Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS

Fast and Effective Method Development Using an Application Kit and a Pesticides Compound Parameter Database

Application Note

Food Safety and Environmental

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Abstract

The analysis of pesticide residues in food and environmental samples is challenging due to the low concentrations and large number of analytes that need to be monitored and quantified. In addition, method development for Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) with a triple quadrupole instrument is laborious and time consuming because of the compound dependent parameters that need to be optimized. This application note describes how pesticide residue LC/MS/MS methods can be set up quickly and efficiently using the Agilent Pesticides Application Kit. This Application Kit contains a pesticide test mix, a 600-compound pesticide MRM database, a quick start guide and several dynamic Multiple Reaction Monitoring (MRM) methods, which can easily be incorporated into a specific method for pesticide residue analysis. The Pesticides Dynamic MRM database contains compounds commonly monitored around the world and provides fast, customized method development of the analysts' list of pesticides. Results from a 100 and 300-compound mixture are demonstrated with an Agilent 1200 SL Series Rapid Resolution LC and the Agilent 6460 Series Triple Quadrupole LC/MS System with Agilent Jet Stream Technology. The 300-compound mixture was also analyzed using an Agilent 1290 Infinity Ultra High Pressure Liquid Chromatograph (UHPLC) and a 6460 LC/MS. With the higher pressure capabilities of the Agilent 1290 Infinity UHPLC, rapid separations with higher peak capacity and less peak overlap than the Agilent 1200 Series RRLC were produced. Using a spinach matrix spiked with 16 pesticides, the performance of a complete method with the SampliQ extraction and dispersive SPE kits and the Agilent LC/MS/MS triple quadrupole on a typical food matrix was

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Introduction

The analysis of target pesticide residues has traditionally been performed using Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) methods. Because of the number of pesticides used and the sensitivity needed for monitoring hundreds of pesticides in a single analysis, both techniques are a requirement. GC/MS is needed for the less polar, more volatile pesticides and LC/MS for pesticides that are more polar or thermally labile and there is much overlap between them. However, many of the pesticides developed over the last 20 years are most amenable to LC/MS. The method of choice for trace analysis in complex matrices uses a triple quadrupole (QQQ) mass spectrometer incorporating multiple reaction monitoring (MRM). During an MRM analysis the QQQ monitors the product ions produced by collisions of precursor ions in the central quadrupole (the collision cell) of the mass spectrometer, as seen in Figure 1. An MRM analysis can generate a very sensitive and specific analysis of target compounds.

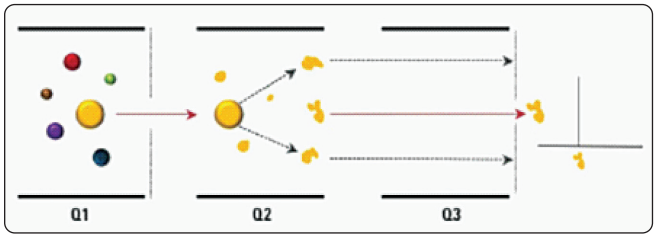


Figure 1. A schematic diagram of MRM mode on a triple quadrupole instrument. The precursor ion is selected in Q1, fragmentation occurs in Q2, and the product is selected by Q3. Since two stages of mass selectivity are used, there is very little interference from background matrix resulting in excellent sensitivity.

Over time regulating agencies have continually increased the number of pesticides and residues that must be monitored. It is now common that hundreds of residues need to be analyzed in a single LC/MS analysis. To address this challenge the MRM transitions that need to be monitored are switched using programmed time segments. This is called time segmented MRM. It is accomplished by programming the QQQ to monitor specific product ions in time segments during the LC/MS analysis. However, the method requires well defined elution time boundaries and must avoid time segment switches when compounds elute from the LC. If a time segmented MRM analysis is generated for a sample that contains hundreds of residues, the time segmented MRM analysis becomes subject to cycle and dwell time limitations that affect the sensitivity and specificity of the analysis. A new technique, Dynamic Multiple Reaction Monitoring (MRM) alleviates these limitations and also allows easier method development and future modifications of the method, such as the addition of new pesticides to be analyzed. Using Dynamic MRM, analyte ions are only monitored while they are eluting from the LC. This significantly improves the MS duty cycle time for very complex samples when compared with the time segment method and improves the sensitivity and specificity of an analysis.[1]

One of the challenges in developing an MRM method, whether it is a time segment or Dynamic MRM, is creating the time sequence of MS/MS events and mass spectrometer conditions necessary to maximize sensitivity and specificity. It is essential to generate a list of two or more MRM transitions and compound specific parameters, fragmentor voltage and collision energy for each compound being analyzed. The availability of a database containing over 600 pesticides with the MS/MS instrumental information that can be used with all Agilent triple quadrupoles eliminates the need to create this information via tedious manual procedures. The database allows easy import of selected compounds into the user's analytical method. A portion of this database is shown in Figure 2. In addition to creating custom methods, the read-only database allows the user to copy their customized database to meet his or her specific needs. A technical note describes this database in detail. [2] The Agilent Pesticides Application Kit also includes a pesticide test mixture that is used to demonstrate the performance of the system and pre-tested methods, allowing faster method development. Neither the kit nor the test mixture diminishes the need for each laboratory to define suitable QC/QA procedures and perform validation. Each laboratory must have QC tests fit-for-purpose and run analytical standards to validate analytical results.

This application note will demonstrate the use of the Agilent Pesticide Application Kit with a 600-compound parameter database and Dynamic MRM for the analysis of complex pesticide mixtures. The liquid chromatographic separations are performed using an Agilent 1200 SL Series RRLC or an Agilent 1290 Infinity UHPLC with an Agilent 6460 QQQ incorporating Jet Stream technology.[3] The methods described in the note are straightforward to generate using the Agilent MassHunter data analysis software and the Pesticide Dynamic MRM Database. Some limits of detection (LOD) of 100 fg or less were achieved using these methods with the Agilent 6460 Series QQQ LC/MS system. These methods are also compatible with all Agilent 6400 series LC/MS systems.

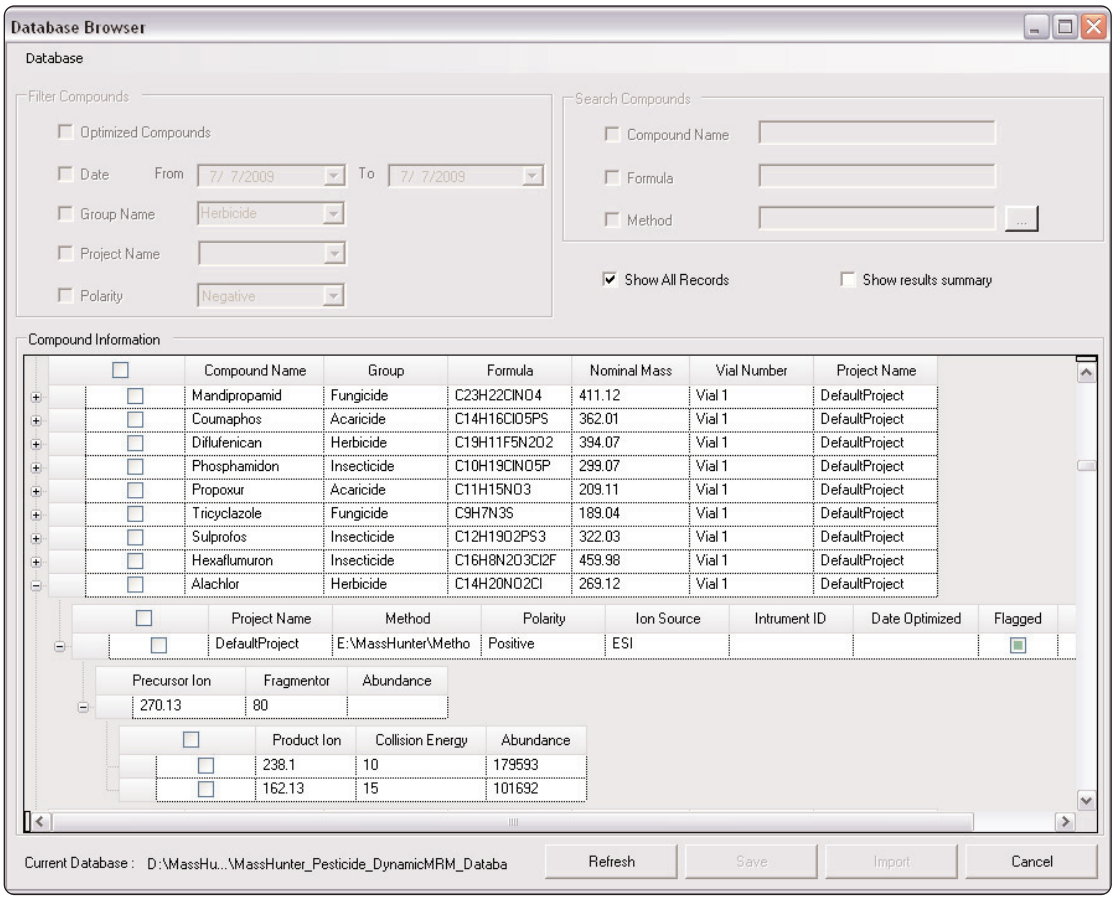


Figure 2. Compound Parameter Database with over 600 pesticides entries.

