Optimizing the Performance of the Agilent GC/MS/MS Pesticide Analyzer

Technical Overview

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Introduction

In order to successfully transition multi-residue pesticide methods from elemental-selective gas chromatograph (GC) detection or selective ion monitoring (SIM) mode using a gas chromatograph/single quadrupole mass spectrometer over to multiple reaction monitoring (MRM) mode using a gas chromatograph/triple quadrupole mass spectrometer, many aspects of the analysis must be considered.

First of all, the detection limit (or calibration range), in general, shifts lower by two orders of magnitude. Quantitative analysis of food or environmental samples for organic contaminants at parts-per billion (ppb) or parts-per-trillion (ppt) levels, places additional emphasis on all parts of the GC/MS/MS system (inlet, capillary column, and mass spectrometer ion source, in particular) to be sufficiently inert to allow the detection and confirmation of hundreds of trace target compounds in a single GC run.

One of the most important aspects is the use of matrix-matched calibration standards, that is, authentic reference materials at known concentrations prepared in blank matrix of the sample types to be analyzed.

This technical note discusses the benefits of using matrix-matched calibration standards and other topics that lead to the successful implementation and optimization of multi-residue pesticide methods with the Agilent GC/MS/MS Pesticide Analyzer.

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The requirement for matrix-matched standards

Within any single or triple quadrupole GC/MS system there are many potential hardware components, which may have activity that will lead to the quantitative loss of target analytes. The most likely sources of active sites are the GC inlet liner, capillary column and the mass spectrometer ion source itself. While performing GC/MS analyses at parts-per-million (ppm) levels, minute losses due to activity have less impact on results than when analyses are performed at the ppb or ppt level. Recent developments in deactivation techniques for GC inlet liners (such as the Agilent Ultra-Inert range of inlet liners) have improved GC performance characteristics but it is still feasible that ultra-trace levels of active analytes may be lost during the injection phase of the analysis.

Typically, solvent standards, that is, authentic reference materials at known concentration prepared in a pure organic solvent, are employed for GC/MS analyses at ppm or high-ppb levels where the total gravimetric amount of all the analytes in any calibration standard compensates for minor losses due to activity within the GC/MS system. However, trace (or ultra-trace) detection and confirmation of analytes at ppb or ppt levels can be heavily influenced (impaired) by even the smallest level of activity within a GC/MS/MS system.

One of the key components of multi-residue pesticides analysis by GC/MS/MS is the sample preparation. Sample extraction and cleanup must be appropriate to the analysis. Too little, or nonoptimized, sample preparation can lead to the preparation of very dirty samples that contain high levels of co-extracted matrix materials. These co-extracted materials can quickly contaminate the inlet liner and lead to rapid loss in system performance. However, an overly aggressive sample preparation procedure may result in a very clean extract, but actually remove target analytes from the final extracted sample. The Appendix contains additional information related to sample preparation requirements.

While these co-extracted materials may seem to be more foe than friend, they can, in fact, play a very positive role in the overall performance of the analytical method. The co-extracted materials can perform a role of analyte protectant [1, 2] whereby they blanket the active sites that the target analytes would otherwise be exposed to during the injection into the GC liner. The amount of protection afforded varies from analyte-to-analyte and from matrix-to-matrix type. It is common to select a combination of analyte protectants to cover their volatility range [3].

In order to demonstrate the matrix protectant effect, samples of ethyl acetate extracts of lettuce, onion and satsuma (mandarin orange) were spiked with 23 organo-phosphorous (OP) and pyrethroid insecticides at a concentration of 50 ppb (pg/µL). The list of insecticides is shown in Table 1. A solvent standard was also prepared at 50 ppb in ethyl acetate.

Table 1. Insecticides Spiked at 50 ppb in Lettuce, Onion and Satsuma Extracts

Dichlorvos	Methacrifos	Sulfotep	Fonofos	Propetamphos	Diazinon
Etrimfos	Dichlofenthion	Chlorpyriphos methyl	Fenchlorphos	Pirimiphos methyl	Chlorpyriphos
Bromophos methyl	Pirimiphos ethyl	Chlorfenvinphos	Bromophos ethyl	Ethion	Carbophenothion
Azinphos methyl	Azinphos ethyl	Permethrin I	Permethrin II	Deltamethrin	

A sequence of 120 injections (over a period of 54 hours) was run and the order of injections is shown in Figure 1. One-microliter injections were made using a multi-mode inlet (MMI) in cold splitless mode and the column was backflushed for 3 minutes at the end of each analysis (post-column configuration). The Agilent 7000 Triple Quadrupole GC/MS system was run in EI MRM mode with two MS/MS transitions for each analyte.

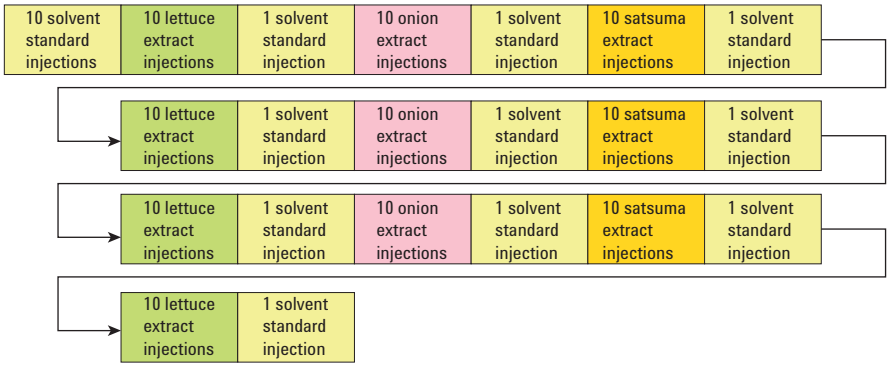

Figure 1. Sequence of 120 solvent standard and spiked matrix injections.

