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National Food Institute
Technical University of Denmark

Appendix 6

Validation Report 12

Determination of pesticide residues in hay by LC-MS/MS and GC-MS/MS

(QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with LC-MS/MS and GC-MS/MS. The method was validated for 75 pesticides and degradation products in hay matrix.

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]. The method were slightly modified due to the dry texture of the hay.

2. Principle of analysis

Sample preparation: The hay samples was is grinded on a cutting Mill SM 2000 from Retsch equipped with a sieve at 1 mm (see foto below).

Extraction: One gram of sample was added 10 ml of water and left for 30 min. Then the samples were added ml acetonitril and the samples was shaken. Salt and buffer mixture was added and the sample was shaken again.

Clean-up: After centrifugation the supernatant was 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. For the LC-MS/MS analysis the extraction is followed by adding internal standard and the extract is filtered into HPLC vials.

Quantification and qualification:

LC-MS/MS: The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The validation includes pesticides determined with both positive and negative ESI. $^{13}C_6$-carbaryl was used as internal standard for quantification. All pesticides were detected in the multiple reaction monitoring mode (MRM). For each pesticide precursor ion and 2 product ions were determined. One product ion for quantification and one for qualification. The MRM transitions for the pesticides and degradation products sought validated are given in **Appendix 1**.

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 transistion 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 93 pesticides or degradation products in hay. The validation was performed on 5-6 replicates on each cereals commodity at each of the three spiking levels; 0.01, 0.02 and 0.1 mg/kg. A blank sample of hay was included.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at 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 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 LC-MS/MS and GC-MS/MS are presented in **Figure 1**. Examples of calibration curves are presented in **Figure 2**.

