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アプリケーションノート

Minimizing the Impact of the Sample Matrix During Routine Pesticide Residue Analysis in Food

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Abstract

This application note describes the application of ultra sensitive detection to minimize the impact of matrix effects during analysis of 81 pesticide residues in a range of food products and also the use of a novel MRM acquisition mode that allows direct monitoring of the matrix background in each sample injected.

Benefits

- Detection of pesticides in complex food matrices using large multi-residue methods to below the required regulatory concentrations.
- Ability to monitor changes in the sample matrix between samples and batches.
- Reduction of matrix concentration to minimize matrix effects while maintaining detection.

Introduction

One of the biggest challenges in ensuring the safety of our food supplies is the measurement of hazardous ultra trace level components in the presence of a highly complex sample matrix. For the analysis of pesticides in food matrices, increased use of liquid chromatography systems, coupled with tandem quadrupole mass spectrometers has allowed progress in reducing the problems caused by the sample matrix. However, difficulties remain when trying to discriminate against matrix components that exhibit similar physiochemical properties. Unawareness of these difficulties in each unique sample can lead to poor quality results, and can impact a laboratory’ s performance and reputation.

Understanding the matrix challenge of each injected sample is clearly beneficial as is the ability to monitor changes in the sample matrix between samples and batches. This capability can lead to the continuous improvement of analytical quality in the laboratory. Conventional LC tandem quadrupole systems do not allow the direct monitoring of the sample matrix during high sensitivity MRM quantitation and it is only recently with the newest generation of instruments that this has become possible.

Problems caused by the sample matrix can include disruption to chromatography, increased chemical noise, and most notably, ionization suppression.[1-4] In highly complex matrices such as herbs and spices, these problems can be found in combination to make determination of pesticide residue concentration very difficult.

In addition to problems caused by the sample matrix, there are also pesticides that, by nature, are more difficult to analyze using LC-MS/MS due to a poor (relative) response factor. Successful analysis of these compounds to the regulatory concentration limits is difficult when considering the practicality of increasing sample amount and the balance of extracted matrix concentration. A much more practical solution is to use increased instrument sensitivity to maximize performance at these required

concentrations. Also, if enough sensitivity is available, then the reduction of matrix concentration injected onto the system becomes possible.

Described here is the application of ultra sensitive detection to minimize the impact of matrix effects during analysis of 81 pesticide residues in a range of food products. Also described is the use of a novel MRM acquisition mode that allows direct monitoring of the matrix background in each sample injected.

Mass spectrometer acquisition

Quanpedia generated MRM parameters (a full MRM list can be found in Appendix 1) were used as the basis of RADAR-enabled mass spectrometer acquisition method. RADAR is an information-rich acquisition approach that allows measurement of target analytes with precision in MRM mode, while simultaneously scanning the background for all other components.

Figure 1 shows a RADAR-enabled mass spectrometer acquisition method with time scheduled MRMs for target pesticides and a simultaneous full scan (MS2) acquisition.

Figure 1. Mass spectrometer experiment showing RADAR acquisition mode.

Experimental

Waters DisQuE (EN 15662:2008) Extraction Kit (QuEChERS) was used to prepare spiked extracts of grape, avocado, marjoram, and ginger. Sample matrix concentrations were 1g/mL for grape and avocado and 0.1 g/mL for marjoram and ginger. The final acetonitrile extracts from QuEChERS were diluted 10x into mobile phase and 10 µL were injected onto the analytical system (referred to as original sample). Subsequent dilutions of this were then made to reduce matrix effects.

LC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY BEH C_{18} 100 mm x 2.1 mm, 1.7 µm
Mobile phase A:	0.1% HCOOH in H_2O
Mobile phase B:	0.1% HCOOH in MeOH
Run time:	10.00 min

UPLC gradient:

Time (min)	Flow (mL/Min)	%A	%B
-	0.5	90	10
0.25	0.5	90	10
7.75	0.5	2	98
8.5	0.5	2	98
8.51	0.5	90	10

MS conditions

MS system:	Xevo TQ-S
Ionization mode:	ES positive
Capillary voltage:	0.60 kV
Source temp:	130 °C
Desolvation temp:	650 °C

Cone gas flow:	150 L/hr
Desolvation gas flow:	1200 L/hr

Results and Discussion

Detection to below regulatory limits

European Union (EU) regulations to control pesticide exposure from food consumption are among the toughest in the world. In order to import food and food commodities into Europe, the level of pesticide contamination must be below the stated maximum residue limits (MRLs) for that product.[5] Confirmation of positive results requires good quantitative performance well below these concentrations, which can be very challenging in more complex matrices.