The peak areas for one of the OP analytes, ethion, across the entire sequence of 120 injections are shown in Figure 2.

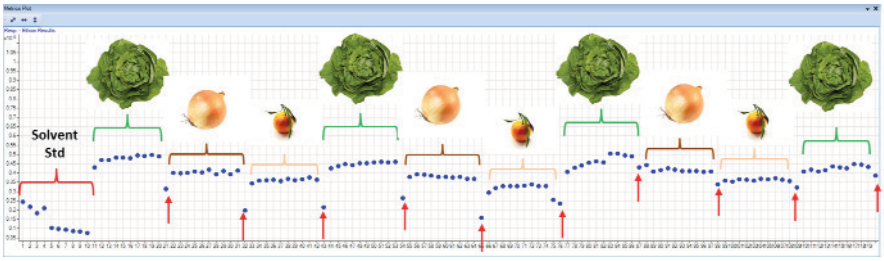

Figure 2. Ethion peak areas for 120 consecutive injections of solvent standards and matrix standards. (Repeat solvent standard injections denoted with red arrows).

The trend of the ethion peak area for the first 10 solvent injections is downwards. The peak area for ethion in the first lettuce matrix injection increases immediately by a factor of 4 and the response stabilizes across the 10 lettuce matrix injections. The peak area for ethion in the first repeat solvent standard drops significantly compared to the tenth lettuce matrix injection, but the peak area is significantly higher than the tenth solvent standard injection. This demonstrates the passivation effect of the lettuce matrix on the injection port liner and the protection effect of the matrix on the analyte. This pattern of response is repeated across the entire sequence of injections with the peak area for the ethion in the repeat solvent standard injections dependent upon which matrix type they follow. Clearly, the use of solvent based standards for quantitation of analytes within this sequence would lead to inaccurate results.

The reproducibility of peak area responses (expressed as %RSD) for all 23 pesticides in the lettuce (n = 40), onion (n = 30) and satsuma (n = 30) matrices are shown in Figure 3. For ethion, the %RSD is about 6–8%.

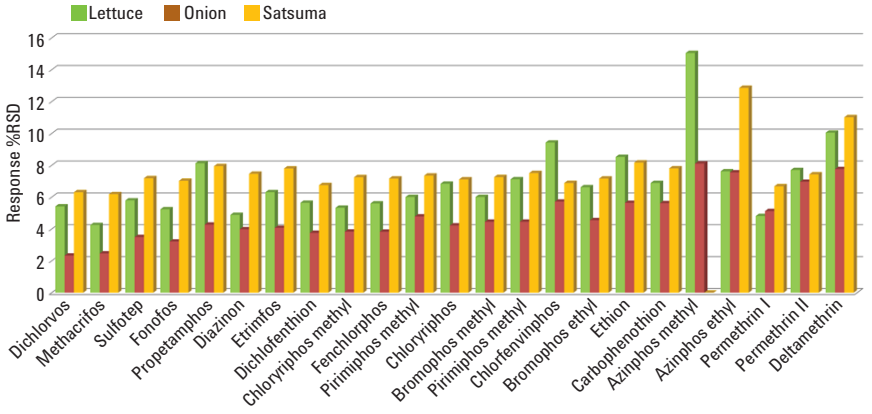

Figure 3. Reproducibility of peak areas for 23 pesticides spiked into lettuce (green bars), onion (brown bars) and satsuma (orange bars) extracts at 50 ppb.

The majority of the pesticides gave peak area %RSD values of less than 10% across the 120 injections of the 54-hour sequence. Azinphos methyl was not measured in the satsuma extract since the two MS/MS transitions chosen for this analyte suffered interference from the sample matrix. The solution to this problem is to use the alternative MS/MS transitions provided in the Agilent G9250AA Pesticides and Environmental Pollutants MRM database as discussed in a later section of this technical note.

Another major factor concerning the influence of matrix on quantitative results relates to the construction and use of calibration curves. The slope of the calibration curve is affected by the nature (and relative concentration) of matrix components injected with the target analytes. The relative slopes of calibration curves for a solvent standard compared to a matrix-matched standard is shown in Figure 4. The consequence of the increased slope for the matrix-matched calibration curve is an over estimation of the quantitative result if a sample response in matrix were to be measured against a solvent based calibration curve.

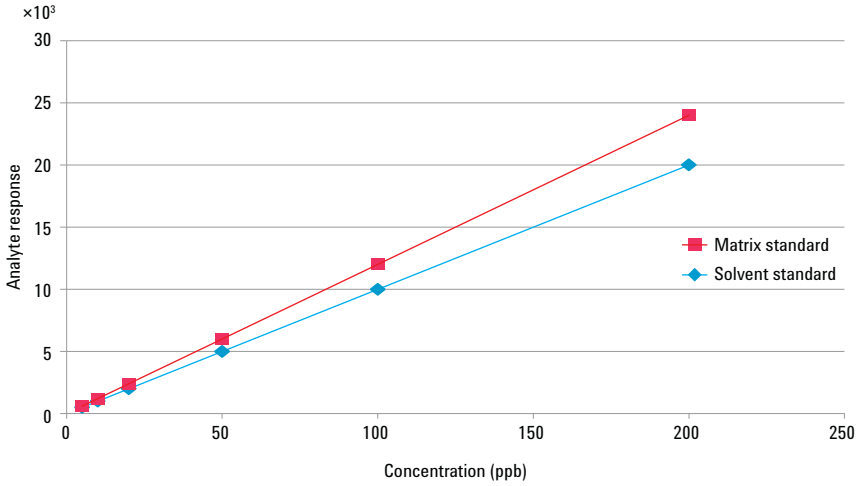

Figure 4. Increased slope of the calibration curve for an analyte in matrix (red squares) compared to the same analyte in a solvent standard (blue diamonds).

