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Validation Report 21

Determination of pesticide residues in maize for livestock feed by GC-MS/MS and LC-MS/MS

(QuEChERS method)

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December 2016

CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS and LC-MS/MS. The method was sought validated for 80 pesticides in maize for livestock feed (in-house standard mixture B). The QuEChERS method is an extraction method 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].

2. Principle of analysis

Sample preparation: The samples were milled with a sieve size of 1 mm.

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

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

The principle of the extraction and clean-up procedure is outlined in **Appendix 3**.

Quantification and qualification: The final extract was analysed by GC/MS/MS and LC-MS/MS.

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

LC-MS/MS: The pesticide residues were 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. All pesticides were detected in the MRM mode. 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 1b**.

3. Validation design

The method was south validated for 80 pesticides or degradation products in maize (see **Table 1)**. The validation was performed on 5-6 replicates at each of four spiking levels; 0.005, 0.01, 0.02 and 0.1 mg/kg. A blank sample of maize was included in each analytical batch.

Table 1. Pesticides included in the recovery experiments.

Pesticides included in recovery experiments			
Acetamiprid	EPN	Iprovalicarb	Phoxim
Acrinathrin	Ethoprophos	Isofenphos-methyl	Profenofos
Aldicarb	Etofenprox	Lufenuron	Propargit
Aldicarb sulfon	Fenamiphos	Mepanipyrim	Propyzamide
Bitertanol	Fenamiphos sulfoxide	Methamidophos	Prothiofos
Bromopropylate	Fenamiphos sulfone	Methidathion	Pyridaben
Bromuconazole	Fenarimol	Methiocarb	Pyriproxyfen
Bupirimate	Fenazaquin	Methiocarb sulfone	Spinosyn A
Buprofezin	Fenoxycarb	Methiocarb sulfoxide	Spinosyn D
Cadusafos	Fenpropathrin	Monocrotophos	Spirodiclofen
Carbendazim	Fenthion	Myclobutanil	Tau-Fluvalinate
Chlorfenapyr	Fenthion oxon	Oxadixyl	Tebufenpyrad
Chlorobenzilate	Fenthion oxon-sulfon	Oxamyl	Tefluthrin
Clofentezine	Fenthion oxon-sulfoxid	Paraoxon-methyl	Tetraconazole
Dicloran	Fenthion sulfone	Parathion-methyl	Tetradifon
Dimethomorph	Fenthion sulfoxide	Pencycuron	Thiabendazole
Diphenylamine	Flufenoxuron	Phenthoate	Tolclofos-methyl
DMF	Fosthiazate	Phosalone	Tolylfluanid
DMPF	Hexythiazox	Phosmet	Triflumuron
DMST	Indoxacarb	Phosmet oxon	Zoxamide

4. Chromatograms and calibration curves

The calibration curves were based on at least 4 calibration levels, i.e. 0.003, 0.01, 0.033 and 0.1 µg/ml. The calibration curves were in general best fitted to a linear curve. 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 and calibration curves obtained when analysing extracts by GC-MS/MS and LC-MS/MS are presented in **Figure 1-2** and **Figure 3-4**, respectively.

