EURL for Cereals and Feeding stuff
National Food Institute
Technical University of Denmark

Validation Report 14

Determination of pesticide residues in feed by GC-MS/MS

(QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS on feed for laying hens, which were cereals based. The QuEChERS method has an extraction and clean-up step, which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[1]. This validation includes only results from validation experiment on feeds.

2. Principle of analysis

Sample preparation: If samples are in pellets or whole grains, the samples is milled with a sieve at 1.0 mm.

Extraction: The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.

Clean-up: After centrifugation the supernatant is transferred to a tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube with PSA and $MgSO_4$. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the calibration standards.

Quantification and qualification: The final extract is analysed GC/MS/MS. The pesticide residues are separated on a DB5-MS column and detected by tandem mass spectrometry (MS/MS) operating with electron energy at 70 eV, source temperature at 180°C and transfer line at 250°C. The injection volume was 4 µl. All pesticides were detected in the multiple reaction monitoring mode (MRM). For each pesticide two transitions were determined. One for quantification and one for qualification. The MRM transitions for the pesticides and degradation products are given in **Appendix 1**.

3. Validation design

The method was south validated for 68 pesticides or degradation products in feeds for laying hens. The feed material used for validation was a mixture of different feeds, that comprised different kind of cereals, different kind of vegetable fat (soya, rape oil) and minerals. The validation was performed on 5-6 replicates at each of the two spiking levels; 0.01and 0.1 mg/kg. A sample of the feed commodity without spiked pesticides was included.

The methods has later been used to analyse EUPT test material, EUPT-CF7.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels, i.e. 0.003, 0.01, 0.033 and 0.1 µg/ml. The calibration curves were best fitted to a linear curves. The quantification was performed from the mean of two bracketing calibration curves. The majority of the correlation coefficients (R) were higher or equal to 0.99. Examples of chromatograms obtained when analysing the extracts by GC/MS/MS and LC/MS/MS are presented in **Figure 1** . Examples of calibration curves are presented in **Figure 2**.