The variation in matrix effect (ME) can be demonstrated by comparing the slopes of calibration curves created with solvent standards and matrix matched standards using the following equation:

$$ME(\%) = \left(\left(\frac{\text{slope of calibration curve in matrix}}{\text{slope of calibration curve in solvent}} \right) - 1 \right) \times 100$$

Seven-point calibration curves were prepared for 44 pesticides in solvent and in four different tea extracts: green, red, black and chamomile and then analyzed using the GC/MS/MS Pesticide Analyzer. The slope variation percentage for the different analytes in each of the 4 tea matrices is shown in Figure 5. The majority of analytes show increased response in the presence of matrix with the increase also dependent upon the matrix type. Thus the use of matrix-matched calibration standards for the trace analysis of pesticides in extracted samples by GC/MS/MS is a definite requirement.

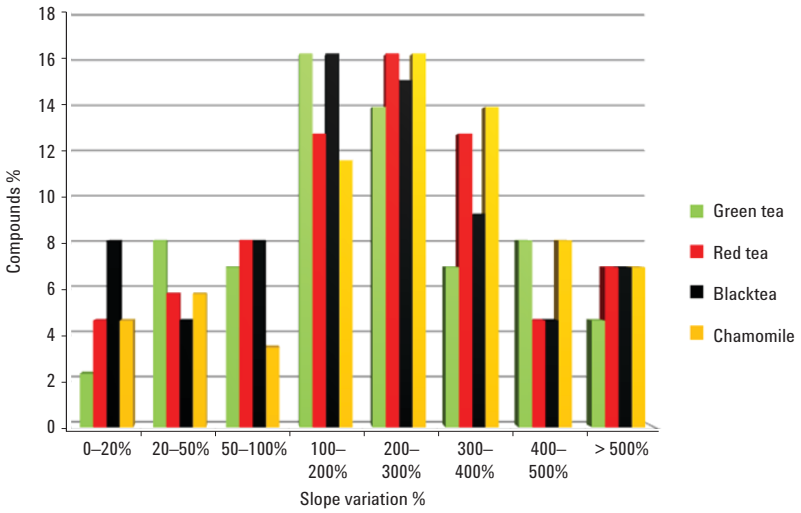

Figure 5. Effect on calibration curve slopes for 44 pesticides in four different tea matrices. (Data by permission of Prof. Amadeo R. Fernández-Alba, EU-RL Fruits and Vegetables, University of Almeria, Spain).

Internal Standard (ISTD) vs. External Standard (ESTD)

Besides using matrix-matched standards, another common practice to improve quantitation accuracy is to use internal standard (ISTD). An internal standard is a chemical substance that is added in a constant amount to samples, the blank, and calibration standards in a chemical analysis. This substance can then be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This ratio is usually called the response factor (RF) or relative response factor (RRF), indicating that the target compound response is calculated relative to that of the internal standard.

The ISTD is used to correct for the loss of analyte during sample preparation or sample introduction. The ISTD can be a generic chemical compound or isotope labeled analyte [2]. The principal disadvantage of using ISTD is that the internal standards must be compounds that are not found in the samples to be analyzed and they must produce an unambiguous response on the chromatographic detector system.

In external standard (ESTD) quantitation, known data from a calibration standard and unknown data from the sample are combined to generate a quantitative report. It is called external standard because the standard or known material is separate or external to the unknown material. External standard calibration is one of the most common approaches to calibrations. It involves a simple comparison of instrument responses from the sample to the responses from the target compounds in the calibration standards.

The advantages of ESTD calibration are that it is simple to perform this type of calibration and it can be applied to a wide variety of methods. The primary disadvantage is that it is greatly affected by the stability of the chromatographic system and the presence of chromatographic interferences in the sample extract.

Table 2 shows a comparison of ISTD quantitation results versus ESTD quantitation results. The %RSD quantitation results are based on a sequence of 30 injections of spiked tea extracts at 50 ppb. The first five injections of the sequence were treated as liner passivation injections and then the statistics were calculated with injections 6–30, inclusive.

Table 2. Comparison of ISTD and ESTD Quantitative Results (Expressed as %RSD Values) from 25 Repetitive Injections of Tea Extracts Spiked with Pesticides at a Concentration of 50 ppb