Figure 2 shows a selection of extracted MRM chromatograms for pesticides spiked into avocado at 0.005 mg/kg. Quantitative and confirmatory transitions are both detected at this level, which is 10x below the European MRL (except zoxamide, which is 4x below). This includes parathion, which has a relatively poor response factor when analyzed using electrospray ionization. Comfortable quantitation of pesticides at these low concentrations allows high confidence when reporting results around maximum residue limits.

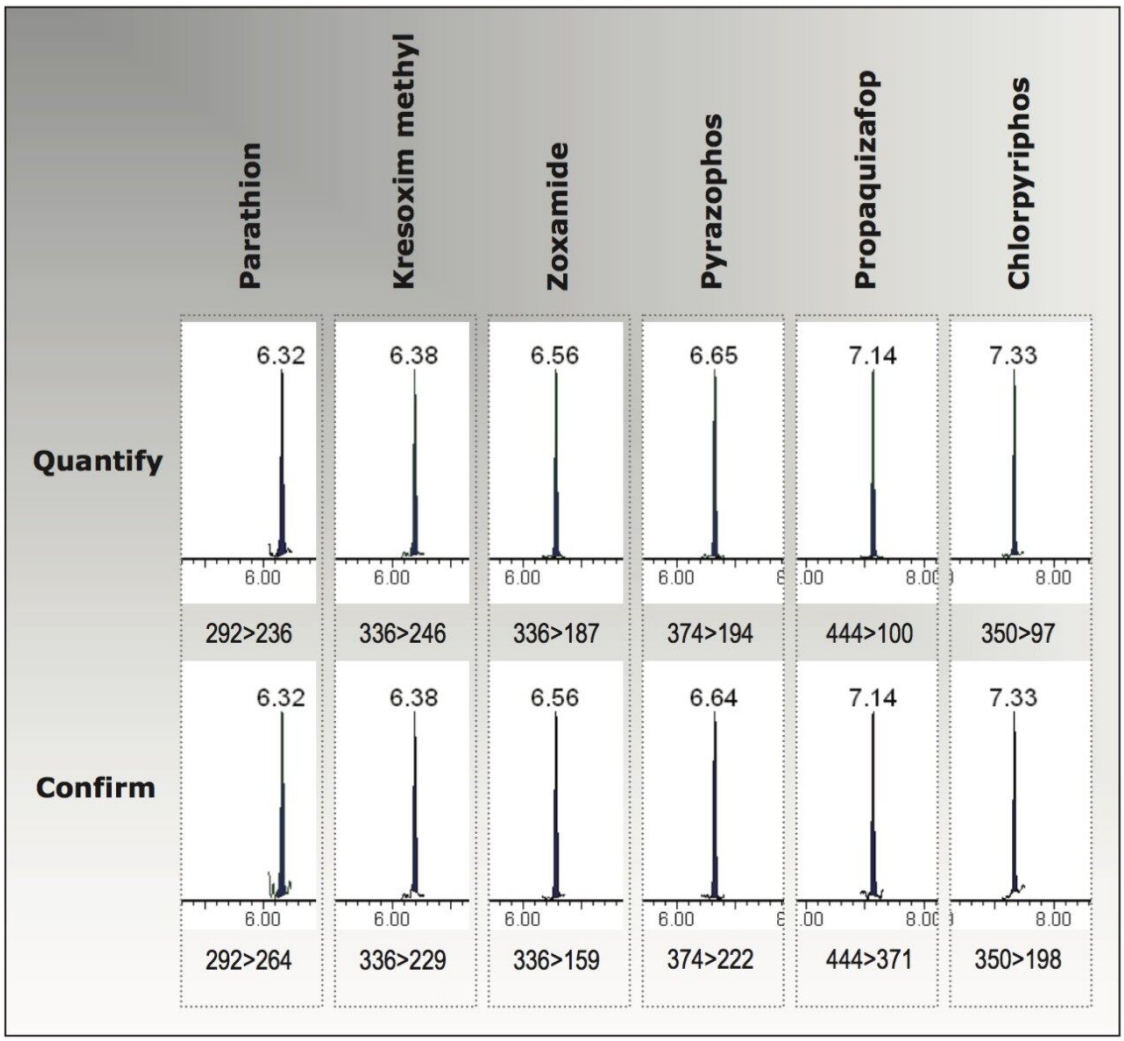

Figure 2. Quantitative and confirmatory MRM transitions for pesticides spiked into avocado at 0.005 mg/kg.

Monitoring matrix complexity

Each sample analyzed had full scan data available along with the MS/MS data. This was due to the RADAR functionality of the Xevo TQ-S being enabled. These data were used to monitor the complexity of the sample matrix background in each sample.

Differences in the co-extracted background for grape, avocado, marjoram, and ginger were observed by plotting the base peak intensity (BPI) chromatogram. For ginger and marjoram, 10x less sample was extracted using QuEChERS to give a 0.1 g/mL matrix, as opposed to the usual 1 g/mL matrix for grape and avocado. This is due to the extremely high complexity of the sample matrix, as well as to aid extraction of these drier samples. Figure 3 shows base peak intensity (BPI) chromatograms overlaid with MRM chromatograms for pesticides spiked at 1.0 x 10^5 g/kg for each matrix.