Experimental

Reagents and Chemicals

- Agilent Pesticide Test Mix, p/n 5190-0469 acid and base diluted separately as instructed to 10 ppb in 10% acetonitrile/90% water
- An Agilent SampliQ QuEChERS AOAC Extraction kit, p/n 5982-5755. Agilent SampliQ QuEChERS AOAC Dispersive SPE kits for Highly Pigmented Fruits and Vegetables, p/n 5982-5321 (2 mL) and p/n 5982-5356 (15 mL)
- Multiple pesticide standards were obtained from Sigma, Chemservice, and Dr. Erhenstofer

Instrument Settings

- *Appendix I: LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples
- Appendix II: LC/MS/MS Conditions for a 100 Pesticide Methods
- *Appendix III: LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL
- Appendix IV: LC/MS/MS Conditions for the 300-Pesticide Methods using the Agilent 1290 Infinity LC
- Appendix V: LC/MS/MS Conditions for Pesticides in Spinach using QuEChERS Extraction.
- *Appendix VI: LC/MS/MS Conditions for the 165-Pesticide Methods using the Agilent 1200 Series SL
- *Appendix VII: LC/MS/MS Conditions for the 224-Pesticide Methods using the Agilent 1200 Series SL
- Appendix VIII: LC/MS/MS Conditions for the 224-Pesticide Methods using Agilent 1290 Infinity LC

*Each of these methods are included with the Application Kit

Spinach Sample Preparation

- Weigh 15 g (±0.1 g) of homogenized spinach sample.
- Spike standards or IS solution if necessary.
- Vortex 30 s.
- Add 15 mL of 1% acetic acid in acetonitrile.
- Add 1 bag of extraction kit (p/n 5982-5982-5755) buffered QuEChERS extraction tubes, AOAC Method 2007.01 to 6 g $MgSO_4$ and 1.5 g NaAc.
- Cap and hand-shake vigorously for 1 min.
- Centrifuge at 4000 rpm for 5 min.
- Transfer 1 mL or 8 mL upper layer to the dispersive SPE kit (p/n 5982-5321 or p/n 5982-5356) for highly pigmented fruits and vegetables.
- Vortex 1 min.
- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15 mL tubes at 4000 rpm for 5 min.
- Transfer 200 µL of the upper layer to the autosampler vial.
- Add 800 µL of water or appropriate standard spiking solution.
- Vortex 1 min, to prepare for LC/MS/MS analysis.

Results and Discussion

Positive and Negative Ion Test Mix

In addition to the 600-compound database, the Agilent Application Kit for pesticide residue analysis also includes a positive and negative ion test mix, with their analysis methods shown in Appendix I. The methods contain compound names, MRM transitions, fragmentor voltages, collision energies, and retention times for the Dynamic MRM. The test mix and the supplied method allow the analyst to demonstrate that the system is operating properly for pesticide analysis immediately after installation. The LC/MS/MS extracted ion chromatograms (EIC) from the test mix analyzed in the positive and negative ion mode using Dynamic MRM is shown in Figures 3 and 4.

The Application Kit Quick Start Guide [4] shows the analyst how to run the test mixes and create a Dynamic MRM method. To create new methods, standards are analyzed at higher concentrations with a one segment MRM method. The data is processed using the Agilent MassHunter Quantitative Data Analysis software to generate a custom report that now includes analyte retention times. A Dynamic MRM method is generated by importing the results from the custom report and specifying a delta retention time window. This process will be automated in the near future. Table 1 shows a partial listing of

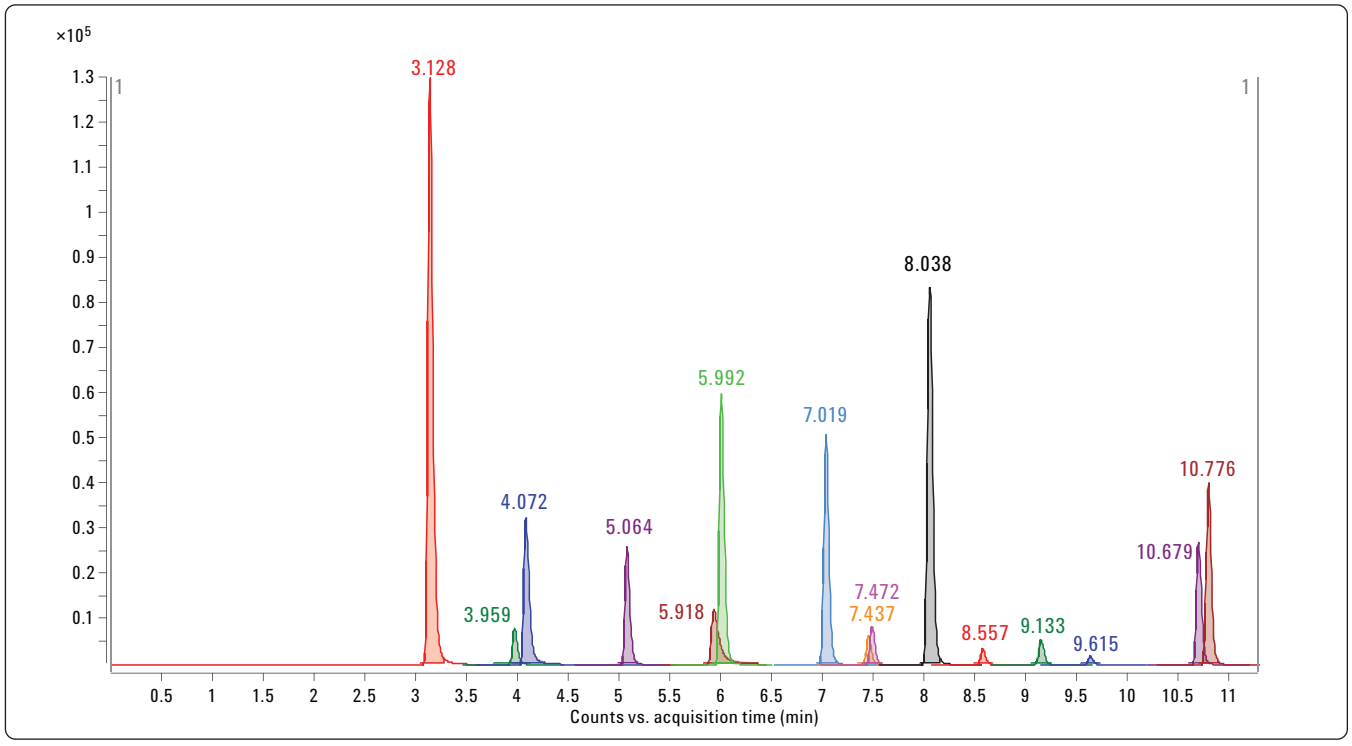


Figure 3. Positive ion test mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

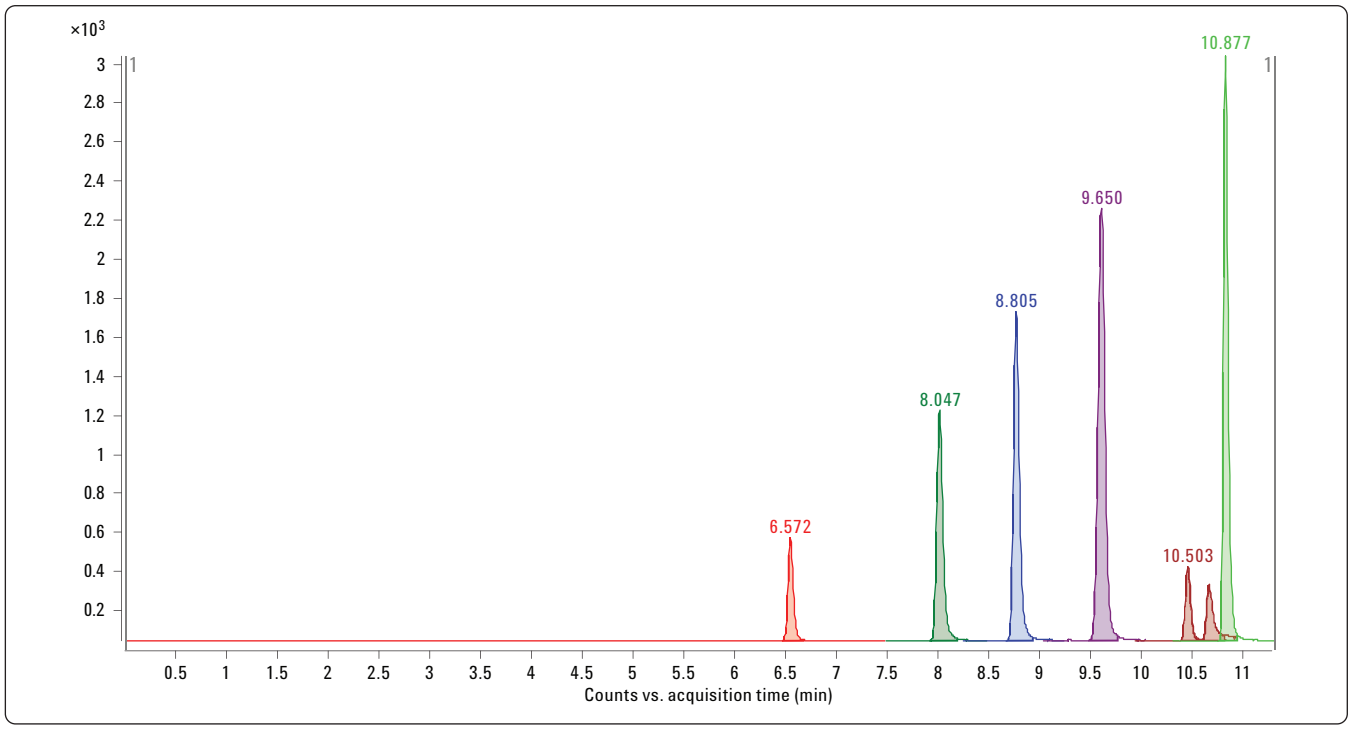


Figure 4. Negative Ion Test Mix extracted ion chromatogram (see Appendix 1 for list of compounds matching retention times given in chromatogram).

the acquisition parameters from a Dynamic MRM method. Note in this example the retention time window (Delta RT) is 2 min which is large for narrow peaks. A window this wide can be used to run standards where retention times have shifted and need to be updated in the users' customized method.

