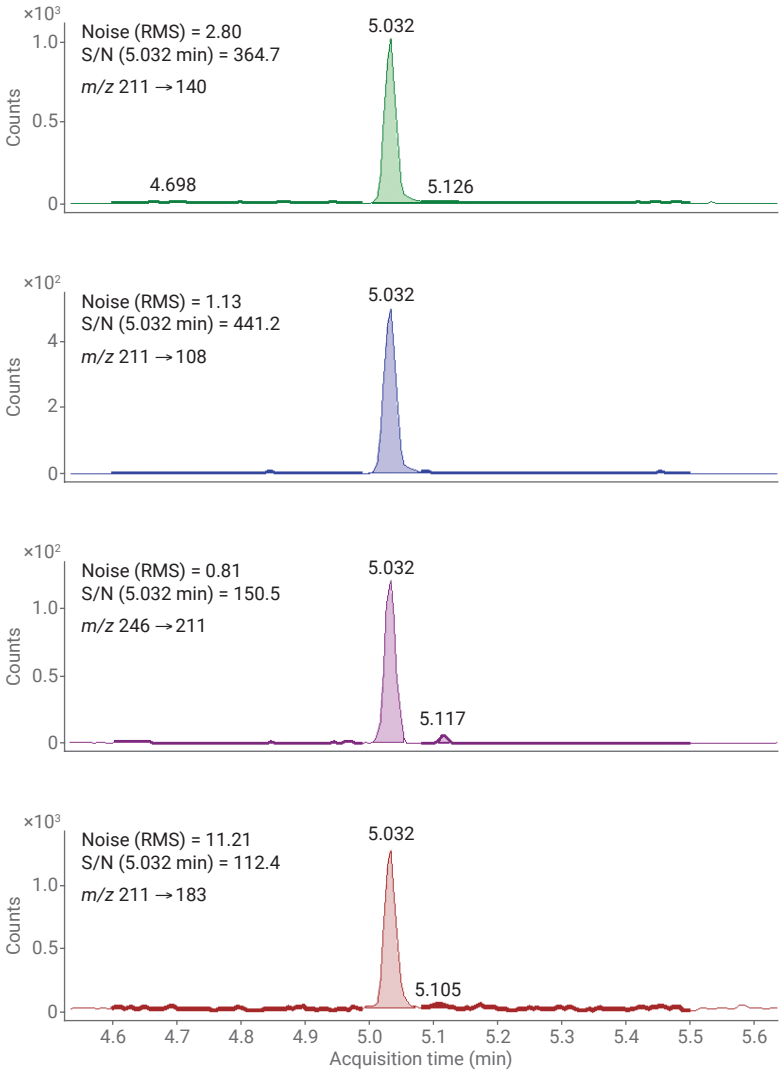



**Figure 29**. Comparison of noise (RMS) and S/N values obtained for etridiazole MRMs 211 → 140, 211 → 108, 246 → 211, and 211 → 183 at 10 ng/g in broccoli.

### 7.1.6 Time segmented MRM program

Once the MRMs are optimized and selected for each analyte, the MRM time segment program can be created using the MassHunter Optimizer for GC/TQ following these steps:

1) Create a CSV (comma-separated values) file of MRM information. This is typically done by using the Pesticides and Environmental Pollutants MRM Database or exporting the MRM table from a previous method.
2) Create or find a GC/MS method for the pesticide analysis. This method will be specified in the MRM Optimizer as the GC component of the method.
3) Launch the Optimizer for GC/TQ.
4) Specify the acquisition method. If the method was not previously an MRM method, change the method type in Optimizer to MRM. You may be prompted about importing the MRMs. Choose "No".
5) Click the Import Compound Info ribbon and select the CSV File item. Now, select the CSV file of MRM information that was created in step one.
6) Click the Results button. Choose the MRM method tab and specify a method name. Then, click the button to create a time segmented MRM method.

The software uses overlapping time segments (that is, inclusion of an analyte in more than just one time segment) and automatically generates the acquisition method. The method must be checked for potentially missing or misidentified analytes, which can happen for isomers and compounds sharing the same transitions or having interferences, such as those coming from septum bleed or solvent/inlet contamination.

Alternatively, it is possible to create the MRM time segments manually. If a relatively small number of analytes have to be included in the method, or the analytes are well separated or grouped in somewhat isolated elution clusters, then the time segments can simply end/start between them. For a larger number of analytes, especially those with similar retention behavior in GC, setting segments between analytes or their groups may be difficult. This can potentially lead to too many analytes in one window (thus very short dwell times or long cycle times) or can cut off peaks because they elut too close to the end/start of the time segment. For these reasons, the use of overlapping time segments is recommended for a larger number of analytes,[20] such as in pesticide residue analysis. This is especially true in complex food matrices that can induce matrix-dependent retention time shifts, leading to peak cut-offs or even nondetection if analytes elute too close to the segment end.

The cycle time can be calculated by multiplying the number of MRMs with their dwell times, plus inter-scan delays (times needed for switching between MRMs). It is possible to keep both the dwell times and cycle times (data point density) constant across each extracted ion peak by keeping both dwell times and the number of MRMs constant for each MRM and overlapping time segments, respectively. An example method was created, which uses a dwell time of 10 ms for each MRM and a very similar number of MRMs (approximately 26) in each segment, except for the last segment, which does not need to be overlapped. This

contains only 10 MRMs (with 28 ms dwell times) for the least volatile analytes on the given target list, that is, fenvalerate, tau-fluvalinate, and deltamethrin isomers, which are sufficiently separated from the previous group of analytes. As a result, a data acquisition speed of approximately 3.5 cycles is obtained in each time segment. This provides, on average, more than 15 data points above the baseline, as demonstrated in **Figures 30 and 31**, for the parathion-ethyl example, showing two different ways to obtain data points in the MassHunter Qualitative Analysis software. The issues concerning the number of data points across a peak and over dwell times are discussed further in **Chapter 8 (Page 66)**.

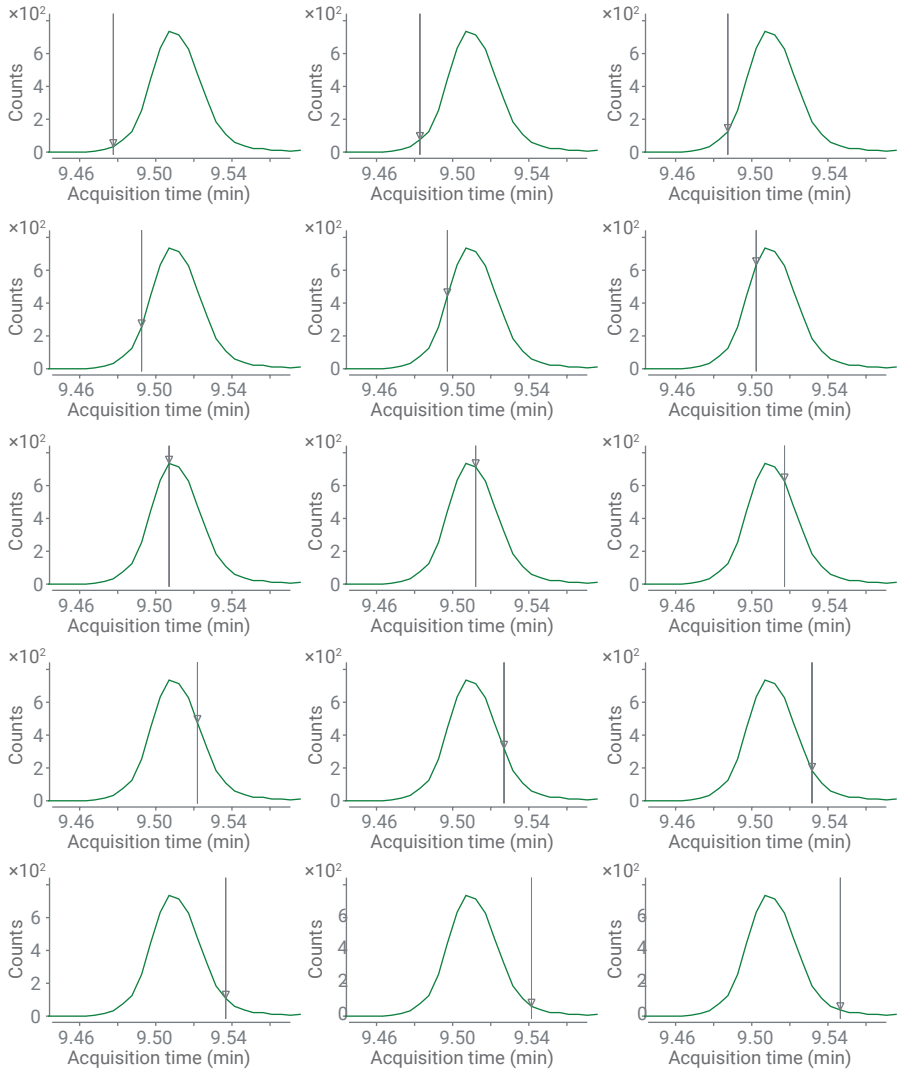



**Figure 30**. Determination of the number of data points (above baseline) across a peak of parathion-ethyl using the walk chromatogram option in the MassHunter Qualitative Analysis software.

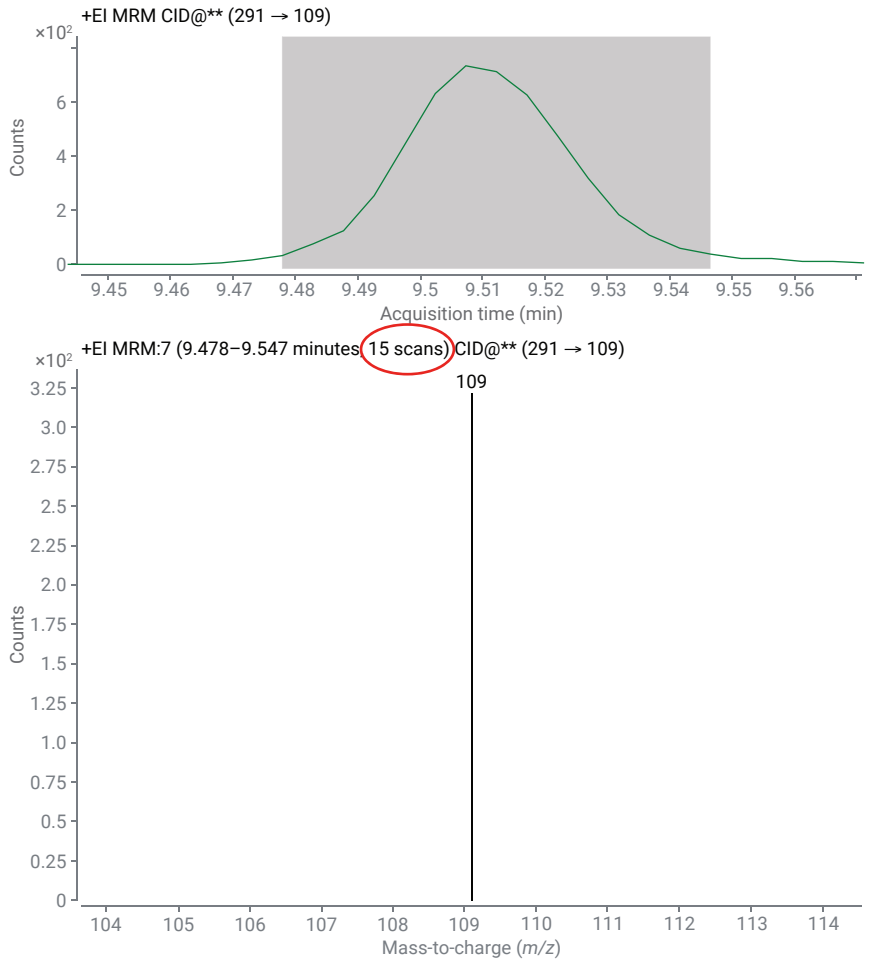



**Figure 31**. Determination of the number of data points (above baseline) across a peak of parathion-ethyl by extracting an average MS spectrum from the selected chromatogram range in the MassHunter Qualitative Analysis software.

### 7.1.7 Dynamic MRM (dMRM) program

There are some limits to what can be accomplished with time segmented methods. As the number of analytes in a method increases, so too will the number of concurrent MRM transitions in each segment. It will be necessary to either reduce the dwell times for these transitions or to increase the cycle time for each MS scan. Reducing dwell times (the amount of time required for the triple quadrupole to analyze a single MRM transition) can compromise MS data integrity by introducing collision cell cross-talk (insufficient clearing of the collision cell between individual MRM experiments so that some product ions from a previous MRM may be detected in the subsequent MRM). Maintaining the same dwell time but increasing the overall MS cycle time may mean that not enough data points are collected during the elution of a very narrow peak to allow for reliable quantitation. Both of these factors can lead to compromises in data quality. There is an additional challenge using time segments. To not compromise any data, the change from one segment to the next must occur during a time when no peaks are eluting from the column. In complex analyses, such as pesticide analysis, where many coeluting peaks are monitored at almost every time point during the chromatogram, this can be a formidable challenge.[66]

The dynamic MRM mode can address these challenges. Ion transitions and a retention time window for each analyte are stored in a method. MRM transition lists are then built dynamically throughout an MS run, based on the retention time window for each analyte. In this way, analytes are only monitored while they are eluting and valuable MS duty cycle is not wasted by monitoring them when they are not expected. An added benefit of this approach is that MassHunter MS Optimizer software can readily determine and store optimal transition ions for each target analyte, greatly simplifying dynamic MRM method setup.[66]

The Agilent MassHunter acquisition software for GC/MS systems (B.07.05) introduced the dynamic MRM (dMRM) feature (available for 7000 series and 7010 series GC/TQ). This streamlines the creation of the MRM program using overlapping analyte acquisition windows (set based on analyte retention times) with constant cycles and dynamically changing dwell times. This approach was used to develop the MRM program in the example method discussed in this section. The method includes 1,113 MRMs (369 analytes, plus three internal standards), with a maximum of 107 concurrent MRMs, resulting in a minimum dwell time of 2.02 ms at 3.3 cycles (on average more than 10 above-baseline data points across peaks). The left retention time delta parameter was set at 0.2 minutes, and the right at 0.5 minutes, except for the cypermethrin and cyfluthrin four-isomer peak windows, which were based on the first isomer retention time and a larger right delta of 0.7 minutes to safely capture all four isomers.

**Dynamic MRM/Scan (dMRM/Scan) mode** is a new acquisition mode available on the Agilent GC/TQ systems starting from 7000E and 7010C. This acquisition mode allows for the collection of dynamic MRM data and scan data simultaneously in one analytical run. The simultaneous dMRM/scan capability enables identification of the unknown compounds and retrospective analysis, while maintaining sensitivity and dynamic range of the method comparable to a conventional dMRM analysis. Additionally, scan data enables more confidence in compound identification by library spectrum matching. Finally, the full scan data allow the analyst to evaluate the sample matrix to ensure the most efficient performance of the GC/TQ system.[68]

To achieve acquisition in two modes simultaneously, the GC/MS/MS is required to switch rapidly between the dynamic MRM and scan modes during acquisition. As such, some loss in instrument performance is expected compared to a dedicated dMRM mode. In practice, this drop in performance is often minimal and overall performance is comparable between the two modes of acquisition. An example method using dMRM/Scan mode can be found in the Agilent application note, “Dynamic MRM/Scan Mode: Adding More Confidence to Sensitive Quantitation in Complex Foods by Triple Quadrupole GC/MS (GC/TQ)” (5994-4966EN).[68]

## 7.2 Multimode inlet – solvent vent and cold splitless optimization

The Multimode inlet (MMI), as its name indicates, can be used in multiple different modes, including hot or cold split or splitless (without or with a pressure pulse) and solvent vent.[56,57]

The solvent vent mode enables solvent elimination from the inlet prior to the analyte transfer to the column. Therefore, larger volumes can be injected to increase sensitivity. Effective solvent elimination (venting) is very important in the case of acetonitrile (the QuEChERS extraction solvent), even when relatively small volumes (such as 2 µL in the **Annex IV method**) are being injected. As opposed to other solvents that have been used in GC analysis of pesticides (such as ethyl acetate, acetone, toluene, isooctane, or hexane),[10] acetonitrile does not wet the surface of the relatively nonpolar stationary phase well, and forms droplets rather than a continuous film upon recondensation in GC. Therefore, **recondensation of acetonitrile needs to be avoided to prevent peak splitting or fronting**, especially in the case of early eluting analytes, because their focusing can be negatively affected by the excessive amount of acetonitrile in the GC column.[10,21] **Figure 32A** demonstrates the effect of an excessive amount of acetonitrile on the peak shape of early eluting dichlorvos (multiple small peaks eluting as different fractions before the main dichlorvos peak) and somewhat later eluting hexachlorocyclohexane (HCH) isomers (fronting peaks). For comparison, peak shapes and intensities obtained for these analytes under optimized PTV solvent vent conditions are shown in **Figure 32B**.