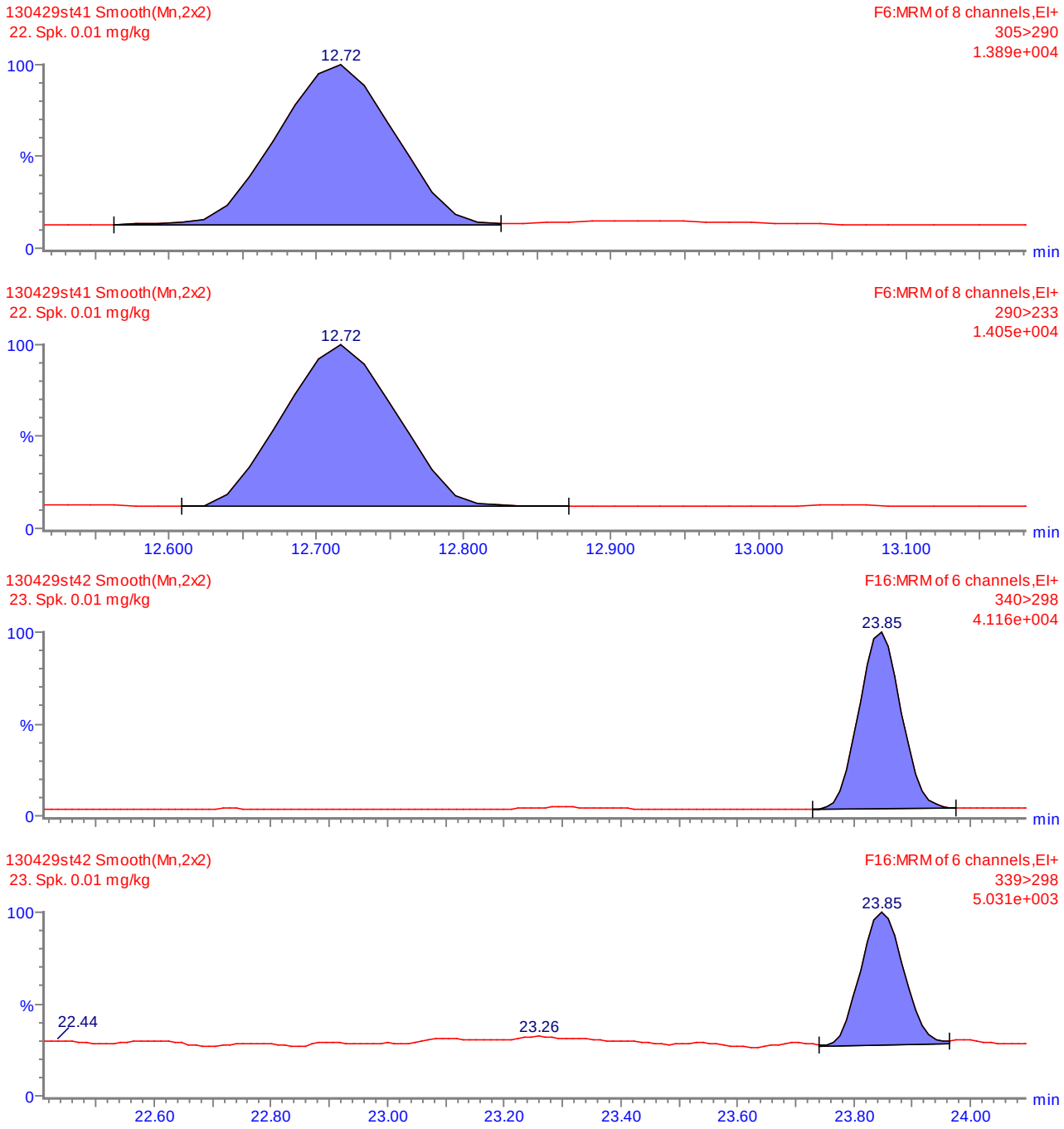

Figure 1: Examples of chromatograms for pirimiphos-methyl and fluquinconazole obtained when analysing extract spiked with 0.01 mg/kg (two MRM transitions are shown for each pesticide).

