LC-MS/MS and GC-MS/MS Multi Residue Pesticide Analysis in Fruit and Vegetable Extracts on a Single Tandem Quadrupole Mass Spectrometer

by Kari Organtini[1], Gareth Cleland[1], Eimear McCall[2], and Simon Hird[2]
[1]Waters Corporation, Milford, MA, USA
[2]Waters Corporation, Wilmslow, UK

Hundreds of pesticides are commercially available and are approved for use on various fruit and vegetable plants to prevent pest infestation and improve shelf-life of fresh produce. Maximum Residue Levels (MRLs) are set at the highest level of pesticide that the relevant regulatory body would expect to find in that crop when it has been treated in accordance with good agricultural practice. In the EU, if a pesticide is not explicitly mentioned in the MRL legislation, a default MRL is used for enforcement. This default value is set to be equal to the limit of quantification (LOQ) achievable with the analytical methods used for analysis. National authorities control and enforce MRLs by testing samples for pesticide residue levels using analytical surveillance programs. These programs check for compliance with MRLs, assess dietary exposure, and check for use of unauthorised pesticides. The food industry also carries out its own due diligence analyses.

Mass spectrometry (MS) coupled with both gas chromatography (GC) and liquid chromatography (LC) is needed to provide comprehensive analysis of a wide range of pesticide residues with sufficient sensitivity to meet global MRL regulations. The use of Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) sample extraction and clean up has streamlined analytical efficiencies for multi residue analyses [1]. The advantage of ultra high performance liquid chromatography (UHPLC) coupled with tandem quadrupole mass spectrometry (MS/MS) for multi residue pesticide analysis is widely reported [2]. More recently the use of GC-MS/MS utilising atmospheric pressure ionisation (APGC) has been shown to offer significant improvements in performance over EI for challenging pesticides, in terms of selectivity, specificity, and speed of analysis [3,4].

The APGC source ionises compounds using a corona discharge at atmospheric pressure in an APCI-like manner. Therefore, this ionisation mechanism is a much softer technique than classic electron impact (EI) ionisation and produces larger amounts of intact parent ions, especially in the case of fragile or easily fragmented compounds. APGC ionisation can occur using two mechanisms; proton transfer (wet source) or charge transfer (dry source). In proton transfer ionisation, $[M+H]^+$ ions are formed, whereas in charge transfer ionisation, $M^{+\cdot}$ ions are formed.

In this work, a single workflow for the multi residue analysis of pesticides is demonstrated on a variety of fruit and vegetable samples. Utilising the universal source of Waters Xevo® TQ-S micro mass spectrometer allows for LC (electrospray ionisation) and GC (atmospheric pressure ionisation) analyses to be completed on the same tandem quadrupole MS instrument, with less than 30 minutes needed to switch between chromatographic inlets. The performance of the method will be highlighted in terms of sensitivity, repeatability, and linearity for both LC and GC in compliance with the SANTE guidelines (11945/2015) for pesticide analysis [5].

Methods

The LC and GC suites of pesticides analysed in this study (listed in appendix tables) were chosen to cover a wide range of different pesticide classes and chemistries. The multi residue MS/MS methods were generated using the Quanpedia™ database, with separate databases utilised for generation of the LC and GC methods. Each database contains MRMs and retention time information for each compound. When the MS method is generated the MRM function windows are automatically set for each compound. For the LC method, a window of 1 minute was placed around each compound's expected retention time. For the GC method, a window of 30 seconds was used due to the narrower peak widths exhibited in GC analysis. In addition to the MS methods, the TargetLynx™ software data processing methods and LC inlet method were also generated through the Quanpedia database.

Sample Extraction and Cleanup

Celery, lemon, corn, and kale samples were purchased at a local grocery store. Samples were chosen to be representative of different types of matrix complexity from

different commodity groups, including high water content (celery and kale), high acid content (lemon), and high starch/ protein with low water content (corn). Samples were immediately homogenised in a food processer and frozen until sample preparation was performed. QuEChERS extraction was performed according to the official AOAC method 2007.01 using Waters DisQuE™ Dispersive Solid Phase Extraction (d-SPE) product [6]. Figure 1 highlights the sample extraction.

Table 1: dSPE clean up conditions used for each sample matrix.