Table 1. Dynamic MRM Screen Capture of Acquisition Parameters

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time
Acephate	☐	184	Unit	125	Unit	80	10	1.212	2
Aminocarb	☐	209	Unit	137	Unit	120	20	1.251	2
Atrazine	☐	216	Unit	132	Unit	120	20	7.602	2
Azinphos-methyl	☐	318	Unit	132	Unit	80	10	9.346	2
Carbofuran	☐	222	Unit	123	Unit	120	15	7.13	2
Chlorpyrifos methyl	☐	322	Unit	125	Unit	80	15	12.168	2
Diazinon	☐	305	Unit	153	Unit	160	20	11.822	2
Dimethoate	☐	230	Unit	171	Unit	80	10	4.645	2
Imazalil	☐	297	Unit	159	Unit	160	20	6.498	2

Dynamic MRM Parameters

Cycle Time 500 ms

Fast and effective screening of a 100-compound pesticide mix using Dynamic MRM

A 100-compound mix of pesticides was used to demonstrate the effectiveness of the Dynamic MRM. Appendix II contains the LC/MS/MS conditions and a partial listing of the Dynamic MRM method used to analyze a 100-pesticide mixture at the 100 pg/compound level. Note that the column used was 50 mm in length so faster analysis and less efficiency is obtained. The LC/MS/MS extracted ion chromatogram shown in Figure 5 illustrates the performance of the system. The complete LC analysis took less than 15 minutes. Figure 6 shows a 1-min time window where 11 compounds (22 MRM's) are eluting. Figure 7 shows the 1-min delta retention time window for each Dynamic MRM transition. Note the many peak overlaps in the chromatograms. This necessitates the use of dynamic transitions instead of time segmented transitions in order to achieve the needed cycle time so that each peak can have enough data points to adequately describe the peak for quantitation. Furthermore time segmented MRM has an inherent "dead time" data loss when monitoring analyte peaks eluting near or between time segment boundaries. Time segmented MRM methods may require duplicate monitoring of specific analytes which elute over adjoining time segments. In addition, Dynamic MRM maximizes the dwell times for overlapping peaks enhancing the signal-to-noise while maintaining constant cycle time. Note that the cycle time selected should ideally provide about 20 data points across a peak with a minimum of 64 data points in the retention time window (Delta Ret Window).

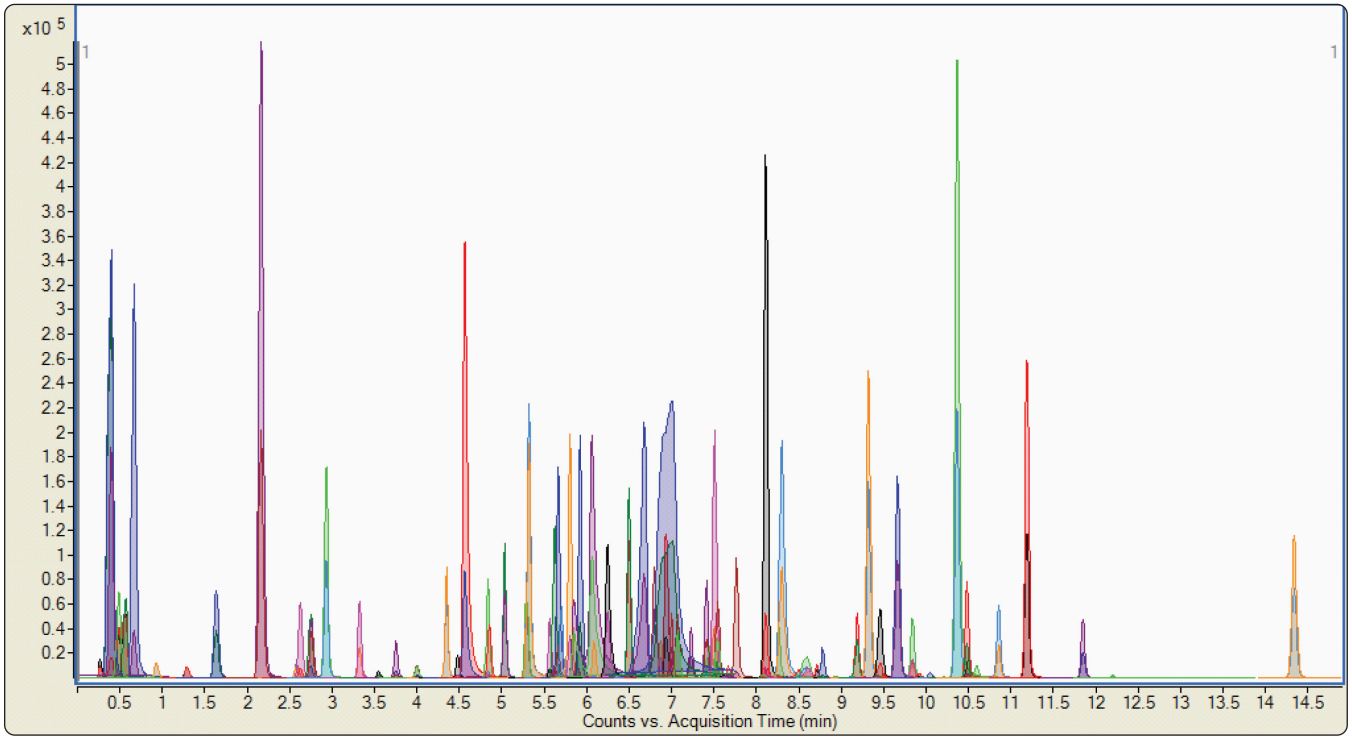


Figure 5. Extracted Ion Chromatograms of 100 compound pesticide mixture (100 pg level).

Compound name	Precursor ion	Product ion	Retention time
Cinosulfuron	414.1	183	5.579
Cinosulfuron (Q)	414.1	157	5.579
Chlorotoluron	213.1	72	5.642
Chlorotoluron (Q)	213.1	140	5.642
Atrazine	216.1	174	5.682
Atrazine (Q)	216.1	132	5.682
Carbaryl	202.1	145	5.736
Carbaryl (Q)	202.1	117	5.734
Carboxin	236.1	143	5.836
Carboxin (Q)	236.1	87	5.836
Chlorsulfuron	358.0	167	5.896
Chlorsulfuron (Q)	358.0	141	5.896
Ethiofencarb	226.1	107	5.937
Ethiofencarb (Q)	226.1	164	5.936
Dodemorph	283.3	116	6.073
Dodemorph (Q)	282.3	98	6.074
Diuron (Q)	233.0	160	6.101
Cyprodinil	226.1	108	6.245
Cyprodinil (Q)	226.1	93	6.246
Difenoxurone	287.1	123	6.509
Difenoxurone (Q)	287.1	72	6.509

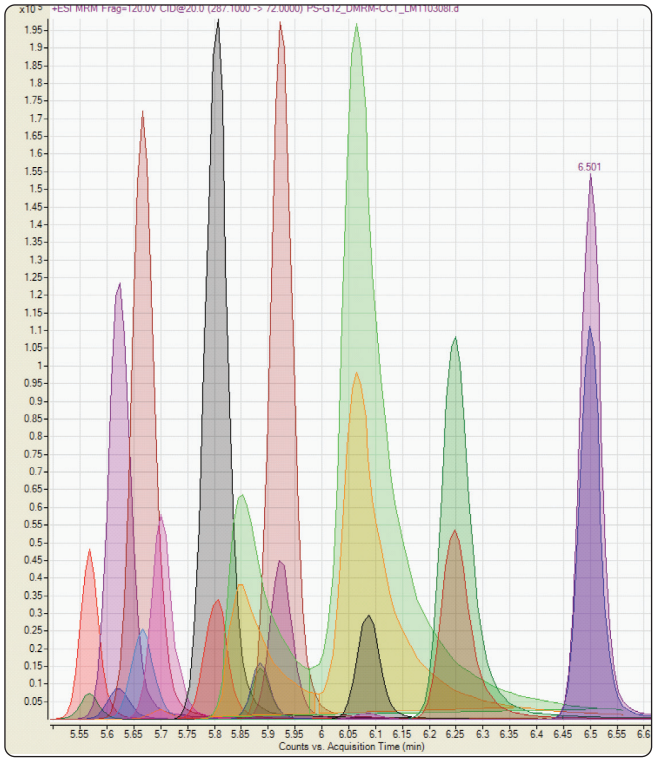


Figure 6. Left: Table of 11 compounds monitored during a 1 minute time window. Right: Dynamic MRM of compounds being monitored.