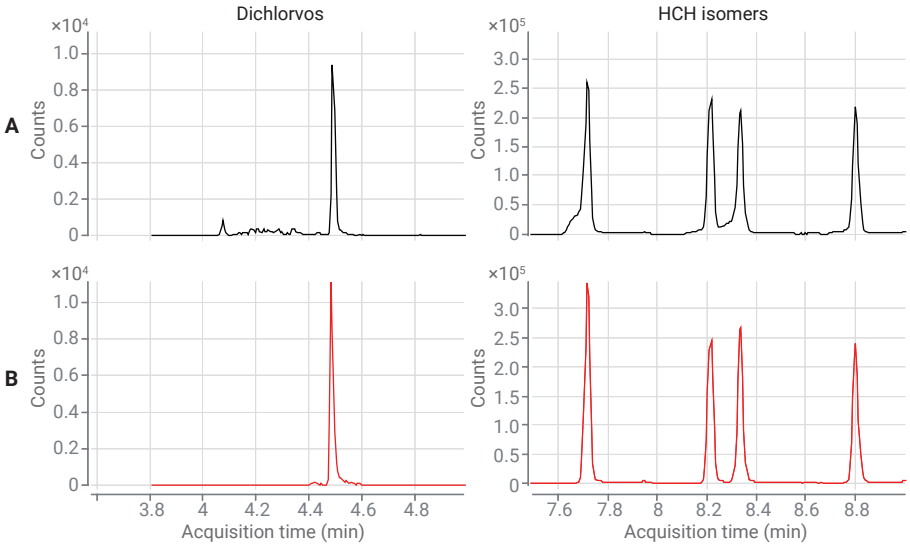



**Figure 32**. Peak shapes of dichlorvos and HCH isomers obtained (A) in the presence of an excessive amount of acetonitrile in the column and (B) under optimum PTV solvent venting conditions. Reprinted with permission.[21]

Careful optimization of the inlet conditions, including selection of an appropriate liner, is necessary for successful PTV solvent vent injection and overall long-term system performance.[38,39] The Annex IV method uses a dimpled liner, which has been tested to provide an adequate surface for the initial retention of several microliters (for example, 5 µL) of acetonitrile. Moreover, the dimples serve as a good physical barrier for the postinjection retention of less volatile and nonvolatile matrix components (see **Figure 33**), thus providing good column protection.[21] The dimpled liner has an internal diameter of 2 mm, resulting in a small internal volume, which supports fast analyte transfer onto the column. Another possibility is the use of an Agilent Ultra Inert inlet liner, packed with a thoroughly deactivated glass wool plug (such as Agilent p/n 5190-2293) that can also provide a surface for acetonitrile retention and protection of the column against deposits of nonvolatile matrix components.[58]

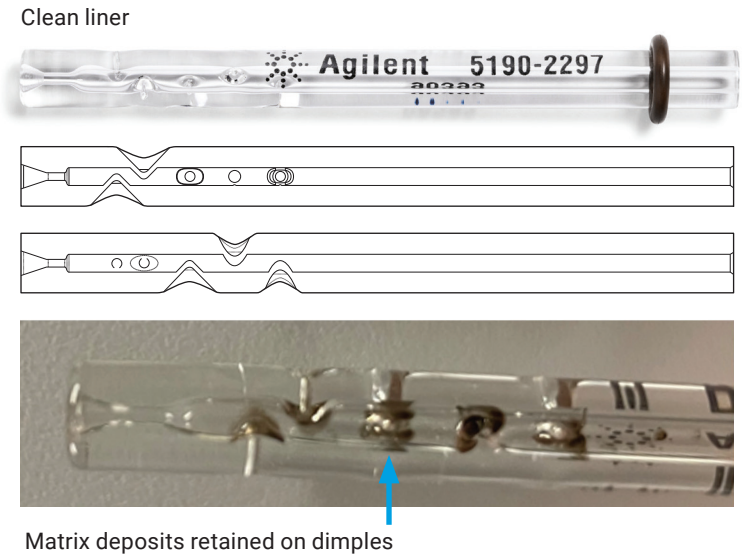



**Figure 33**. A dimpled liner with nonvolatile matrix deposits retained mostly on the dimples, thus minimizing column contamination.

The solvent vent injection consists of three main stages (periods), for which the following parameters need to be optimized (or set) in the acquisition method:

### Solvent vent period:

- Inlet temperature
- Vent time
- Vent flow
- Vent pressure

### Analyte transfer period:

- Inlet temperature program
- Splitless time

### Post-transfer period:

- Purge flow
- Gas saver flow and time

The solvent vent parameters are interdependent and should be optimized together in relation to each other.[38,39,58] The data acquisition software for GC/MS includes a Solvent Elimination Calculator, which can provide a starting point, and other useful information for the optimization of the solvent vent period, based on the solvent type (its boiling point), injection volume, and boiling point (if known) of the first eluting analyte.[56]

A higher inlet temperature and vent flow lead to faster solvent elimination (thus potential losses of analytes), whereas higher vent pressure decreases the solvent elimination rate. The inlet temperature needs to be at least 5 to 10 °C below the boiling point of the injection solvent (that is, not above 72 to 77 °C in acetonitrile with a boiling point of 82 °C) to retain the injected sample in the inlet without analyte losses. The lower the initial inlet temperature, the more effective the trapping of the liquid sample in the inlet, making evaporation conditions milder. However, lower venting temperatures lead to longer venting times and longer inlet re-equilibration times; therefore, inlet temperatures <50 °C are not practical for routine analysis (even if an active inlet cooling is used).

For the optimization of solvent vent period parameters, peak shapes and areas of early eluting analytes should be monitored as indicators of optimum conditions for just enough venting to provide effective solvent elimination, while preventing analyte losses. Conversely, monitoring late-eluting analytes is important for the optimization of the analyte transfer from the inlet onto the column (mainly final inlet temperature and time of the transfer, that is, splitless period). In addition to the inlet parameters, the initial oven temperature is also important for focusing and peak shapes of early eluting analytes. An example of the step-wise optimization approach for MMI parameters in PTV solvent vent mode is described in Agilent application note 5991-1196EN.[58] As mentioned above, the vent parameters are interrelated, so different optimization starting points or preferences (such as the initial inlet temperature) may result in different sets of optimum parameters providing similar results. Similar to the MS/MS optimization, it is important to test the inlet and other optimized GC conditions using representative matrix extracts, which may affect transfer of less volatile analytes from the inlet, or analyte focusing.

For the analysis of fruits and vegetable QuEChERS extracts using an Agilent 7000 series GC/MS/MS, an injection volume of 2 µL is typically sufficient in practice.[20] The inlet conditions in the **Annex IV** method were originally optimized for 5 µL injections of acetonitrile QuEChERS extracts, containing dichlorvos as the most volatile analyte.[21] They were applied as safe venting conditions for the 2 µL injection to the list of analytes included in the Annex IV method, which starts with dichlobenil as the most volatile analyte. Therefore, more volatile pesticides, such as dichlorvos, can be included in the **Annex IV** method without any inlet parameter modifications for 5 µL injections in acetonitrile. If dichlorvos or other pesticides more volatile than dichlobenil were to be analyzed using a lower injection volume than 5 µL, the vent time should be verified and, if necessary, optimized (decreased) to prevent their losses while still effectively eliminating acetonitrile. This can be done by monitoring peak areas and shapes for Column backflushing optimization dichlorvos and other early eluting pesticides to prevent

peak splitting and fronting, while minimizing losses of volatile analytes (shown in **Figure 21**). Similarly, it is possible to increase the injection volume above 5 µL, and the vent time would need to be extended. Other conditions (such as inlet temperature or injection speed) could be kept the same for injection volumes up to approximately 10 µL, but may need to be re-optimized if larger injection volumes are used.

The method in **Annex V** uses a cold splitless injection of 0.5 µL. The decreased injection volume is enabled by increased detection sensitivity of the Agilent 7010 series GC/MS/MS instrument. The inlet conditions of the PTV solvent vent and cold splitless injections in the Annex IV and V methods (respectively) are very similar. The difference is that the injection solvent is not vented, therefore the vent flow and pressure are not applied, and the split vent is closed during the first stage of the injection process.

Another consideration when changing the injection volume involves the concentration of analyte protectants, which should be adjusted to provide a similar amount of protectants introduced into the GC system.[59] For example, it is recommended to increase the analyte protectant concentration in the final extract (given in **Annex II (Page 85)**) four-fold when decreasing the injection volume from 2 to 0.5 µL.

## 7.3 Column backflushing optimization

As discussed in **Chapter 5.5 (Page 38)**, there are two basic types of backflushing: postrun and concurrent. The **optimization of postrun backflushing** is more straightforward because it starts after the last analyte is detected. The effectiveness of postrun backflushing should be optimized or at least verified by the following procedure:

1. Analyze a representative matrix extract using the given backflushing conditions (oven temperature, column flow, and backflushing time).
2. Inject a solvent (acetonitrile) blank right after the preceding matrix run using a full scan MS (*m/z* 45–650) method, without backflushing and with an extended hold (for example, an additional 30 minutes) at the final oven temperature. This is to check for any potential matrix peaks that may be detected in the solvent blank run but originate from the previous matrix injection.
3. If there are matrix peaks detected in the subsequent solvent blank analysis, extend the backflushing duration or re-optimize (increase) the oven temperature or column flow (or both).

The representative matrix (several different matrices would be even better) should be selected for their higher content of less volatile matrix components (typically sterols) to represent worse-case scenarios for backflushing. **Adding an extra 1 to 2 minutes to the optimum postrun backflushing time** is recommended to ensure rugged backflushing operation in routine practice.

**Figure 34** shows full scan total ion chromatograms comparing GC/MS analysis of milk extracts without and with postrun backflushing. The chromatograms demonstrate the effectiveness of backflushing through the absence of any matrix components in the subsequent solvent blank.[43] In this example, an additional 33 minute bake-out period at 320 °C was needed after the last analyte eluted to remove the less volatile milk component from the column without the use of backflushing. The postrun backflush eliminated those compounds effectively in just 7 minutes and at a lower oven temperature of 280 °C, significantly reducing the cycle time (by more than 30%) and extending the life of the column. Moreover, those components are backflushed out of the GC system through a split vent, and do not reach the MS to contaminate it.

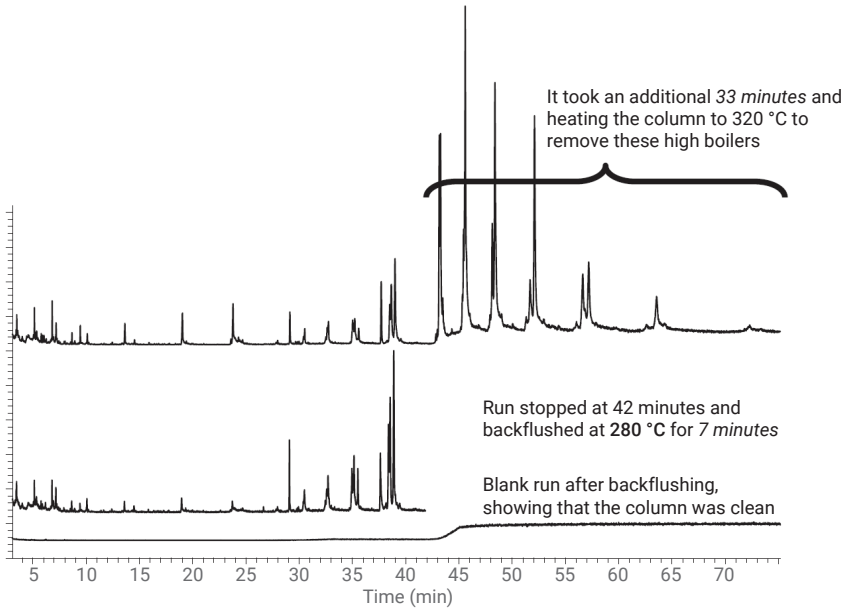



**Figure 34**. Full-scan total ion chromatograms comparing GC/MS analysis of milk extracts without backflushing (top trace) and with post run backflushing (middle trace) demonstrating the effectiveness of backflushing by the absence of any matrix components in the subsequent solvent blank.[43]

The **optimization of concurrent backflushing** is slightly more complicated because the start of the backflush needs to be determined experimentally as the time when the last analyte of interest safely elutes from the first to the second column. The effectiveness of concurrent backflushing also needs to be evaluated and optimized by the same subsequent solvent blank injection experiments, described above for the postrun backflushing optimization.

The **Annex IV** and **V** methods use a concurrent backflushing setup with a short, 5 m capillary column, and a 15 m analytical column of the same column diameter, stationary phase type (HP-5ms UI), and film thickness. The PUU is installed between the two columns and its pressure (helium flow) is controlled by either a pneumatics control module (PCM), an auxiliary (AUX) EPC module, or a pneumatic switching device (PSD). All backflushing parameters (timing and flows) are easily set and controlled using the MassHunter acquisition software for GC/MS systems. **Figure 35** shows the flow programs for the two columns, illustrating the two basic phases of the analytical run with concurrent backflushing: (A) elution of analytes from the first column and (B) backflushing of the first column to remove less volatile matrix components and prevent contamination of the second column and the MS source.

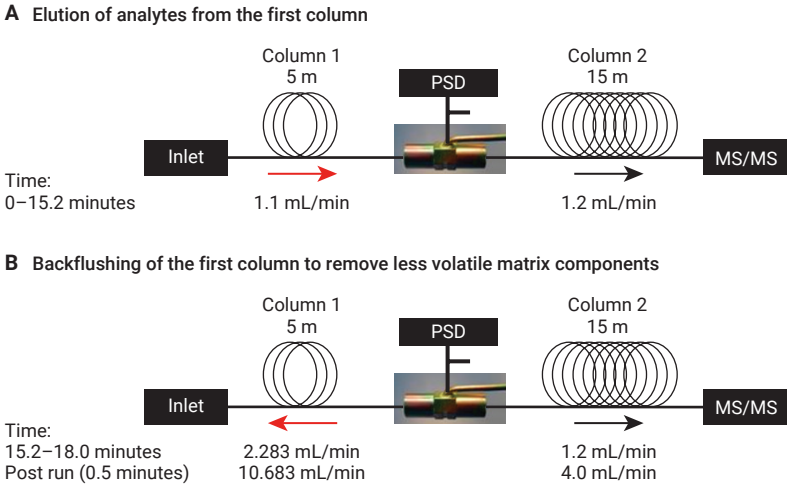



**Figure 35**. Illustration of two phases of the column flow program used in the Annex IV method concurrent backflushing setup: A) elution of the analytes from the first column, and B) backflushing of the first column to remove less volatile matrix components.

The start of the concurrent backflushing in the Annex IV method (at 15.2 minutes) was determined experimentally by testing different backflushing start times and monitoring the peak area of the last analyte (deltamethrin), see **Figure 36**.

To account for potential matrix-related retention time shifts, 0.2 minutes was added as a safety margin to the shortest time that showed no deltamethrin loss (15.0 minutes in **Figure 36**). Similarly, the start of the concurrent backflushing in the **Annex V** method (at 15.5 minutes) was determined experimentally for the last analyte included in that method (dimethomorph II). After the start time of the concurrent backflush, the inlet temperature was increased to 300 °C to support backflushing (elimination through the split vent) and prevent deposits of less volatile matrix components in the inlet.

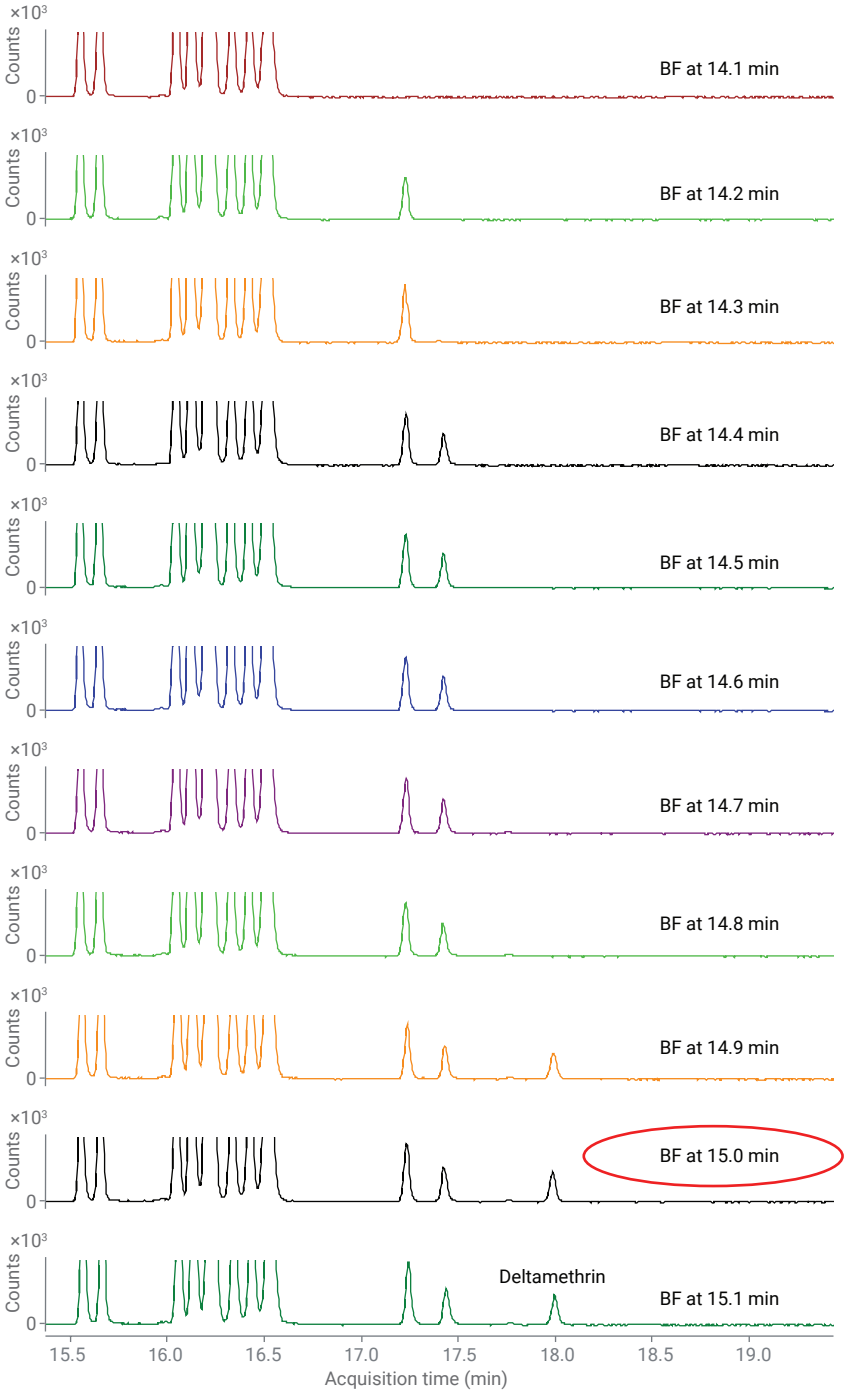



**Figure 36**. Optimization of the concurrent backflushing start by monitoring peak areas of late eluting analytes (especially the last analyte deltamethrin), thus their transfer from the first to the second column, at different backflushing (BF) start times (14.1–15.1 minutes).