Sample	$MgSO_4$	PSA	GCB	Volume
Celery	150 mg	25 mg	7.5 mg	1 mL
Lemon	150 mg	25 mg	-	1 mL
Corn	150 mg	25 mg	-	1 mL
Kale	900 mg	150 mg	150 mg	6 mL

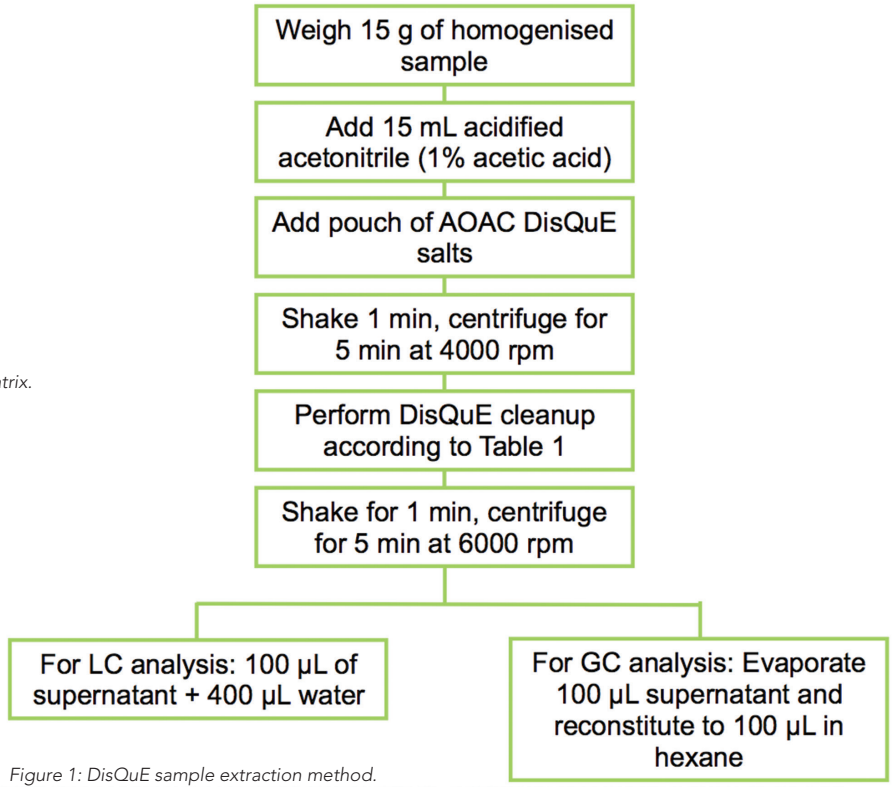


Figure 1: DisQuE sample extraction method.

Experimental

LC-MS/MS Conditions

LC system: ACQUITY UPLC H-Class
Column: ACQUITY UPLC BEH C18 1.7 µm, 2.1 x 100 mm
Column temp.: 45°C
Injection volume: 5 µl
Flow rate: 0.45 mL/min
Mobile phase A: Water + 10 mM ammonium acetate
Mobile phase B: Methanol + 10 mM ammonium acetate

Gradient:

Time (min)	% A	% B
0.00	98	2
0.25	98	2
12.25	1	99
13.00	1	99
13.01	98	2
17.00	98	2

MS System: Xevo TQ-S micro
Ionisation mode: ESI+
Capillary voltage: 1 kV
Desolvation temp.: 500°C
Desolvation gas flow: 1000 L/hr
Source temp.: 150°C

GC-MS/MS Conditions

GC System: 7890A
Autosampler: CTC PAL RTC
Column: 30 m x 0.25 mm x 0.25 µm Rxi-5MS
Carrier gas: Helium
Flow rate: 2.0 mL/min

Injection: Splitless
Injector temp: 280°C
Injection volume: 1 µl
Makeup gas: Nitrogen at 250 mL/min
Transfer line temp.: 320°C
Oven program:

Rate (°C/min)	Temp. (°C)	Hold (min)
-	80	1.00
25	150	0.00
8	270	0.00
20	320	4.10

MS system: Xevo TQ-S micro
Ionisation mode: APGC+
Ionisation mechanism: Proton transfer (3 vials of uncapped water in source)
Corona current: 20 µA for first 3.5 min 3.0 µA for rest of run
Cone gas flow: 0 L/hr
Auxiliary gas flow: 250 L/hr
Source temp.: 150°C

Results and Discussion

Method Management Using the Quanpedia Database

Working with methods involving large numbers of compounds can be time consuming when done manually and is prone to errors when setting up time segmented acquisition. Quanpedia is a compound centric database typically used for method generation, but it can also function as a method management tool. Initial methods for this analysis were generated using existing LC and APGC databases (Figure 2). Retention time changes resulting from further method development or method changes were updated in the database. This allowed for immediate and automatic updates to be made in the MS processing methods by re-generating the methods with three simple clicks.