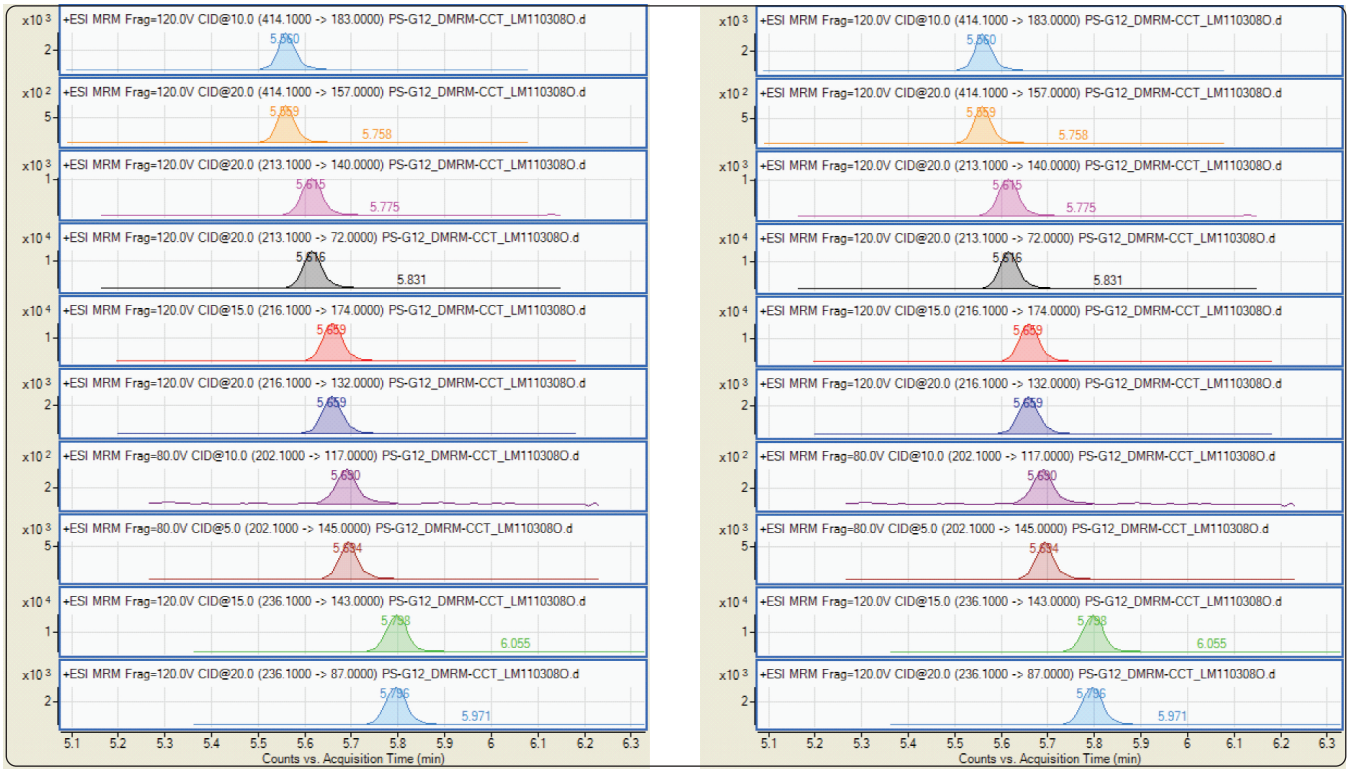


Figure 7. Dynamic MRM windows for each MRM transition.

Typical results achieved with the method are shown in Figure 8. It illustrates the results from one of the compounds, atrazine, in the 100-compound mixture. Note the 20 data points that were collected during the elution of atrazine. This provides a sufficient number of data points to assure quantitative accuracy and shows the effectiveness of Dynamic MRM.

Average signal height:	15,650
Average signal area:	50,966
RSD:	3.2%
Estimated LOQ:	100 fg or less

6–7 data points above FWHM

3 sec FWHM, 6 sec at 10% valley

20 data points baseline-to-baseline

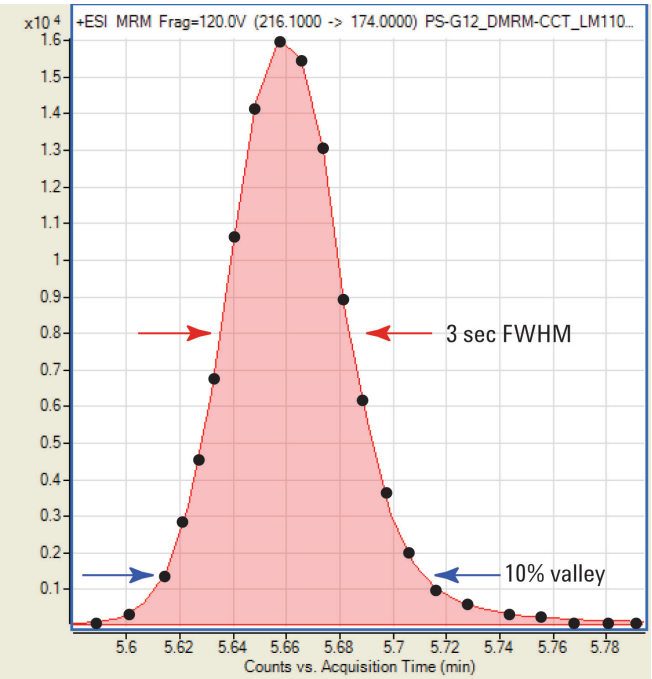


Figure 8. Typical analytical results shown with 10 pg of atrazine visualizing the effectiveness of Dynamic MRM.

The calibration data from four compounds in the mixture are illustrated in Figure 9. $R^{2'}$s = 0.0998 are achieved for each pesticide. With constant cycle time maintained, the quantitative results with Dynamic MRM are excellent.

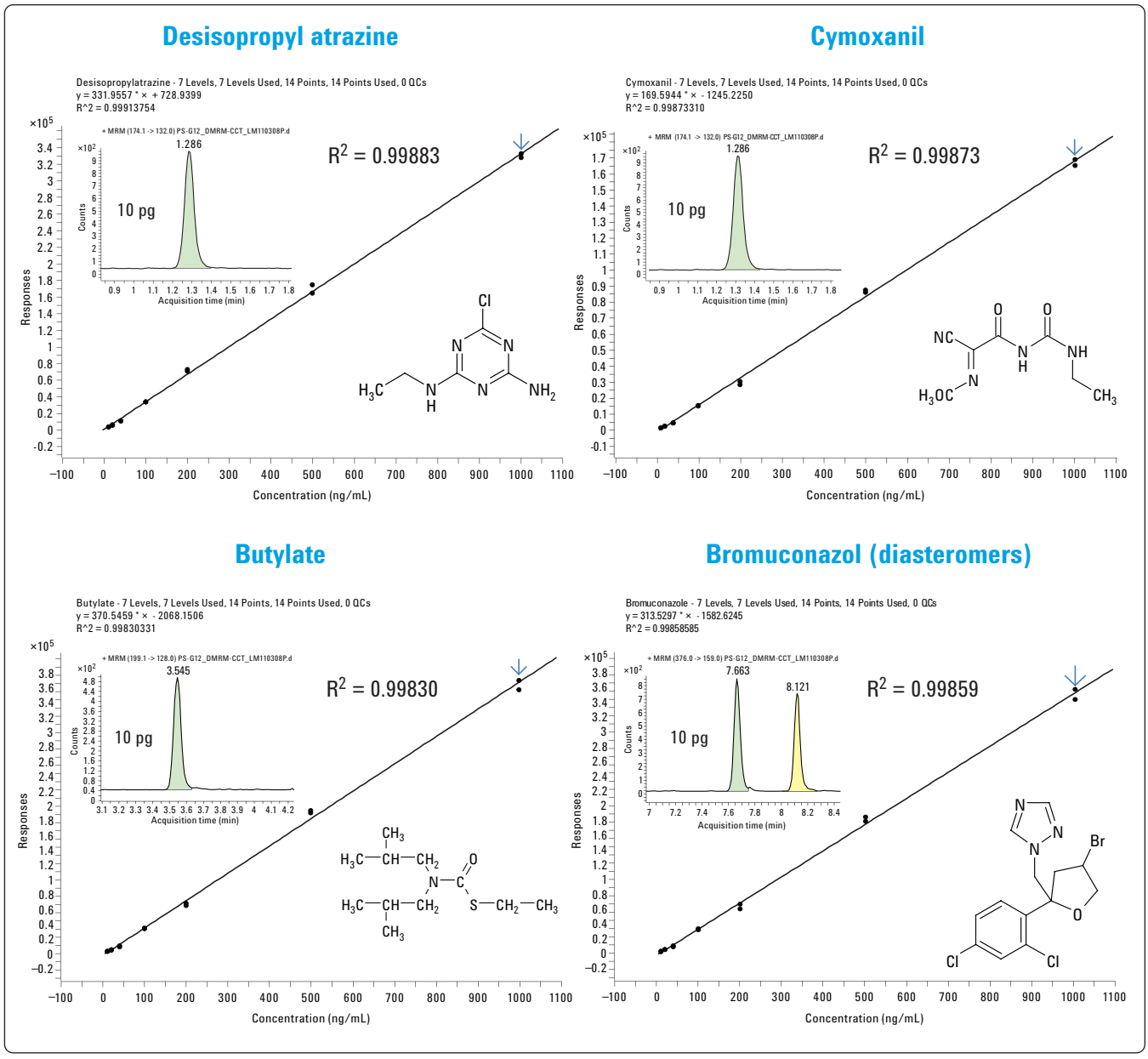


Figure 9. Dynamic MRM Calibration Plots, 10 pg–1 ng (7 levels).

Sharper peaks are produced with a 300-pesticide mix using the new Agilent 1290 Infinity LC

Appendix III contains a partial listing of the Dynamic MRM method used to analyze a 300-pesticide mixture at the 100 pg/compound level. The LC/MS/MS extracted ion chromatogram is shown in Figure 10. The analysis took less than 20 minutes using the Agilent 1200 Series SL RRLC and an Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column at a flow rate of 0.5 mL/min. The same mixture was separated using an Agilent 1290 Infinity UHPLC with an Eclipse-Plus C18, 2.1 mm × 150 mm, 1.8 µm column. Figure 11, an extracted ion chromatogram and Figure 12, an expanded portion of the chromatogram, demonstrate that this complex mixture has been analyzed in about 15 minutes which is approximately 25% faster than with the Agilent 1200 Series SL RRLC. The Agilent 1290 Infinity UHPLC also produced a separation with higher peak capacity and less peak overlap than the Agilent 1200 Series SL RRLC. Typical peak ½ heights using atrazine as an example with the Agilent 1290 Infinity UHPLC are 1.8 s. This is because the longer column provides higher efficiency and the Agilent 1290 Infinity LC can operate at the pressure these conditions incurred (~900 bar).