Analyte	%RSD ISTD	%RSD ESTD	Delta
Trifluralin	3.03	5.58	-2.5
BHC-alpha	4.09	6.25	-2.2
BHC-beta	9.02	10.75	-1.7
Lindane	9.67	11.41	-1.7
d10-Phenanthrene	1.62	4.18	-2.6
BHC-delta	5.56	7.46	-1.9
Chlorothalonil	9.19	10.36	-1.2
Parathion-methyl	2.75	4.80	-2.0
Heptachlor	13.41	14.98	-1.6
Fenitrothion	2.97	5.21	-2.2
Aldrin	2.30	4.36	-2.1
Heptachlor endo-epoxide (isomer A)	2.61	5.02	-2.4
Captan	7.08	7.46	-0.4
Chlordane-*trans* (gamma)	2.52	4.61	-2.1
Endosulfan-alpha	1.98	4.21	-2.2
Chlordane-*cis* (alpha)	3.04	4.90	-1.9
Hexaconazole	1.25	3.75	-2.5
DDE-p,p′	0.91	3.49	-2.6
Dieldrin	2.03	4.56	-2.5
Endrin	7.45	9.48	-2.0
Endosulfan-beta	2.20	4.53	-2.3
DDO-p,p′	1.87	4.29	-2.4
Endosulfan sulfate	4.49	6.22	-1.7
DDT-p,p′	51.86	53.10	-1.2
Dicofol-p,p′	5.69	7.75	-2.1
Bifenthrin	0.78	2.93	-2.2
Cyhalothrin (lambda)	2.11	4.46	-2.3
Permethrin I	0.82	2.97	-2.2
Permethrin II	1.37	3.96	-2.6
Cyfluthrin I-IV	2.05	4.30	-2.3
Cypemethrin I-IV	1.99	4.23	-2.2
Fenvalerate I	2.61	4.07	-1.5
Fenvalerate II	3.02	4.83	-1.8
Deltamethrin	2.74	4.63	-1.9

From the data in Table 2, the numbers are in general very good, with most %RSDs < 5%. All of them are less than 15% except DDT-p,p'. For all analytes, %RSD values are lower for ISTD versus ESTD. The column on the right is the difference between the ISTD and ESTD %RSD values. Although the quantitation results of using ESTD are very good in this example, the %RSD using ISTD is always 1 to 3 percentage points lower than the %RSD using ESTD, for all analytes.

GC/MS/MS Pesticide Analyzer Methods (Constant Flow and Constant Pressure)

The Agilent GC/MS/MS pesticide analyzer provides optimized hardware, application specific consumables (such as columns, liners) and chromatographic methods to enable rapid implementation of multi-residue MRM methods using the Agilent 7000 GC/MS/MS system. The four key features of the GC/MS/MS pesticide analyzer are shown in Figure 6.

Figure 6. Key features of the Agilent GC/MS/MS Pesticide Analyzer.

In order to provide flexibility in analysis and analyte coverage, three different pre-configured methods are available based on two pre-configured systems. A fourth method can be easily set up in a lab for faster analysis. The four methods are retention time locked and all employ capillary flow technology with a purged ultimate union (PUU) to facilitate backflushing. Two of the methods operate in constant flow (CF) mode and two operate in constant pressure (CP) mode. A comparison of the four analyzer method configurations is shown in Figure 7.

	40 Minute CF Method	**20 Minute CF Method**	**40 Minute CP Method**	**20 Minute CP Method**
Column (#1)	15 m × 0.25 mm, 0.25 mm HP-5MSUI	15 m × 0.25 mm, 0.25 mm HP-5MSUI	30 m × 0.25 mm, 0.25 mm HP-5MSUI	15 m × 0.25 mm, 0.25 mm HP-5MSUI
Column (#2)	15 m × 0.25 mm, 0.25 mm HP-5MSUI	15 m × 0.25 mm, 0.25 mm HP-5MSUI	Capillary Restrictor, 0.5 m × 0.15 mm deactivated tubing	Capillary Restrictor, 0.5 m × 0.15 mm deactivated tubing
Backflush type	Midpoint	Midpoint	Post column	Post column
No. target analytes	~300+	~200+	~300+	~200+

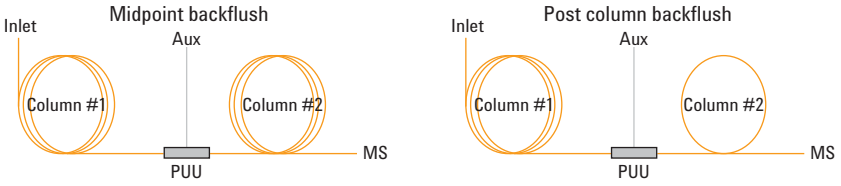

Figure 7. GC/MS/MS Pesticide Analyzer configurations.

The choice of analyzer configuration largely depends upon the required runtime, required detection limits, and the number of target analytes to be analyzed at system installation and allowing for increased numbers of target analytes to be analyzed in the future. The constant flow methods have the advantage of providing the same volumetric flow to the MS ion source throughout the chromatographic run. However, the use of midpoint backflush makes the initial method setup a little more involved. The constant pressure method, using post column backflushing, has the advantage of automated RTL setup but the responses of earlier eluting analytes are slightly impaired by the higher volumetric flow rate at the beginning of the run. In addition, CP configurations allow exact retention time scaling and flow splitting to multiple detectors. By swapping the 30-m column #1 with a 15-m column #1 (no change to the restrictor), the 20-min CP method can be scaled and RT locked to exactly 2 times faster than the 40-min CP method. In other words, all analyte retention times in 20-min CP method are exactly half of the RTs in the 40-min CP method. Both CF and CP methods provide excellent sensitivity for pesticides at the low-ppb and sub-ppb range.

Mode of Injection

As shown in Figure 6, the Agilent Multimode Inlet (MMI) can be used for the full range of hot and cold injection modes. Hot splitless (HSL) mode is best employed using the 4-mm id single-tapered ultra-inert liner with glass wool (p/n 5190-2293). This liner gives excellent performance for pesticides analysis and the glass wool helps to protect the head of the capillary column from nonvolatile matrix material (blocks line-of-sight). All precautions normally associated with HSL injection must be made to ensure that the vapor cloud created by the solvent does not overfill the liner volume and cause loss of analytes through the split vent.