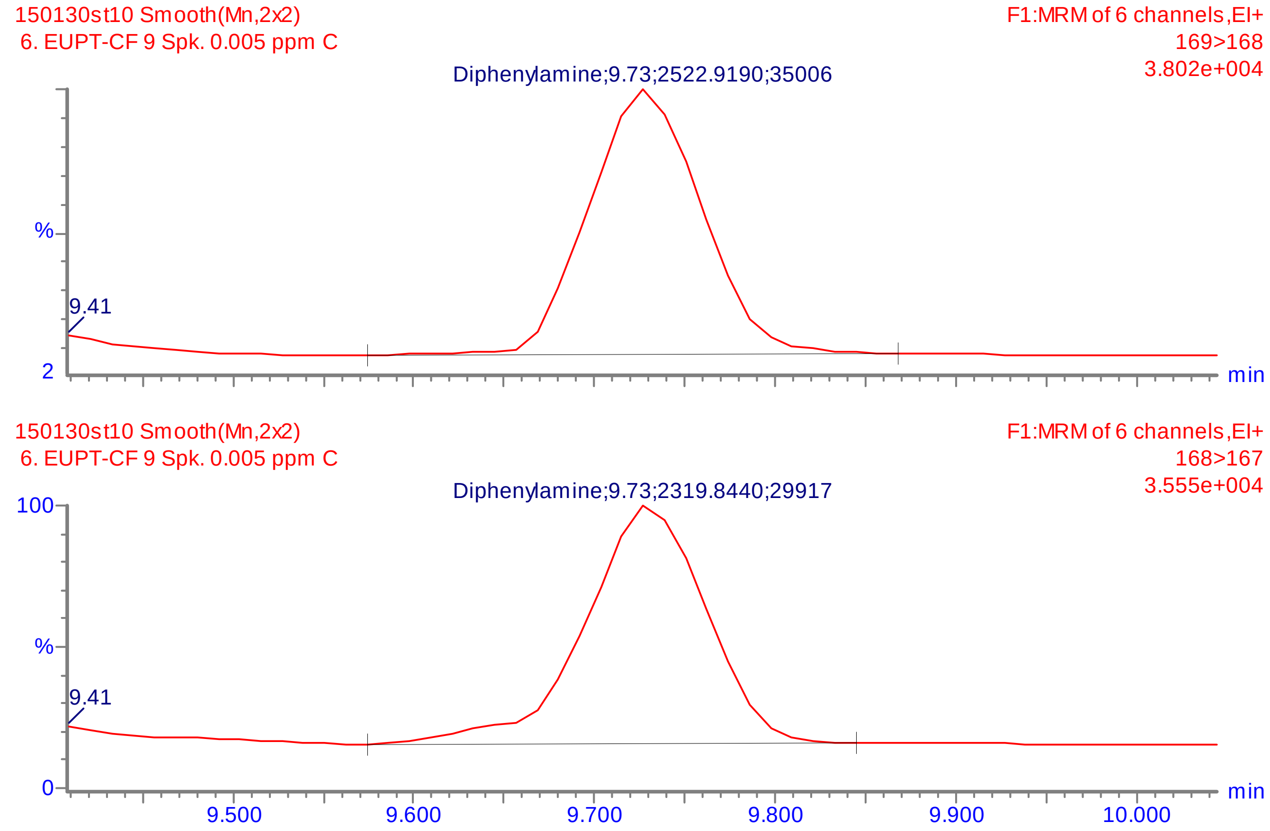

Figure 1: Examples of GC-MS/MS chromatograms for diphenylamine in maize for livestock feed obtained when analysing extract of sample spiked with 0.005 mg/kg (Quantifier and qualifier MRM transitions are shown).