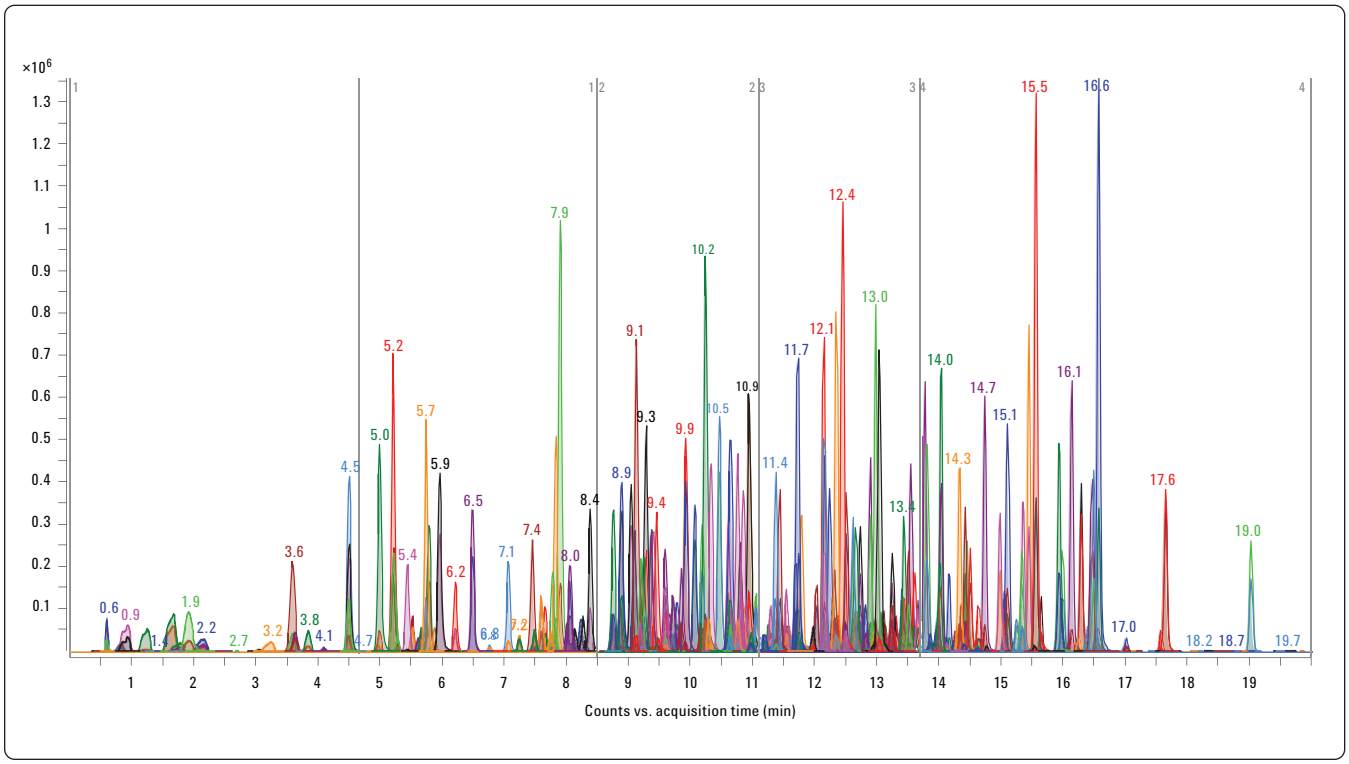


Figure 10. EIC of 300 compound pesticide mixture using an Agilent 1200 Series SL RRLC.

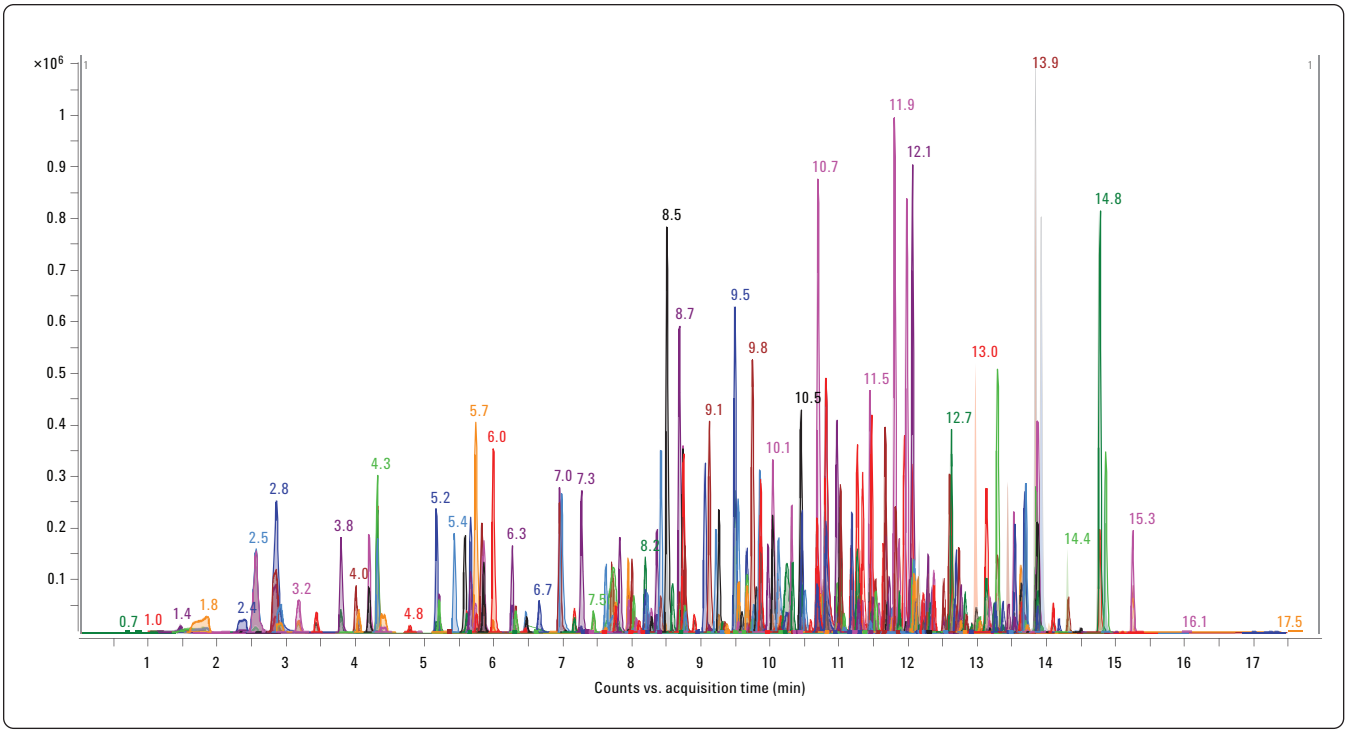


Figure 11. EIC of 300-compound pesticide mixture using the Agilent 1290 Infinity UHPLC.

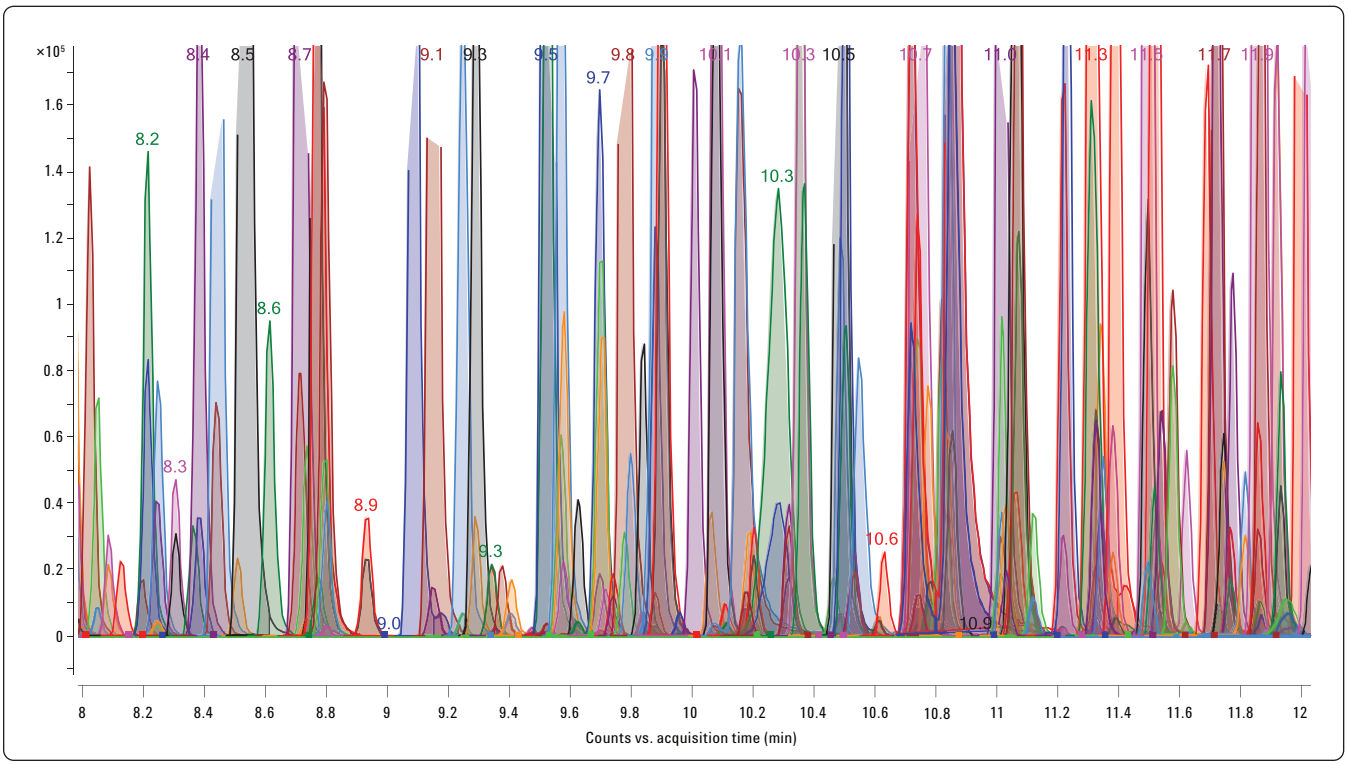


Figure 12. Expanded EIC of 300-compound pesticide mixture using an Agilent 1290 Infinity UHPLC illustrating the high peak capacity of the Agilent 1290 Infinity.

Faster analysis with a 224-pesticide mix using the new Agilent 1290 Infinity LC

Another advantage of the Agilent 1290 Infinity LC with the Agilent 6460 Series QQQ LC/MS is the ability to increase flow and decrease analysis time. Using the 1200 Series SL the analysis of 225 pesticides is performed in 15 min and shown in Figure 13. The method for this analysis is given in Appendix IV. With the Agilent 1290 Infinity LC the flow can be doubled and the gradient completed in half the time. This provides the same separation in less than 7 min as shown in Figure 14. The method for this analysis is given in Appendix V. Analyzing hundreds of pesticides in one run, it is best to obtain the highest peak capacity as shown in the 300-pesticide example. However, if speed of analysis is absolutely necessary, it is shown that the higher pressure capability of the Agilent 1290 Infinity LC and the higher pressure capability of the HD columns provide the performance needed.