Robust and Rapid Data Acquisition

For the successful analysis of large numbers of pesticides and their metabolites, it is important that the mass spectrometer can maintain sufficient sensitivity while acquiring MRM transitions with a fast scan speed in order to provide enough data points across each chromatographic peak (e.g. minimum of 12 points per peak).

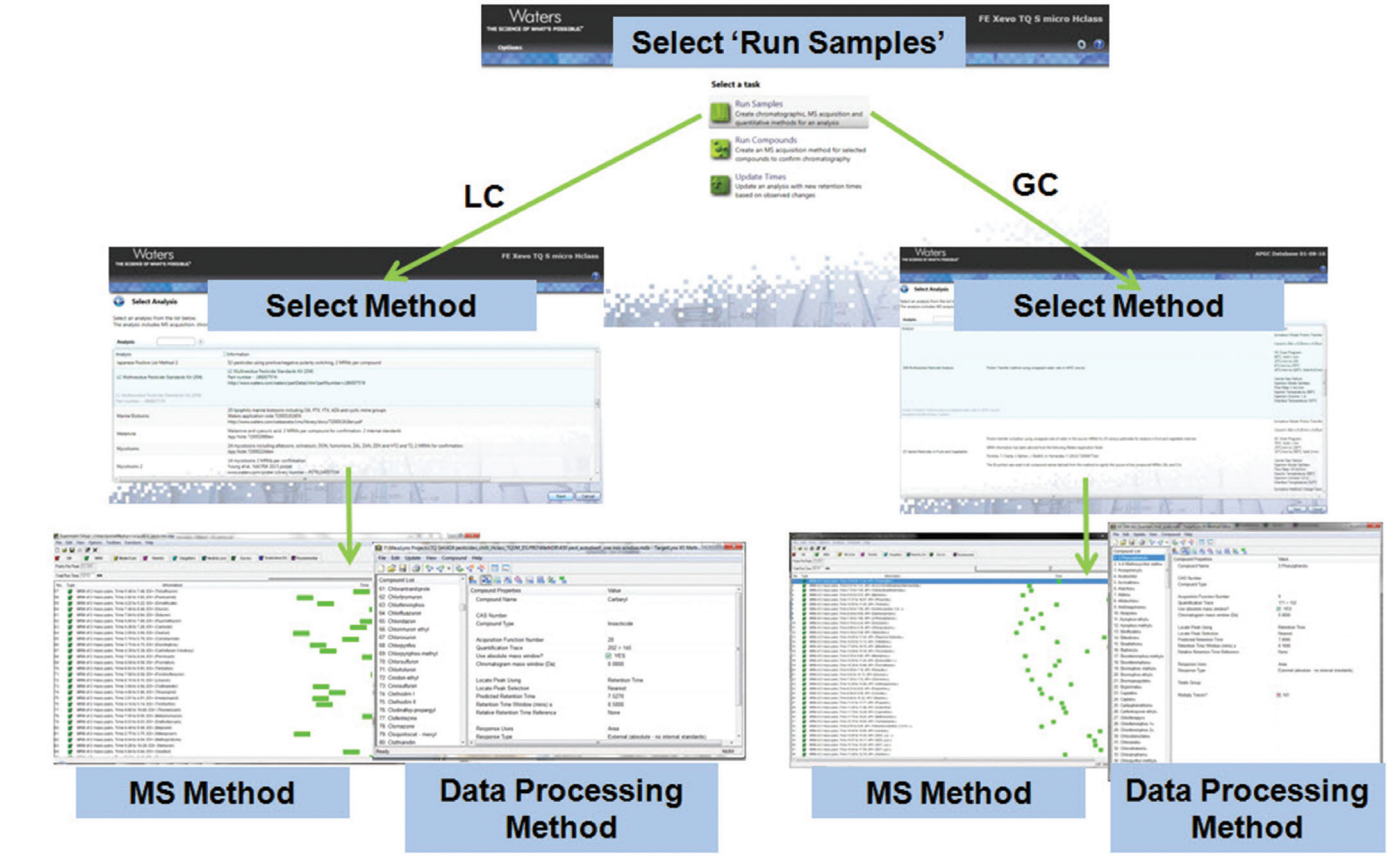
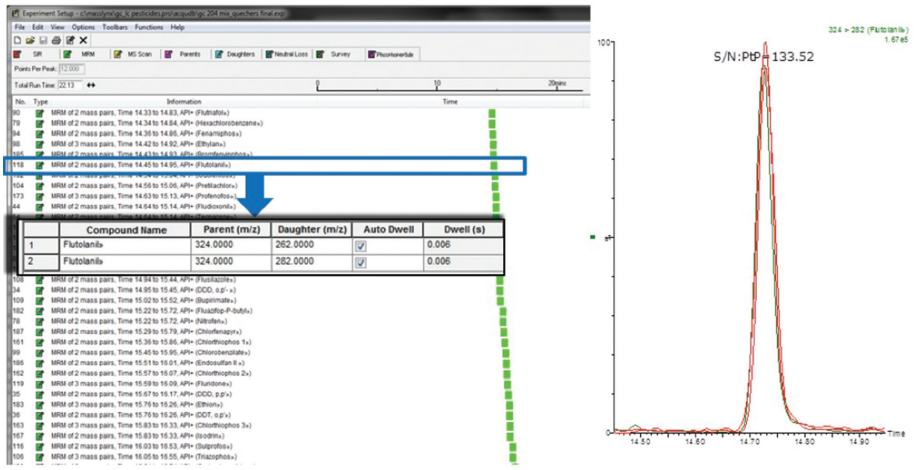