Cold injections may also be used and these are either in cold splitless (CSL) mode or, for larger volume injections, solvent vent (SV) mode.

CSL mode can be employed with either the 4-mm id single-tapered ultra-inert liner with glass wool or with the 2-mm id deactivated multi-baffled liner (p/n 5190-2296). CSL mode has the advantage over HSL mode in that analytes are not subjected to thermal shock from a hot injection port liner and analytes are transferred in a more gentle fashion to the head of the capillary column. In general, thermally labile analytes will give better response with CSL mode than with HSL mode.

If lower detection limits are required, the MMI can be used to perform large volume injections in SV mode. The MassHunter acquisition software includes a solvent elimination calculator, which guides the user through the optimization of large volume injections with the MMI. A guide to using the MMI in HSL, CSL and SV modes is available in a previously published booklet from Agilent [4].

Figure 8 shows a comparison of the responses of a 40-ppb standard solution from three injection modes: HSL, CSL, and SV described above [5]. The bottom total ion chromatogram (TIC) is a typical 2-µL hot splitless injection. Some of the 40-ppb pesticides are barely visible (80 pg each on-column). The middle TIC is from a 10-µL cold splitless injection. The MMI starting temperature was 30 °C. In this TIC, the on-column amount for each analyte is 400 pg. Lastly, the top TIC is from a 25-µL solvent vent injection with MMI starting temperature at 35 °C. In this TIC, the signal-to-noise ratio is significantly better than the TIC from hot splitless injection (bottom TIC). The peak shape and resolution are maintained, even with the 25-µL injection volume. This implies that the solvent was mostly eliminated during the injection.

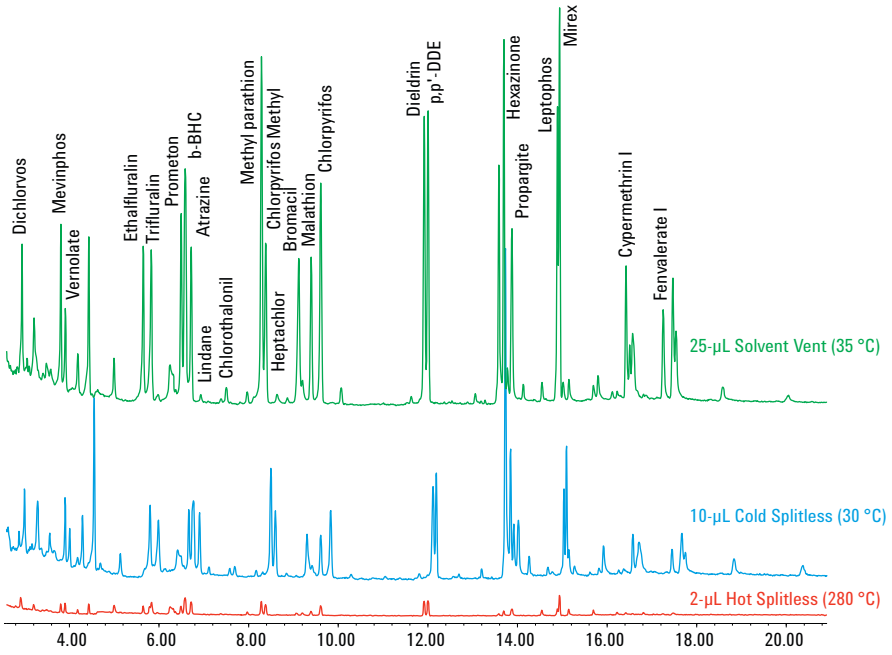

Figure 8. Overlay of total ion chromatograms (TICs) from three injection modes, plotted on the same scale. Each injection was made from a 25-pesticide standard at 40 ppb each in acetone.

Advantages of employing backflushing

The positive advantages of sample matrix have been presented. The presence of co-extracted matrix can cause problems for the trace analysis of pesticides by GC/MS/MS. Higher MW components co-extracted through the extraction process can build up within the injection port liner and cause adsorption or breakdown of target analytes either reducing or completely eliminating analyte, or both, response. This effect can only be rectified by the replacement of the injection port liner as part of routine maintenance of the injection port. The frequency of liner replacement depends upon the nature of the sample matrix and the sample preparation method employed. Typically, replacement occurs after every batch of 60 to 100 samples.

As well as the contamination of the injection port liner, more volatile sample matrix components may pass on to the capillary column. These more volatile matrix components chromatograph along with the target analytes, but inevitably, some higher-boiling matrix components remain behind in the capillary column after the last analyte of interest has eluted into the mass spectrometer. In the past, these unwanted matrix components were removed from the column by employing a bake-out period of the oven temperature program. The bake-out period is detrimental in that it can cause thermal stress for the capillary column (shortening its useful lifetime), it drives the matrix components into the mass spectrometer ion source (causing contamination and the requirement for more frequent ion source cleaning) and it may add a considerable amount of time to the analysis thereby increasing cycle time.

If the matrix components are not removed from the column after each sample injection, they can build up within the column and cause detrimental effects to chromatographic peak shapes and cause retention time shifts. A diagram of the buildup of unwanted matrix components within a capillary column during a sequence of sample injections is shown in Figure 9.