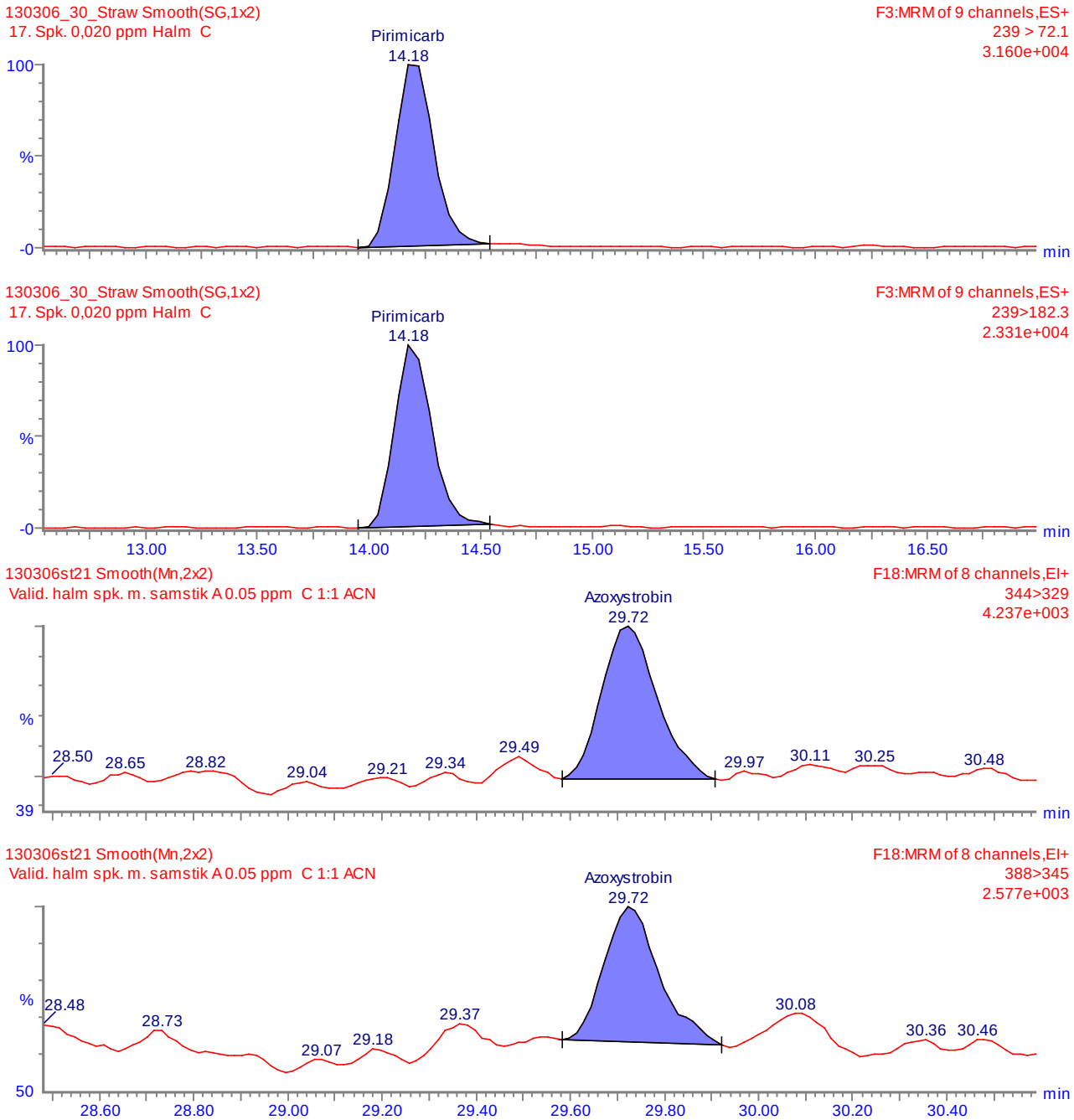

Figure 1: Examples of chromatograms for pirimicarb 0.02 mg/kg on LC-MS/MS and azoxystrobin 0.05 mg/kg on GC-MS/MS (two MRM transitions are shown for each pesticide).