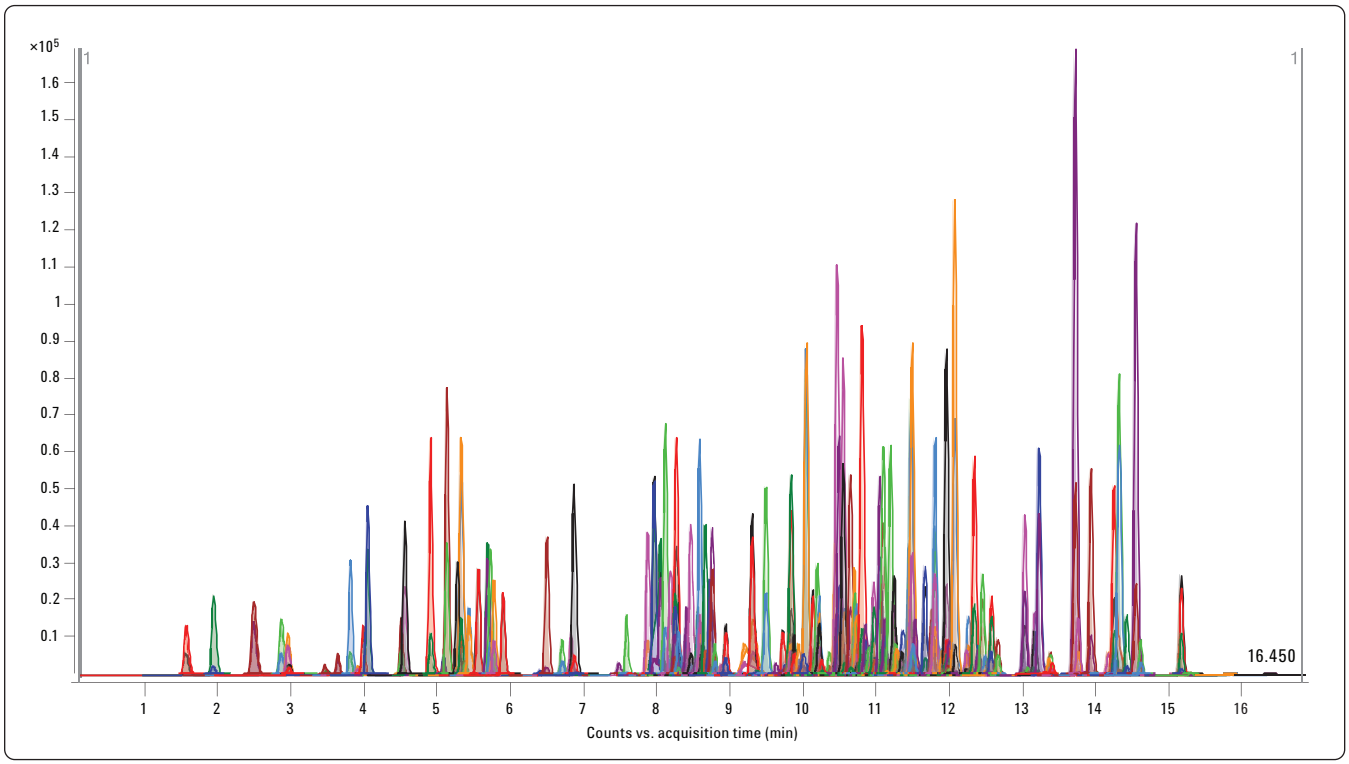


Figure 13. EIC of 224 pesticides using the Agilent 1200 Series SL LC and the Agilent 6460 QQQ LC/MS.

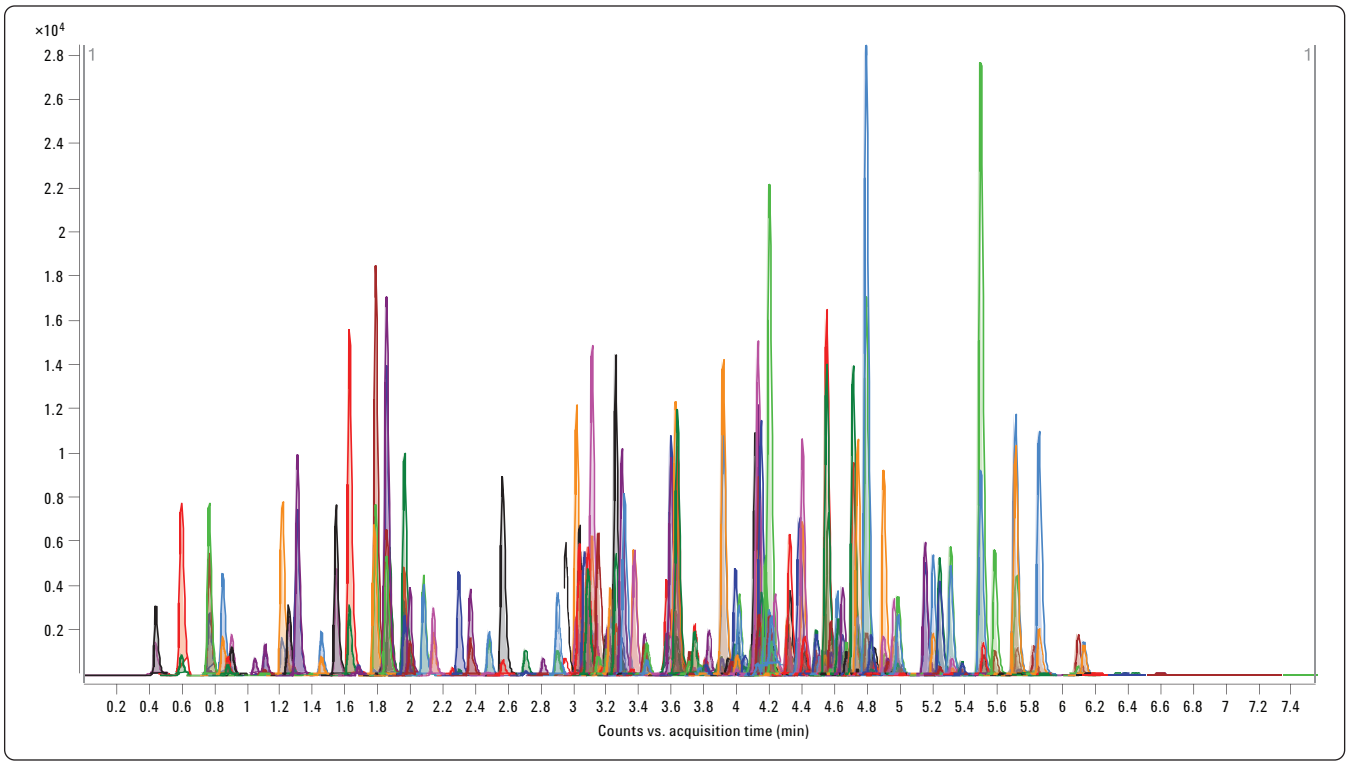


Figure 14. EIC of 224-pesticide mix analyzed with Agilent 1290 Infinity LC and the Agilent 6460 QQQ LC/MS.

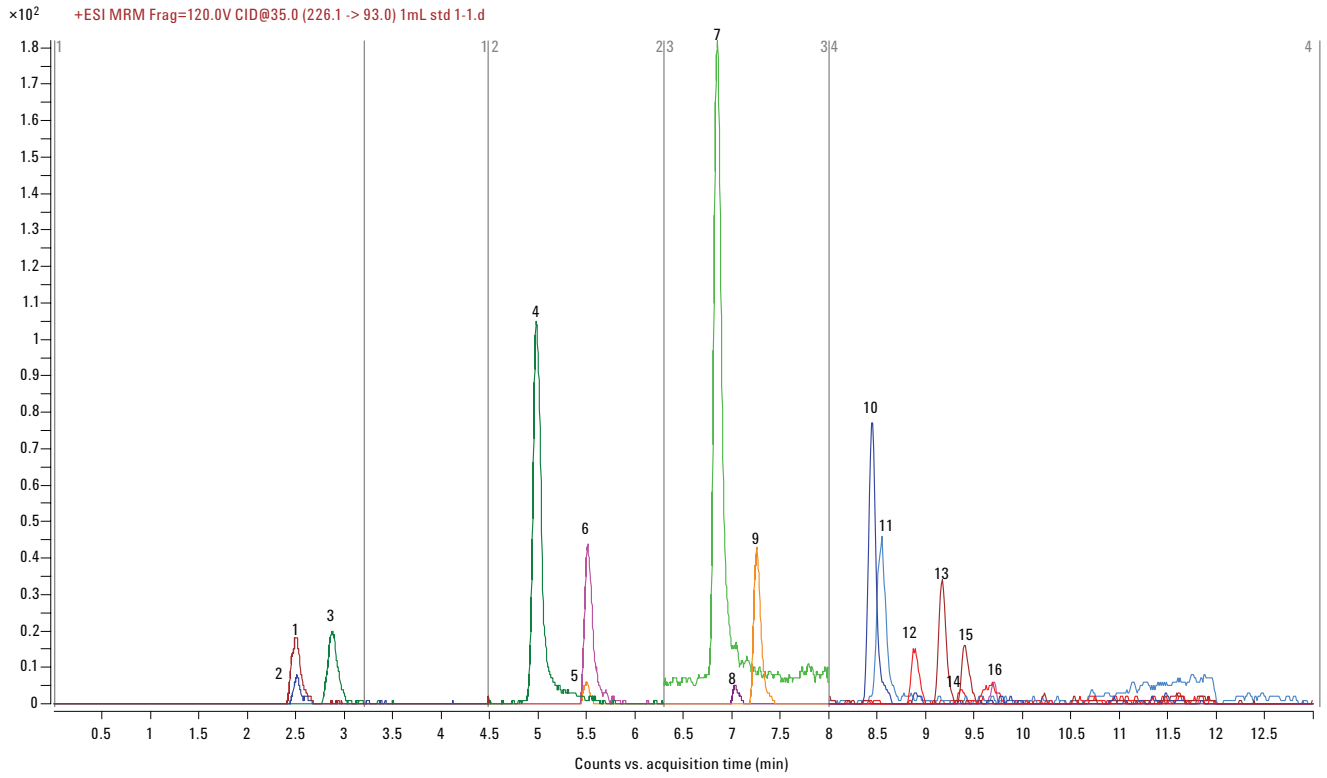
Pesticides Application Kit in a food matrix-Spinach using SampliQ Extraction and Dispersive SPE Kits

To demonstrate the use of the Agilent application kit for the analysis of a typical food product with Agilent's easy to use SampliQ extraction and dispersive SPE kits, a spinach matrix was spiked with 10 ppb of the 16 pesticides listed in Table 2. Triphenylphosphate (TPP) is the internal standard.