The concurrent backflushing flow through the first column is set to achieve 2 psi inlet pressure, while keeping the second column constant until the end of the analysis for optimum analyte detection by MS. For example, the column 1 flow should be –2.283 mL/min during the concurrent backflushing to achieve 2 psi in the inlet while keeping the column 2 flow at 1.2 mL/min. An inlet pressure of 2 psi is the minimum recommended to maintain the septum purge flow of up to 5 mL/min.

After the detection of the last analyte, the run is stopped (at 18 or 19 minutes in the **Annex IV** or **V** methods, respectively) and the column 2 flow is increased postrun (with the filament off) to the maximum recommended flow of 4 mL/min. This results in a –10.683 mL/min backflushing flow rate in column 1 (at the 2 psi inlet pressure), which speeds up the elimination of less volatile compounds from the first column. Under these conditions, a postrun time of 0.5 minutes enables column 1 to be flushed with more than 15 void volumes.

# 8. GC/MS/MS method modification for a different list of analytes

The GC/MS/MS method provided in **Annex IV (Page 93)** includes GC-amenable analytes that were analyzed by the pesticide residue laboratory at the Florida Department of Agriculture and Consumer Services at the time of publication of the first edition of this reference guide. The selectivity of the MRMs has been verified in representative PDP matrices: apple sauce, broccoli, and tangerines. This method was developed as an expansion of a slightly different list of pesticides used to analyze PDP samples at the California Department of Food and Agriculture. The MRM conditions of that method, which was verified in another set of PDP matrices (plums, onions, and snap peas), are shown in the application note 5990-1054EN,[20] with most compounds and their MRMs being a subset of the Annex IV method. The method in **Annex V** (Page 96) covers a significantly expanded list of analytes, which are typically included in pesticide multiresidue methods globally. In addition to compounds preferably analyzed by GC/MS(/MS), the list includes pesticides that can be equally well analyzed by LC/MS/MS and also some analytes that are better suited for LC/MS/MS analysis, but can be monitored by GC/MS/MS for confirmation purposes or even for primary analysis in cases when an LC/MS/MS instrument is not available. The **Annex V** method can be modified to cover a different list of analytes. If the new target list is a subset of the given list of compounds, then the acquisition method can be used as it is, and the excluded analytes can simply be deleted from the quantification method. Alternatively, the extra analytes can also be removed from the acquisition method. This is easily done when using the dMRM option, where compounds can simply be deleted from the list or eliminated from the data acquisition process by unchecking the **Enable** box in the Compound table.

If the new target list includes additional analytes, several steps need to be followed to add them to the method:

1. Obtain retention times and suitable MRMs for the additional analytes (see below).
2. For a time segmented method, create a new time segment program using acceptable dwell times and data acquisition speed (cycles). For a dynamic MRM method, add the new analytes to the dMRM table.
3. If acceptable dwell times or data acquisition speed cannot be achieved, limit the number of MRMs per analyte. If necessary, you may have to split the total number of analytes into two analytical runs. Alternatively, it is possible to adjust the GC oven program (analyte separation), but this option is not recommended if you want to use an MRM database with locked retention times to add analytes to the method.
4. Verify/adjust the inlet parameters and backflushing conditions for the volatility range of the target analytes (if different from the original analyte list).

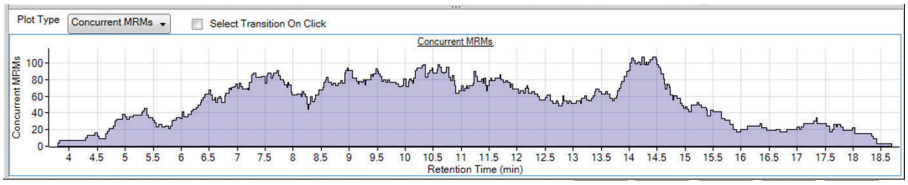



**Figure 37**. A histogram of MRMs included in the dMRM program in the Annex V method.

As the **first step**, suitable MRMs can be obtained using the Pesticides and Environmental Pollutants MRM Database (G9250AA)[76] or going through the optimization process described in **Chapter 7.1.3. (Page 47).** The best way to obtain retention times of additional compounds is to inject their higher-level reference standard solution in full scan mode using the given GC conditions.

Ideally, this is done using individual standard solutions (not mixes), in which case the full scan analysis can also help identify potential impurities or degradation products present in that standard, or being formed during the GC injection or separation. In analyte mixes, the use of known MRMs (from the MRM database or another reliable source) can help find the analyte retention times faster, but the full scan analysis is still recommended to avoid potential misidentification.

The Pesticides and Environmental Pollutants MRM Database 4.0 (P&EP 4.0)[76] is a GC/MS/MS database, where an average of eight MRM transitions are archived for more than 1,100 compounds including pesticides, phthalates, PBDEs, PAHs, PCB congeners, and SVOCs. Use of this database can significantly increase method development speed, particularly when dealing with a large list of analytes.

In the **second step**, the data acquisition speed (cycles) dictates how many data points are acquired across an analyte peak of a given peak width. It is determined as 1/(cycle time), with the cycle time calculation given in **Chapter 7.1.4 (Page 49)** as the number of MRMs in the given time segment, multiplied by their dwell times plus interscan delays (approximately 1 ms in the Agilent 7000/7010 instruments). Therefore, if we want to increase the number of MRMs in a time segment/ acquisition window without changing the peak width, we need to reduce the dwell time or decrease the data acquisition rate, or both.[33] The Agilent 7010 triple quadrupole GC/MS offers a minimum MRM dwell time of 0.5 ms.

Very low dwell times can increase noise, leading to lower signal to noise ratios and higher RSDs. Thus, a minimum dwell time of approximately 2 ms is recommended in practice.

Another option is to decrease the data acquisition rate to a minimum speed that still provides enough data points across a peak. The intricate question is how many data points are needed to define a chromatographic peak and provide adequate quantification. There are many discrepancies in the literature concerning this question. Some sources indicate 15 to 20 points or 10–20, whereas others state that 8–10, 5–6, or as little as 3–4 points should be enough to meet quantitative needs.[22] Moreover, it is not always clear if full width at half maximum (FWHM) or full peak widths at baseline are used in the discussions, or if the baseline points at the beginning and end of the peak should be counted or not.[33] A practical, performance-based approach is to determine an acceptable

minimum number of data points experimentally by evaluating the repeatability (RSDs) of peak areas and heights, as demonstrated in **Figure 38**, which shows an example of a GC/MS analysis using a single quadrupole in selected ion monitoring (SIM).[60] **Figure 38** shows that as few as six data points should not significantly increase peak area RSDs compared to 10 or even more. The number of data points here were calculated to include the baseline point, so would correspond to approximately five data points above the baseline.[33] Based on experience with fast GC/MS using a single quadrupole, **seven data points above the baseline should more likely serve as a safe minimum**.[33]

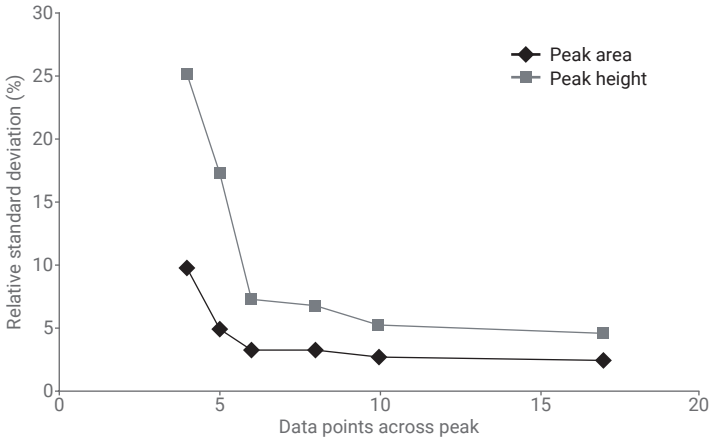



**Figure 38.** The measured relationship between data points across a peak versus RSD of peak area and height in a GC/MS analysis using a single quadrupole system. Reprinted with permission.[60]

Using a data acquisition rate of 3.3 cycles in the **Annex V** method, on average more than 10 data points above the baseline were obtained across peaks. **Figure 37** shows a histogram of MS/MS transitions included in the **Annex V** method, demonstrating a fairly good distribution of 1,113 MRMs (369 analytes, plus three internal standards). As is typical for pesticide multiresidue methods, the majority of GC-amenable pesticides elute in the mid-volatility region, whereas the early eluting (up to approximately 6.5 minutes) and late eluting (after approximately 15 minutes) regions have fewer analytes, thus fewer concurrent MRMs. The largest number (107) of concurrent MRMs appears in the 14 to 14.5 minute window, resulting in a minimum dwell time of 2.02 ms for that region. Modifying the data acquisition rate to 2.7 cycles would provide more than 8 to 9 data points above the baseline. It would also enable the addition of at least 24 MRMs, even in the busiest part of the chromatogram, while keeping the minimum dwell time at approximately 2 ms. This means that even more MRMs could be included in other parts of the chromatograms. Alternatively, the number of MRMs per compound could be decreased because the Annex V method typically uses at least three MRMs per analyte. However, this is not expected because the method already incorporates a significant number of pesticides, especially those important for inclusion in GC/MS(/MS).

# 9. GC/MS/MS routine analysis

The **fourth step,** which involves verification or adjustment of the inlet parameters and backflushing conditions, needs to be done if more or fewer volatile analytes are added to the method than the originally included analytes. Dimethomorph II is the last (least volatile) analyte in the **Annex V** method. There are only a few GC-amenable pesticides less volatile than this compound; the Pesticides and Environmental Pollutants MRM Database (G9250AA) lists just six less volatile pesticides, all of which are better suited for LC/MS analysis. Addition of those analytes would require re-optimization of the backflushing start time, as described in **Chapter 7.3 (Page 61).** Also, the GC oven temperature program would most likely need to be extended to elute these analytes from the column. For example, the least volatile analyte in the database is temephos, with a retention time of 19.62 minutes using the Annex V method conditions. This would require extension of the GC oven temperature program (the final temperature hold time) by another minute, resulting in a run time of 20 minutes.

As for the cold splitless and PTV solvent vent conditions, the addition of less volatile, GC-amenable pesticides may not require any changes in the inlet parameters, but it is advisable to check the efficiency of analyte transfer at the final inlet temperature of 280 °C. Some compromise between transfer efficiency and final inlet temperature can be made to prevent volatilization or pyrolysis of less volatile matrix components at higher inlet temperatures. As for the more volatile analytes, the PTV solvent vent conditions in the Annex IV method were optimized for 5 µL injections of pesticides in acetonitrile extracts, containing dichlorvos as the most volatile analyte.[21] It was applied as a method with very safe venting conditions to the list of analytes included in the **Annex IV** method, which starts with dichlobenil as the most volatile analyte, but would enable the inclusion of more volatile pesticides, such as dichlorvos. In any event, potential losses of more volatile analytes can be evaluated using the procedure discussed in **Chapter 7.2 (Page 58)**.

This chapter discusses important aspects of routine pesticide residue analysis using the GC/MS/MS methods provided in **Annexes IV** and **V** and the GC/MS(/MS) technique in general. It provides recommendations for routine maintenance of the GC/MS/MS system and related method updates.

The chapter also summarizes routine quality control (QC) requirements and guidelines in pesticide residue analysis, with a focus on GC/MS(/MS) measurement, calibration, and quantification.

## 9.1 Routine GC/MS/MS system maintenance

Once a GC or GC/MS method is developed, tested, and in routine use, regular maintenance of the system is essential for continued success. Consumables such as syringes, septa, inlet liners, and columns need to be replaced to ensure that the GC system is leak-free, inert, and resolving analytes at the optimum resolution. GC intelligence in the Agilent 8890 GC system and Agilent Intuvo 9000 GC includes features such as early maintenance feedback (EMF) counters, guided maintenance procedures, diagnostic tests, troubleshooting guidance, system performance monitoring, and an extensive help and information section to ensure analysts have everything they need to replace consumables appropriately.

EMF counters and guided maintenance procedures, both found in the Maintenance menu on the touch screen or the browser interface, allow the analyst to set up timers or counters to monitor the use of each consumable item. The intelligent maintenance procedures guide both new and experienced analysts in these processes, identifying the tools required, cooling heated zones as necessary, demonstrating the procedure, and calling diagnostic tests required for verifying that the procedures are completed correctly.

Diagnostic tests and troubleshooting guidance, found in the Diagnostics menu on the touch screen and browser interface, allow the analyst to call upon a wide variety of diagnostics to ensure that inlets and detectors are leak-free and operating correctly. Troubleshooting guidance queries sets the analyst on a logical path (assisted by the diagnostic tests) to quickly identify and correct problems.

Advanced analysts with established methods can use the advanced intelligence features, such as GC Performance monitoring (Blank Evaluation and Peak Evaluation – both found in the Diagnostics menu), to monitor key chromatography attributes against a reference standard. This ensures that maintenance can be completed as consumables are exhausted and begin to affect the quality of chromatographic peaks.

All of these features are backed up by an extensive Help and Information collection (accessed via the GC Browser Interface), which provides supporting guidance, video content, and manuals to ensure the analyst has everything they need to maintain their Agilent GC system.

Using the **Annex IV** method and configuration, the daily routine maintenance of the GC/MS/MS system (after injection of a typical analytical batch of approximately 50 samples) should involve replacement of the dimpled liner and trimming the tip (approximately 1 to 5 cm or more if needed) of the first column. It is recommended to clean the MMI injection port with a swab dipped in methanol approximately every two months, or as deemed necessary, for example, when matrix deposits are observed on the outside of the liner. The first, shorter column should be replaced as needed (typically after one to two months of daily operation, which corresponds to approximately 1,500 to 3,000 injections), whereas the second column should last considerably longer (six months of daily operation or more) due to the use of concurrent backflushing. When using the Annex IV method and backflushing configuration for the analysis of fruits and vegetables, the MS source should require minimum cleaning. It is recommended to clean the MS source at the same time as the second column is being replaced

or as a part of the preventive maintenance, so approximately every six months. The parameters that can be considered for replacing the columns include decreased chromatographic separation of closely eluting analytes with the same MS/MS transitions (for example, *beta*-HCH and *gamma*-HCH isomers or *p,p'*-DDD and *o,p'*-DDT) or decreased sensitivity due to peak broadening or tailing.[21]

The **Annex V** method employs a 7010 GC/TQ instrument with a four-fold lower injection volume, resulting in a reduced introduction of the sample matrix into the GC/MS/MS system. This should lead to less frequent maintenance, thus a less frequent liner replacement, column trimming, column replacement, and MS source cleaning.

## 9.2 Routine update of the GC/MS/MS method

Routine trimming of the first column (or its replacement) changes the column length, which affects analyte retention times, unless the column flow program is adjusted in the acquisition method. There are two basic approaches that can be used to deal with this issue, and update the GC/MS/MS method routinely.

One approach is to keep the column flows the same but adjust the MS/MS segment or dMRM retention times, as well as backflushing start time ensuring that all analytes are safely detected. The Optimizer for GC/TQ can be used in *Start with MRMs* workflow. The Update retention times functionality available in the Optimizer allows retention time updating without user intervention. It is recommended to review the updated results if multiple compounds share the same MRM transitions.

Another approach involves the use of retention time locking (RTL), which keeps the retention times very similar (locked) by adjusting the column flow (pressure) program. To use RTL, it is important to select a suitable analyte, which will be used as the locking compound. This compound should elute roughly in the middle of the analytical run and not elute at oven ramp transitions. Chlorpyrifos-methyl, with a retention time of 9.143 minutes, was chosen for the **Annex VI and VII** method locking because it fulfills these requirements and has been used in PDP laboratories as the process control compound. This means that it should be present in all sample extracts and standard solutions. To lock the method, it is necessary to initially do three to five calibration runs (after a cleanout run) at different column flow rates. This data is then used to create a calibration curve of flow rates plotted against the observed retention times of the locking compound at the different flow rates. This calibration can then be used to determine the exact flow rate that will give a targeted retention time for the locking compound. This can be caliculated with a spreadsheet or it can be done automatically using MassHunter Acquisition 13.0 for GC/MS software which has an updated user-friendly and intuitive interface (**Figure 42**). It allows for semi-automated or manual compound selection and features both a visual and quantitative assessment of the calibration curve fit, while providing the tool to maintain excellent precision of the retention times even after column trimming. The column flows used for creating a retention time locking calibration should be selected so that the target retention time for the locking compound can be achieved with the column flow within the tested flow range.