Figure 2: Quanpedia databases used to manage the methods used for both LC and GC analyses demonstrating the three click workflow of method generation



The fast scanning speeds of the Xevo TQ-S micro provide this robust and rapid data acquisition while maintaining large retention time windows to accommodate any shift in retention time due to column maintenance (GC) or chromatography changes caused by the different matrices [6]. Figure 3 highlights one of the busiest sections of the APGC MS method. In this example, flutolanil is just one of approximately 30 pesticides (set across 30 channels, each acquiring at least two transitions per compound) eluting in a 1.5 minute time window.

Figure 3: Demonstration of the rapid scanning capabilities of the Xevo TQ-S micro showing the retention of peak quality at a fast scan time.

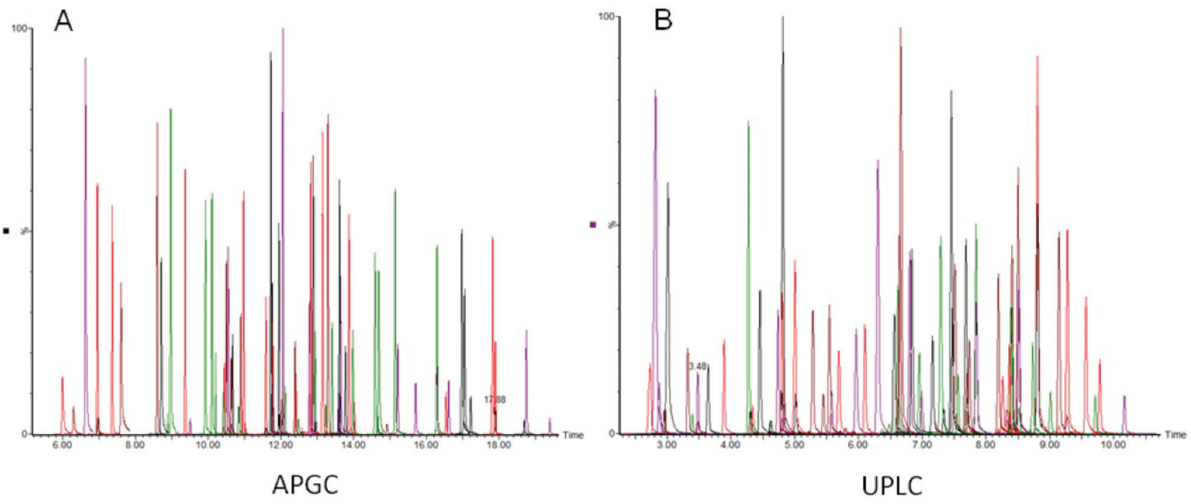


Figure 4: Overlay of a selection of pesticides at 0.010 mg/kg analysed in a celery extract on A. APGC, and B. UHPLC.