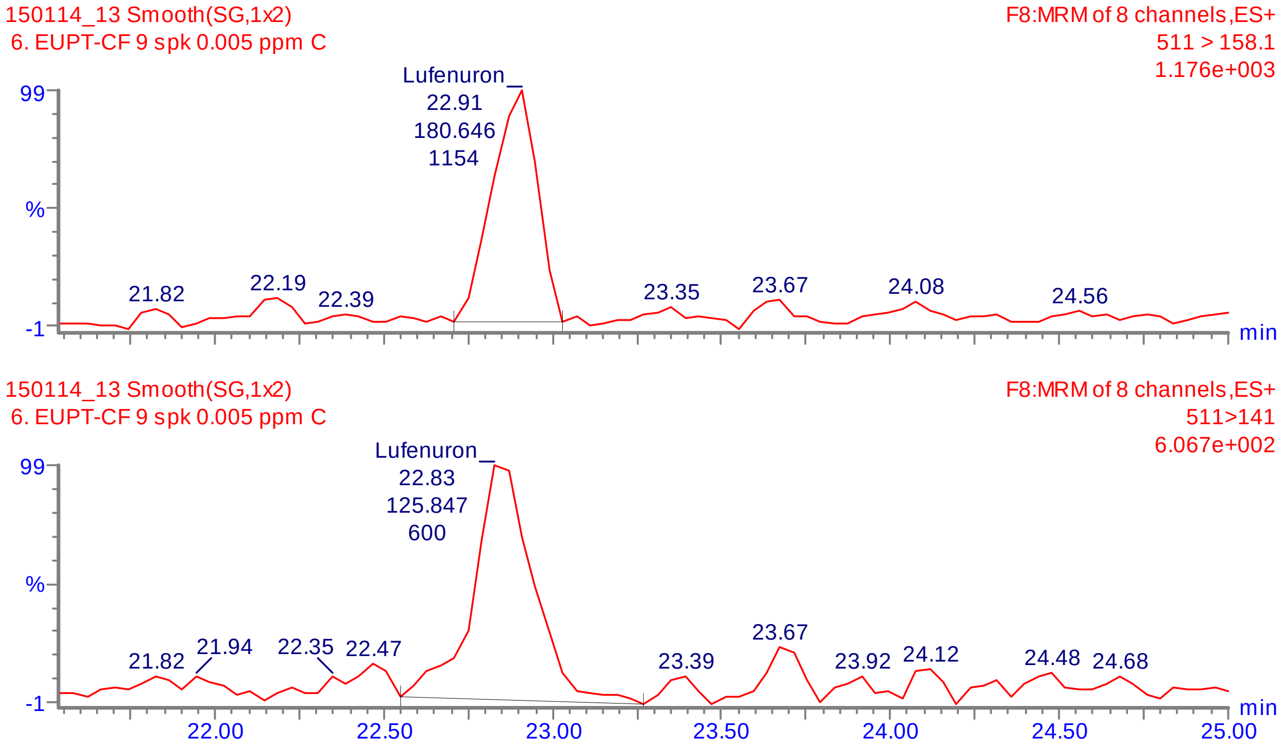

Figure 2: Examples of LC-MS/MS chromatograms lufenuron in maize for livestock feed obtained in positive mode when analysing extract of sample spiked with 0.005 mg/kg (Quantifier and qualifier MRM transitions are shown).

Compound name: Diphenylamine
Correlation coefficient: r = 0.997576, r^2 = 0.995159
Calibration curve: 2.15397e+006 * x + -307.883
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

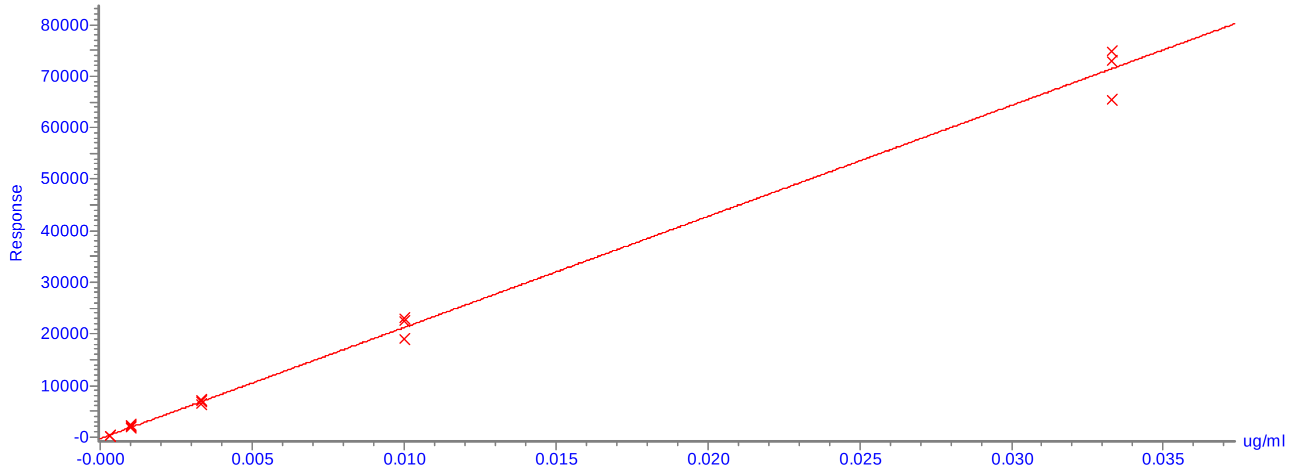

Figure 3. Examples of GC-MS/MS calibration curves for diphenylamine matrix matched with maize for livestock feed (concentrations from 0.001-0.100 µg/ml)

Compound name: Lufenuron
Correlation coefficient: r = 0.997610, r^2 = 0.995225
Calibration curve: 23.6916 * x + 0.00116182
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