If the first column is just being trimmed, then the previous RTL calibration table can be used, and only one run is necessary to obtain the new flow conditions to relock the method. If you are relocking a method, enter the flow and RT from your relocking run.

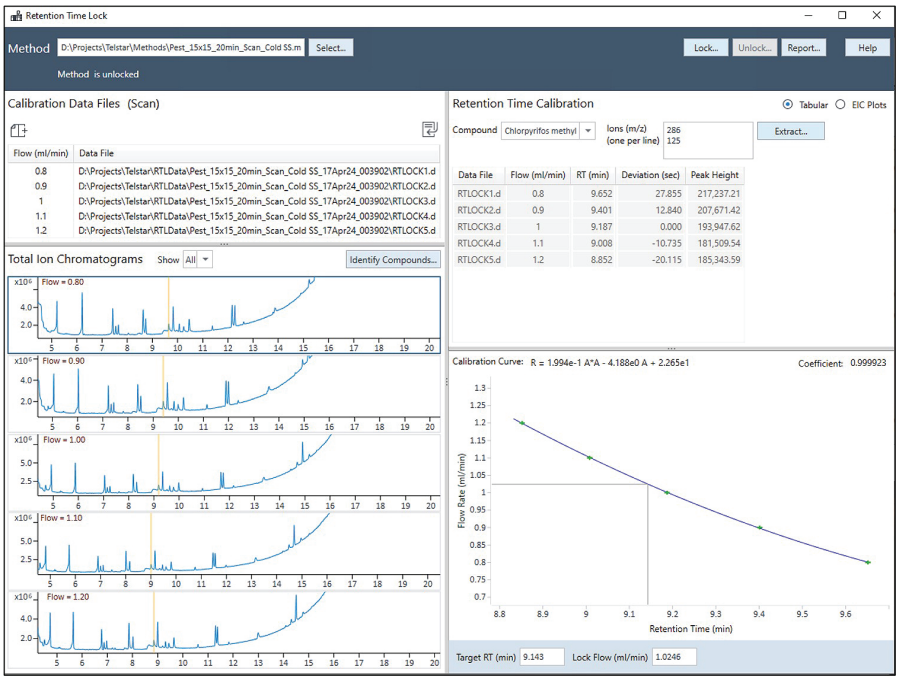



**Figure 39**. Retention time locking software in Agilent MassHunter Acquisition 13.0 for GC/MS.

## 9.2.1 Updating the gain curve

The electron multiplier (EM) gain factor is a parameter that is an understandable, predictable, and consistent way of maintaining the electron multiplier (EM) voltage setting. Adjusting the gain factor adjusts the signal sensitivity of the GC/MS detector. To achieve a consistent detector response with the gain factor, a gain curve is generated each time the GC/MS instrument is tuned. As the detector EM is used, the relationship between gain factor and EM voltage can drift out of calibration and may need to be updated. One clue that the gain curve may need to be updated is if the GC/MS detector response drifts downward and the baseline response is not recovered after standard inlet and column maintenance. The most common way to update the gain curve is to retune the GC/MS. However, performing a GC/MS tune can make the existing quantifier and qualifier ion ratios change. Performing a GC/MS tune also requires that the instrument be recalibrated or at least that the calibration be verified with the new tune. Due to this, we need to be able to update the gain curve without completely retuning the GC/MS. This can be done from the GC/MS driver without performing a complete tune either manually (as shown in **Figure 40**) or via a keyword in the run sequence (as shown in **Figure 41**) .

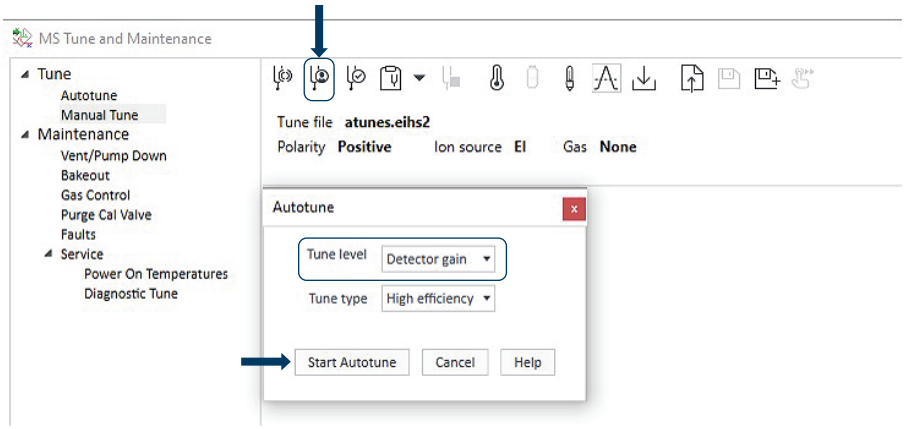



**Figure 40.** Manual Update of the Gain Curve from the GC/MS Driver.

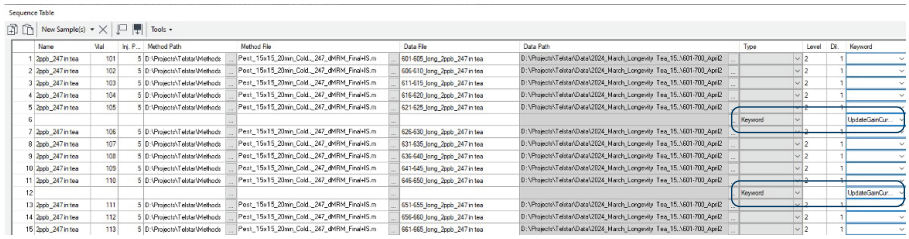


**Figure 41** Update of Gain Curve in GC/MS sequence.

## 9.3 Quality control requirements

For pesticide residue analysis, the analytical methods need to be validated to demonstrate that they are fit for purpose. **Method validation** involves, at a minimum, determination of analyte mean recoveries (as a measure of trueness or bias), precision (repeatability and reproducibility), and the limit of quantitation (LOQ). Other validation parameters include linearity, specificity, robustness, and evaluation of matrix effects.

For determination of mean spike recoveries in representative matrices, the SANTE guidance document[29] requires "being capable of providing acceptable mean recovery values at each spiking level and for at least one representative commodity from each of the relevant commodity groups [...]. Mean recoveries from initial validation should be within the range 70 to 120%, with an associated repeatability RSDr ≤20%, for all analytes within the scope of a method. In exceptional cases, mean recovery rates outside the range of 70 to 120% can be accepted if they are consistent (RSD ≤20%) and the basis for this is well established (e.g. due to analyte distribution in a partitioning step), but the mean absolute recovery should not be lower than 30% or above 140%."[29] The **mean recoveries of 70 to 120% and RSDr ≤20% represent widely acceptable validation criteria in pesticide residue analysis**, but other criteria might be used and justified depending on the purpose of the analysis.

For example, the Pesticide Data Program (PDP) requires mean recoveries of 50 to 150% as the validation criteria for methods used when analyzing PDP samples.[19] This is because the main aim of the program is to provide exposure data and, ideally, include as many pesticides as possible in multiresidue methods.

Routine recovery determination with each batch of samples is a typical part of **on-going quality control (QC)** in pesticide residue analysis. Acceptable limits for a single recovery result may be determined as mean recovery ±2x RSDr, based on initial validation data or on-going routine recovery results (from control charts).[29] The SANTE document also suggests that a general acceptability range of 60 to 140% may be used for recoveries obtained in routine multiresidue analysis.

Similarly, the PDP program QC criteria require the routine spike recoveries to be within a statistically calculated range or between 50 to 150%.[19] However, it is acknowledged that with a large number of analytes in a spike, a few compounds may be outside of the control limits due to matrix variability among actual samples, compared to the limited matrix set used during the method validation. Therefore, recoveries outside of the acceptability range may not require a corrective action (such as batch reanalysis) in certain justified cases, especially if the recoveries are high but there are no residues in the samples.

As discussed in **Chapter 4 (Page 23)**, it is recommended to **use ISTDs and quality/process control standards to improve precision and ensure correct execution of the entire procedure for each individual sample in the batch**. For example, the PDP laboratories add chlorpyrifos-methyl to each sample before extraction as a process control compound for GC-amenable pesticides and compare its recovery against statistically calculated criteria or absolute range criteria of 50 to 150% (see **Chapter 4**). Other ongoing QC criteria relating to the calibration are discussed in the following section.

## 9.4 Calibration and sample injection sequence considerations

As highlighted in **Chapter 5.3 (Page 31)**, matrix-matched calibration is the most widely used calibration approach in pesticide residue analysis to routinely compensate for matrix effects. For rugged GC/MS(/MS) analysis, it is recommended to **add analyte protectants to both the matrix-matched standards and samples**[20] because analyte protectants can help compensate for variability in the GC system activity, and also for sample-to-sample variability when it comes to matrix composition.

Depending on the purpose of the analysis and other factors, the SANTE document allows the use of single-level calibration, interpolation between two levels, or a calibration curve (for three or more calibration levels).[29] If a calibration curve is used, the fit should be evaluated by individual residuals (%difference between calculated versus known standard concentration), especially in the concentration region relevant to the detected residue, therefore not relying only on correlation coefficient values. The **individual residuals should be within ±20%** of the theoretical value.

For comparison, the calibration curve fitness for the PDP sample analysis should be demonstrated in the same injection sequence used to report the data by one of the following accepted methods.[19]

- Correlation coefficient (where R >0.995/R2 >0.990),
- Percentage relative standard deviation (where %RSD ≤20), or
- Percentage difference of calculated versus known standard concentration in the curve (where difference, the residual, is within 20%).

A suggested concentration range for PDP calibrations is 1x LOQ to 10x LOQ. Second-order curves (that is, quadratic) may be used, providing that a sufficient number of points (a minimum of five) is used to define the curve.

For rugged quantitative analysis, it is important that calculated concentrations are consistent throughout the entire analytical sequence. This is ensured in routine practice by checking calibration integrity (or response drift). Calibration integrity can be calculated as percentage difference (%D) using **Equation 1.**[19]

$$\%D = \frac{C_1 - C_2}{C_1} \times 100$$

**Equation 1**.

$C_1$ is the known concentration of the analyte in a calibration standard, and $C_2$ is the concentration of that standard calculated using the calibration curve.

PDP specifies that **%D should be less than or equal to 20%, so the relative back-calculated concentrations (%accuracies)** in all calibration standards and postextraction QCs **should be within 80 to 120% of the theoretical (known) values.**

To meet the calibration integrity requirements in routine GC/MS/MS analysis of pesticides, it is important to have a rugged and well-optimized GC/MS/MS method, and use ISTDs, backflushing, and analyte protectants.[20] Also, the sample injection sequence should be designed to provide adequate calibration and QC frequency. For a typical batch in PDP analysis, containing 31 samples of the same matrix type, matrix blank, matrix spike, and a reagent blank, it was demonstrated that, using the **Annex IV** method, a **matrix-matched calibration set injected in the middle of the sequence should provide acceptable calibration integrity throughout the entire sequence**.[20] Additional calibration sets (or subsets) can be injected at the beginning and end of the sequence to serve as post-extraction QCs. This is to check the calibration integrity, but it can also be used for bracketing the calibration if needed. It is recommended to start the sequence by injecting a matrix blank to prime the system before the injection of calibration standards, QCs, or samples. Multiple matrix blank injections are typically not necessary when analyte protectants are used.

For illustration purposes, **Figure 42** shows examples of calibration curves (calibration points shown as black dots) for representative analytes, which were constructed using a matrix-matched standard set injected in the middle of a typical PDP batch of plum samples (using the **Annex IV** method).[20] The QC results (depicted as blue triangles in the charts) are analyte responses obtained in QC samples injected throughout the sequence, and in calibration standards analyzed at the beginning and end of the sequence. Excellent calibration integrity was obtained for all analyzed pesticides throughout the sequence, demonstrated in **Figure 43**. This shows the accuracy of results obtained in calibration standards and QC samples at the 2x LOQ level, which is the concentration level recommended for a routine recovery check in PDP sample analysis.

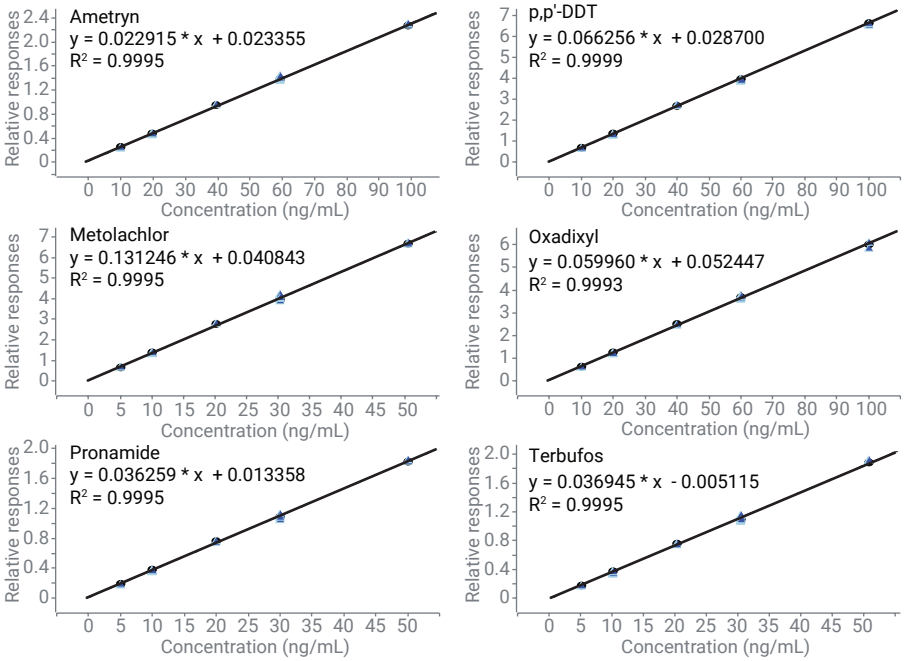



**Figure 42**. Calibration curves (calibration points shown as black dots) and QC results (depicted as blue triangles in the charts) obtained for representative pesticides in plum matrix within a typical PDP sample batch.[20]

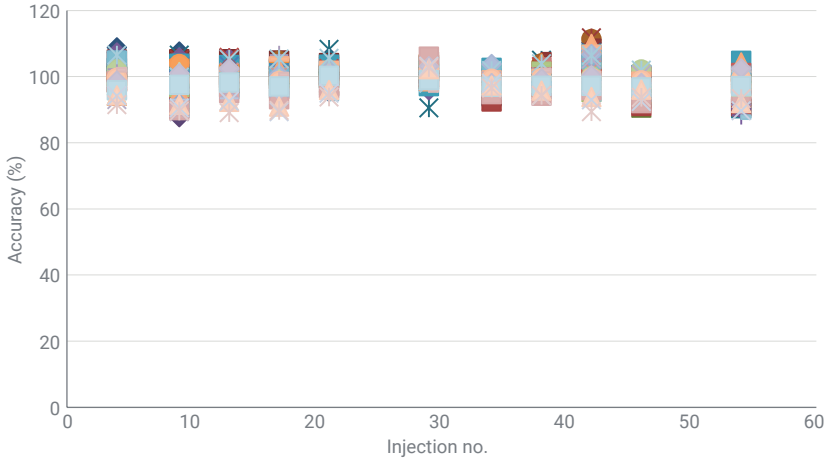



**Figure 43**. Accuracy (%) obtained for all tested analytes (>70 pesticides) at the 2x LOQ concentration level in calibration standards and QC samples injected throughout a typical sequence of PDP plum samples.[20]