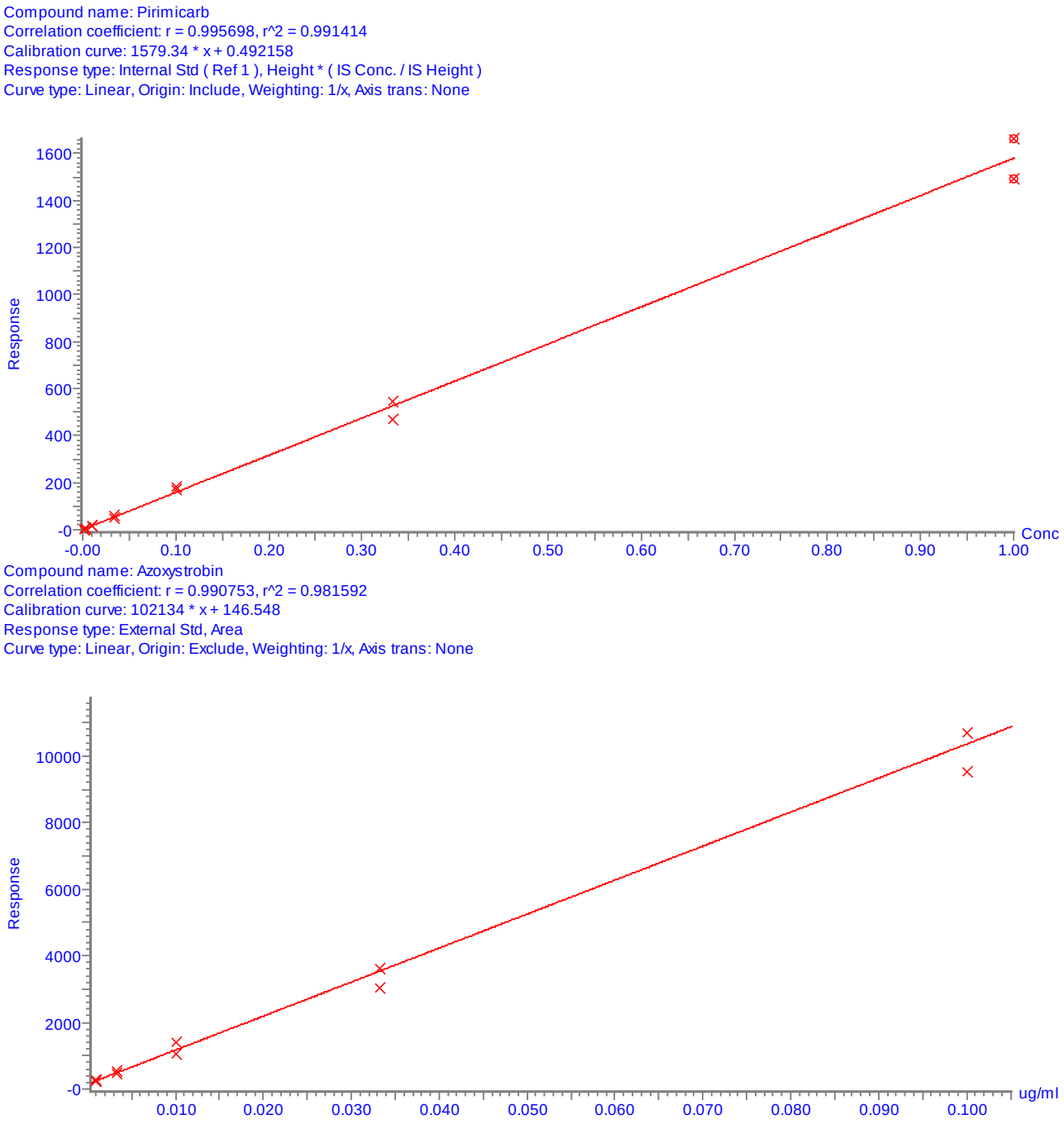

Figure 2. Examples of calibration curves for pirimicarb (LC-MS/MS, concentrations from 0.003-1.0 µg/ml) and azoxystrobin (GC-MS/MS, concentrations from 0.003-0.1 µg/ml) .

5. Validation parameters

Precision – repeatability

Repeatability was calculated for all pesticides and degradation products on all three spiking levels. Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time. Repeatability in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Appendix 3 shows the relative repeatability for the validated pesticides and degradation products.

Accuracy – Recovery

The accuracy was determined by recovery, samples were spiked at three 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 three spiking levels (0.01 mg/kg, 0.02 mg/kg and 0.1 mg/kg). Recoveries is listed in **Appendix 3**.

Robustness

The QuEChERS method has earlier by Anastassiades et al. 2003[1] in connection with the development of the method been shown to be robust.

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 3**.

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 must be less than or equal to the standard deviation proposed by Horwitz[3].
2. The average relative recovery must be between 70 and 120%[4].

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

7. Results and discussion

The method was sought validated for 94 compound. Two different detection system was used, LC/MS/MS and GC/MS/MS. In general, the compounds analysed on LC/MS/MS could be detected

at all three spike levels, while it was the case for only one fifth of the compounds measured on GC/MS/MS. Ten compounds were measured on both LC/MS/MS and GC/MS/MS and for these compound the results from LC/MS/MS was better and more pesticide were validated. Some of the compound measured on the GC/MS/MS can be analysed on LC/MS/MS but were analysed on GC/MS/MS for historical reasons. Furthermore, a more sensitive GC system would allow more pesticides.

In total, 76 compounds were validated, 47 at all spike levels, 67 at the two highest spike level and 76 at the highest level. The relative repeatability (RSD_r) varied between 2-31 %, however most of the values were below 20%. For the majority of the pesticides the recovery was in the range of 70-110% at all three concentration levels. But in general the recoveries for cycloxydim was low for all commodities. The LOQs were in the range of 0.01-0.15 mg/kg.

The results for the pesticides are listed in Appendix 3.

8. Conclusions

In conclusion, 76 pesticides and degradation products were validated on hay matrice for the QuEChERS method using LC-MS/MS and GC-MS/MS for the detection.

9. References

1 http://www.quechers.com/ or Anastassiades et al., J. AOAC Int., vol. 86, no. 2, p. 412, 2003

2 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 W. Horwitz, Anal. Chem., 1982; 54, 67A.

4 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.

5 EU Pesticides database available at http://ec.europa.eu/sanco_pesticides/public/index.cfm

Appendix 1. MRM transitions for the validated pesticides analysed by LC/MS/MS.