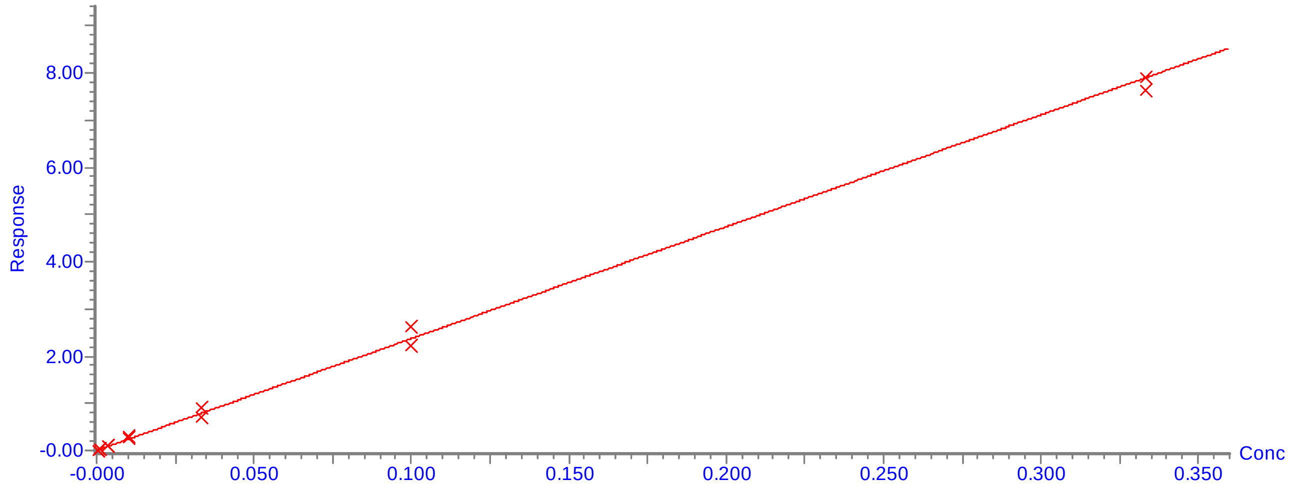

Figure 4. Examples of LC-MS/MS calibration curves for lufenuron matrix matched with maize for livestock feed (concentrations from 0.001-0.333 µg/ml).

5. Validation parameters

Precision – repeatability

Repeatability was calculated for all pesticides and degradation products on four spiking levels (0.005, 0.01, 0.02 and 0.1 mg/kg). Repeatability is the relative standard deviation on the results from two or more analysis of replicate samples, prepared by the same technician, on the same instrument and within a short period of time.

Repeatability (RSD_r) in this validation was calculated from the 5-6 replicate determinations as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three levels (0.005, 0.01 mg/kg, 0.02 and 0.1 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

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

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) were meet.

The obtained results including recovery, $RSD_{r,}$ combined uncertainty (U_c) and limit of quantification (LOQ), are presented 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%[3].
2. The average relative recovery must be between 70 and 120%[3].

If the above mentioned criteria have been meet, the quantification limits, LOQs is stated.

The combined uncertainty given by:

$$U_c = \sqrt{Bias^2 + RSD^2}$$

Where Bias is the measurement uncertainty of the method (100 - Recovery (%)) and RSD is the standard deviation on the bias or recovery.

7. Results and discussion

Overall validation

All 80 compounds included in the study were possible to validate using QuEChERS combined with GCMSMS or LCMSMS. 35 compounds were possible to validate using GCMSMS and 52 using LCMSMS and thus seven of the compounds where possible to validate using both LCMSMS and GCMSMS.

Of the 35 compounds amenable to GCMSMS a LOQ of 0.005 mg/kg was obtained for 31 compounds and a LOQ of 0.01 mg/kg for 4 compounds. For 6 of the GCMSMS amenable compounds the results needs to be corrected for recovery in order to obtain acceptable precision. Of the 52 compounds amendable to LCMSMS a LOQ of 0.005 mg/kg were obtained for 39 compound, and a LOQ of 0.01 mg/kg for 8 compounds, LOQ of 0.02 mg/kg for two compounds and an LOQ of 0.1 mg/kg for the last three compounds. For 13 of the LCMSMS amenable compounds the results needs to be corrected for recovery in order to obtain acceptable precision (see **Appendix 2)**.

Low recoveries were found for fenamiphos sulfoxide and oxadixyl (51-56%). The validation data was accepted despite these low recoveries, because the recovery was at the same low level for all spike levels and the standard deviation on the results were acceptable.

8. Conclusions

In conclusion 80 pesticides were successfully validated on maize for livestock feeding using the QuEChERS method and GC-MS/MS or/and LC-MS/MS. LOQs at 0.005 mg/kg were obtained for 64 of the 80 compounds.

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 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 Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Document No SANTE/11945/2015, 01/01/2016. Published by European Commission Directorate-General for health and food safety.

Appendix 1a. MRM transitions GC-MS/MS.