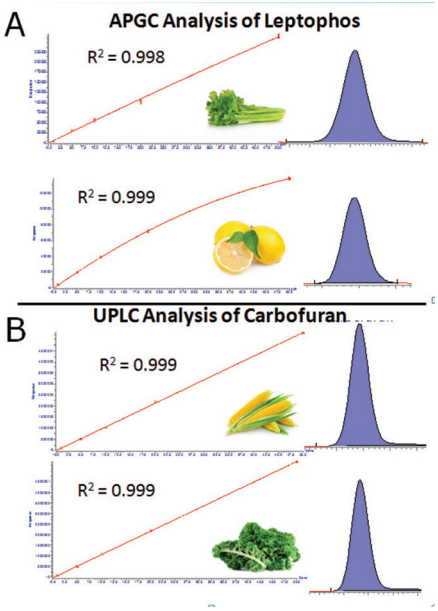


Figure 5: Matrix matched calibration curves and chromatograms for standards at 0.001 mg/kg for peaks from: A. GC analysis of leptophos in celery and lemon, and B. LC analysis of carbofuran in corn and kale.

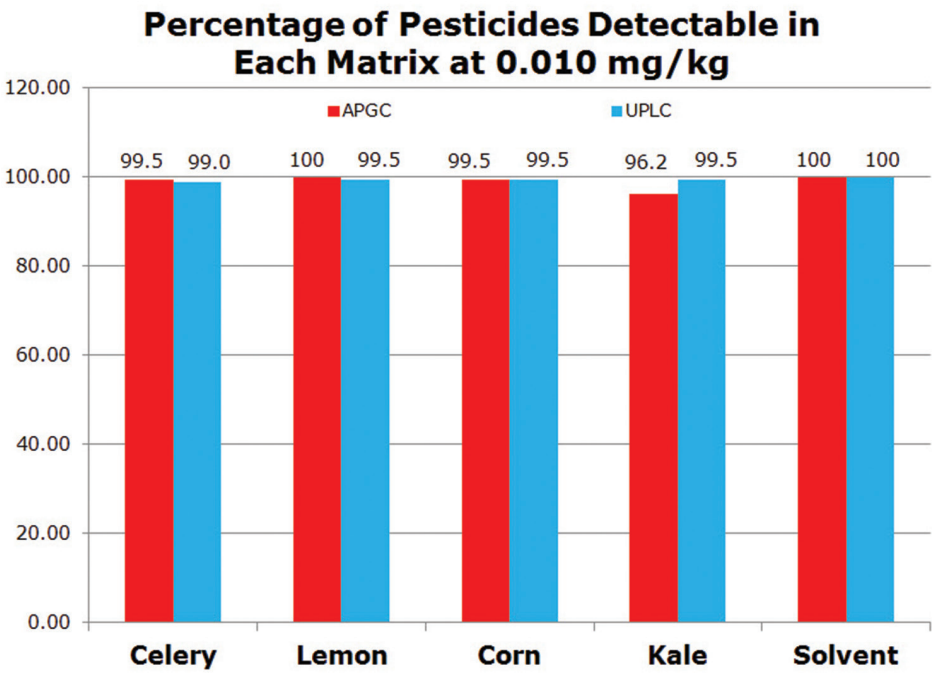


Figure 6: The percentage of pesticides detected in the 0.01 mg/kg standard for each matrix using both GC and LC.

The dwell time calculated for this compound using the autodwell function was 0.006 s. The resulting chromatogram of three replicate injections of 0.010 mg/kg of flutolanil in a celery matrix can be seen in Figure 3. Even with the fast scanning speed, 19 points were collected across the peak and the RSD of three consecutive injections in matrix was 5.2%. The same is true for the LC method used for this analysis.

Pesticides in Matrix

Matrix matched standards were prepared in celery, lemon, corn, and kale over a range of 0.001 to 0.050 mg/kg, and replicate injections made using the LC and GC methods. A TIC overlay for a selection of pesticides is shown in Figure 4, with 0.010 mg/kg in celery extract from both the A. APGC, and B. UHPLC analyses. The data were fitted with the best fit calibration: for the UHPLC data, the response was shown to be linear, whereas the APGC response over the range investigated was non-linear and so it was fitted with a quadratic calibration. A majority of the compounds in both analysis methods had correlation coefficient (R^2) values of 0.995 or greater. Figure 5 shows the matrix matched calibration curves and the peak response at 0.001 mg/kg of a representative pesticide from each analysis method in the four matrices. Residuals from triplicate injections at each calibration point were within ±20%. Ion ratios were also shown to be within 30% tolerance of the reference values.