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# Annex I Major chemical classes of insecticides, fungicides and herbicides and preferred determinative technique (GC/MS or LC/MS) for their analysis

| Pesticide group | Chemical class | Representative compounds | Preferred technique |
|---|---|---|---|
| Insecticides | Avermectin | abamectin, doramectin, emamectin | LC/MS |
| | Benzoylurea | chlorfluazuron, diflubenzuron, flufenoxuron, lufenuron, novaluron, teflubenzuron, triflumuron | LC/MS |
| | Carbamate | alanycarb, aldicarb, bendiocarb, benfuracarb, butocarboxim, butoxycarboxim, carbaryl, carbofuran, carbosulfan, ethiofencarb, fenobucarb, fenoxycarb, formetanate, isoprocarb, methiocarb, methomyl, oxamyl, pirimicarb, thiodicarb, thiofanox | LC/MS; some (for example, carbaryl, ethiofencarb, or methiocarb) can be analyzed by GC/MS; pirimicarb is preferred by GC/MS |
| | Diacylhydrazine | chromafenozide, halofenozide, methoxyfenozide, tebufenozide | LC/MS |
| | Organochlorine | aldrin, chlordane, DDD, DDE, DDT, dicofol, dieldrin, endosulfan, endrin, heptachlor, lindane, methoxychlor, mirex | GC/MS |
| | Organophosphorus | acephate, azinphos-ethyl, azinphos-methyl, bromophos, bromophos-ethyl, cadusafos, chlorethoxyfos, chlorpyrifos, chlorpyrifos-methyl, coumaphos, cyanophos, demeton-S-methyl, diazinon, dichlofenthion, dichlorvos, dicrotophos, dimethoate, disulfoton, EPN, ethion, ethoprophos, fenamiphos, fenitrothion, fenthion, fosthiazate, heptenophos, isofenphos-methyl, malathion, mecarbam, methamidophos, mevinphos, monocrotophos, naled, omethoate, oxydemeton-methyl, parathion, parathion-methyl, phorate, phosalone, phosmet, phosphamidon, phoxim, pirimiphos-methyl, profenofos, propetamphos, prothiophos, quinalphos, temephos, terbufos, thiometon, triazophos, trichlorfon | GC/MS or LC/MS suitable for most; GC/MS for less polar OPs (for example, bromophos-Et/Me, chlorpyrifos-Et/Me, fenitrothion, parathion-Et/Me, or prothiophos); LC/MS for more polar or otherwise problematic OPs (for example, acephate, azinphos-Et/Me, coumaphos, dicrotophos, dimethoate, methamidophos, monocrotophos, naled, omethoate, or phosmet) |
| | Neonicotinoid | acetamipirid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam | LC/MS |
| | Pyrethrum | cinerin I and II, jasmolin I and II, pyrethrin I and II | LC/MS (and GC/MS) |
| | Pyrethroid | acrinathrin, allethrin, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, cyphenothrin, deltamethrin, esfenvalerate, etofenprox, fenpropathrin, fenvalerate, flucythrinate, flumethrin, methothrin, permethrin, phenothrin, prallethrin, resmethrin, *tau*-fluvalinate, tefluthrin, tetramethrin, tralomethrin, transfluthrin | GC/MS or LC/MS (GC/MS for halogenated; LC/MS for non-halogenated) |
| | Spinosyn | spinetoram, spinosad | LC/MS |
| Fungicides | Anilinopyrimidine | cyprodinil, mepanipyrim, pyrimethanil | GC/MS (or LC/MS) |
| | Carbamate | iprovalicarb, propamocarb, thiophanate, thiophanate-methyl | LC/MS |
| | Chlorine-substituted aromatics | chloroneb, chlorothalonil, dicloran, hexachlorobenzene, quintozene (PCNB), tecnazene (TCNB), tolclofos-methyl | GC/MS |
| | Dithiocarbamate | ferbam, mancozeb, maneb, metiram, propineb, thiram, zineb, ziram | LC/MS for individual compounds; GC/MS for group analysis as carbon disulfide |
| | Dicarboximide | chlozolinate, iprodione, procymidone, vinclozolin | GC/MS |
| | Imidazole | benomyl, carbendazim, fuberidazole, imazalil, pefurazoate, prochloraz, thiabendazole, triflumizole | LC/MS (or GC/MS for some) |
| | Phenylamide | benalaxyl, furalaxyl, metalaxyl, ofurace, oxadixyl | LC/MS or GC/MS |

| Pesticide group | Chemical class | Representative compounds | Preferred technique |
|---|---|---|---|
| | Strobilurin | azoxystrobin, dimoxystrobin, famoxadone, fenamidone, fluoxastrobin, kresoxim-methyl, orysastrobin, pyraclostrobin, trifloxystrobin | LC/MS (or GC/MS) |
| | N-Trihalomethylthio | captafol, captan, folpet, dichlofluanid, tolylfluanid | GC/MS (LC/MS for dichlofluanid and tolylfluanid) |
| | Triazole | azaconazole, bitertanol, bromuconazole, cyproconazole, difenoconazole, diniconazole, epoxiconazole, fenbuconazole, flusilazole, flutriafol, hexaconazole, ipconazole, metconazole, myclobutanil, penconazole, propiconazole, prothioconazole, tebuconazole, triadimefon, triadimenol, triticonazole | GC/MS or LC/MS |
| Herbicides | Acetamide | diphenamid, napropamide | GC/MS or LC/MS |
| | Aryloxyphenoxypropionate | cyhalofop-butyl, diclofop-methyl, fenoxaprop-ethyl, fluazifop, fluazifop-butyl, haloxyfop, haloxyfop-methyl, quizalofop, quizalofop-ethyl | LC/MS or GC/MS for esters; LC/MS for free acids |
| | Carbamate | chlorpropham, propham | GC/MS (or LC/MS) |
| | Chloroacetamide | acetochlor, alachlor, butachlor, dimethachlor, dimethenamid, metazachlorctofen, metolachlor, propachlor | GC/MS or LC/MS |
| | Cyclohexanedione oxime | alloxydim, clethodim, cycloxydim, sethoxydim, tralkoxydim | LC/MS |
| | Dinitroaniline | benfluralin, butralin, dinitramine, ethalfluralin, oryzalin, pendimethalin, trifluralin | GC/MS |
| | Diphenyl ether | acifluorfen, aclonifen, bifenox, fluoroglycofen-ethyl, fomesafen, lactofen, oxyfluorfen | GC/MS |
| | Imidazolinone | imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin, imazethapyr | LC/MS |
| | Quaternary ammonium | diquat, mepiquat, paraquat | LC/MS |
| | Phenoxycarboxylic acid | 2,4-D, 2,4-DB, clomeprop, dichlorprop, MCPA, MCPB, mecoprop, 2,4,5-T | LC/MS |
| | Phenylurea | chlorotoluron, diuron, fenuron, isoproturon, linuron, metoxuron, monolinuron, neburon | LC/MS |
| | Pyridazinone | chloridazon, norflurazon | LC/MS |
| | Pyridinecarboxylic acid | clopyralid, fluroxypyr, picloram, triclopyr | LC/MS |
| | Quinolinecarboxylic acid | quinclorac, quinmerac | LC/MS |
| | Sulfonylurea | amidosulfuron, azimsulfuron, bensulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, ethoxysulfuron, foramsulfuron, halosulfuron-methyl, metsulfuron-methyl, pirisulfuron-methyl, rimsulfuron, sulfometuron-methyl, thifensulfuron-methyl, triasulfuron, triflusulfuron-methyl | LC/MS |
| | Thiocarbamate | butylate, cycloate, di-allate, EPTC, molinate, pebulate, thiobencarb, tri-allate, vernolate | GC/MS |
| | Triazine | ametryn, atrazine, cyanazine, prometon, prometryn, propazine, simazine, simetryn, terbumeton, terbutryn | LC/MS or GC/MS |
| | Triazinone | hexazinone, metamitron, metribuzin | LC/MS or GC/MS |
| | Triazolinone | carfentrazone-ethyl, sulfentrazone | GC/MS or LC/MS |
| | Triazolopyrimidine | cloransulam-methyl, diclosulam, florasulam, flumetsulam, metosulam | GC/MS |
| | Uracil | bromacil, lenacil, terbacil | LC/MS or GC/MS |

# Annex II Example of a QuEChERS sample preparation protocol for GC/MS/MS analysis of pesticides in fruits and vegetables

This QuEChERS protocol example for sample preparation of fruits and vegetables is based on the AOAC 2007.01 method with acetate buffering.[11] It includes pre-extraction addition of a process control standard (chlorpyrifos-methyl) and postextraction addition of internal standards and analyte protectants (added to both samples and matrix-matched standards).

## A. Apparatus and material

(a) **GC/MS/MS system:** Agilent 7890A GC coupled to a 7000 or 7010 series triple quadrupole, equipped with a multimode inlet (for additional configuration see Annex IV or V, respectively).

(b) **Sample processing equipment:** Capable of chopping and blending to provide homogeneous fruit and vegetable samples.

(c) **Centrifuges:** Capable of achieving at least 1,500 rcf and holding 50 mL centrifuge tubes used for extraction, and 2 mL minitubes used for dSPE.

(d) **Analytical balances:** Accurate to at least three and four decimal places (1.0 and 0.1 mg).

(e) **Freezer and refrigerator:** Capable of continuous operation at or below –20 °C and approximately +4 °C, respectively.

(f) **Shaker (optional):** Capable of shaking 50 mL centrifuge tubes.

(g) **Vibrational device (optional):** For example, a vortex mixer.

(h) **Automatic pipettes:** Capable of accurately transferring volumes of 10 to 1,000 µL (preferably positive displacement pipettes, suitable for handling organic solvents).

(i) **50 mL centrifuge tubes:** For example, disposable 50 mL polypropylene or reusable 50 mL fluorinated ethylene propylene centrifuge tubes with screw caps (to be used for sample extraction).

(j) **Spatula/spoon:** For transferring sample into centrifuge tubes.

(k) **Solvent dispenser:** For transferring 15 mL of 1% acetic acid in acetonitrile.

(l) **Assorted laboratory glassware:** For example, volumetric flasks, volumetric pipettes, beakers, or funnels.

(m) **Amber glass autosampler vials (2 mL, screw cap):** For automated injection into the GC/MS/MS system.

## B. Reagents

(a) **Acetonitrile:** Quality must be of sufficient purity that is free of interfering compounds (HPLC-grade or better).

(b) **Water:** Quality must be of sufficient purity that is free of interfering compounds (HPLC-grade or better).

(c) **Acetic acid:** Glacial; quality must be of sufficient purity that is free of interfering compounds (ACS-grade or better).

(d) **Preweighed salt mixture for the AOAC buffered QuEChERS method:** 6 g of anhydrous magnesium sulfate ($MgSO_4$) and 1.5 g of sodium acetate (NaOAc), such as Agilent p/n 5982-6755 or 5982-7755.

(e) **Preweighed sorbent mixture for the dispersive SPE cleanup:** Containing 150 mg anhydrous $MgSO_4$, 50 mg primary secondary amine (PSA), and 50 mg $C_{18}$ in 2 mL centrifuge tubes, such as Agilent p/n 5982-5122.

(f) **L-gulonic acid γ-lactone** (L-gulonolactone), CAS # 1128-23-0: >95% purity.

(g) **D-Sorbitol**, CAS # 50-70-4: >95% purity.

(h) **Helium:** UHP, used as GC carrier gas.

(i) **Nitrogen:** UHP, used as GC/MS/MS collision gas.

(j) **Toluene (optional):** Quality must be of sufficient purity that is free of interfering compounds for preparation of individual stock solutions of pesticides with limited solubility or stability in acetonitrile.

(k) **Pesticide standards:** High-purity reference standards of the pesticide analytes, obtained as neat materials, individual solutions, or composite solutions in suitable solvents.

(l) **Process control standards:** High-purity reference standards of suitable process control compounds to be added to the sample before the extraction, for example, chlorpyrifos-methyl (CAS no. 5598-13-0).

(m) **Internal standards (ISTDs):** High-purity reference standards of compounds suitable to serve as ISTDs or QC standards, for example, triphenyl phosphate (TPP, CAS no. 115-86-6), $d_{10}$-parathion, or $^{13}C_{12}$-*p,p'*-DDT.

(n) **Blank matrix samples:** Verified to be free of analytes above the detection limit; used for preparation of matrix blanks, spikes, and matrix-matched standards.

## C. Reagent solution preparation

**Notes:** Store solutions (b)–(i) in a freezer at or below –20 °C, and solutions (j)–(l) in a refrigerator at approximately +4 °C. Matrix-matched standards should be prepared together with the sample batch and analyzed the same day (store in a refrigerator at approximately +4 °C if necessary).

(a) **1% Acetic acid in acetonitrile:** Add 10 mL of glacial acetic acid to a 1,000 mL volumetric flask. Bring to volume with acetonitrile and mix thoroughly. This solution is used for the sample extraction and preparation of certain standard solutions.

(b) **Pesticide individual stock solutions (optional):** Prepare or obtain individual stock solutions of the pesticide analytes at concentrations that allow the preparation of composite solutions, such as at 2,000 to 5,000 µg/mL. Appropriate solvents should be used that are compatible with the sample preparation method and analyte in terms of solubility and stability (preferably acetonitrile or toluene).

(c) **Pesticide composite stock solutions:** Prepare or obtain composite stock solutions of the pesticide analytes at concentrations that allow the preparation of an intermediate composite standard solution (for example, at 10 µg/mL) of all analytes to be used for the preparation of working standard solutions. Appropriate solvents should be compatible with the sample preparation method and analyte in terms of solubility and stability (preferably acetonitrile or toluene). The acetonitrile composite solutions, containing base-sensitive pesticides, should be acidified at 1% with acetic acid.

(d) **Process control and ISTD individual stock solutions:** Prepare individual stock solutions of the selected compounds at 500 to 5,000 µg/mL in acetonitrile (or toluene if needed).

(e) **Process control working solutions:** Prepare a process control working solution (for example, at 10 µg/mL) in acetonitrile to be added to the samples before extraction.

(f) **Pesticide composite intermediate (spiking) solution:** Using the pesticide composite stock solutions and the process control stock solution, prepare a composite stock solution of the pesticide analytes (including the process control compound) in acetonitrile (or acetonitrile with 1% acetic acid if base-sensitive analytes are in the mixture) at a concentration (for example, at 10 µg/mL) that allows the preparation of working standard solutions.
**Note:** Uniform concentration level is recommended for every pesticide to simplify the standard preparation and data processing/reporting.

(g) **ISTD composite stock and intermediate solutions:** If more than one ISTD is used, prepare an ISTD composite stock solution (for example, at 50 µg/mL) and use it to assemble an intermediate ISTD solution (for example, at 5 µg/mL) in acetonitrile with 1% acetic acid.

(h) **ISTD working solution:** Prepare an ISTD working solution (for example, at 500 ng/mL) in 1% acetic acid in acetonitrile to be added to the sample extracts before the GC/MS/MS analysis (postextraction and cleanup).

(i) **Pesticide working solutions:** Prepare pesticide working solutions in 1% acetic acid in acetonitrile to be used for the preparation of matrix-matched standards. The working solutions should contain ISTDs at a constant concentration (for example, at 500 ng/mL), and pesticide analytes and process control compounds at appropriate concentration levels.

The following table gives an example of the preparation of a set of pesticide working standards (10 mL in 1% acetic acid in acetonitrile) at 100 to 1,000 ng/mL (with ISTDs at 500 ng/mL) that are used to prepare matrix-matched standards at levels corresponding with 10 to 100 ng/g in the sample (see section **C(m)**, below). Add the following volumes of the pesticide composite intermediate solution (concentration = 10 µg/mL) and ISTD intermediate solution (concentration = 5 µg/mL) to a 10 mL volumetric flask. Bring to volume with 1% acetic acid in acetonitrile and mix thoroughly.

| Pesticide working solution | Pesticide composite intermediate solution (10 µg/mL) | ISTD intermediate solution (5 µg/mL) |
|---|---|---|
| Concentration (ng/mL) | Volume (µL) | Volume (µL) |
| 100 | 100 | 1,000 |
| 200 | 200 | 1,000 |
| 400 | 400 | 1,000 |
| 600 | 600 | 1,000 |
| 1,000 | 1,000 | 1,000 |

(j) **L-Gulonolactone stock solution:** Weigh approximately 500 mg of L-gulonolactone in a 10- mL volumetric flask. Add 4 mL of water then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(k) **D-Sorbitol stock solution:** Weigh approximately 500 mg of D-sorbitol in a 10- mL volumetric flask. Add 5 mL of water then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(l) **Analyte protectant (AP) solution (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol composite solution):** Add 4 mL of the L-gulonolactone stock solution and 2 mL of the D-sorbitol stock solution into a 10- mL volumetric flask and bring to volume with acetonitrile.

(m) **Matrix-matched standards:** Prepare a blank extract as described in the sample preparation procedure. Add appropriate pesticide working solutions (to obtain desirable concentration levels) and an AP solution (the volume depends on the matrix-matched standard final volume and GC injection volume).

The following table gives an example of preparation of a set of matrix-matched standards at levels corresponding with 10 to 100 ng/g in the sample. The AP solution volume is based on the 2 µL injection volume in the **Annex IV** method **(Page 93)**.

| Matrix-matched standard | Pesticide working solution | | Blank extract | AP solution |
|---|---|---|---|---|
| Concentration (ng/g) | Concentration (ng/mL) | Volume (µL) | Volume (µL) | Volume (µL) |
| 10 | 100 | 25 | 250 | 10 |
| 20 | 200 | 25 | 250 | 10 |
| 40 | 400 | 25 | 250 | 10 |
| 60 | 600 | 25 | 250 | 10 |
| 100 | 1000 | 25 | 250 | 10 |

**Note:** If the pesticide working solutions do not contain ISTDs, they need to be added separately to the matrix-matched standards at this point, using the same volume and concentration of the ISTD working solution as added to the final sample extracts (for example, 25 µL of a 500 ng/mL ISTD working solution). The matrix concentration (dilution) of the sample extracts and matrix-matched standards need to be the same, so the sample extract volume must be adjusted by adding acetonitrile (25 µL in this example) if the ISTD working solution is added to matrix-matched standards separately.