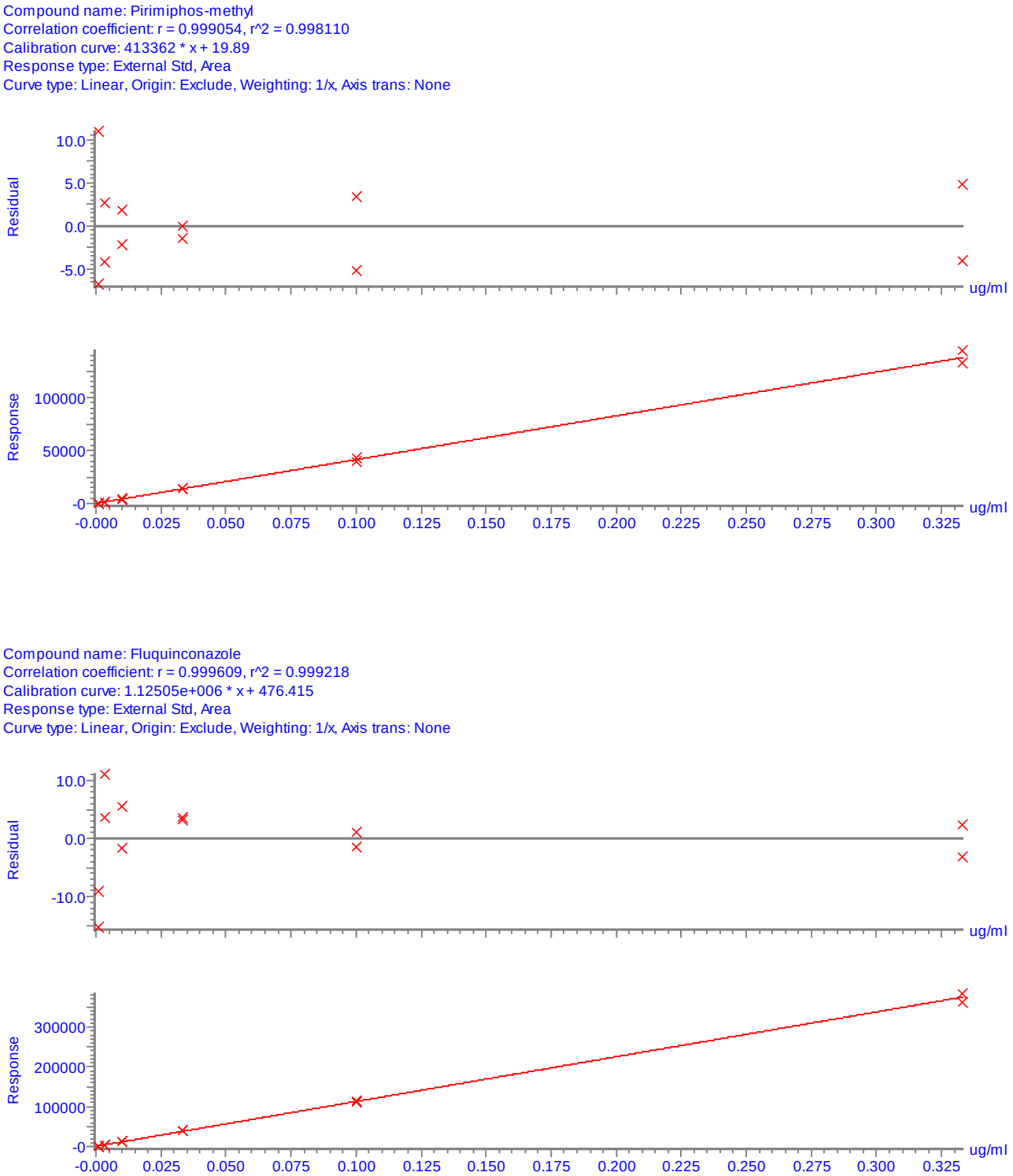

Figure 2. Examples of calibration curves and residual for pirimiphos-methyl and fluquinconazole. Concentrations from 0.003-0.333 µg/ml.

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all two spiking levels. Repeatability is given as the relative standard deviation on the result from four or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time. The internal Reproducibility is not calculated as feed was only analyzed once.

Appendix 2 shows the relative repeatability for the validated pesticides and degradation products.

Accuracy – Recovery

The accuracy was determined by recovery, samples were spiked at two concentration levels. In appendix 2 and 3 recovery, repeatability and limit of quantification (LOQ) are given for the validated pesticides, isomers and degradation products for all two spiking levels (0.01 mg/kg and 0.1 mg/kg). Recoveries is listed in **Appendix 2**.

Robustness

The method is not tested for robustness

Limit of quantification, LOQ

Quantification limits (LOQ) are calculated from the results at the lowest accepted spike level, as 6 times the standard deviation (absolute recovery). The quantification limits are given in **Appendix 2**.

6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability should be ≤ 20%[2].
2. The average relative recovery must be between 70 and 120%[2].

If the above mentioned criteria have been meet, the detection limits have been calculated.

7. Results

Spike experiments:

The 55 pesticides were validated with the recovery was in the range of 70-120% at both concentration levels. However, for cyproconazole, penconazole and trifloxystrobin the recoveries were slightly over 120% at the highest spike level but fine at the lowest spike level. Likewise the recovery for trifluralin was below 70% at the highest spike level but fin at the lowest spike level. The relative repeatability (RSD_r) varying between 1-24 % and the LOQs were in the range of 0.01-0.08 mg/kg. Dimethoate, fenvalerate, lambda-cyhalothrin and permethrin were not validated on the lowest spike level. The results for the pesticides which were validated are listed in **Appendix 2**.

Thirteen pesticides were not validated, due to different problems, e.g. elution in partly outside the chromatographic window and many of them had recoveries around 125% and consequently above the criteria. The pesticides were: 2-Phenylphenol, captan, carbofuran, carboxin, chlorothalonil, dichlorvos, epoxiconazole, fenbuconazole, fenpropidin, fenpropimorph, metconazole, tebuconazole and thiamethoxam. A re-validation of these compound will probably result in a validation on both spiking levels.

EUPT-CF7 test material:

The method was used to analyse the EUPT-CF7 test material with good result. z-scores were calculated based on the Assigned values, and were between -0.7 and 0.6, see **Table 1**.

Table 1. Results from analysis of EUPT-CF7 test material, the assigned values and calculated z-scores.

Pesticide	Mean, mg/kg	Assigned values, mg/kg	z-score
Azoxystrobin	0.106	0.115	-0.3
Boscalid	0.132	0.149	-0.5
Chlorpyrifos-methyl	0.244	0.252	-0.1
Cypermethrin	0.236	0.282	-0.6
Endosulfan-alpha	0.232	0.220	0.2
Endosulfan-sulfate	0.312	0.307	0.1
Epoxiconazole *)	0.129	0.121	0.3
Fenpropidin *)	0.101	0.171	-1.6
Fenpropimorph *)	0.352	0.333	0.2
Fluquinconazole	0.099	0.107	-0.3
Flutriafol	0.317	0.308	0.1
Iprodione	0.322	0.325	0.0
Kresoxim- methyl	0.070	0.060	0.6
Lindane	0.286	0.279	0.1
Malathion	0.259	0.316	-0.7
Propiconazole	0.213	0.217	-0.1
Tebuconazole *)	0.070	0.076	-0.3
Triadimenol *)	0.236	0.230	0.1
Trifloxystrobin	0.053	0.057	-0.3

Four of the non-validated pesticides were also present in the test material. Also these four pesticides, showed good results with z-scores between -1.6 and 0.3. This indicate that a re-validation of these compound will be possible.