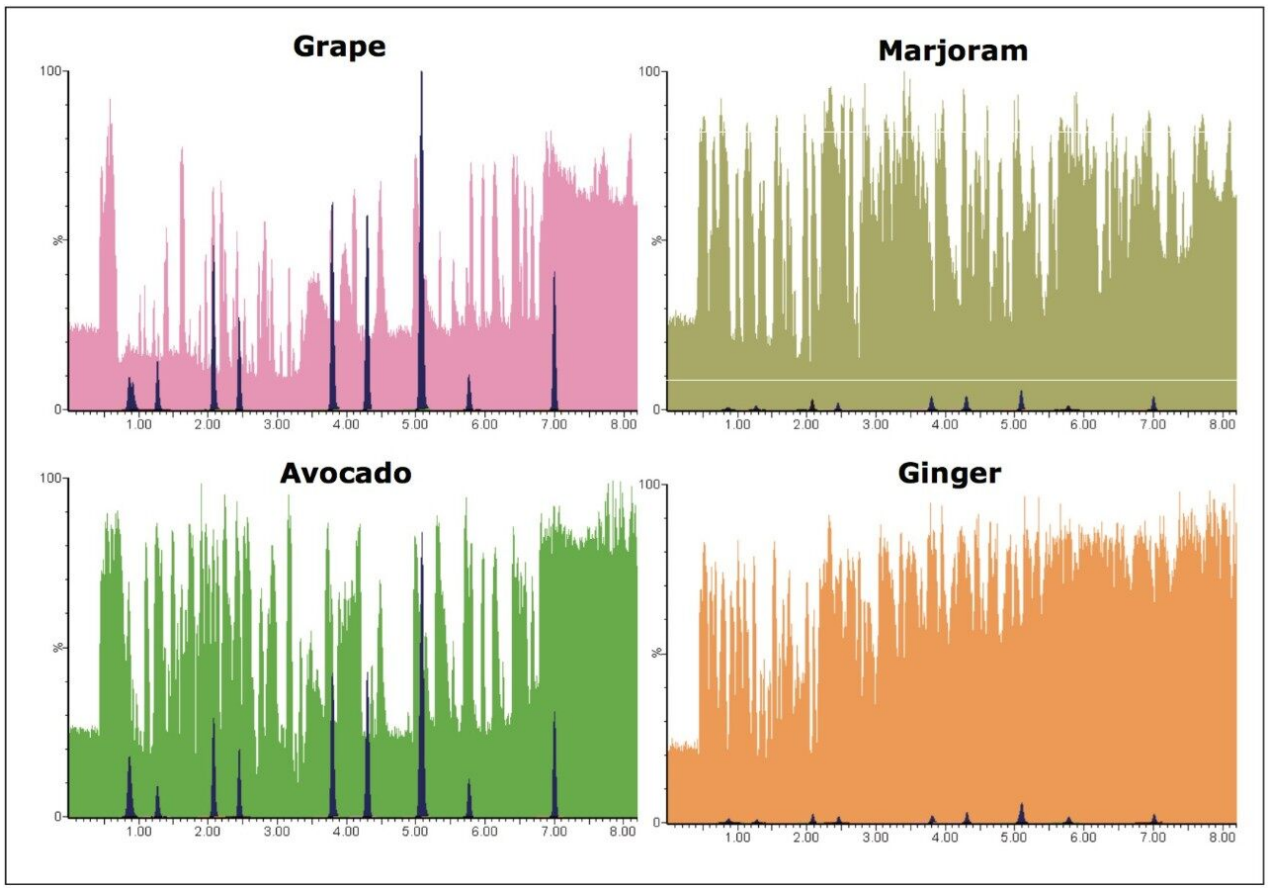

Figure 3. BPI chromatograms overlaid with MRM chromatograms for pesticides spiked at 0.01 mg/kg into grape (1.0 g/mL matrix), avocado (1.0 g/mL), marjoram (0.1 g/mL), and ginger (0.1 g/mL).

Despite the reduction in matrix concentration, the ionizable background is high in marjoram and ginger samples, compared with grape and avocado; as a consequence, the likelihood for analyte ion suppression (and enhancement) may be higher for these types of samples.

With simultaneous full scan it is also possible to observe specific components that co-elute with target analytes. Figure 4 shows BPI and MRM mass chromatograms for a grape sample spiked with dimethoate at 0.01 mg/kg. Full scan spectra from the elution region of dimethoate were combined and the most intense ion from the mass spectrum extracted into another chromatogram (XIC), revealing a discrete peak that co-elutes with dimethoate, as shown in Figure 4.