GC-MS/MS	**Retention time**	**Precursor ion-1**	**Product ion-1**	**CE**	**Precursor ion-2**	**Product ion-2**	**CE**
Acrinathrin-1	23.68	208	181	10	181	152	15
Acrinathrin-2	24.1	208	181	10	181	152	15
Bitertanol	24.88	170	141	18	141	115	14
Bromopropylate	21.73	341	185	15	183	155	15
Bromuconazole-1	21.54	295	173	10	293	173	15
Bromuconazole-2	22.37	295	173	10	293	173	15
Cadusafos	10.59	159	131	5	158	114	5
Chlorfenapyr	18.28	328	247	15	364	248	20
Chlorobenzilate	18.48	251	139	10	139	111	10
Clofentezine	6.86	102	75	10	137	102	10
Dicloran	11.1	206	124	22	206	176	10
Diphenylamine	9.9	169	168	10	168	167	10
EPN	21.73	185	157	6	169	77	13
Fenamiphos	17	303	195	5	154	139	10
Fenarimol	23.86	330	139	15	219	107	10
Fenpropathrin	22.05	181	152	10	208	181	5
Fenthion	14.46	278	109	15	278	124	20
Flufenoxuron	12.01	331	268	15	268	241	15
Fosthiazate	15.07	195	103	5	283	103	15
Indoxacarb	30.15	264	176	10	527	264	5
Isofenphos-methyl	15.3	199	121	5	241	199	10
Mepanipyrim	16.7	222	207	15	223	207	20
Methidathion	16.28	145	85	5	302	145	5
Monocrotophos	10.47	127	109	10	192	127	10
Myclobutanil	17.67	179	125	14	179	152	6
Oxadixyl	18.92	163	132	8	163	117	18
Parathion-methyl	13.16	263	109	10	263	127	10
Pencycuron	10.59	180	125	10	125	89	10

GC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
Phenthoate	15.86	274	121	8	274	125	16
Phosalone	22.93	367	182	5	182	111	15
Profenofos	17.29	339	269	10	374	339	5
Propyzamide	11.7	173	145	12	173	109	22
Prothiofos	17.19	309	239	12	267	239	5
Tau-Fluvalinate-1	28.97	250	200	20	208	181	15
Tau-Fluvalinate-2	29.11	250	200	20	208	181	15
Tefluthrin	12.1	177	127	15	197	141	10
Tetraconazole	14.75	171	136	10	337	220	15
Tetradifon	22.61	356	229	15	229	201	15
Tolclofos-methyl	13.29	265	250	15	267	252	10
Tolylfluanid	15.67	238	137	15	137	91	20

Appendix 1b. MRM transitions for LC-MS/MS.

LC-MS/MS	**Retention time**	**Precursor ion-1**	**Product ion-1**	**CV**	**CE**	**Precursor ion-2**	**Product ion-2**	**CV**	**CE**
Acetamiprid	9.36	223	126	27	20	223	90	27	35
Acrinathrin	24.12	540	372	45	11	540	513	45	20
Aldicarb	11.18	213	89	29	13	213	116	50	12
Aldicarb sulfon	7.64	240.4	148	30	13	240.4	86.2	21	21
Bitertanol	20.37	338.2	99	31	17	338.2	70	31	17
Bupirimat	19.35	317	166	27	23	317	108	25	25
Buprofezin	22.33	306	201	10	11	306	106.2	10	23
Cadusafos	21.8	271.3	159	50	13	271.3	97	50	40
Carbendazim	10.27	192	160	55	18	192	132.3	30	29
Clofentezine	21.69	303	138	20	20	303	102.1	20	20
Dimethomorph	16.73/17.48	388	301	45	20	388	165	23	30
Diphenylamine	19.35	170	93.1	25	20	170	151.9	25	20
DMF (N-2,4-dimethylphenylformamide)	12.5	150	132.2	34	35	150	106.8	34	20
DMPF (2.4 dimethylphenyl-N-methylformamidine)	8.73	164	123	10	20	164	133	10	20
DMST (dimethylaminosulfutoluidide)	13.8	232	106	30	20	232	150.9	30	20
Ethoprophos	19.28	243	97	50	23	243	131	50	31
Etofenprox	26.83	394	177.3	25	20	394	107.1	25	20
Fenamiphos	18.93	304	217.1	31	23	304	202	31	33
Fenamiphos sulfone	13.37	336	188	50	31	336	266	50	23
Fenamiphos sulfoxide	12.88	320	171	50	13	320	292	50	40
Fenazaquin	25.07	307	161.2	55	17	307	131.1	52	14
Fenoxycarb	20.29	302.3	88.2	42	31	302.3	116.2	42	31
Fenthion	20.96	279	169.1	25	20	279	247.1	25	20
Fenthion oxon	17.03	263	231	20	30	263	216	20	15
Fenthion oxon-sulfon	9.8	295	217	33	20	295	78	33	20
Fenthion oxon-sulfoxid	9.41	279	264	20	15	279	104	20	20
Fenthion sulfone	14.24	311	125	25	20	311	108.9	25	20
Fenthion sulfoxide	13.61	295	279.7	25	15	295	108.9	25	20