LC-MS/MS ESI-		Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CE	CV
1	3-Hydroxy carbofuran	255.3	163	30	13	255.3	107.2	30	25
2	Acephat	183.78	143.01	30	8	183.78	125	30	20
3	Carbaryl	219.3	145.27	29	13	219.3	127.2	29	37
4	Cyprodinil	226	93.2	16	33	226	77.2	16	40
5	Dementon-S-methyl sulfoxid	247	169	33	10	247	127	18	25
6	Demeton-S-methyl sulfon	263	169	55	15	263	127	45	28
7	Epoxiconazol	330.11	121.1	45	23	330.11	91.2	45	41
8	Fenhexamid	302.28	97.3	30	21	302.28	55.5	30	40
9	Flusilazole	316.17	247.1	20	17	316.17	165.1	51	20
10	Imazalil	297.4	159.2	29	21	297.4	201.2	29	20
11	Imidacloprid	256	209	21	15	256	175	20	20
12	Isoproturon	207.07	72.2	38	23	207.07	165.2	17	13
13	Kresoxim-methyl	314	116	30	30	314	131	30	20
14	Linuron	249.24	160.2	21	21	249.24	182.2	21	13
15	Malaoxon	315	127	48	10	315	99.1	33	21
16	Methacrifos	258.09	209	17	11	258.09	125	21	25
17	Methomyl	163.15	106.2	29	13	163.15	88.3	29	5
18	Metribuzin	215.06	187.1	52	23	215.06	84.3	21	20
19	Omethoat	214	183	10	11	214	143	10	17
20	Paclobutrazole	294.3	70.2	20	31	294.3	125.2	20	31
21	Pendimethalin	282.12	212	33	10	282.12	194	33	10
22	Pirimicarb	239	72.1	25	16	239	182.3	25	14
23	Pirimicarb-desmethyl	225.11	72.3	22	21	225.11	168.1	22	15

LC-MS/MS ESI-		Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CE	CV
24	Pirimiphos-methyl	306	164	20	20	306	108	20	20
25	Prothioconazole_desthio	312	70	50	30	314	127	50	35
26	Pyraclostrobin	388.15	194	24	11	388.15	163	24	25
27	Pyrimethanil	200	107	30	25	200	82.2	33	27
28	Spiroxamin	298.26	144.13	51	20	298.26	100.2	35	30
29	Tebufenozide	353.23	133.05	24	17	353.23	297	24	5
30	Thiacloprid	253.204	126.115	10	23	253.204	186	10	40
31	Thiodicarb	355	88	27	15	355	108	27	15
32	Triazophos	314.312	119	42	40	314.312	162	42	13
33	Tricyclazole	189.99	162.97	50	21	189.99	135.98	50	27
34	Triticonazole	318.04	70.3	22	15	318.04	125	22	25
35	Clothianidin *)	247.923	58.134	50	13	247.923	150	50	13
36	Diflubenzuron *)	308.78	156	46	15	308.78	93.1	46	40
37	Fipronil *)	435.22	330.2	42	13	435.22	250.1	42	23
38	Fludioxanil *)	247	180	22	27	247	126	22	33

*) Pesticides analysed by negative ESI

Appendix 2. MRM transitions for the validated pesticides analysed by GC/MS/MS

GC-MS/MS		Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CV
1	2-Phenylphenol	8.76	141	115	15	170	169	20
2	Azinphos-methyl	22.16	160	77	15	132	77	10
3	Azoxystrobin	29.75	344	329	15	388	345	15
4	Bifenthrin	20.92	181	166	10	165	115	20
5	Boscalid	26	342	140	15	167	139	20
6	Captan	15.11	149	70	12	149	105	2
7	Carbofuran	7.34	149	121	5	164	149	10
8	Carboxin	16.96	235	143	5	143	87	5
9	Chlorfenvinphos		323	267	15	295	267	5
10	Chlorothalonil	11.8	266	133	18	266	231	10
11	Chlorpropham	9.79	213	127	15	213	171	5
12	Chlorpyrifos	13.82	197	169	10	314	258	12
13	Chlorpyrifos-methyl	12.53	286	93	20	125	79	5
14	Cyfluthrin	25.46	226	206	10	163	91	10
15	Cypermethrin	25.86	163	127	10	181	152	20
16	Cyproconazole	17.41	222	125	15	139	111	15
17	Cyprodinil	14.61	226	225	15	223	208	15
18	Deltamethrin	28.61 and 29.04	181	152	10	253	174	10
19	Diazinon	11.27	304	179	10	276	179	10
20	Dichlorvos	7.02	109	79	5	187	93	10
21	Difenoconazole	28.38 and 28.52	323	265	15	325	267	15
22	Dimethoate	10.67	229	87	7	125	79	6
23	Endosulfan-alpha	19.09	195	159	5	339	159	20
24	Endosulfan-beta	15.89	195	159	5	339	159	20
25	Endosulfan-sulfate	17.74	272	236	20	387	252	10
26	Epoxiconazole	20.26	192	138	10	206	165	5
27	Ethion	18.07	384	231	5	231	203	15