Table 2. List of 16 Pesticides and Instrument Parameters Spiked into Spinach Matrix at 10 ppb

Analyte	MRM channel (*m/z*) Quantifier	Qualifier	Fragmentor (V)	Collision energy (V) Quantifier	Qualifier	Retention Time (min)
Acephate	184.0 > 94.9	184.0 > 110.0	60	3	15	2.55
Methamidophos	142.0 > 94.0	142.0 > 124.9	60	8	8	2.54
Pymetrozine	218.1 > 105.0	218.1 > 78.0	115	20	50	2.97
Carbendazim	192.1 > 160.0	192.1 > 105.0	95	18	40	5.07
Imidacloprid	256.1 > 209.1	256.1 > 175.0	60	12	18	5.53
Thiabendazole	202.1 > 175.0	202.1 > 131.0	110	27	38	5.65
Propoxur	210.1 > 111.0	210.1 > 92.9	50	12	15	6.89
Thiophanate methyl	343.1 > 151.0	343.1 > 117.9	105	17	65	7.08
Carbaryl	202.0 > 145.0	202.0 > 115.0	50	3	40	7.30
Ethoprophos	243.1 > 130.9	243.1 > 172.9	80	15	15	8.50
Imazalil	297.1 > 158.9	297.1 > 200.9	80	22	15	8.52
Penconazole	284.1 > 158.9	284.1 > 172.9	80	32	32	8.95
Cyprodinil	226.1 > 93.0	226.1 > 108.0	120	35	35	9.23
Dichlorfluanid	333.0 > 123.0	333.0 > 223.9	85	28	5	9.40
Kresoxim methyl	314.0 > 222.1	314.0 > 235.0	70	10	10	9.44
Tolyfluanid	347.0 > 136.9	347.0 > 238.0	60	25	3	9.73
TPP (IS)	327.1 > 77.0	327.1 > 151.9	70	45	45	9.49

Figure 15 shows the EIC of the spinach sample spiked at the 10-ppb pesticide level. All the pesticides are easily detected at this level with a total analysis time less than ten minutes.



10 ppb of pesticides spiked into the spinach matrix.

Peak identification:

1. Acephate
2. Methamidophos
3. Pymetrozine
4. Carbendazim
5. Imidacloprid
6. Thiabendazole
7. Propoxur
8. Thiophanate methyl
9. Carbaryl
10. Ethoprophos
11. Imazalil
12. Penconazole
13. Cyprodinil
14. Dichlorfluanid
15. Kresoxim methyl,
16. Tolyfluanid

Internal standard peak (TPP) is not shown to get clear peak profile of other small peaks.

Figure 15. EIC of 10 ppb pesticides into spinach matrix..

An example of the linearity achieved for the spiked spinach matrix is shown in Figure 16. The calibration range was 5 – 250 ng/g and seven levels were used to generate the curve, 5, 10, 25, 100, and 250 ng/g. The curve was generated by plotting the ratio of the analyte peak area, carbaryl, to the internal standard (IS) peak area with the ratio of the analytes concentration to IS concentration. The $R^2 = 0.998$.

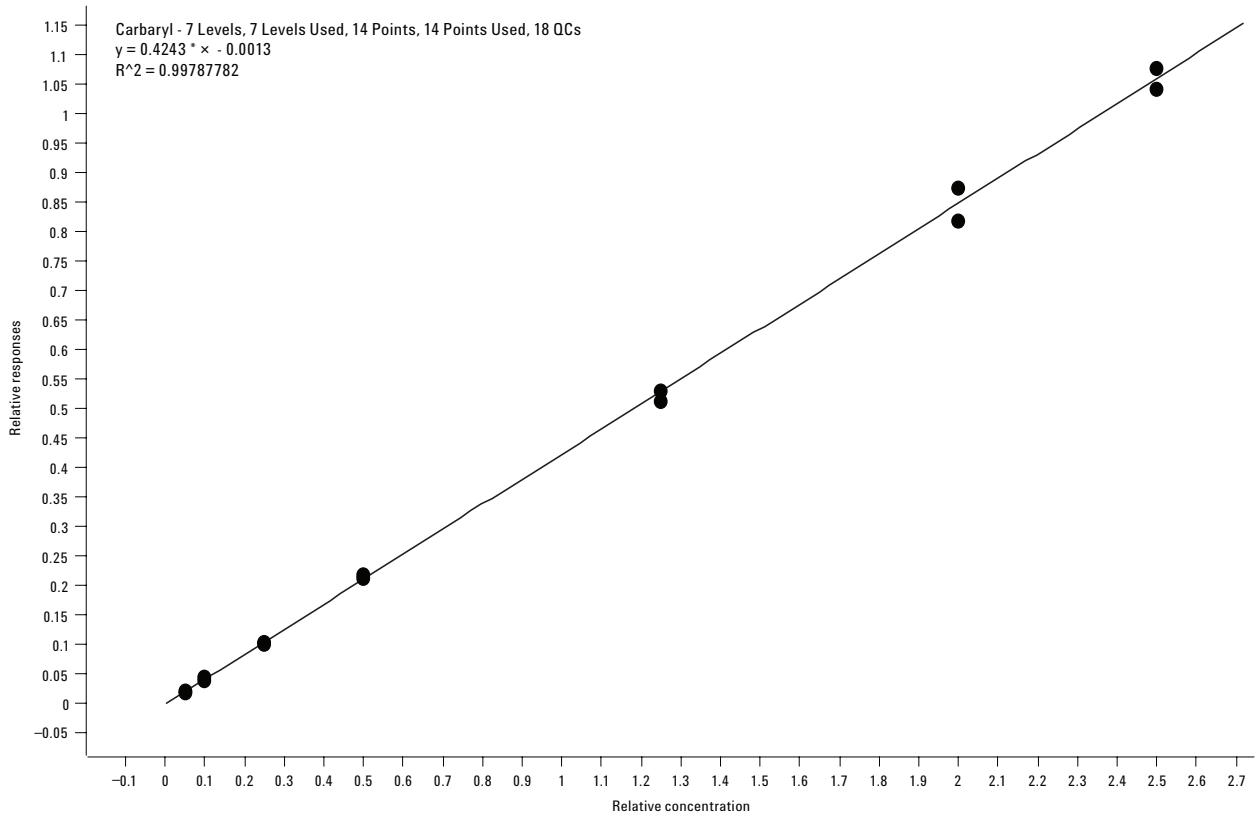


Figure 16. Carbaryl calibration curve.

Conclusions

The **Agilent Pesticide Application Kit** for LC/QQQ provides the user with fast method development for hundreds of pesticides with multiple transitions and the ability to develop those methods customized to his or her specific analytical needs.

This application note demonstrates the use of the Agilent Application Kit for Pesticides using several Agilent technologies for screening large numbers of compounds. The following technologies are used:

- ***600 compound pesticide MRM database*** and the **Agilent MassHunter Data Acquisition and Analysis software.** The combination gives users the ability to generate acquisition and analysis methods quickly. The methods can be easily customized and rapidly modified to meet the needs of future analyses.
- **Dynamic MRM** which maximizes the detection capability of the QQQ when hundreds of residues are being analyzed.
- **Agilent 1200 Series SL RRLC** interfaced to the **Agilent 6400 series triple quadrupoles** for fast and high resolution LC/MS/MS analysis. Use of the Agilent 6460 QQQ with Agilent's Jet Stream Electrospray Ion Source ensures lowest levels of detection of the pesticides. However, any of the Agilent 6400 series LC/QQQ will provide excellent results.
- Easy to use **SampliQ QuEChERS sample preparation kits** included in the Application Kit provide a fast and reproducible method to extract pesticide residues from complex food matrixes in a few simple steps.
- **Ready to use methods** with retention times for Dynamic MRM using the Agilent 1200 Series SL LC system. See all * Appendix methods.[4]

Use of these technologies allows methods to be quickly developed and enables screening of complex matrices containing hundreds of potential residues at femtomole concentrations.

This kit is compatible with all Agilent 1200 Series LC and 6400 series QQQ MS systems and will enable the user to quickly get started running multi-residue pesticides. For the most demanding analyses, the Agilent 1290 Infinity LC with the 6460 QQQ should be considered. Additional methods for this system should be available in the near future.

References

1. Application Note 5990-3595EN, New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Samples.
2. Technical Note 5990-4255EN Pesticide Dynamic Multi-reaction monitoring Database.
3. Technical Note 5990-3494EN Agilent Jet Stream Thermal GradienFocusing Technology.
4. Agilent Publication 5990-4262EN Pesticide analysis with DRMRM database quick start guide.