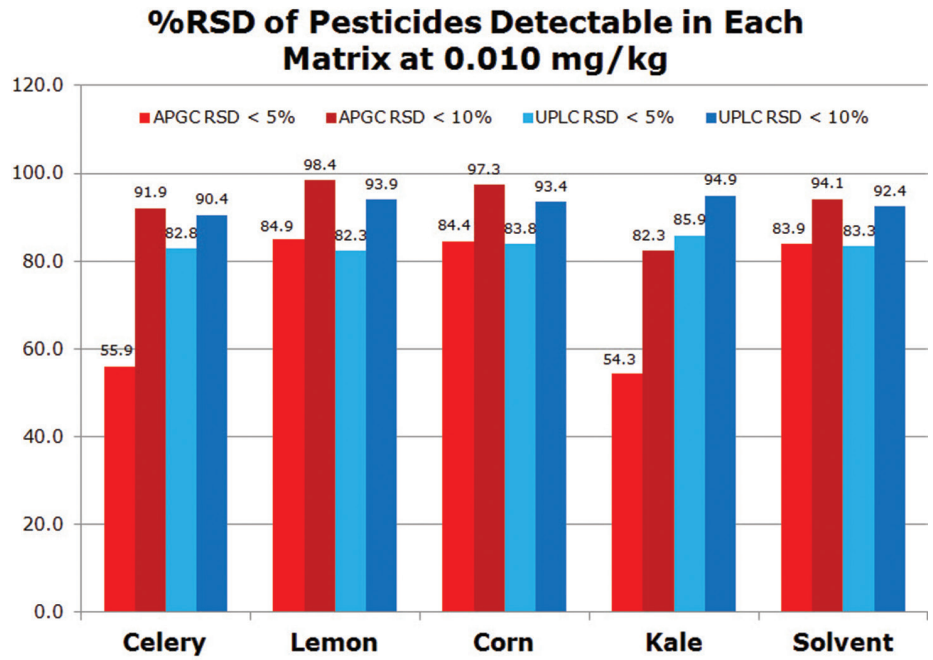


Figure 7: Percentage of compounds detected at 0.01 mg/kg in each matrix and associated RSDs.

For convenience, all sample extracts were spiked at the default MRL of 0.01 mg/kg. Figure 6 demonstrates the number of pesticides in each method detected in the spiked matrices at 0.01 mg/kg. However many pesticides could also be detected at 0.001 mg/kg as demonstrated in Figure 5 which shows leptophos (APGC compound) and carbofuran (UHPLC compound) in the different matrices. The precision of the measurements was excellent with more than 90% of the detected pesticides exhibiting RSDs of peak area of <10% (n=3). The exception was the APGC analysis of the kale matrix, which had more than 80% of pesticides exhibiting RSDs of <10% (Figure 7).

Conclusions

Complex multi residue pesticide analysis was demonstrated using both LC and GC analysis on the same tandem quadrupole instrument. Analysis methods were generated and maintained using Quanpedia databases, making method generation and maintenance fast and simple. Although the multi residue methods contained approximately 200 compounds each, the reliable scanning speed of the tandem quadrupole mass spectrometer employed (Xevo TQ-S micro) produced accurate and precise measurements. The performance for the determination of pesticide residues analysed in four matrices of varying complexity complied with the SANTE guidelines for pesticide residue analysis. Detection at the EU default maximum residue limit of 0.01 mg/kg was easily achieved for >99% of the pesticides analysed with good precision (RSDs <10%) for most analytes in the food samples. Having the flexibility of the MS Universal Source architecture to provide access to both LC-MS/MS and GC-MS/MS on the same instrument, allows for an increase of laboratory efficiency, while maintaining required sensitivity and repeatability.

References

1. D Shah, E McCall, G Cleland. Single LC-MS/MS Method for Confirmation and Quantification of Over 400 Pesticides in a Complex Matrix Without Compromising Data Quality. Waters Application Note no. 720005559EN. January, 2016.

2. T Kovalczuk, M Jech, J Poustka, J Hajslova. UPLC-MS/MS: A novel challenge in multiresidue pesticide analysis in food, Analytica Chimica Acta, 577, 2006.