8. Conclusions

In conclusion 55 pesticides and degradation products were validated on feed for laying hens for QuEChERS method and GC-MS/MS detection.

9. References

1 EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

2 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/10684/2010, 01/01/2010, European Commission, Brussels, 2010.

Appendix 1. MRM transitions for the pesticides included in the experiments.

	Retention time	Transition 1	Collision energy 1	Transition 2	Collision energy 2
2-phenylphenol	8.45	141>115	15	171>142	20
Azinphos-methyl	21.38	160>77	15	132>77	10
Azoxystrobin	28.88	344>329	15	388>345	15
Bifenthrin	20.22	181>166	10	165>115	20
Boscalid	25.21	342>140	15	167>139	20
Captan		149>70	12	149>105	2
Carbofuran	7.13	149>121	5	164>149	10
Carboxin	16.29	235>143	5	143>87	5
Chlorfenvinphos		323>267	15	295>267	5
Chlorothalonil	11.24	266>133	18	266>231	10
Chlorpropham	9.41	213>127	15	213>171	5
Chlorpyrifos	13.25	197>169	10	314>258	12
Chlorpyriphos-methyl	12.02	286>93	20	125>79	5
Cyfluthrin	24.52-24.87	226>206	10	163>91	10
Cypermethrin	25.09-25.45	181>152	20	163>127	10
Cyproconazole	16.73	222>125	15	139>111	15
Cyprodinil	14.01	226>225	15	223>208	15
Deltamethrin	27.8-28.18	253>174	10	181>152	10
Diazinon	10.84	304>179	10	276>179	10
Dichlorvos	10.25	109>79	5	187>93	10
Dicloran	6.78	206>124	22	206>176	10
Difenoconazole	27.63-27.77	323>265	15	325>267	15
Dimethoate	10.25	229>87	7	125>79	6
Endosulfan alfa	18.37	195>159	5	339>159	20
Endosulfan beta	15.24	195>159	5	339>159	20
Endosulfan sulfate	17.04	272>235	20	387>289	10
Epoxiconazole	19.54	192>138	10	206>165	5
Ethion	17.41	384>231	5	231>203	15
Fenbuconazole	24.52	198>129	10	129>102	15
Fenitrothion	12.72	277>260	5	277>109	15
Fenpropidin	12.72	98>70	10	99>71	10
Fenpropimorph		303>128	5	117>115	10
Fenvalerate	26.78-27.18	167>125	10	125>99	10
Fipronil	14.52	367>213	20	367>255	15
Fluquinconazole	23.85	340>298	15	339>298	15
Flutriafol	15.52	219>123	15	123>95	10
HCH, alpha	9.98	217>181	10	219>183	10
HCH, beta	10.56	217>181	10	219>183	10
Hexaconazole	15.7	231>175	10	214>172	15
Iprodione	19.86	314>245	10	216>187	5
Isoprothiolane	15.86	290>118	10	290>204	2
Kresoxim-methyl	16.5	206>116	4	206>131	10
Lambda-cyhalothrin	27.72-22.08	197>141	10	208>181	10