## D. Sample preparation procedure

1. Weigh 15.0 ±0.3 g of thoroughly homogenized sample into a 50- mL centrifuge tube. **Note:** Fruit and vegetable samples should be extracted frozen or when in the process of thawing.
2. Add an appropriate volume of the **process control working solution to each test sample** (for example, add 75 µL of 10 µg/mL process control working solution to fortify the sample at 50 ng/g with chlorpyrifos-methyl). For the **spike recovery samples**, add an appropriate volume of the pesticide composite intermediate (spiking) solution (for example, add 75 µL of 10 µg/mL pesticide spiking solution to fortify the sample at 50 ng/g with the analytes; do not add any process control solution if the pesticide spiking solution contains the process control compound (or compounds)). Vortex the mix briefly, and leave standing at room temperature for approximately 15 minutes to ensure the pesticide-sample interaction occurs. **Do not add any process control or pesticide spiking solution to the matrix blanks to be used for matrix-matched calibration standards.**
3. Using a solvent dispenser, add 15 mL of 1% acetic acid in acetonitrile to each tube.
4. Shake vigorously for approximately 1 minute by hand or use a suitable shaker.
5. Add 6 g of anhydrous $MgSO_4$ and 1 g of NaOAc to each tube and seal the tube well. To prevent leaking, ensure that the salts do not get into the screw threads or rim of the tube.

6. Immediately after the salt addition, start shaking/vortexing each tube for several seconds to ensure that crystalline agglomerates (formed by $MgSO_4$ in the presence of water) are broken up sufficiently. Then, shake the tubes vigorously by hand or vortex/shake for approximately 1 minute (this can be done in parallel for the entire batch).
7. Centrifuge the tubes at >1,500 rcf for approximately 5 minutes.
8. Transfer 1 mL of the acetonitrile extract (upper layer) to a dispersive SPE tube containing 150 mg $MgSO_4$ + 50 mg PSA + 50 mg $C_{18}$. **Notes:** A small amount of graphitized carbon black (GCB), for example, 7.5 mg per mL extract, can be added for matrices with a high content of chlorophyll or carotenoids. If needed or desirable (for example, for the cleanup of blank extracts used for preparation of several matrix-matched standards), scale up this step and use 150*X mg $MgSO_4$ + 50*X mg PSA + 50*X mg $C_{18}$ (+ 7.5*X mg GCB) per X mL of the extract.
9. Seal the dispersive SPE tube well and shake/mix by hand or use a vortex mixer for approximately 30 seconds. Avoid prolonged contact of the extract with the sorbents.
10. Centrifuge the dispersive SPE tube at >1,500 rcf for approximately 1 minute.
11. Immediately transfer 250 µL of the final extract (supernatant without any particles) from the dispersive SPE tube to an amber glass autosampler vial. **Note:** Different volumes than 250 µL can be transferred, but the volumes of the ISTD and AP solutions added to the extract have to be adjusted accordingly.
12. For **test samples, spikes, reagent blanks, and matrix blanks** (to be analyzed as blanks), add 25 µL of the 500 ng/mL ISTD working solution in 1% acetic acid in acetonitrile. **Note:** This will result in an ISTD concentration equivalent to 50 ng/g in the sample, which is the same concentration as in the matrix-matched standard set example provided in **Annex II, section C, step (m)** (Page 88). For **matrix-matched calibration standards**, add 25 µL of the appropriate pesticide working solution to the blank extract (see section **Annex II, section C, step (m)** for the matrix-matched standard preparation procedure).
13. Add 10 µL of the AP protectant solution to all extracts (samples, spikes, blanks, and matrix-matched standards) in the amber glass autosampler vials.
14. Cap the vials and vortex briefly. Analyze by GC/MS/MS using the **Annex IV** or **Annex V** method.

# Annex III Examples of pesticides that require special consideration when using QuEChERS

The following table provides examples of compounds that can be analyzed by the QuEChERS method but require special considerations in certain matrices (such as acidic compounds in neutral/less acidic matrices, basic compounds in acidic matrices, or lipophilic pesticides in matrices with a higher fat content), when using certain dSPE sorbents (for example, planar pesticides with GCB or acidic pesticides with PSA) or in general, such as base- or acid-sensitive pesticides.

| Pesticide type | Examples of compounds | Problem | Solution |
|---|---|---|---|
| Acidic | Aryloxyphenoxypropionate free acids (cyhalofop, diclofop, fenoxaprop, haloxyfop, fluazifop, quizalofop), dicamba, imidazoline acidic herbicides (imazamethabenz, imazamox, imazapic, imazapyr, imazaquin, imazethapyr), phenoxycarboxylic acids (2,4-D, 2,4-DB, clomeprop, dichlorprop, MCPA, MCPB, mecoprop, 2,4,5-T), pyridinecarboxylic acids (clopyralid, fluroxypyr, picloram, triclopyr), quinolinecarboxylic acids (quinclorac, quinmerac) | Potential losses during the partition step in neutral/less acidic matrices | Use buffering during the extraction/partition step<br>Note: To release free acids from conjugated forms (for full compliance with certain residue definitions), use alkaline hydrolysis prior to the QuEChERS extraction61. |
| | | Retention by PSA | Avoid the use of PSA; skip the cleanup step and analyze acidic pesticides directly in the diluted raw extract by LC/MS(/MS) in negative ESI (derivatization for GC/MS(/MS) also possible). |
| Basic | Aminocarb, carbendazim, imazalil, pymetrozine, thiabendazole | Potential losses during the partition step in acidic matrices | Use buffering during the extraction/partition step |
| Acid-sensitive | Amitraz, benfuracarb, carbosulfan, sulfonylurea herbicides (amidosulfuron, azimsulfuron, bensulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, ethoxysulfuron, foramsulfuron, halosulfuron-methyl, metsulfuron-methyl, pirisulfuron-methyl, rimsulfuron, sulfometuron-methyl, thifensulfuron-methyl, triasulfuron, triflusulfuron-methyl) | Degradation in the presence of acids (at lower pH) | Eliminate the use of acid in the procedure, especially the addition of acetic or formic acid (at approximately 0.1 %) to the final extract for stabilization of base-sensitive pesticides<br>**Notes:** Amitraz has a common moiety residue definition in the EU (amitraz and metabolites containing the 2,4-dimethylaniline moiety), thus its main metabolites N-2,4-dimethylphenyl-N-methyl-formamidine (DMPF) and 2,4-dimethylformanilide (DMF, 2,4-dimethylphenylformamide) should be monitored in multiresidue methods.<br>Benfuracarb and carbosulfan degrade to carbofuran in acidic conditions. All three pesticides have separate MRLs in the EU, thus an additional analysis of non-acidified extract should be performed if carbofuran is found in the sample. In the US, only carbofuran has a tolerance set for carbofuran and its carbamate and phenolic metabolites. |

| Pesticide type | Examples of compounds | Problem | Solution |
|---|---|---|---|
| Base-sensitive | Captan, chlorothalonil, dichlofluanid, dicofol, folpet, tolylfluanid | Degradation in the presence of basic compounds (at higher pH); unstable even in acetonitrile | Use buffering during the extraction/partition step and acidify the final extract (at approximately 0.1 % of acetic or formic acid) and all solutions in acetonitrile (prepare stock solutions in toluene)[10]<br><br>**Notes:** N-trihalomethythio fungicides (captan, folpet, dichlofluanid, tolylfluanid) and dicofol are also known to degrade in the GC system, thus their analysis is complicated in general.[10] Monitor their degradation products 1,2,3,6-tetrahydrophthalimide (THPI), phthalimide, N',N'-dimethyl-N-phenylsulfonyldiamid (DMSA), N',N'-dimethyl-N-p-tolylsulfonyldiamide (4-dimethylaminosulphotoluidide, DMST), and 4,4'-dichlorobenzophenone, respectively.<br><br>Unfortunately, these degradation products/ metabolites are not part of respective residue definitions, except for THPI in captan residue definition in animal commodities in the US or DMST in the EU tolyfluanid residue definition. Thus in commodities with set MRL/tolerance, monitoring of these compounds can mostly serve only as an indication of the use of a given pesticide and not for compliance purposes. |
| Lipophilic | Aldrin, chlordane, DDD, DDE, DDT, dicofol, dieldrin, endosulfan, endrin, heptachlor, hexachlorobenzene, mirex, pentachloroaniline, pentachloroanisole, pentachlorothioanisole (MPCPS), permethrin, prothiophos, quintozene | Potential losses due to partition between the acetonitrile and fat/oil layer | For samples with a higher lipid content, decrease the sample to acetonitrile ratio by reducing sample size to form smaller fat/oil layer (separated from the acetonitrile extract), thus increase the partition of lipophilic pesticides into the acetonitrile extract.[15, 62] Use a suitable QC standard (for example, PCB 138 or 153) to monitor losses of lipophilic pesticides in samples with a higher fat content. Use standard addition procedure for accurate quantitation or adjust the results for lower recoveries, which are typically consistent for a given sample type. |
| Planar | Carbendazim, chlorothalonil, coumaphos, cyprodinil, hexachlorobenzene, pentachloroaniline, pentachlorothioanisole (MPCPS), thiabendazole | Retention by GCB | Avoid using GCB in the dSPE cleanup, especially for matrices that do not contain higher amounts of chlorophyll or carotenoid pigments. For highly pigmented matrices, use only very small amount of GCB (for example, 7.5 mg per mL extract), which still leaves some pigments in the extract but gives acceptable recoveries for planar pesticides. Use a suitable QC standard (for example, d10-anthracene or d10-phenanthrene) to monitor losses of planar compounds if GCB is used. |

# Annex IV Example of a GC/MS/MS legacy method for pesticide multiresidue analysis using a 7000 series instrument

## GC/MS/MS instrument configuration

| | |
|---|---|
| Gas chromatograph | Agilent 7890 or 8890 series GC |
| Mass spectrometer | 7000 series Agilent triple quadrupole MS |
| MS source | EI with extractor |
| Inlet | Multimode inlet (MMI) with air cooling |
| Liner | 2 mm id dimpled liner (p/n 5190-2297) |
| Autosampler | 7693A automatic liquid sampler (ALS) |
| Backflushing | Purged Ultimate union (PUU) controlled by either a pneumatics control module (PCM), AUX EPC module, or pneumatic switching device(PSD) |
| Carrier gas | Helium |
| Column 1 | HP-5ms UI, 5 m × 250 µm, 0.25 µm (p/n G3903-61005 or cut from 15 m, 30 m, or 60 m columns, p/n 19091S-431 UI, 19091S-433 UI, or 19091S-436 UI, respectively)<br>Configured from the MMI to AUX EPC, PCM, or PSD |
| Column 2 | HP-5ms UI, 15 m × 250 µm, 0.25 µm (p/n 19091S-431 UI)<br>Configured from the AUX EPC, PCM, or PSD to vacuum |

## GC injection conditions

| | |
|---|---|
| Mode | Solvent vent |
| Injection volume | 2 µL (syringe size: 5 µL) |
| Solvent washes | Preinjection: 1x solvent A (4 µL) |
| | Postinjection: 5x solvent A and 5x solvent B (4 µL each) |
| Sample wash | 1x 2 µL |
| Sample pumps | 5 |
| Injection speed | Fast |
| MMI temperature program | 60 °C for 0.35 minutes; then 900 °C/min to 280 °C (15 minutes hold); then 900 °C/min to 300 °C (until the end of the analysis) |
| Purge flow to split vent | 50 mL/min at 1.5 minutes |
| Vent flow | 25 mL/min |
| Vent pressure | 5 psi until 0.3 minutes |
| Gas saver | 20 mL/min at 5 minutes |
| Septum purge flow | 3 mL/min |
| Air cooling (Cryo) | On at 100 °C (MMI Liquid N2 option selected on GC for air cooling) |

## GC oven conditions

| | |
|---|---|
| Oven temperature program | 60 °C for 1.5 minutes; then 50 °C/min to 160 °C; then 8 °C/min to 240 °C; then 50 °C/min to 280 °C (2.5 minutes hold); then 100 °C/min to 290 °C (1.1 minutes hold) |
| Run time | 18 minutes |
| Postrun | 0.5 minutes at 290 °C |

## GC column flow conditions

| | |
|---|---|
| Column 1 flow program | 1.1 mL/min for 15.2 minutes; then 100 mL/min per min to −2.283 mL/min (flow balanced with the column 2 flow to achieve 2 psi inlet pressure) until the end of the analysis<br>Postrun: −10.683 mL/min |
| Column 2 flow program | 1.2 mL/min until the end of the analysis Postrun: 4 mL/min |
| (Retention time locking) | Chlorpyrifos-methyl locked at 8.524 minutes |

## MS conditions

| | |
|---|---|
| MS source | EI, −70eV |
| Source temperature | 280 °C |
| Quadrupole temperature | 150 °C |
| Transfer line temperature | 280 °C |
| Solvent delay | 4.0 minutes |
| He quench gas | 2.25 mL/min |
| N2 collision gas | 1.5 mL/min |
| Acquisition mode | Multiple reaction monitoring (MRM) |
| MS1/MS2 resolution | Wide |

## Time segments

| Index | Start Time (min) | Scan Type | Gain |
|---|---|---|---|
| 1 | 4.00 | MRM | 10 |
| 2 | 6.12 | MRM | 10 |
| 3 | 7.28 | MRM | 10 |
| 4 | 7.68 | MRM | 10 |
| 5 | 8.33 | MRM | 10 |
| 6 | 9.20 | MRM | 10 |
| 7 | 9.37 | MRM | 10 |
| 8 | 9.68 | MRM | 10 |
| 9 | 9.99 | MRM | 10 |
| 10 | 10.22 | MRM | 10 |
| 11 | 10.34 | MRM | 10 |
| 12 | 10.44 | MRM | 10 |
| 13 | 10.59 | MRM | 10 |
| 14 | 11.10 | MRM | 10 |
| 15 | 11.50 | MRM | 10 |
| 16 | 11.85 | MRM | 10 |
| 17 | 12.56 | MRM | 10 |
| 18 | 13.41 | MRM | 10 |
| 19 | 13.84 | MRM | 10 |
| 20 | 14.01 | MRM | 10 |
| 21 | 14.32 | MRM | 10 |
| 22 | 14.39 | MRM | 10 |
| 23 | 14.63 | MRM | 10 |
| 24 | 15.14 | MRM | 10 |
| 25 | 16.52 | MRM | 10 |

## Segment details

**Note:** Lines highlighted in gray are duplicated MRMs for isomers, which are excluded from the segments to eliminate double or multiple entries in the acquisition method. Lindane (gamma-HCH) is excluded from segment 2, but kept in segment 3 in this acquisition method example. All isomer peaks that require separate integration and quantitation should be included in the MassHunter quantitation method.

# Annex V Example of a GC/MS/MS legacy method for pesticide multiresidue analysis using a 7010 series instrument

## GC/MS/MS instrument configuration

| | |
|---|---|
| Gas chromatograph | Agilent 7890 or 8890 series GC |
| Mass spectrometer | 7010 Agilent triple quadrupole MS |
| MS source | EI, high-efficiency source (HES) |
| Inlet | Multimode inlet (MMI) with air cooling |
| Liner | 2 mm id dimpled liner (p/n 5190-2297) |
| Autosampler | 7693A automatic liquid sampler (ALS) |
| Backflushing | Purged Ultimate union (PUU) controlled by either a pneumatics control module (PCM), AUX EPC module, or pneumatic switching device (PSD). |
| Carrier gas | Helium |
| Column 1 | HP-5ms UI, 5 m × 250 µm, 0.25 µm (p/n G3903-61005 or cut from a 15 m, 30 m, or 60 m columns, p/n 19091S-431 UI, 19091S-433 UI, or 19091S-436 UI, respectively) Configured from the MMI to AUX EPC, PCM, or PSD |
| Column 2 | HP-5ms UI, 15 m × 250 µm, 0.25 µm (p/n 19091S-431 UI) Configured from the AUX EPC, PCM, or PSD to vacuum |

## GC injection conditions

| | |
|---|---|
| Mode | Cold splitless |
| Injection volume | 0.5 µL (syringe size: 5 µL) |
| Solvent washes | Preinjection: 1x solvent A (4 µL)<br>Postinjection: 5x solvent A and 5x solvent B (4 µL each) |
| Sample wash | 1x 1 µL |
| Sample pumps | 5 |
| Injection speed | Fast |
| MMI temperature program | 60 °C for 0.35 minutes; then 900 °C/min to 280 °C (15 minute hold); then 900 °C/min to 300 °C (until the end of the analysis) |
| Purge flow to split vent | 50 mL/min at 1.5 minutes |
| Gas saver | 20 mL/min at 5 minutes |
| Septum purge flow | 3 mL/min |
| Air cooling (Cryo) | On at 100 °C (MMI Liquid N2 option selected on GC for air cooling) |

## GC oven conditions

| | |
|---|---|
| Oven temperature program | 60 °C for 1.5 minutes; then 50 °C/min to 160 °C; then 8 °C/min to 240 °C; then 50 °C/min to 280 °C (2.5 minute hold); then 100 °C/min to 290 °C (2.1 minutes hold) |
| Run time | 19 minutes |
| Postrun | 0.5 minutes at 290 °C |

## GC column flow conditions

| | |
|---|---|
| Column 1 flow program | 1.1 mL/min for 15.5 min; then 100 mL/min per min to –2.283 mL/min (flow balanced with the column 2 flow to achieve 2 psi inlet pressure) until the end of the analysis<br>Postrun: –10.683 mL/min |
| Column 2 flow program | 1.2 mL/min until the end of the analysis Postrun: 4 mL/min |
| (Retention time locking) | Chlorpyrifos-methyl locked at 8.524 minutes |

## MS conditions

| | |
|---|---|
| MS source | EI, –70eV |
| Source temperature | 280 °C |
| Quadrupole temperature | 150 °C |
| Transfer line temperature | 280 °C |
| Solvent delay | 3.5 minutes |
| He quench gas | 2.25 mL/min |
| N2 collision gas | 1.5 mL/min |
| Acquisition mode | Dynamic multiple reaction monitoring (dMRM) |
| Acquisition rate | 3.3 cycles/s |
| Gain factor | 10 |
| MS1/MS2 resolution | Wide |