3. M Tienstra, T Portoles, F Hernandez, J G J Mol. Fast gas chromatographic residue analysis in animal feed using split injection and atmospheric pressure chemical ionisation tandem mass spectrometry. J. Chrom. A. 1422, October, 2015.

4. L Cherta, T Portoles, J Beltran, E Pitarch, J G Mol, F Hernandez. Application of gas chromatography-mass spectrometry with atmospheric pressure chemical ionisation for the determination of multiclass pesticides in fruits and vegetables. J. Chrom. A. 1314: 224-240, November, 2013.

5. European Commission. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. 2015, rev. 0.

6. AOAC Official Method 2007.01. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate. 2013.

Pesticides in GC Method

2-Phenylphenol	Diclobenil	Oxyfluorfen
4,4'-Methoxychlor olefin	Dicloran	Paclobutrazol
Acetochlor	Dimethachlor	Parathion
Acrinathrin	Diphenamid	Pebulate
Alachlor	Diphenylamine	Penconazole
Allidochlor	Edifenphos	Pendimethalin
Anthraquinone	Endosulfan ether	Pentachloroaniline
Atrazine	Endosulfan II	Pentachlorobenzonitrile
Azinphos-ethyl	Endosulfan sulphate	Pentachlorothioanisole
Azinphos-methyl	Endrin aldehyde	Permethrin, cis-
Benfluralin	EPN	Permethrin, trans-
Bifenthrin	Ethalfluralin	Phenothrin 1
Bioallethrin	Ethion	Phenothrin 2
Biphenyl	Ethylan	Phorate
Bromfenvinphos	Etofenprox	Phosalone
Bromfenvinphos-methyl	Etridazole	Phosmet
Bromophos-ethyl	Fenamiphos	Piperonyl butoxide
Bromophos-methyl	Fenarimol	Pirimiphos-ethyl
Bromopropylate	Fenchlorphos	Pirimiphos-methyl
Bupirimate	Fenitrothion	Prochloraz
Captafol	Fenpropathrin	Procymidone
Captan	Fenson	Prodiamine
Carbophenothion	Fenthion	Profenofos
Carfentrazone ethyl	Fenvalerate 1	Profluralin
Chlorfenapyr	Fenvalerate 2	Propachlor
Chlorfenvinphos	Fipronil	Propanil
Chlorobenzilate	Fluazifop-P-butyl	Propisochlor
Chloroneb	Fluchloralin	Propyzamide
Chlorothalonil	Flucythrinate 1	Prothiofos
Chlorpropham	Flucythrinate 2	Pyraclofos
Chlorpyrifos	Fludioxonil	Pyrazophos
Chlorpyrifos-methyl	Fluquinconazole	Pyridaben
Chlorthal-dimethyl	Flusilazole	Pyridaphenthion
Chlorthiophos 1	Flutolanil	Pyrimethanil
Chlorthiophos 2	Flutriafol	Pyriproxyfen
Chlorthiophos 3	Folpet	Quinalphos
Chlozolinate	Fonofos	Resmethrin 1
Clomazone	Hexachlorobenzene	Sulfotep
Coumaphos	Hexazinone	Sulprofos
Cycloate	Iodofenfos	tau-Fluvalinate 1
Cyfluthrin 1	Iprodione	tau-Fluvalinate 2
Cyfluthrin 2	Isazophos	Tebuconazole
Cyfluthrin 3	Isodrin	Tebufenpyrad
Cyfluthrin 4	Isopropalin	Tefluthrin
Cyhalothrin, lambda-	Lenacil	Terbacil
Cypermethrin 1	Leptophos	Terbufos
Cypermethrin 2	Linuron	Terbutylazine
Cypermethrin 3	Malathion	Tetrachloroaniline, 2,3,5,6-
Cypermethrin 4	Metalaxyl	Tetrachlorvinphos
Cyprodinil	Metazachlor	Tetradifon
DDD, o,p'-	Methacrifos	Tetramethrin 1
DDD, p,p'-	Methoxychlor	Tetramethrin 2
DDE, o,p'-	Methyl parathion	Tolclofos-methyl
DDE, p,p'-	Metolachlor	Tolylfluanid
DDT, o,p'-	Mevinphos	Transfluthrin
DDT, p,p'-	MGK 264 1	Triadimefon
Deltamethrin	MGK 264 2	Triadimenol
Diallate	Myclobutanil	Triallate
Diazinon	N-(2;4-Dimethylphenyl)formamide	Triazophos
Dichlofluanid	Nitralin	Triflumizole
Dichloroaniline, 3,4'-	Nitrofen	Trifluralin
Dichlorobenzophenone, 4,4'-	Oxadiazon	Vinclozolin