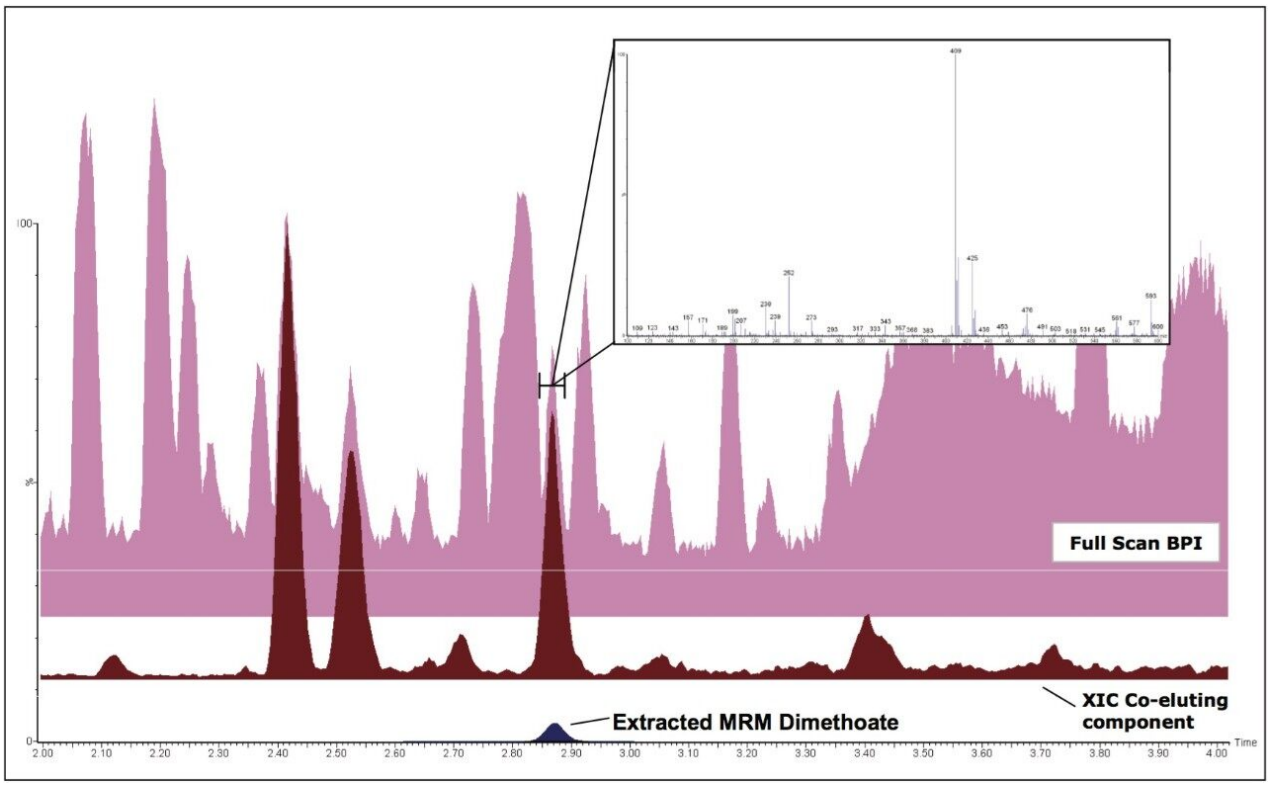

Figure 4. RADAR full scan BPI and MRM mass chromatograms for a grape sample spiked with dimethoate at 0.01 mg/kg. Also shown is the extracted ion chromatogram (XIC) of the co-eluting component with the subtracted mass spectrum inset.

If significant problems are observed with this or any other components in the matrix, the ability to observe them allows for further investigation and necessary remedial action to be carried out. Also, this acquisition mode can help to track the clean-up efficiency of the methodology employed.

Reduction of matrix effects

Minimizing matrix effects allows higher confidence in the quality of analytical data obtained. Reducing matrix concentration injected onto the analytical system is a simple and effective means to do this. When using a standard flow ESI source this can be achieved by reducing the amount of sample to be extracted, reducing the number of sample enrichment steps, or diluting final extracts. In any case, this is only a possibility if enough sensitivity is available to maintain detection at the required concentrations.

Ginger samples showed the highest ionizable background when compared to all other samples, despite having a relatively low matrix concentration (0.1 g/mL), as shown in Figure 3. Matrix effects were observed in the ginger samples with ion suppression and chromatography problems most apparent.

Diluting the ginger extracts 10x allowed recovery of distorted peak shape for cyromazine and reduction in

matrix suppression for a number of pesticides, as shown in Figure 5. Table 1 shows reduction of ion suppression with a 10x dilution of sample. This reduction in suppression is clear when comparing peak area of pesticides in ginger to standards with no matrix present. As the matrix concentration is reduced the peak area response begins to correlate closely with standard peak areas.