GC-MS/MS		Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CV
28	Fenbuconazole	25.31	198	129	10	129	102	15
29	Fenitrothion	13.27	277	260	5	277	109	15
30	Fenpropidin	12.96	98	70	10	99	71	10
31	Fenpropimorph	13.9	303	128	5	117	115	10
32	Fenvalerate	27.56 and 27.96	167	125	10	125	99	10
33	Fipronil	15.21	367	213	20	367	255	15
34	Fluquinconazole	24.59	420	351	10	351	255	15
35	Flutriafol	16.15	383	255	20	255	228	10
36	HCH, -alpha	10.41	217	181	5	181	145	15
37	HCH, -beta	11.11	218	182.5	5	147	147	10
38	Hexaconazole	16.33	231	175	10	214	172	15
39	Iprodione	20.63	314	245	10	216	187	5
40	Isoprothiolane	16.47	290	118	10	290	204	2
41	Kresoxim-methyl	17.12	206	116	4	206	131	10
42	Lambda-cyhalothrin	22.44 and 22.81	197	141	10	208	181	10
43	Lindane	11.11	217	181	10	219	183	10
44	Malathion	13.5	173	99	10	173	127	5
45	Metconazole	21.46	125	89	10	127	89	10
46	Metribuzin	12.42	198	82	15	214	198	5
47	Parathion	13.87	291	109	10	291	81	20
48	Penconazole	14.83	248	157	20	159	123	15
49	Pendimethalin	14.75	281	252	5	252	162	5
50	Permethrin	24.12 and 24.38	183	168	15	183	153	10
51	Phosphamidone	12.27	264	127	10	127	109	10
52	Pirimicarb	11.95	238	166	10	166	96	15
53	Pirimiphos-methyl	13.25	305	290	10	290	233	10
54	Prochloraz	24.71	180	138	10	310	268	5
55	Procymidone	15.32	283	96	6	283	254	10
56	Propiconazole	19.07 and 19.28	173	145	15	259	173	15
57	Pyrimethanil	11.34	199	198	5	198	183	10

GC-MS/MS		Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CV
58	Quinoxyfen	19.03	237	208	20	272	237	15
59	Tebuconazole	19.67	250	125	15	125	89	10
60	Thiamethoxam	14.47	212	139	10	247	182	10
61	Triadimefon	13.93	208	181	5	181	111	10
62	Triadimenol	15.19 and 15.42	168	70	5	128	100	10
63	Triazophos	18.61	257	162	5	285	162	10
64	Trifloxystrobin	19.22	222	190	5	186	145	10
65	Trifluralin	9.88	264	206	5	290	248	10
66	Triticonazole	22.01	235	182	15	217	167	15
67	Vinclozolin	12.52	285	212	5	198	145	15

Appendix 3. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQs) for pesticides validated on Hay.