For More Information

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Appendix I

LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples

Agilent 1200 Series SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:	35
Injection volume:	5
Autosampler temperature:	Ambient
Needle wash:	5 s with methanol
Mobile phase:	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow Rate:	0.3 mL/min
Gradient:	5% B at t = 0 to 95% B at t = 12 min
Stop Time:	12 min
Post:	Time 3 min

Jet Stream Conditions

Gas temperature:	250 °C
Gas flow:	7 L/min
Nebulizer:	40 psi
Sheath gas temperature:	325 °C
Sheath gas flow:	1 L/min
Capillary + ion:	3500 V
Nozzle voltage:	0 V
Capillary – ion:	2500 V
Nozzle voltage:	1500 V

MS/MS Scans for positive ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Aminocarb	❑	209	Unit	137	Unit	120	20	3.128	1	Positive
Imazapyr	❑	262	Unit	217	Unit	160	15	3.959	1	Positive
Thiabendazole	❑	202	Unit	131	Unit	120	30	4.072	1	Positive
Dimethoate	❑	230	Unit	171	Unit	80	10	5.064	1	Positive
Imazalil	❑	297	Unit	159	Unit	160	20	5.918	1	Positive
Metoxuron	❑	229.1	Unit	72.1	Unit	93	14	5.992	1	Positive
Carbofuran	❑	222	Unit	123	Unit	120	15	7.019	1	Positive
Atrazine	❑	216	Unit	132	Unit	120	20	7.437	1	Positive
Metosulam	❑	418	Unit	175	Unit	144	26	7.472	1	Positive
Metazachlor	❑	278.1	Unit	134.1	Unit	75	18	8.038	1	Positive
Molinate	❑	188.1	Unit	55.1	Unit	78	22	9.113	1	Positive
Malathion	❑	331	Unit	99	Unit	80	10	9.615	1	Positive
Pyraclostrobin	❑	388	Unit	163	Unit	120	20	10.679	1	Positive
Diazinon	❑	305	Unit	153	Unit	160	20	10.776	1	Positive

MS/MS Scans for negative ions

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Bentazon	❑	239.1	Unit	132	Unit	80	32	6.572	1	Negative
2,4,5-T	❑	252.9	Unit	194.8	Unit	76	9	8.047	1	Negative
Silvex	❑	266.9	Unit	194.9	Unit	90	5	8.805	1	Negative
Acifluorfen	❑	360	Unit	315.9	Unit	78	5	9.650	1	Negative
Dinoseb	❑	239.1	Unit	207	Unit	154	21	10.503	1	Negative
Hexaflumuron	❑	459	Unit	438.9	Unit	102	5	10.877	1	Negative

Appendix II

LC/MS/MS Conditions for 100-Pesticide Methods

Agilent 1200 Series LC Parameters

Column:		Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 50 mm, 1.8 µm Agilent p/n 959741-902
Column temperature:		35 °C
Injection volume:		1.0 µL
Autosampler temperature:		6 °C
Needle wash:		Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:		A = 0.1% formic acid in water B = 0.1% formic acid in 95:5 acetonitrile:water
Flow rate:		0.6 mL/min
Gradient	Time	%B
	0	10
	10	70B
	15	90B
Stop time	20	10B
Post time	5	

Note that example transitions, fragmentor voltages, and collision energies for this method are shown in Figure 7.

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Agilent 6460A QQQ settings

MS1 and MS2 resolution:	Unit
Time Filtering:	Peak width = 0.03 min
Dynamic MRM transitions:	200
Constant cycle time:	373 ms
Delta EMV:	400 V

Appendix III

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	1.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.5 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
18	No change	600	95
20	No change	600	95
20.01	No change	600	6
Stop time	20 min	10%B	
Post time	5 min		

Jet Stream Conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	6 L/min
Nebulizer gas pressure (nitrogen):	35 psig
Capillary voltage:	4000 V
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	Off

Ten representative MS/MS Transitions from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Promecarb	❑	208.1	Unit	151	Unit	80	5	11.635
Promecarb	❑	208.1	Unit	109	Unit	80	10	11.635
Flurtamone	❑	334.1	Unit	303	Unit	120	20	11.644
Flurtamone	❑	334.1	Unit	247	Unit	120	30	11.644
Isoxaflutole	❑	377.1	Unit	360.1	Unit	100	5	11.669
Isoxaflutole	❑	360.1	Unit	251	Unit	120	10	11.669
Dimethenamide	❑	276.1	Unit	244	Unit	120	10	11.683
Dimethenamide	❑	276.1	Unit	168	Unit	120	15	11.683
Diethofencarb	❑	268.2	Unit	226	Unit	80	5	11.706
Diethofencarb	❑	268.2	Unit	152	Unit	80	20	11.706

Appendix IV

LC/MS/MS Conditions for 300-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1290 LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 150 mm, 1.8 µm RRHD 1200 Series bar columns Agilent p/n 959759-902
Column temperature:	60 °C
Injection volume:	35 µL (stacked injection, 5 µL sample + 30 µL H_2O
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25 + 0.01% formic acid), 10 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = MeOH w/5 mM ammonium formate + 0.01% formic acid
LC flow rate:	0.5 mL/min
LC gradient:	6% B (T = 0) to 98% B (T = 15 min), hold 3 min

MS Parameters

Sheath gas flow:	11 L/min
Sheath gas heater:	375 °C
Charging Electrode:	300 V (pos ion mode)
Capillary voltage:	–4 kV (pos ion mode)
Nebulizer pressure:	35 psig
Drying gas temperature:	325 °C
Drying gas flow:	8 L/min

Ten representative MS/MS Scan Segments from 300-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time
Chloridazon	❑	222	Unit	104	Unit	120	25	5.841
Chloridazon	❑	222	Unit	92	Unit	120	30	5.841
Aminocarb	❑	209.1	Unit	152.1	Unit	120	10	5.841
Aminocarb	❑	209.1	Unit	137	Unit	120	20	5.841
Fluroxypyr	❑	255	Unit	209	Unit	80	10	5.845
Fluroxypyr	❑	255	Unit	181	Unit	80	15	5.845
Acetamiprid	❑	223.1	Unit	126	Unit	80	15	5.858
Acetamiprid	❑	223.1	Unit	56	Unit	80	15	5.858
Vamidothion	❑	288	Unit	146	Unit	80	10	5.996
Vamidothion	❑	288	Unit	118	Unit	80	20	5.996

Appendix V

LC/MS/MS Conditions for Pesticides in Spinach using QuECHERS Extraction

Agilent 1200 Series HPLC conditions

Column:		Agilent ZORBAX Eclipse Plus Phenyl-hexyl, 150 mm × 3 mm, 3.5 µm Agilent p/n 959963-312	
Column temperature:		30 °C	
Injection volume:		10 µL	
Mobile phase:		A = 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H_2O B = 5 mM ammonium acetate, pH 5.0 in ACN	
Needle wash:		1:1:1:1 ACN/MeOH/IPA/H_2O w/0.2% FA	
Gradient:	Time (min)		Flow rate
	(min)	% B	(mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	10.1	20	0.5
	12.0	100	0.5
Stop time:	13.0 min		
Post run:	4 min		
Total cycle time:	17 min		

Agilent 6410 MS conditions

Positive mode	
Gas temperature:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 psi
Capillary:	4000 V

Appendix VI

LC/MS/MS Conditions for 165-Pesticide Methods using the Agilent 1200 Series Infinity SL

Agilent 1200 Series Infinity SL LC Parameters

Column:		Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm 1.8 µm Agilent p/n 959764-902
Column temperature:		35 °C
Injection volume:		5.0 µL
Autosampler temperature:		6 °C
Needle wash:		Flushport (MeOH:H2O 75:25) 5 s
Mobile phase:		A = H2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in methanol
Gradient	Pump Time Table	
	Time (min)	Solv ratio B (%)
	0.00	10
	1.00	10
	18.00	100
	20.00	100
	20.10	10
	25.00	10

Jet Stream Conditions

Spray Chamber Conditions

Gas temperature:	200 °C
Dry gas :	6 L/min
Nebulizer:	35 psi
Sheath gas temperature:	250 °C
Sheath gas flow:	12 L/min
Positive cap voltage:	4000 V
Nozzle voltage:	300 V

Ten Representative MS/MS Transitions from 167-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Ethiofencarb-sulfon	❑	275	Unit	201	Unit	80	0	6.89	1
Ethiofencarb-sulfon	❑	275	Unit	107	Unit	80	10	6.89	1
Clothianidin	❑	250	Unit	169	Unit	90	5	7.064	1
Clothianidin	❑	250	Unit	132	Unit	90	15	7.064	1
Imidacloprid	❑	256.1	Unit	209	Unit	80	15	7.071	1
Imidacloprid	❑	256.1	Unit	175.1	Unit	80	20	7.071	1
Ethiofencarb-sulfoxid	❑	242	Unit	185	Unit	80	15	7.153	1
Ethiofencarb-sulfoxid	❑	242	Unit	107	Unit	80	5	7.153	1
Monalide	❑	257.1	Unit	200.1	Unit	105	4	7.165	1
Monalide	❑	257.1	Unit	137.1	Unit	105	8	7.165	1

Appendix VII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1200 Series SL

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.3 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
14	No change	600	95
17	No change	600	95

Stop time 17 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

Ten representative MS/MS Transitions from 224-Compound Methods

Compound Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window
Buprofezin	❑	306.2	Unit	201.1	Unit	115	4	14.321	1
Buprofezin	❑	306.2	Unit	57.2	Unit	115	16	14.321	1
Sulprofos	❑	323	Unit	247.1	Unit	130	5	14.327	1
Sulprofos	❑	323	Unit	219	Unit	130	12	14.327	1
Eprinomectin B1a	❑	914.6	Unit	468.3	Unit	150	5	14.372	1
Eprinomectin B1a	❑	914.6	Unit	330.3	Unit	150	10	14.372	1
Chlorfluazuron	❑	540	Unit	383	Unit	115	16	14.402	1
Chlorfluazuron	❑	540	Unit	158	Unit	115	16	14.402	1
Fenpyroximat	❑	422.2	Unit	366.2	Unit	130	15	14.428	1
Fenpyroximat	❑	422.2	Unit	135	Unit	130	40	14.428	1

Appendix VIII

LC/MS/MS Conditions for 224-Pesticide Methods using the Agilent 1290 Infinity LC

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus-C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.6 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
7	No change	600	95
10	No change	600	95

Stop time 10 min
Post time 3 min

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	10 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V

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