Pesticides in LC Method

Abamectin	Etoxazole	Nuarimol
Acephate	Famoxadone	Omethoate
Acetamiprid	Fenamidone	Oxadixyl
Acibenzolar-S-methyl	Fenarimol	Oxamyl
Aldicarb	Fenazaquin	Paclobutrazol
Aldicarb sulfone	Fenbuconazole	Penconazole
Aldicarb sulfoxide	Fenhexamid	Pencycuron
Ametryn	Fenobucarb	Phenmedipham
Aminocarb	Fenoxycarb	Picoxystrobin
Amitraz	Fenpropimorph	Piperonyl butoxide
Azoxystrobin	Fenpyroximat	Pirimicarb
Benalaxyl	Fenuron	Procloraz
Bendiocarb	Fipronil	Promecarb
Benfuracarb	Flonicamid	Prometon
Benzoximate	Flufenacet	Prometryn
Bifenazate	Flufenoxuron	Propamocarb
Bitertanol	Fluomethuron	Propargite
Boscalid	Fluoxastrobin	Propham
Bromuconazole I	Fluquinconazole	Propiconazole
Bromuconazole II	Flusilazole	Propoxur
Bupirimate	Flutolanil	Prothioconazole
Buprofezin	Flutriafol	Pymetrozine
Butafenacil	Forchlorfenuron	Pyracarbolid
Butocarboxim	Formetanate HCL	Pyraclostrobin
Butoxycarboxim	Fuberidazole	Pyridaben
Carbaryl	Furalaxyl	Pyrimethanil
Carbendazim	Furathiocarb	Pyriproxifen
Carbetamide	Hexaconazole	Quinoxyfen
Carbofuran	Hexythiazox	Rotenone
Carbofuran-3-hydroxy	Hydramethylnon	Secbumeton
Carboxin	Imazalil	Siduron
Carfentrazone-ethyl	Imidacloprid	Simetryn
Chlorantraniliprole	Indoxacarb	Spinetoram
Chlorfluazuron	Ipconazole	Spinosad A
Chloroxuron	Iprovalicarb I	Spinosad D
Chlortoluron	Iprovalicarb II	Spirodiclofen
Clethodim I	Isocarbofos	Spirotetramat
Clofentezine	Isoprocarb	Spiroxamine I
Clothianidin	Isoproturon	Spiroxamine II
Cyazofamid	Kresoxim-methyl	Sulfentrazone
Cycluron	Linuron	Tebuconazole
Cymoxanil	Lufenuron	Tebufenozide
Cyproconazole I	Mandipropamid	Tebufenpyrad
Cyproconazole II	Mefenacet	Tebuthiuron
Cyprodinil	Mepanipyrim	Teflubenzuron
Cyromazine	Mepronil	Temephos
Desmedipham	Mesotrione	Terbumeton
Diclobutrazol	Metaflumizone	Terbutryn
Dicrotophos	Metalaxyl	Tetraconazole
Diethofencarb	Metconazole	Thiabendazole
Difenoconazole	Methabenzthiazuron	Thiacloprid
Diflubenzuron	Methamidophos	Thiamethoxam
Dimethoate	Methiocarb	Thidiazuron
Dimethomorph I	Methomyl	Thiobencarb
Dimethomorph II	Methoprotryne	Thiophanate-methyl
Dimoxystrobin	Methoxyfenozide	Triadimefon
Diniconazole	Metobromuron	Triadimenol
Dinotefuran	Metribuzin	Trichlorfon
Dioxacarb	Mevinphos I	Tricyclazole
Diuron	Mevinphos II	Trifloxystrobin
Emamectin benzoate	Mexacarbate	Triflumizole
Epoxiconazole	Monocrotophos	Triflumuron
Etaconazole	Monolinuron	Triticonazole
Ethiofencarb	Myclobutanil	Vamidothion
Ethiprole	Neburon	Zoxamide
Ethirimol	Nitenpyram	
Ethofumesate	Novaluron	