# Annex VI Example of a GC/MS/MS method for pesticide multiresidue analysis using a 7000 or 7010 series instrument using helium carrier gas[63]

| GC | |
|---|---|
| Agilent 8890 with fast oven, auto injector, and tray | |
| Inlet | Multimode inlet (MMI) |
| Mode | Splitless |
| Purge Flow to Split Vent | 60 mL/min at 0.75 min |
| Septum Purge Flow | 3 mL/min |
| Septum Purge Flow Mode | Switched |
| Injection Volume | 1.0 µL |
| Injection Type | Standard |
| L1 Airgap | 0.2 µL |
| Gas Saver | On at 20 mL.min after 3 min |
| Inlet Temperature | 60 °C for 0.1 min, then to 280 °C at 600 °C/min |
| Post Run Inlet Temperature | 310 °C |
| Post Run Total Flow | 25 mL/min |
| Carrier Gas | Helium |
| Inlet Liner | Agilent Ultra Inert 2mm dimpled liner (p/n 5190-2297) |
| **Oven** | |
| Initial Oven Temperature | 60 °C |
| Initial Oven Hold | 1 min |
| Ramp Rate 1 | 40 ° C/min |
| Final Temp 1 | 170 °C |
| Final Hold 1 | 0 min |
| Ramp Rate 2 | 10 °C |
| Final Temp 2 | 310 °C |
| Final Hold 2 | 2.25 min |
| Total Run TIme | 20 min |
| Post Run Time | 1.5 min |
| Equilibration Time | 0.25 min |

| Column 1 | |
|---|---|
| Type | Agilent HP-5ms UI (p/n 19091S-431UI-KEY) |
| Length | 15 m |
| Diameter | 0.25 mm |
| Film Thickness | 0.25 µm |
| Control Mode | Constant flow |
| Flow | 1.016 mL/min |
| Inlet Connection | Mulitmode inlet (MMI) |
| Outlet Connection | PSD (PUU) |
| PSD Purge Flow | 5 mL/min |
| Post Run Flow (Backflushing) | –7.873 |
| **Column 2** | |
| Type | Agilent HP-5ms UI (p/n 19091S-431UI-KEY) |
| Length | 15 m |
| Diameter | 0.25 mm |
| Film Thickness | 0.25 µm |
| Control Mode | Constant flow |
| Flow | 1.216 mL/min |
| Inlet Connection | PSD (PUU) |
| Outlet Connection | MSD |
| Post Run Flow (Backflushing) | 8.202 |

| MSD | |
|---|---|
| Model | Agilent 7000E or 7010C |
| Source | Inert Extractor Source with a 3 mm lens or HES |
| Vacuum Pump | Performance turbo |
| Tune File | Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xmml |
| Solvent Delay | 3 min |
| Quad Temperature (MS1 and MS2) | 150 °C |
| Source Temperature | 280 °C |
| Mode | dMRM or Scan |
| He Quench Gas | 2.25 mL/min |
| $N_2$ Collision Gas | 1.5 mL/min |
| **MRM Statistics** | |
| Total MRMs (dMRM Mode) | 614 |
| Minimum Dwell Time | 6.85 ms |
| Minimum Cycle Time | 69.8 ms |
| Maximum Concurrent MRMs | 52 |
| EM Voltage Gain Mode | 10 |
| **Scan Parameters** | |
| Scan Type | MS1 Scan |
| Scan Range | 45 to 450 *m/z* |
| Scan Time (ms) | 22 |
| Step Size | 0.1 amu |
| Threshold | 0 |
| EM Voltage Gain Mode | 1 |

# Annex VII Example of a GC/MS/MS method for pesticide multiresidue analysis using a 7000 or 7010 series instrument using hydrogen carrier gas[69]

| GC | |
|---|---|
| Model | Agilent 8890 with fast oven, auto injector, and tray |
| Inlet | Multimode inlet (MMI) |
| Mode | Solvent Vent |
| Purge Flow to Split Vent | 60 mL/min at 2.56 min |
| Septum Purge Flow | 3 mL/min |
| Vent Flow | 100 mL/min |
| Vent Pressure | 5 psi until 0.06 min |
| Septum Purge Flow Mode | Switched |
| Cryo | On (Air) |
| Cryo Use Temperature | 200 °C |
| Injection Volume | 2.0 µL |
| L1 Airgap | 0.2 µL |
| Gas Saver | Off |
| Inlet Temperature | 60 °C for 0.06 min, then to 280 °C at 600 °C/min |
| Post Run Inlet Temperature | 310 °C |
| Post Run Total Flow | 25 mL/min |
| Carrier Gas | Hydrogen |
| Inlet Liner | Agilent Ultra Inert 2 mm dimpled liner |
| Inlet Liner Part Number | 5190-2297 |
| **Oven** | |
| Initial Oven Temperature | 60 °C |
| Initial Oven Hold | 1 min |
| Ramp Rate 1 | 40 ° C/min |
| Final Temp 1 | 170 °C |
| Final Hold 1 | 0 min |
| Ramp Rate 2 | 10 °C/min |
| Final Temp 2 | 310 °C |
| Final Hold 2 | 2.25 min |

| Oven | |
|---|---|
| Total Run TIme | 20 min |
| Post Run Time (Backflush Duration | 1.5 min |
| Equilibration Time | 0.5 min |
| **Column 1** | |
| Type | Agilent HP-5ms UI (p/n 19091S-577UI) |
| Length | 20 m |
| Diameter | 0.18 mm |
| Film Thickness | 0.18 µm |
| Control Mode | Constant Flow |
| Flow | 1.0 mL/min (nominal before retention time locking) |
| Inlet Connection | Mulitmode inlet (MMI) |
| Outlet Connection | PSD (PUU) |
| PSD Purge Flow | 5 mL/min |
| Post Run Flow (Backflushing) | –6.260 mL/min |
| **Column 2** | |
| Type | Agilent HP-5ms UI (p/n 19091S-431UI-KEY) |
| Length | 15 m |
| Diameter | 0.25 mm |
| Film Thickness | 0.25 µm |
| Control Mode | Constant flow |
| Flow | 1.216 mL/min |
| Inlet Connection | PSD (PUU) |
| Outlet Connection | MSD |
| Post Run Flow (Backflushing) | 8.202 |
| **MSD** | |
| Model | Agilent 7000E or 7010C |
| Source | Inert Extractor Source with a 3 mm lens or HES |
| Vacuum Pump | Performance turbo |

| MSD | |
|---|---|
| Tune File | Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xmml |
| Solvent Delay | 3 min |
| Quad Temperature (MS1 and MS2) | 150 °C |
| Source Temperature | 280 °C |
| Mode | dMRM or Scan |
| He Quench Gas | 2.25 mL/min |
| $N_2$ Collision Gas | 1.5 mL/min |
| **MRM Statistics** | |
| Total MRMs (dMRM Mode) | 614 |
| Minimum Dwell Time | 6.85 ms |
| Minimum Cycle Time | 69.8 ms |
| Maximum Concurrent MRMs | 52 |
| EM Voltage Gain Mode | 10 |
| **Scan Parameters** | |
| Scan Type | MS1 Scan |
| Scan Range | 45 to 450 *m/z* |
| Scan Time (ms) | 22 |
| Step Size | 0.1 amu |
| Threshold | 0 |
| EM Voltage Gain Mode | 1 |
| Agilent MassHunter Workstation revisions 10 or above | – MassHunter Acquisition software for GC/MS systems<br>– MassHunter Quantitative Analysis<br>– Unknowns Analysis Quantitative Analysis<br>– MassHunter Qualitative Analysis |

# Annex VIII Rapid analysis of pesticides in food using LC/MS/MS and GC/MS/MS consumable workflow ordering guide

Pigmented fresh fruits and vegetables contain highly abundant natural pigments, such as chlorophyll and lutein from green vegetables, anthocyanidins and anthocyanins from red, blue, purple, and black fruits, and carotenoids and xanthophylls from orange and yellow fruits and vegetables. Enhanced cleanup to remove pigment co-extractives before direct injection on analytical instruments is vital to avoid matrix effects, such as ion suppression on LC/MS/MS, matrix interferences on GC/MS/MS, and matrix deposition on the detection flow path and MS source.

Agilent Carbon S sorbent, an advanced hybrid carbon material with optimized carbon content and pore structure, provides equivalent or better pigment removal from plant sample matrices compared to graphitized carbon black (GCB). As a result, Carbon S sorbent delivers a better balance between analyte recovery and matrix pigment removal efficiency (**Figure 1**).

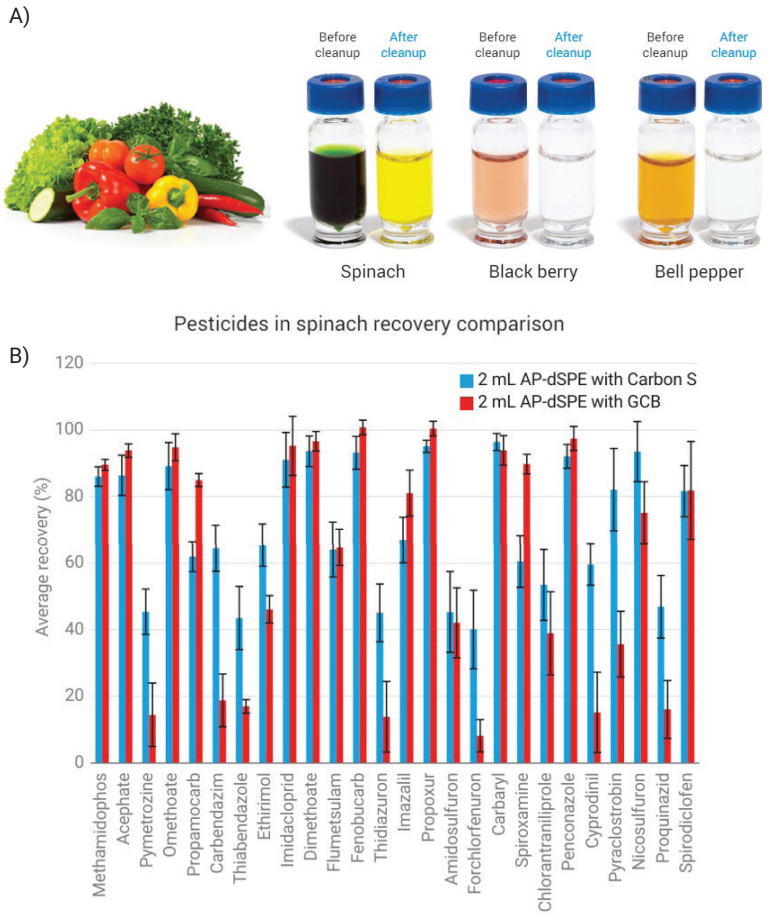


**Figure 1**. Carbon S products demonstrate a better balance between analyte recovery and matrix pigment removal efficiency. A) Efficient pigment removal for pigmented fresh fruits and vegetables.1 B) Improved planar pesticides recovery.[2]

Agilent dSPE kits with Carbon are a direct and easy replacement of dSPE kits with GCB. AOAC pigment dSPE with Carbon S kits are recommended as replacements for current dSPE cleanup of high chlorophyll leafy vegetables and Universal dSPE with Carbon S kits are recommended for general pigmented fresh produce.

Compared to traditional dSPE cleanup, passthrough cleanup provides simplified workflow steps (**Figure 2**), such as the elimination of uncapping and capping the dSPE tubes, vortexing, and centrifugation, while delivering highly efficient and selective matrix/pigment removal, improved target recovery and reproducibility, and reduced matrix effect and interferences.

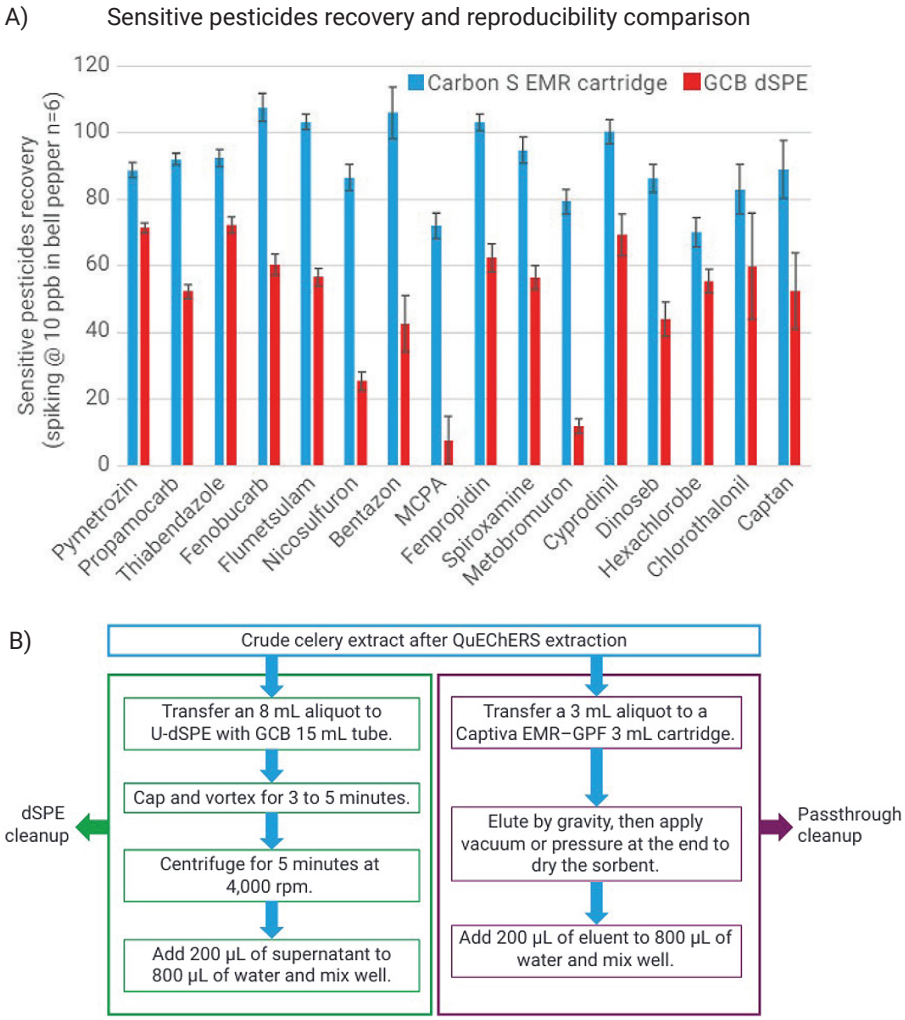


**Figure 2**. Compared to traditional dSPE cleanup, Captiva EMR Carbon S passthrough cleanup demonstrates significant improved recoveries for (A) sensitive pesticides and (B) a simplified workflow.[3,4]

A detailed description of all the Captiva EMR cartridges and their recommendations for plant-origin matrices are shown in **Table 1.**

**Table 1.** Agilent Captiva EMR cartridges and their recommendations for pesticide analysis of various plant-origin matrices.[5]

| Agilent Product Name | Sorbents | Sample Loading Volume | Recommendations Based on Sample Matrices | Examples of Applicable Sample Matrix |
|---|---|---|---|---|
| Captiva EMR–Lipid | Carbon EMR-Lipid | 2.5 to 3 mL for 3 mL cartridges<br>5 to 6 mL for 6 mL cartridges | High fatty oily matrices | Edible oil |
| Captiva EMR–HCF1 | Carbon S/$NH_2$ | 3 mL | High chlorophyll fresh leafy vegetables | Spinach, parsley, alfafa |
| Captiva EMR–HCF2 | Carbon S/PSA | 3 mL | High chlorophyll fresh leafy vegetables | Spinach, parsley, alfafa |
| Captiva EMR–GPF | Carbon S/PSA/EC-C18 | 3 mL | General pigmented fresh plant-origin matrix | Berries, peppers, broccoli, grapes |
| Captiva EMR–GPD | Captiva EMR–Lipid/PSA/EC-C18/Carbon S | 2.5 to 3 mL | General pigmented dry plant-origin matrix | Spices, tea, coffee |
| Captiva EMR–LPD | Captiva EMR–Lipid/PSA/EC-C18/Carbon S | 2.5 to 3 mL | Low/none pigmented dry plant-origin matrix | Nuts, light pigmented spices, tobacco |

The passthrough cleanup can be done by gravity elution or using a vacuum manifold. For analysis using LC/MS/MS, the sample eluent can then be directly injected onto the LC/TQ instrument or diluted further with water before injection.

When using GC/MS/MS for analysis, the sample eluent needs to be further dried using anhydrous $MgSO_4$ powder. The addition of $MgSO_4$ can be as simple as a small spatula of anhydrous $MgSO_4$ powder (~200 to 300 mg) from the Agilent Bond Elut QuEChERS EMR–Lipid polish pouch.[5]

The use of Carbon S products, especially the Captiva EMR cartridges, for pesticide analysis in pigment fresh fruits and vegetables demonstrates efficient matrix/pigment removal, higher pass rates for large panel pesticides analysis (**Figure 3**), reduced matrix ion suppression in LC/MS/MS detection, and cleaner matrix background in GC/MS/MS detection.[4] All these improvements make the multiple class multiresidue pesticides quantitative analysis in pigmented fresh fruits and vegetables more reliable and consistent.