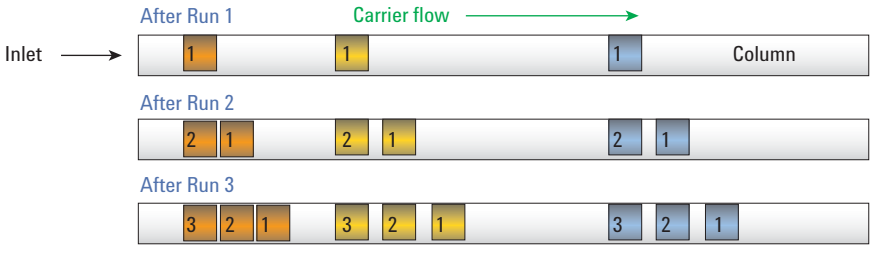

Figure 9. Diagram showing buildup of unwanted matrix components during a sequence of sample injections into a GC capillary column.

A far more efficient means of removing these matrix components from the column is to employ backflushing. At the end of each run, the pressure at the PUU is increased while the pressure at the injection port is dropped to a value of 1–2 psig. This reverses the flow in the column #1 (Figure 7) and efficiently removes the matrix components, least volatile first, out through the split vent. This efficient backflushing process can achieve the effects of a high temperature bakeout in just a few minutes.

Another variation of the Analyzer method uses a shorter (5 m) column #1 in the constant flow configuration. The mid-column configuration allows user to do concurrent backflushing. This mode of backflushing refers to backflushing column #1 while the chromatographic separation continues in column #2. Concurrent backflushing can start as soon as the last analyte of interest moves from column #1 to column #2, which are connected by the purged ultimate union. Not only does backflushing start sooner, but the shorter (5-m) column #1 requires less time to backflush compared to the 15-m column #1. Several other applications use this configuration with concurrent backflushing [2,6]. Although concurrent backflushing is more difficult to optimize, it does reduce the overall cycle time by reducing the time of a post-run segment in the method. However, as shown in Figure 7, the shorter column #1 configuration may not be suitable for a method with 300 or more target compounds. Also, the 5-m HP-5MS UI column is not an off-the-shelf item so the user must manufacture this column by cutting a 5-m length from a 15-m or longer column.

In summary, the use of backflushing provides:

- Consistent analyte retention times and responses
- Robust chromatography and consistent analyte chromatographic peak shapes
- Prevention of high boiling matrix from contaminating the MS ion source
- Extended column life-time and reduced cycle times by removing the need for high-temperature bakeout between runs

An example of the consistent responses of pesticide analytes in a tea extract when post-run backflush is used is shown in Figure 10. A sequence was performed of 160 injections of blank tea matrix spiked with pesticides at 0.1 mg/kg (equivalent to a concentration of 20 pg/µL). After five injections of the matrix, which passivated the liner *in-situ*, the responses of the analytes remain stable for more than 150 injections.

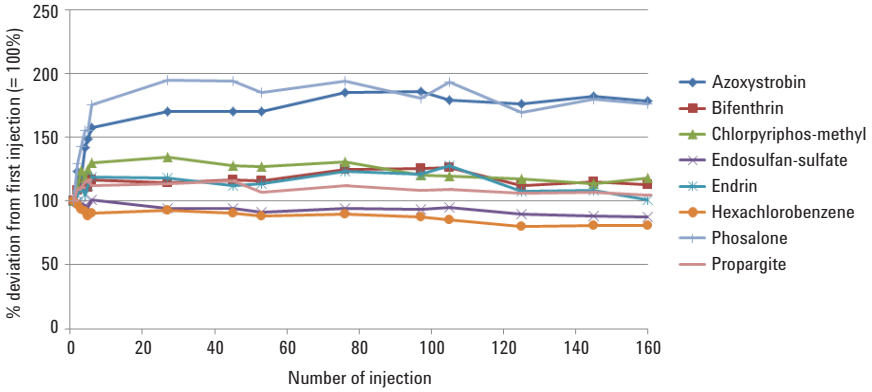

Figure 10. Long-term stability of GC/MS/MS responses for the injection of pesticides in tea extracts. Each point corresponds to the injection of a matrix-matched standard at 0.1 mg/kg concentration (equivalent to 20 pg/µL) in a sequence.

Using the G9250AA Pesticides and Environmental Pollutants MRM Database

The G9250AA Pesticides and Environmental Pollutants MRM database is supplied with the GC/MS/MS Pesticide Analyzer or may be purchased as a stand-alone product. Full details of the MRM database are given in a previously published Agilent application note [7].

The MRM database provides locked retention times that match those generated by the three GC/MS/MS preconfigured analyzer methods documented in this technical note. As discussed previously, 20-min CP method is exactly twice as fast as the 40-min CP method. Therefore, the 20-min CP method can take full advantage of the G9250AA directly by using half of the 40-min CP method RTs in building the acquisition method. The database also provides two means of generating customized multi-residue MRM MassHunter acquisition methods. These are either using the Excel-based macros provided within the MRM database or exporting the analytes to the MassHunter Compound List Assistant (CLA) software utility tool.

Excel based macros

The G9250AA MRM database is supplied with built-in macros to create MRM methods by assigning segment boundaries based on finding a suitable gap or flat baseline between target analyte peaks. This process is better suited to the creation of MRM methods with smaller numbers (for example, less than 100) of target analytes or when there are many flat baseline areas in the chromatogram where no target peaks are eluting. The macro optimizes the cycle time in each time segment by adjusting transition dwell times.