LC-MS/MS	Retention time	Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CV	CE
Flufenoxuron	24.06	489.6	158.1	22	13	489.6	141	22	40
Hexythiazox	22.78	353	228	45	11	353	168	45	27
Iprovalicarb	17.76	321	119.1	45	17	321	91.2	45	48
Lufenuron	22.43	511	158.1	33	21	511	141	33	41
Lufenuron	23.23	509	339	13	13	509	175	13	31
Mepanipyrim	18.39	224	106.1	17	23	224	77.27	17	38
Methamidophos	6.45	142	94	40	12	142	124.9	40	12
Methiocarb	17.36	243.4	169.3	30	13	243.4	121.2	30	21
Methiocarb sulfone	9.59	275	122	33	25	275	107.2	33	40
Methiocarb sulfoxide	8.73	242	185	33	10	242	122	33	30
Monocrotophos	8.13	241	127	21	10	241	193	10	20
Oxadixyl	10.72	279	219.1	17	17	279	132.1	17	30
Oxamyl	7.64	237.4	72.4	21	13	237.4	90.3	21	8
Paraoxon-methyl	11.23	265	202	51	35	265	127	40	19
Phosmet	15.89	335	160	17	17	335	133	17	45
Phosmet oxon	11.1	302	160.2	29	21	302	133.2	29	35
Phoxim	21.27	299	77.1	10	23	299	115	17	17
Propargit	23.18	368	175.1	24	15	368	231	24	11
Pyridaben	24.36	365	147.2	38	23	365	309.1	0	0
Pyriproxyfen	22.89	322	96	55	20	322	185	27	23
Spinosyn A	21.72	733	142	30	20	733	98	30	20
Spinosyn D	22.59	746.5	142	30	40	746.5	98.2	30	45
Spirodiclofen	24.39	411	313	20	20	411	71	20	20
Tebufenpyrad	22.08	334.5	147	55	23	334.5	117	55	30
Thiabendazole	11.24	201.8	175	45	24	201.8	131.2	45	29
Tolylfluanid	19.91	347	137	40	25	347	238	40	25
Triflumuron	20.57	359	155.9	21	25	359	139	20	30
Zoxamide	21.21	336.4	187	36	40	336.4	159	36	20

Appendix 2. Recoveries, repeatability (RSD$_r$), Combined Uncertainty (Comb. U.) and Limit of Quantification (LOQ) for pesticides validated on maize for livestock feed.