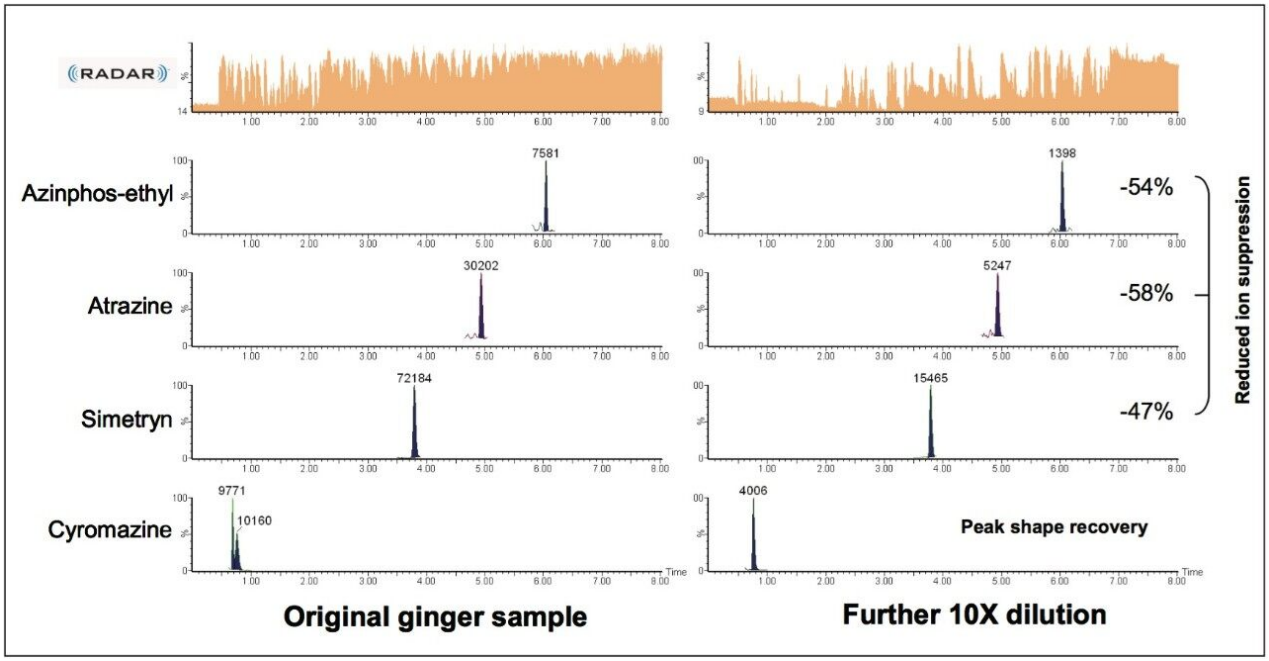

Figure 5. Effects of reducing sample matrix concentration by dilution for ginger. The full scan RADAR background is shown in the top chromatogram with MRM chromatograms for a selection of pesticides below.

	% Peak area recovery to standard	
	Original Extract	Diluted Extract
Thiabendazole	89.2	105.2
Atrazine-desisopropyl	71.6	100.8
Aldicarb	36.4	91.2
Desmetryn	49.2	97.0
Prometon	85.8	109.2
Simazine	63.1	103.4
Hexazinone	80.0	98.7
Demeton S Methyl	69.7	117.0
Tebuthiuron	79.1	96.3
Ametryn	66.7	103.4
Terbutryn	81.7	102.8
Azinphos Methyl	58.1	91.8
Trietazine	46.8	91.6
Azinphos Ethyl	60.5	86.1

Table 1. Reduction of ion suppression for a ginger extract upon 10x dilution of original samples. Calculated as percent peak area recovery to a standard injection with no matrix present.

Conclusion

- Xevo TQ-S allows detection of pesticides in complex food matrices using large multi-residue methods to below the required regulatory concentrations. This includes compounds with poor relative response factors.
- The RADAR mode of acquisition enables the collection of spectral information on background components in the sample matrix while simultaneously collecting MRM data. This can help identify areas of potential ion suppression, observe untargeted contaminants, and aid in the development of matrix reduction strategies.
- Where matrix effects are observed, the high sensitivity offered by Xevo TQ-S allows matrix concentration in samples to be reduced to counteract these effects. This is possible while maintaining detection at regulatory concentrations and allows higher confidence in reported data.