		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
Detection system	**Hay - QuEChERS**	**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
GC-MS/MS	**2-Phenylphenol**							84	22		0.06
LC-MS/MS	**3-Hydroxycarbofuran**				90	22		89	9		0.06
LC-MS/MS	**Acephat**				73	11		91	10		0.02
GC-MS/MS	**Azinphos-methyl**										
GC-MS/MS	**Azoxystrobin**	70	18		73	20		88	21		0.01
GC-MS/MS	**Bifenthrin**	95	15		85	8		95	5		0.01
GC-MS/MS	**Boscalid**	95	21		83	10		86	8		0.01
GC-MS/MS	**Captan**										
LC-MS/MS	**Carbaryl**	113	26		106	26		99	11		0.04
GC-MS/MS	**Carbofuran**										
GC-MS/MS	**Carboxin**	107	23		75	23		80	12		0.02
GC-MS/MS	**Chlorfenvinphos**										
GC-MS/MS	**Chlorothalonil**										
GC-MS/MS	**Chlorpropham**				109	27		120	13		0.04
GC-MS/MS	**Chlorpyrifos**				86	12		93	15		0.02
GC-MS/MS	**Chlorpyrifos-methyl**	110	23		83	24		104	17		0.02
LC-MS/MS	**Chlothianidin *)**	92	19		112	12		102	23		0.02
GC-MS/MS	**Cyfluthrin**										
GC-MS/MS	**Cypermethrin**										
GC-MS/MS	**Cyproconazole**	103	20		99	14		98	12		0.01
LC-MS/MS	**Cyprodinil**	88	13		81	14		87	4		0.01
GC-MS/MS	**Cyprodinil**				101	26		104	9		0.04
GC-MS/MS	**Deltamethrin**										

		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
Detection system	**Hay - QuEChERS**	**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
LC-MS/MS	**Demeton-S-methylsulfon**	88	9		93	6		100	6		0.01
LC-MS/MS	**Desmethyl pirimicarb**	82	5		93	7		103	5		0.01
GC-MS/MS	**Diazinon**							80	14		0.03
GC-MS/MS	**Dichlorvos**										
GC-MS/MS	**Difenoconazole**	71	15		74	11		76	9		0.01
LC-MS/MS	**Diflubenzuron *)**	91	25		115	23		72	17		0.07
GC-MS/MS	**Dimethoate**										
GC-MS/MS	**Endosulfan sulfate**										
GC-MS/MS	**Endosulfan-alpha**										
GC-MS/MS	**Endosulfan-beta**										
LC-MS/MS	**Epoxiconazole**	75	7		85	11		92	10		0.01
GC-MS/MS	**Epoxiconazole**	83	31		85	13		95	5		0.02
GC-MS/MS	**Ethion**				93	6		81	5		0.01
GC-MS/MS	**Fenbuconazole**				77	10		87	8		0.01
LC-MS/MS	**Fenhexamid**	80	14		80	5		87	9		0.01
GC-MS/MS	**Fenitrothion**	117	26		97	12		93	11		0.02
GC-MS/MS	**Fenpropidin**										
GC-MS/MS	**Fenpropimorph**							83	19		0.05
GC-MS/MS	**Fenvalerate**				82	16		85	9		0.02
LC-MS/MS	**Fipronil *)**	85	22		76	21		76	18		0.02
GC-MS/MS	**Fipronil**				97	8		87	7		0.01
LC-MS/MS	**Fludioxonil *)**	80	18		111	18		120	15		0.11
GC-MS/MS	**Fluquinconazole**	87	9		83	7		83	7		0.01
LC-MS/MS	**Flusilazole**	83	6		89	5		90	4		0.01
GC-MS/MS	**Flutriafol**	87	17		84	24		96	11		0.01

		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
Detection system	**Hay - QuEChERS**	**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
GC-MS/MS	**HCH, -alpha**				72	22		84	23		0.02
GC-MS/MS	**HCH, -beta**							105	18		0.06
GC-MS/MS	**Hexaconazole**										
LC-MS/MS	**Imazalil**	86	18		85	8		89	9		0.02
LC-MS/MS	**Imidacloprid**	77	24		85	18		94	12		0.02
GC-MS/MS	**Iprodione**				99	10		85	9		0.03
GC-MS/MS	**Isoprothiolane**				83	20		89	8		0.05
LC-MS/MS	**Isoproturon**	100	6		93	10		90	7		0.01
LC-MS/MS	**Kresoxim-methyl**	115	20		93	20		86	10		0.03
GC-MS/MS	**Kresoxim-methyl**				86	26		93	18		0.07
GC-MS/MS	**Lambda-cyhalothrin**										
GC-MS/MS	**Lindane**							93	19		0.11
LC-MS/MS	**Linuron**	85	10		95	5		101	5		0.01
LC-MS/MS	**Malaoxon**	83	6		89	8		97	3		0.01
GC-MS/MS	**Malathion**				96	17		98	8		0.05
GC-MS/MS	**Metconazole**							71	16		0.07
LC-MS/MS	**Methacrifos**	85	12		85	16		89	18		0.01
LC-MS/MS	**Methomyl**				101	14		118	13		0.04
LC-MS/MS	**Metribuzin**	102	13		92	11		81	12		0.02
GC-MS/MS	**Metribuzin**				94	20		92	10		0.06
LC-MS/MS	**Omethoat**	70	18		78	8		92	4		0.02
LC-MS/MS	**Oxydemeton-methyl**	73	11		81	7		93	8		0.01
LC-MS/MS	**Paclobutrazole**	93	16		89	9		96	4		0.02
GC-MS/MS	**Parathion**				82	22		82	10		0.05
GC-MS/MS	**Penconazole**	114	15		95	16		95	10		0.05