Numbers in italic is outside 70-120% recovery or above 20% RSD

		Spike level mg/kg 0.005			Spike level mg/kg 0.01			Spike level mg/kg 0.02			Spike level mg/kg 0.1				
		Recovery %	**RSDr, %**	U_c	**Recovery %**	**RSDr, %**	U_c	**Recovery %**	**RSDr, %**	U_c	**Recovery %**	**RSDr, %**	U_c	**LOQ**	**Reco-very corr.**
LC	Acetamiprid	108	15	17	101	15	15	99	9	9	103	7	7	0.005	No
GC	Acrinathrin	113	11	17	101	9	10	96	7	8	100	4	4	0.005	No
LC	Aldicarb	75	14	*29*	94	9	10	103	8	9	107	4	8	0.005	Yes
LC	Aldicarb sulfon	102	14	15	102	9	9	106	6	8	104	5	6	0.005	No
GC	Bitertanol	109	8	12	108	4	9	99	3	3	106	4	7	0.005	No
LC	Bitertanol	91	*34*	*35*	89	*26*	*28*	77	11	*25*	98	7	7	0.02	Yes
GC	Bromopropylate	104	7	8	101	5	5	94	5	7	100	3	3	0.005	No
GC	Bromuconazole	111	9	14	103	6	7	96	5	7	102	3	4	0.005	No
LC	Bupirimate	116	10	19	119	7	20	119	3	19	119	6	20	0.005	No
LC	Buprofezin	98	7	8	98	8	8	106	6	9	114	5	15	0.005	No
GC	Cadusafos	101	16	16	79	10	*23*	93	13	15	100	12	12	0.005	No
LC	Cadusafos	105	9	10	100	8	8	107	8	11	114	5	14	0.005	No
LC	Carbendazim	*52*	20	*52*	*63*	4	*38*	*68*	7	*33*	74	6	*27*	0.010	Yes
GC	Chlorfenapyr	117	8	19	101	12	12	105	4	7	104	6	7	0.005	No
GC	Chlorobenzilate	109	6	11	106	4	7	100	4	4	104	2	4	0.005	No
GC	Clofentezine	87	9	16	86	6	15	86	7	16	88	5	13	0.005	No
GC	Dicloran	113	7	15	92	3	9	95	7	9	99	3	4	0.005	No
LC	Dimethomorph	112	9	16	107	8	11	107	4	8	108	7	10	0.005	No
GC	Diphenylamine	112	9	15	95	5	7	92	7	10	93	4	8	0.005	No
LC	DMF	105	*44*	*44*	93	15	16	90	10	15	92	9	12	0.01	No
LC	DMPF	99	*48*	*48*	99	10	10	102	14	14	*51*	14	*51*	0.01	No
LC	DMST	65	*37*	*51*	87	11	17	110	13	17	115	6	16	0.01	No
GC	EPN	111	12	16	101	4	4	93	3	7	97	2	4	0.005	No
LC	Ethoprophos	106	10	11	109	4	10	104	5	7	108	7	11	0.005	No
LC	Etofenprox	86	20	*24*	78	8	*24*	79	8	*22*	88	6	14	0.005	Yes
GC	Fenamiphos	117	4	17	97	7	8	99	4	4	99	4	4	0.005	No
LC	Fenamiphos	104	7	8	105	7	8	114	7	16	*122*	5	*22*	0.005	No

LC	Fenamiphos sulfone	110	9	14		105	11	12		101	5	5		99	9	9		0.005	No
LC	Fenamiphos sulfoxide	*51*	12	*51*		*52*	15	*51*		*51*	9	*50*		*51*	10	*50*		0.005	Yes
GC	Fenarimol	107	6	9		101	5	5		95	4	7		99	3	4		0.005	No
LC	Fenazaquin	93	6	9		98	7	7		103	3	4		105	6	8		0.005	No
LC	Fenoxycarb	98	11	12		94	4	8		98	9	9		101	3	3		0.005	No
GC	Fenpropathrin	114	6	15		100	6	6		97	5	6		100	3	3		0.005	No
GC	Fenthion	113	8	16		101	5	5		96	8	9		105	4	6		0.005	No
LC	Fenthion	104	13	13		98	16	16		100	16	16		99	7	7		0.005	No
LC	Fenthion oxon	108	8	11		107	9	11		107	5	9		107	7	9		0.005	No
LC	Fenthion oxon-sulfon	response not detectable				*123*	*29*	*37*		98	*22*	*23*		110	7	13		0.1	No
LC	Fenthion oxon-sulfoxid	113	8	15		110	11	15		108	5	10		107	6	9		0.005	No
LC	Fenthion sulfone	*133*	*38*	*50*		94	*25*	*25*		87	15	20		116	6	17		0.020	No
LC	Fenthion sulfoxide	109	10	14		104	5	7		105	9	11		114	5	15		0.005	No
GC	Flufenoxuron	104	14	15		91	14	17		78	26	*34*		94	8	10		0.005	No
LC	Flufenoxuron	76	11	*27*		83	19	*26*		104	16	16		110	12	16		0.005	Yes
GC	Fosthiazate	*124*	11	*26*		104	11	12		93	10	12		95	5	7		0.01	Yes
LC	Hexythiazox	90	9	14		92	14	16		99	9	9		106	5	7		0.005	No
GC	Indoxacarb	*136*	11	*38*		102	9	9		104	6	7		101	4	4		0.01	Yes
LC	Iprovalicarb	112	7	14		104	5	7		104	5	7		103	6	7		0.005	No
GC	Isofenphos-methyl	*123*	3	*23*		106	4	7		102	6	6		105	3	5		0.01	Yes
LC	Lufenuron	*123*	7	*24*		114	5	15		102	8	8		110	7	12		0.01	No
GC	Mepanipyrim	117	6	18		105	7	9		98	8	8		106	3	6		0.005	No
LC	Mepanipyrim	90	8	13		98	8	8		100	5	5		103	6	6		0.005	No
LC	Methamidophos	76	*33*	*41*		*66*	10	*36*		*60*	7	*40*		*67*	6	*33*		0.010	Yes
GC	Methidathion	*123*	5	*23*		97	3	5		88	11	16		92	3	8		0.01	Yes
LC	Methiocarb	84	11	19		93	8	10		94	13	14		99	4	4		0.005	No
LC	Methiocarb sulfone	88	17	*21*		87	12	18		88	12	17		107	12	14		0.005	Yes
LC	Methiocarb sulfoxide	105	6	8		95	8	9		94	8	10		99	6	6		0.005	No
LC	Monocrotophos	96	6	7		86	9	17		92	8	11		97	6	7		0.005	No
GC	Myclobutanil	113	5	14		111	4	12		102	4	4		103	3	5		0.005	No
GC	Oxadixyl	113	7	15		102	2	3		101	3	4		105	2	5		0.005	No
LC	Oxadixyl	*54*	17	*50*		*55*	13	*47*		*56*	6	*45*		*55*	6	*45*		0.005	Yes
LC	Oxamyl	90	8	13		90	11	15		88	12	17		91	5	10		0.005	No