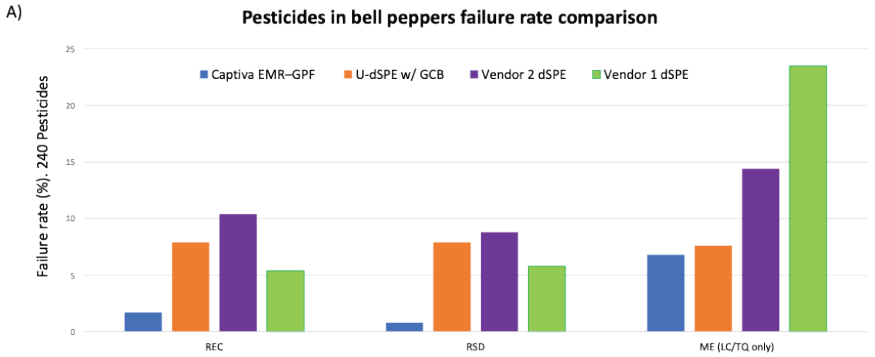


**Figure 3**. Captiva EMR Carbon S passthrough cleanup demonstrates a lower failure rate for larger panel pesticides quantitation.

## Easy selection and ordering information

To order items listed in the tables from the Agilent online store, add items to your Favorite Products list by clicking the MyList header links. Then, enter the quantities of the products you need, click Add to Cart and proceed to checkout. Your list will remain under Favorite Products for your use with future orders.

If this is your first time using Favorite Products, you will be asked to enter your email address for account verification. If you have an existing Agilent account, you will be able to log in. However, if you do not have a registered Agilent account, you will need to register for one. This feature is valid only in regions that are eCommerce enabled. All items can also be ordered through your regular sales and distributor channels.

**MyList 1:** Columns and supplies for analysis of pesticides in food using LC/MS/MS [2,6]

| | Description | Part Number |
|---|---|---|
| **Sample preparation** | **Extraction** [1] | |
| | Agilent Bond Elut QuEChERS extraction kit, AOAC 2007.01, with or without ceramic homogenizers | 5982-5755CH<br>5982-5755 |
| | Agilent Bond Elut QuEChERS EN extraction kit, with or without ceramic homogenizers | 5982-5650CH<br>5982-5650 |
| | Sample crude extract cleanup | |
| **For none or light pigmented fresh produce** | Agilent Bond Elut Dispersive kit, general fruits and vegetables, 15 mL, Agilent Bond Elut Dispersive kit, general fruits and vegetables, 2 mL | 5982-5058CH<br>5982-5122CH |
| **For general pigmented fresh produce** | Agilent Captiva EMR–GPF cartridge, 3 mL | 5610-2090 |
| **For high chlorophyll fresh vegetables** | Agilent Captiva EMR–HCF1, with NH2 or EMR–HCF2 with PSA, 3 mL[2] | 5610-2088<br>5610-2089 |
| **For low pigmented dry plant-origin food and essential oils** | Agilent Captiva EMR–LPD cartridge, 6 mL | 5610-2092 |
| **For general pigmented dry plant-origin food** | Agilent Captiva EMR–GPD cartridge, 6 mL | 5610-2091 |
| **For animal-origin food and oils** | Agilent Captiva EMR–Lipid cartridge, 3 mL<br>Agilent Captiva EMR–Lipid cartridge, 6 mL | 5910-1003<br>5910-1004 |
| | **Traditional alternative dSPE cleanup** | |
| **For general pigmented fresh produce** | Agilent Bond Elut QuEChERS Universal dispersive SPE kit, 2 mL, with Carbon S, 50 mg PSA, 50 mg C18, 7.5 mg Carbon S, 150 mg $MgSO_4$ | 5610-2058 |
| | Agilent Bond Elut QuEChERS Universal dispersive SPE kit, 15 mL, with Carbon S, 400 mg PSA, 400 mg C18, 45 mg Carbon S, 1,200 mg $MgSO_4$ | 5610-2060 |
| **For high chlorophyll fresh vegetables** | Agilent Bond Elut QuEChERS AOAC Pigmented Fruits and Vegetables dSPE kit with Carbon S, 2 mL | 5610-2062 |
| | Agilent Bond Elut QuEChERS Pigmented Fruits and Vegetables dSPE kit with Carbon S, 15 mL | 5610-2064 |
| | **Sample postfiltration** | |
| | Filter vial, nylon, 0.2 µm, 100/pk<br>Filter vial, RC, 0.2 µm, 100/pk | 5191-5936<br>5610-2125 |

| | Description | Part Number |
|---|---|---|
| | **Other sample preparation consumables** | |
| | Ceramic homogenizers, 50 mL tube, 100/pk [3] | 5982-9313 |
| | Centrifuge tube and cap, polypropylene, 50 mL, 25/pk | 5610-2049 |
| | Agilent positive pressure manifold-48 processor | 5191-4101* |
| | SPE cartridge rack, 6 mL, for PPM-48 | 5191-4104* |
| | SPE cartridge rack, 3 mL for PPM-48 | 5191-4103* |
| | Collection rack for 16 x 100 mm tubes | 5191-4108* |
| **Standards** | Ready-to-use 254-compound standards mix, 8 x 1 mL, 100 µg/mL each | 5190-0551 |
| **HPLC columns** | Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm column | 695775-902 |
| | InfinityLab Poroshell 120 EC-C18, 2.1 x 5 mm, 2.7 µm, guard column, 3/pk | 821725-911 |
| | Agilent ZORBAX Eclipse Plus C18 column, 2.1 x 100 mm, 1.8 µm | 959758-902 |
| | Agilent ZORBAX Eclipse Plus C18 column, UHPLC guard, 2.1 x 5 mm, 1.8 µm | 821725-901 |
| **HPLC supplies** | Agilent 1290 Infinity inline filter, 0.3 µm | 5067-6189 |
| | InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC | 5067-5957 |
| | InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC | 5067-6166 |
| | InfinityLab Quick Turn fitting, for column outlet | 5067-5966 |
| | InfinityLab Quick Turn capillary, 0.12 x 280 mm, for connection from column to detector | 5500-1191 |
| | Kit of Stay Safe waste cap GL45, with 4 ports and waste can, 6 L | 5043-1221 |
| | Charcoal filter with time strip for waste container | 5043-1193 |
| | Stainless steel solvent inlet filter, 10 µm pore size | 01018-60025 |
| **Solvent filtration assembly** | InfinityLab solvent filtration assembly, includes glass funnel, 250 mL, membrane holder glass base, glass flask, 1 L, and aluminum clamp | 5191-6776 |
| **Solvents and reagents** | InfinityLab Ultrapure LC/MS acetonitrile | 5191-4496 |
| | InfinityLab Ultrapure LC/MS methanol | 5191-4497 |
| | InfinityLab Ultrapure LC/MS water | 5191-4498 |
| | Formic acid reagent-grade, 99.5% purity, 5 mL | G2453-85060 |
| | MS solution, formic acid, 99.5% purity, 10 mL | US-700002341 |
| | 5 M ammonium formate solution | G1946-85021 |
| **Vials and caps** | Agilent A-Line certified amber (screw top) vials; 100/pk | 5190-9590 |
| | Agilent deactivated vial inserts; 500 µL, 500/pk | 5183-2086 |
| | Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm; 500/pk | 5190-7024 |

*1 Both AOAC and EN extraction kits work equivalently. The selection of extraction kits is based on customer preference.*
*2 Both Captiva EMR–HCF1 and EMR–HCF2 cartridges work equivalently. The selection of passthrough kits is based on customer preference.*
*3 Only needed when extraction kits without ceramic homogenizers are used.*
**One-time purchase.*

**MyList 2**: Columns and supplies for analysis of pesticides in pigmented fresh fruits and vegetables using GC/MS/MS

| | Description | Part Number |
|---|---|---|
| **Sample preparation** | **Extraction** [1] | |
| | Agilent Bond Elut QuEChERS EN extraction kit, with or without ceramic homogenizers | 5982-5650CH<br>5982-5650 |
| | Agilent Bond Elut QuEChERS extraction kit, AOAC 2007.01, with or without ceramic homogenizers | 5982-5755CH<br>5982-5755 |
| | **Sample crude extract cleanup** | |
| **For none or light pigmented fresh produce** | Agilent Bond Elut Dispersive kit, general fruits and vegetables, 15 mL, Agilent Bond Elut Dispersive kit, general fruits and vegetables, 2 mL | 5982-5058CH<br>5982-5122CH |
| **For general pigmented fresh produce** | Agilent Captiva EMR–GPF cartridge, 3 mL | 5610-2090 |
| **For high chlorophyll fresh vegetables** | Agilent Captiva EMR–HCF1 with NH2 or EMR–HCF2 with PSA, 3 mL [2] | 5610-2088<br>5610-2089 |
| **For low pigmented dry plant origin food and essential oils** | Agilent Captiva EMR–LPD cartridge, 6 mL | 5610-2092 |
| **For general pigmented dry plant origin food** | Agilent Captiva EMR–GPD cartridge, 6 mL | 5610-2091 |
| **For animal origin food and oils** | Agilent Captiva EMR–Lipid cartridge, 3 mL<br>Agilent Captiva EMR–Lipid cartridge, 6 mL | 5910-1003<br>5910-1004 |
| | **Sample postdrying** | |
| | Agilent Bond Elut QuEChERS EMR–Lipid polish pouch, 3.5 g anhydrous $MgSO_4$ | 5982-0102 |

| | Description | Part Number |
|---|---|---|
| | **Other sample preparation consumables** | |
| | Ceramic homogenizers, 50 mL tubes, 100/pk [3] | 5982-9313 |
| | Centrifuge tube and cap, polypropylene, 50 mL, 25/pk | 5610-2049 |
| | Agilent positive pressure manifold-48 processor | 5191-4101* |
| | SPE cartridge rack, 6 mL, for PPM-48 | 5191-4104* |
| | SPE cartridge rack, 3 mL, for PPM-48 | 5191-4103* |
| | Collection rack for 16 x 100 mm tubes | 5191-4108* |
| **Standards** | Ready-to-use 254-compound standards mix, 8 x 1 mL, 100 µg/mL each | 5190-0551 |
| | InfinityLab Ultrapure LC/MS acetonitrile | 5191-4496 |
| **GC column** | Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm film thickness (two) | 19091S-431UI |
| | Agilent DB-5ms UI, 15 m × 0.25 mm, 0.25 µm (two) (recommended) | 122-5512UI |
| **GC supplies** | Fritted liner, splitless, UI, low, 870 µL, 4 mm, 1/pk** | 5190-5112 |
| | Inlet liner, UI, splitless, single taper, glass wool | 5190-2293 |
| | Agilent Blue Line syringe, PTFE-tip plunger, tapered, 10 µL | G4513-80203 |
| | Agilent Advanced Green septum, nonstick, 11 mm | 5183-4759 |
| **Vials and caps** | Agilent A-Line certified amber vial, screw top, 100/pk | 5190-9590 |
| | Agilent deactivated vial insert, 100/pk | 5181-8872 |
| | Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm, 500/pk | 5185-5862 |

*1 Both AOAC and EN extraction kits work equivalently. The selection of extraction kits is based on customer preference.*
*2 Both Captiva EMR–HCF1 and EMR–HCF2 cartridges work equivalently. The selection of passthrough kits is based on customer preference.*
*3 Only needed when extraction kits without ceramic homogenizers are used.*
**One time purchase.*
***Fritted liners provided similar responses to the splitless wool liners, but tended to have better retention of peak areas with increased matrix injections across 70 matrix-matched injections than the wool liners.[7]*

## Other food matrices

Agilent has developed and verified an optimized method in accordance with the EU analytical guidance document SANTE/11312/2021 using three food matrix types: tomato and onion (high water content), wheat (high starch content), honey (high sugar content), olive oil (high fat content), and difficult commodities (black pepper) to analyze 510 pesticides in 20 minutes using an Agilent 6470 LC/TQ system.[8-10]

The comprehensive workflow guide includes a consistent sample preparation technique, an optimized UHPLC separation method with predefined consumables and ready-to-use standard mixes, a dMRM acquisition method, data analysis, and reporting supported by onsite and online training.

**MyList 3:** Columns and supplies for analysis of pesticides in difficult or unique commodity groups using LC/MS/MS[10]

| | Description | Part Number |
|---|---|---|
| **Sample preparation** | Agilent Bond Elut QuEChERS EN extraction kit | 5982-5650 |
| | Agilent Captiva EMR—GPD, general pigmented dry | 5610-2091 |
| | Agilent Captiva EMR–GPF cartridge, 3 mL | 5610-2090 |
| | Ceramic homogenizers, 50 mL tubes, 100/pk | 5982-9313 |
| | Centrifuge tube and cap, polypropylene, 50 mL, 25/pk | 5610-2049 |
| | Agilent positive pressure manifold-48 processor | 5191-4101* |
| | SPE cartridge rack, 6 mL, for PPM-48 | 5191-4104* |
| | SPE cartridge rack, 3 mL, for PPM-48 | 5191-4103* |
| | Collection rack for 16 x 100 mm tubes | 5191-4108* |
| **Standards**** | Ready-to-use 254-compound standards mix, 8 x 1 mL, 100 µg/mL each | 5190-0551 |
| **HPLC column** | ZORBAX Eclipse Plus C18, 2.1 x 150 mm, 1.8 µm, 1200 bar | 959759-902 |
| | ZORBAX Eclipse Plus C18 guard, 2.1 mm id, 1.8 µm, 3/pk | 821725-901 |
| **HPLC supplies** | Agilent 1290 Infinity inline filter, 0.3 µm | 5067-6189 |
| | InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC | 5067-5957 |
| | InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC | 5067-6166 |
| | InfinityLab Quick Turn fitting, for column outlet | 5067-5966 |
| | InfinityLab Quick Turn capillary 0.12 x 280 mm, for connection from column to detector | 5500-1191 |
| | Kit of Stay Safe waste cap GL45, with 4 ports and waste can, 6 L | 5043-1221 |
| | Charcoal filter with time strip for waste container | 5043-1193 |
| | Stainless steel solvent inlet filter, 10 µm pore size | 01018-60025 |

| | Description | Part Number |
|---|---|---|
| **Solvent filtration assembly*** ** | InfinityLab solvent filtration assembly, includes glass funnel, 250 mL, membrane holder glass base, glass flask, 1 L, and aluminum clamp | 5191-6776 |
| | Regenerated cellulose filter membrane, 47 mm, 0.20 µm, 100/pk | 5191-4340 |
| **Solvents and reagents** | InfinityLab Ultrapure LC/MS acetonitrile | 5191-4496 |
| | InfinityLab Ultrapure LC/MS methanol | 5191-4497 |
| | InfinityLab Ultrapure LC/MS water | 5191-4498 |
| | Formic acid reagent-grade, 99.5% purity, 5 mL | G2453-85060 |
| | MS solution, formic acid, 99.5% purity, 10 mL | US-700002341 |
| | 5 M ammonium formate solution | G1946-85021 |
| **Vials and caps** | Agilent A-Line certified amber vial, screw top, 100/pk | 5190-9590 |
| | Agilent deactivated vial inserts, 500 µL, 500/pk | 5183-2086 |
| | Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm, 500/pk | 5190-7024 |

**One time purchase.*
*** Please contact Agilent for custom, premixed pesticide standards.*
***** If using solvents other than those listed in this table, use the InfinityLab solvent filtration assembly to filter before analysis.*

## Annex VIII References


1. Your clear choice for pigment removal: Agilent Carbon S sample preparation products. Agilent Brochure, **5994-4892EN**.
2. Analysis of Pesticide Residues in Spinach Using AOAC Pigmented dSPE with Carbon S Cleanup and LC/MS/MS. Agilent Application Note, **5994-4769EN**.
3. Determination of Multiclass, Multiresidue Pesticides in Bell Peppers Using Captiva EMR–GPF passthrough cleanup by LC/MS/MS and GC/MS/MS. Agilent Application Note, **5994-4767EN**.
4. Determination of Multiclass, Multiresidue Pesticides in Spring Leaf Mix Agilent Application Note, **5994-4765EN**.
5. Determination of Over 300 Pesticides in Cayenne Pepper Using Captiva EMR-GPD passthrough cleanup and LC/MS/MS and GC/MS/MS. Agilent Application Note, **5994-5630EN**.
6. Determination of Over 300 Pesticides in Cumin Powder Using Captiva EMR-LPD Passthrough Cleanup and LC/MS/MS and GC/MS/MS Detection. Agilent Application Note, **5994-6882EN**.
7. Analysis of Multiclass Multiresidue Pesticides in Milk Using Agilent Captiva EMR-Lipid with LC/MS/MS and GC/MS/MS. Agilent Application Note, **5994-2038EN**.
8. Multiresidue Pesticide Analysis in Food Matrices with an Ultra Inert Splitless Glass Frit Liner by GC/MS/MS. Agilent Application Note, **5994-1473EN**.
9. Comprehensive LC/MS/MS Workflow of Pesticide Residues in Food Using the Agilent 6470 Triple Quadrupole LC/MS System-Pesticides residue workflow in high water content, high oil content, and high starch content samples. Agilent Application Note, **5994-2370EN**.
10. Analysis of 510 Pesticide Residues in Honey and Onion on an Agilent 6470 Triple Quadrupole LC/MS System - Pesticides residue workflow for high sugar content and high water content samples. Agilent Application Note, **5994-3573EN**.

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