Compound List Assistant tool

Use of the Compound List Assistant (CLA) tool is particularly suited to the creation of MRM methods that contain very large numbers of target analytes (for example, more than 200). A key parameter in CLA is the RT Window. This value is the time in minutes both before and after the compound's RT value in which a compound's peak must be found to be identified. Based on the RT Window parameter, CLA will assign MRM transitions across segment boundaries, where appropriate. This is most likely to be required in those portions of the chromatogram where many target analytes are eluting in a short time period. However, CLA does not optimize dwell or cycle times within or across time segments so this must be achieved by balancing transition dwell times manually in the MassHunter MRM acquisition table to achieve consistent cycle times. Achieving cycle times within each time segment of 4–5 cycles/second generally provides sufficient data points across target analyte peaks to achieve good quantitation statistics.

Both the Excel based macro and CLA approaches to creating customized MRM methods are shown and discussed in detail in the Building MRM Method video that is supplied on the G9250AA MRM Database CD and also available to view online at www.youtube.com.

Other analyzer configurations (for example, shorter column #1) [2] or analytical columns (for example VF or DB type columns) can still take advantage of this MRM Database. The user must determine the retention times (RTs) of all target analytes from full-scan analyses and enter the RTs into the database in order to use MRM transitions in the database.

Conclusions

Multi-residue pesticide analysis of environmental or food samples by GC/MS/MS places many demands on the analyst, the analytical methodology, and the analytical instrument. Appropriate sample preparation methodology is key to achieving successful results, as is the optimization of analytical methods.

Accurate quantitation of trace levels of target analytes in complex sample extracts requires the use of matrix-matched calibration standards.

Employing capillary flow technology and backflushing facilitates the creation of more robust chromatographic methods with reduced cycle times and less frequent system maintenance.

The Agilent GC/MS/MS Pesticide Analyzer provides an excellent starting point for multi-residue pesticides analysis in food or environmental samples with preconfigured, retention time locked constant flow or constant pressure methods and application specific consumables. Combining the analyzer hardware and chromatographic methods with the most flexible and complete G9250AA MRM database of more than 1000 organic compounds facilitates rapid generation of customized GC/MS/MS methods and rapid system implementation in a customer laboratory.

Appendix I – Sample Preparation (QuEChERS)

Through the years the efficacy of pesticides has increased. In response to this enhanced toxicity, regulators continually reduce the maximum recommended limit (MRL) thereby increasing the challenges faced by analysts: trace level detection in complex matrices. Successful analysis of pesticide residues in foodstuffs requires extraction of residues from foodstuffs to produce a sample suitable for analysis by GC and LC techniques.

To meet more stringent reporting limits, researchers must extract larger sample volumes to increase the mass of target analytes injected into the analytical system. Food sample extracts contain pesticide residues plus organic components (matrix) co-extracted with the residues of interest. Matrix components can contaminate GC inlet systems, affect chromatographic efficiency, and interfere with ion response in the mass spectrometer.

The simplest preparation procedures - homogenization in an organic solvent followed by liquid-liquid extraction, blow down and reconstitution in GC friendly solvent - extracts and concentrates pesticide residues but does little to remove matrix interferences and yields a sample suitable for analysis by GC with selective detectors. Unfortunately, this technique also concentrates matrix components that can interfere with GC/MS and GC/MS/MS analyses.

Removal of matrix interferences requires additional sample preparation. The QuEChERS sample preparation technique was first introduced for pesticide analysis in foods by USDA scientists in 2003 [8]. It has been rapidly accepted worldwide for multi-residue pesticide analysis due to its special features known as Quick, Easy, Cheap, Effective, Rugged, and Safe. The QuEChERS extracts can be analyzed by LC and GC combined with MS to determine a wide range of pesticide residues. The QuEChERS process can also substantially decrease costs per sample. AOAC International and the European Committee for Standardization (CEN) have defined QuEChERS solvent extraction and solid phase extraction (SPE) cleanup procedures for a variety of matrices (Table 3).

Table 3. QuEChERS Extraction Methods

Guidance	Method	Composition	Agilent p/n
AOAC	2007.01	6 g $MgSO_4$, 1.5 g Na acetate	5982-5755
CEN	EN15662	4 g $MgSO_4$, 1 g NaCl, 1 g Na citrate, 0.5 g disodium citrate sesquihydrate	5982-5650
Original QuEChERS	10-g Sample, Non-buffered	4 g $MgSO_4$, 1 g NaCl	5982-5550
Original QuEChERS	15-g Sample, Non-buffered	6 g $MgSO_4$, 1.5 NaCl	5982-5555

The choice of sample cleanup following a QuEChERS extraction is dependent upon the nature of the sample matrix, which may have a high degree of pigmentation, wax or fat content. Pigmented samples with a high fat content will require more stringent cleanup than a traditional fruit or vegetable sample to remove organics co-extracted with the pesticide residues. Table 4 and Table 5 below outline the cleanup procedures recommended by the CEN and AOAC respectively.