LC	Paraoxon-methyl	response smaller than lowest calibration				28	68	99		57	35	55		114	18	*23*		0.1	Yes
GC	Parathion-methyl	119	8	20		96	8	9		94	11	12		103	3	4		0.005	No
GC	Pencycuron	111	7	13		99	3	3		96	9	10		98	4	5		0.005	No
GC	Phenthoate	117	8	19		98	7	7		97	9	9		103	3	5		0.005	No
GC	Phosalone	117	9	20		101	11	11		98	8	8		101	2	2		0.005	No
LC	Phosmet	*57*	*23*	*48*		74	12	*29*		90	10	14		98	5	5		0.01	Yes
LC	Phosmet oxon	110	8	12		103	6	7		102	3	4		100	5	5		0.005	No
LC	Phoxim	107	12	14		107	8	10		105	3	6		106	7	9		0.005	No
GC	Profenofos	120	10	*23*		104	10	11		95	6	8		95	5	7		0.005	Yes
LC	Propargite	100	5	5		96	8	9		97	3	5		97	7	8		0.005	No
GC	Propyzamide	117	13	*21*		104	17	18		94	7	10		100	5	5		0.005	Yes
GC	Prothiofos	88	8	14		88	9	15		85	5	16		91	3	10		0.005	No
LC	Pyridaben	77	10	*25*		89	11	15		101	12	12		104	15	16		0.005	Yes
LC	Pyriproxyfen	95	10	11		93	8	10		91	4	10		94	6	9		0.005	No
LC	Spinosyn A	106	7	9		100	7	7		106	7	9		117	5	17		0.005	No
LC	Spinosyn D	103	18	18		103	10	10		104	8	9		108	6	10		0.005	No
LC	Spirodiclofen	*135*	6	*35*		112	12	17		106	10	12		107	5	9		0.01	No
GC	Tau-Fluvalinate	105	10	11		92	15	18		94	13	14		100	3	3		0.005	No
LC	Tebufenpyrad	105	12	13		101	9	9		102	5	5		103	6	7		0.005	No
GC	Tefluthrin	108	13	15		105	9	11		98	10	10		107	4	8		0.005	No
GC	Tetraconazole	115	4	15		114	15	20		100	7	7		100	3	3		0.005	No
GC	Tetradifon	100	12	12		85	13	20		93	7	10		94	5	8		0.005	No
LC	Thiabendazole	77	5	23		81	7	20		85	2	15		92	8	11		0.005	Yes
GC	Tolclofos-methyl	116	8	18		101	8	8		95	10	11		103	4	5		0.005	No
LC	Tolylfluanid	response not detectable												101	5	5		0.1	No
LC	Triflumuron	86	11	18		96	10	11		101	4	4		101	7	7		0.005	No
LC	Zoxamide	106	16	17		95	13	14		99	10	10		105	6	8		0.005	No

Appendix 3: Principles of the QuEChERS method for feed extraction

QuEChERS for cereals
(FP417)

Weigh 5 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.