References

1. J M Marín *et al. Journal of Chromatography A*. 1216: 9, 1410-1420. 27 February 2009.

2. Hajšlová & Zrostlíková. *Journal of Chromatography A*. 1000:1-2, 181-197. 6 June 2003.

3. Gosetti *et al . Journal of Chromatography A*. 1217: 25, 3929-3937, 18 June 2010.

4. Kruve *et al. Journal of Chromatography A*. 1187: 1-2, 58-66, 11 April 2008.

5. website: http://ec.europa.eu/sanco_pesticides/public/index.cfm

Appendix 1 Pesticide MRM Parameters

	Precursor ion	Product ion	Collison (V)
Acephate	206	64	10
	206	117	12
Acetamiprid	223	56	28
	223	126	12
Aldicarb	213	89	14
	213	116	19
Ametryn	228	68	15
	228	186	10
Atrazine	216	96	34
	216	174	16
Atrazine-desethyl	188	79	21
	188	146	17
Atrazine-desisopropyl	174	79	25
	174	96	15
Azamethiphos	325	112	16
	325	139	16
Azinphos Ethyl	368	132	22
	368	160	35
Azinphos Methyl	340	132	15
	340	160	10
Azoxystrobin	404	329	15
	404	372	10
Buturon	237	84	28
	237	126	14
Cadusafos	271	131	15
	271	159	28
Carbaryl	202	117	20
	202	145	15
Chlorbromuron	293	182	22
	293	204	12
Chlorpyrifos	350	97	15
	350	198	20
Chlorpyrifos Methyl	322	125	25
	322	290	15
Chlortoluron	213	46	15
	213	72	15
Clodinafop-propargyl	350	91	15
	350	266	16
Coumaphos	363	289	30
	363	307	15
Cyanazine	241	96	22
	241	214	14
Cyromazine	167	60	23
	167	108	15
Demeton S Methyl	253	61	17
	253	89	17
Demeton S methyl sulfone	263	121	28
	263	169	14
Desmetryn	214	82	28
	214	172	19
Dicrotophos	238	112	10
	238	193	10
Difenoxuron	287	72	18
	287	123	18
Diflubenzuron	311	141	30
	311	158	15
Dimefuron	339	72	24
	339	167	18
Dimethoate	230	125	18
	230	199	10
Dimethomorph	388	165	28
	388	301	18
Disulfoton	297	61	32
	297	89	12
Diuron	233	46	13
	233	72	16
Ethoprophos	243	97	29
	243	131	18
Fenuron	165	46	13
	165	72	15
Flamprop-methyl	336	77	46
	336	105	15
Fluazafop-P-butyl	384	282	20
	384	328	15
Flufenacet	364	152	18
	364	194	10
Fluomethuron	233	46	16
	233	72	16
Heptenophos	251	125	13
	251	127	13
Hexazinone	253	71	28
	253	171	15

	Precursor ion	Product ion	Collison (V)
Imazapyr	262	69	24
	262	86	24
Imazaquin	312	86	26
	312	267	18
Imidacloprid	256	175	18
	256	209	14
Isoproturon	207	46	15
	207	72	20
Isoxaben	333	107	56
	333	165	16
Kresoxim Methyl	336	229	15
	336	246	15
Linuron	249	160	15
	249	182	15
Malaoxon	315	99	22
	315	127	11
Metalaxyl	280	192	16
	280	220	12
Metamitron	203	104	20
	203	175	15
Methamidophos	142	94	12
	142	125	12
Metobromuron	259	148	14
	259	170	18
Metosulam	418	140	50
	418	175	26
Mevinphos	225	127	14
	225	193	9
Monolinuron	215	99	32
	215	126	20
Monuron	199	72	15
	199	126	23
Omethoate	214	125	20
	214	183	10
Parathion	292	236	12
	292	264	10
Phoxim	299	129	15
	299	153	7
Pirimiphos-ethyl	334	182	23
	334	198	21
Pirimiphos-methyl	306	108	30
	306	164	20
Prometon	226	86	26
	226	184	16
Propaquizafop	444	100	15
	444	371	15
Pymetrozine	218	79	28
	218	105	18
Pyraclostrobin	388	163	23
	388	194	11
Pyrazophos	374	194	30
	374	222	20
Quinmerac	222	141	28
	222	204	14
Quizalofop-ethyl	373	91	30
	373	299	16
Siduron	233	94	23
	233	137	15
Simazine	202	96	22
	202	124	16
Simetryn	214	96	23
	214	124	18
Spiroxamine	298	100	30
	298	144	19
Sulfotep	323	97	30
	323	171	14
Tebuthiuron	229	116	24
	229	172	16
Terbuthylazine	230	96	26
	230	174	15
Terbutryn	242	186	15
	242	200	15
Tetrachlorvinphos	365	127	15
	365	239	18
Thiabendazole	202	131	26
	202	175	24
Trietazine	230	71	28
	230	99	21
Zoxamide	336	159	36
	336	187	23

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