		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
Detection system	**Hay - QuEChERS**	**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
LC-MS/MS	**Pendimethalin**	92	28		93	19		104	19		0.03
GC-MS/MS	**Pendimethalin**							91	17		0.09
GC-MS/MS	**Permethrin**				76	20		98	18		0.04
GC-MS/MS	**Phosphamidone**				117	11		103	23		0.04
LC-MS/MS	**Pirimicarb**	93	11		100	10		104	4		0.01
GC-MS/MS	**Pirimicarb**				89	19		102	7		0.05
LC-MS/MS	**Pirimiphos-methyl**	92	4		91	4		89	3		0.01
GC-MS/MS	**Pirimiphos-methyl**							87	8		0.04
GC-MS/MS	**Prochloraz**							83	20		0.10
GC-MS/MS	**Procymidone**	120	18		93	17		95	12		0.05
GC-MS/MS	**Propiconazole**				72	18		102	21		0.04
LC-MS/MS	**Prothioconazole-desthio**	100	24		83	11		82	9		0.03
LC-MS/MS	**Pyraclostrobin**	70	8		84	6		96	5		0.01
LC-MS/MS	**Pyrimethanil**	102	7		87	8		87	7		0.01
GC-MS/MS	**Pyrimethanil**	113	9		103	8		101	7		0.02
GC-MS/MS	**Quinoxyfen**	93	24		79	13		95	19		0.03
LC-MS/MS	**Spiroxamine**	98	8		99	9		100	5		0.01
GC-MS/MS	**Tebuconazole**				73	9		88	22		0.02
LC-MS/MS	**Tebufenozide**	77	26		90	22		103	10		0.02
LC-MS/MS	**Thiacloprid**	88	13		90	12		98	4		0.01
GC-MS/MS	**Thiamethoxam**							117	12		0.08
LC-MS/MS	**Thiodicarb**	82	9		77	7		80	2		0.01
GC-MS/MS	**Triadimefon**							105	23		0.15
GC-MS/MS	**Triadimenol**				94	13		87	18		0.04
LC-MS/MS	**Triazophos**	77	13		87	9		97	6		0.01

		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		Spike level mg/kg	Horwitz, %		
		0.01	32		0.02	29		0.1	23		
Detection system	**Hay - QuEChERS**	**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**Recovery, %**	**RSDr, %**		**LOQ**
GC-MS/MS	**Triazophos**				77	24		83	14		0.06
LC-MS/MS	**Tricyclazole**	93	11		83	10		84	23		0.01
GC-MS/MS	**Trifloxystrobin**										
GC-MS/MS	**Trifluralin**										
LC-MS/MS	**Triticonazole**	93	6		91	11		89	9		0.01
GC-MS/MS	**Vinclozolin**				117	16		108	10		0.06

*) Pesticides analysed by negative ESI
Pesticides marked with red colour: Not validated
Pesticides marked with green colour: Validated on both LC-MS/MS and GC-MS/MS. However, for most of the pesticides the validation done by LC/MS/MS is the best.

Appendix 4: Principles of the QuEChERS method for cereal extraction

QuEChERS for cereals (FP417)

Weigh 1 g (±0.05 g) of flour into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80˚C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 ˚C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.