	Retention time	Transition 1	Collision energy 1	Transition 2	Collision energy 2
Lindane	10.59	217>181	10	219>183	10
Malathion	12.96	173>99	10	173>127	5
Metconazole	20.67	125>89	10	127>89	10
Metribuzin	11.9	198>82	15	214>198	5
Parathion	13.29	291>109	10	291>81	20
Penconazole	14.21	248>157	20	159>123	15
Pendimethalin	14.15	281>252	5	252>162	5
Permethrin	23.40-23.65	183>168	15	183>153	10
Phosphamidone	11.79	264>127	10	127>109	10
Pirimicarb	11.48	238>166	10	166>96	15
Pirimiphos-methyl	12.72	305>290	10	290>233	10
Prochloraz	23.96	180>138	10	310>268	5
Procymidone	14.7	283>96	6	283>254	10
Propiconazole	18.39-18.58	173>145	10	259>173	15
Pyrimethanil	10.87	199>198	5	198>183	10
Quinoxyfen	18.29	237>208	20	272>237	15
Tebuconazole	18.92	250>125	15	125>89	10
Thiametoxam	13.86	212>139	10	247>182	10
Triadimefon	13.35	208>181	5	181>111	10
Triadimenol	14.56-14.77	168>70	5	128>100	10
Triazophos	17.93	257>162	5	285>162	5
Trifloxystrobin	18.58	222>190	5	186>145	10
Trifluralin	9.52	264>206	5	290>248	10
Triticonazole	21.23	235>182	15	217>167	15
Vinclozolin	11.99	285>212	5	198>145	15

Appendix 2. Recoveries and, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on feed.

Numbers in italic is outside 70-120% recovery

Feed	Detection	Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.1	23		
		Recovery, %	**RSD_r, %**		**Recovery, %**	**RSD_r, %**		**LOQ**
Azinphos-methyl	GC	79	11		93	13		0.01
Azoxystrobin	GC	112	14		106	5		0.01
Bifenthrin	GC	109	17		110	10		0.01
Boscalid	GC	115	11		112	6		0.01
Chlorfenvinphos	GC	118	9		90	3		0.01
Chlorpropham	GC	107	19		96	8		0.01
Chlorpyrifos	GC	89	9		94	6		0.01
Chlorpyrifos-methyl	GC	81	9		81	3		0.01
Cyfluthrin	GC	101	11		105	6		0.01
Cypermethrin	GC	77	17		106	11		0.01
Cyproconazole	GC	119	11		*127*	9		0.01
Cyprodinil	GC	113	9		107	9		0.01
Deltamethrin	GC	77	15		85	5		0.01
Diazinon	GC	77	7		75	2		0.01
Dicloran	GC	104	13		101	7		0.01
Difenoconazole	GC	97	12		102	4		0.01
Dimethoate	GC				108	3		0.05
Endosulfan alfa	GC	110	11		98	10		0.01
Endosulfan beta	GC	120	21		106	6		0.01
Endosulfan sulfate	GC	107	24		100	5		0.02
Ethion	GC	100	9		100	3		0.01
Fenitrothion	GC	103	11		96	1		0.01
Fenvalerate	GC				115	11		0.02
Fipronil	GC	118	13		120	6		0.01
Fluquinconazole	GC	106	13		111	6		0.01
Flutriafol	GC	106	15		119	6		0.01
HCH, alpha	GC	74	18		72	2		0.01
HCH, beta	GC	91	11		98	8		0.01
Hexaconazole	GC	98	17		118	11		0.01
Iprodione	GC	94	10		101	3		0.01
Isoprothiolane	GC	107	13		113	5		0.01
Kresoxim-methyl	GC	120	16		118	5		0.01
Lambda-cyhalothrin	GC				119	8		0.06
Lindane	GC	95	16		87	6		0.01
Malathion	GC	104	15		105	8		0.01
Metribuzin	GC	97	16		99	6		0.01
Parathion	GC	103	11		98	2		0.01

Feed	Detection	Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.1	23		
		Recovery, %	**RSD_r, %**		**Recovery, %**	**RSD_r, %**		**LOQ**
Penconazole	GC	114	11		*121*	9		0.01
Pendimethalin	GC	96	10		90	4		0.01
Permethrin	GC				120	11		0.08
Phosphamidone	GC	97	9		95	4		0.01
Pirimicarb	GC	86	11		86	5		0.01
Pirimiphos-methyl	GC	96	9		97	3		0.01
Prochloraz	GC	117	19		117	11		0.01
Procymidone	GC	105	9		108	4		0.01
Propiconazole-sum	GC	83	20		115	6		0.01
Pyrimethanil	GC	92	10		88	4		0.01
Quinoxyfen	GC	113	13		119	10		0.01
Triadimefon	GC	111	12		104	5		0.01
Triadimenol	GC	94	20		*121*	6		0.01
Triazophos	GC	104	13		107	4		0.01
Trifloxystrobin	GC	117	18		*122*	5		0.01
Trifluralin	GC	75	11		*63*	5		0.01
Triticonazole	GC	111	13		*125*	8		0.01
Vinclozolin	GC	89	11		85	3		0.01