Table 4. CEN Cleanup Recommendations

Matrix	Removes	Extract Volume	Composition	Agilent p/n
General Fruits & Vegetables	Polar organic acids, some sugars and lipids	2 mL	25 mg PSA 150 mg $MgSO_4$	5982-5021 100/pk
		15 mL	150 mg PSA 900 mg $MgSO_4$	5982-5056 50/pk
Fruits & Vegetables with Fats and Waxes	Polar organic acids, some sugars, more lipids and steroids	2 mL	25 mg PSA 25 mg C18EC 150 mg $MgSO_4$	5982-5121 100/pk
		15 mL	150 mg PSA 150 mg C18EC 900 mg $MgSO_4$	5982-5156 50/pk PN
Pigmented Fruits & Vegetables	Polar organic acids, some sugars and lipids, plus carotenoids and chlorophyll; not for use with planar pesticides	2 mL	25 mg PSA 2.5 mg GCB 150 mg $MgSO_4$	5982-5221 100/pk
		15 mL	150 mg PSA 15 mg GCB 885 mg $MgSO_4$	5982-5256 50/pk
Highly Pigmented Fruits & Vegetables	Polar organic acids, some sugars and lipids, plus high levels of carotenoids and chlorophyll; not for use with planar pesticides	2 mL	25 mg PSA 7.5 mg GCB 150 mg $MgSO_4$	5982-5321 100/pk
		15 mL	150 mg PSA 45 mg GCB 855 mg $MgSO_4$	5982-5356 50/pk

Table 5. AOAC Cleanup Recommendations

Matrix	Removes	Extract Volume	Composition	Agilent p/n
General Fruits and Vegetables	Polar organic acids, some sugars and lipids	2 mL	50 mg PSA 150 mg $MgSO_4$	5982-5022, 100/pk
		15 mL	400 mg PSA 1.2 g $MgSO_4$	5982-5058, 50/pk
Fruits and Vegetables with Fats and Waxes	Polar organic acids, some sugars, more lipids and steroids	2 mL	50 mg PSA 50 mg 18EC 150 mg $MgSO_4$	5982-5122 100/pk
		15 mL	400 mg PSA 400 mg C18EC 1.2 g $MgSO_4$	5982-5158 50/pk
Pigmented Fruits and Vegetables	Polar organic acids, some sugars and lipids, plus carotenoids and chlorophyll; not for use with planar pesticides	2 mL	50 mg PSA 50 mg GCB 150 mg $MgSO_4$	5982-5222 100/pk
		15 mL	400 mg PSA 400 mg GCB 1.2 g $MgSO_4$	5982-5258 50/pk
Fruits and Vegetables with Pigments and Fats	Polar organic acids, some sugars and lipids, plus carotenoids and chlorophyll; not for use with planar pesticides	2 mL	50 mg PSA 50 mg GCB 150 mg $MgSO_4$ 50 mg C18EC	5982-5421 100/pk
		15 mL	400 mg PSA 400 mg GCB 1.2 g $MgSO_4$ 400 mg C18EC	5982-5456 50/pk

The USEPA has recognized four classes of chemical pesticides: organophosphorus, organochlorine, carbamate, and pyrethroid pesticides [9]. Due to differences in stability, polarity and other chemical properties, analysts must take into account the target compound list and subsequent analytical method when considering QuEChERS or an alternate sample preparation method. Selecting an improper extraction procedure will greatly affect recovery of target analytes and may lead to incorrect results. Reconstitution of extracts into a solvent incompatible with GC analysis will negatively affect analytical results. Considerable research has been done to evaluate procedures for the extraction and extract cleanup for pesticides residues analysis by GC/MS and GC/MS/MS. Agilent Technologies can provide guidance on extraction procedures for use with its pesticide residue analyzers.

Appendix II – Sample Preparation Resources

Agilent offers a number of solutions for preparing samples for pesticide analysis by GC/MS/MS. These include solid phase extraction (SPE) and easy-to-use QuEChERS kits designed to make sample preparation easy. Each sample preparation option has its advantages and disadvantages. For additional assistance in finding the right sample preparation method for the specific needs of the method, the user can contact their account manager or sales engineer for more information.

Additional information about the range of Agilent sample preparation solutions is available.

- Agilent Sample Preparation Products for Chromatography 2013-2014 Catalog (Coming Soon)
- Agilent Sample Preparation Solutions for Food Analysis poster: 5990-9454EN
- Explore Agilent sample preparation and consumables solutions for Food Analysis at Planet Agilent: http://www.agilent.com/chem/PlanetAgilent

Solid Phase Extraction (SPE)

SPE is a highly selective sample preparation technique that can be used to clean up samples in advance of GC/MS/MS analysis. Agilent SPE products come in a broad range of formats and chemistries, and a number of application notes detailing the use of these products for either single residue or multi-residue methods. Silica, polymeric, specialty and inorganic SPE configurations can be matched to the goals of the application. Learn more about the range of Agilent SPE products through these resources:

- Agilent Bond Elut Silica-Based SPE Selection Guide 5990-8591EN
- Agilent Bond Elut Plexa and Polymeric SPE Selection Guide 5990-8589EN
- Agilent Bond Elut Specialty, Disc, and Bulk Selection Guide 5990-8592EN

Agilent Bond Elut QuEChERS Sample Preparation Products

Several useful references are listed below to help you get started on QuEChERS sample prep protocol:

- QuEChERS Resources: http://www.chem.agilent.com/en-US/promotions/Pages/QuEChERSappnotes.aspx

  Download QuEChERS application notes, watch Agilent's video on the use of QuEChERS for sample cleanup, and explore Agilent's QuEChERS extraction kits and dispersive SPE cleanup kits, which demonstrate excellent recoveries for frequently used pesticides in different food matrices.

- QuEChERS Webinar: QuEChERS_101_E-seminar_Sep_2011.pdf

  This webinar discusses in detail the QuEChERS extraction procedure from the point of product arrival through extraction, dispersive SPE, and on to analysis. Factors that affect recovery and increase throughput are presented in addition to future applications for QuEChERS.

- QuEChERS selection Guide: 5990-8590EN

  Pre-packaged Agilent QuEChERS Kits are an easy way to capture the time-saving benefits of QuEChERS sample preparation.

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