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

Validation Report 26

Determination of pesticide residues in fish feed

by LC-MS/MS

(modified QuEChERS method)

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CONTENT:

1. Introduction

This report describes the validation of a modified QuEChERS method for fish feed with high fat content combined with LC-MS/MS for 80 pesticides and metabolites and isomers of pesticides. The pesticides sought validated has been evaluated as relevant for fish feed because they may be used in the production of cereals and other matrices of plant origin which may be used in feed production. The aim of the validation was to achieve LOQs at 0.01 mg/kg or below. However fish feed is a complex matrix of high fat content an LOQ of 0.01 mg/kg or less may be difficult to achieve for some analytes.

2. Principle of analysis

Samples and sample preparation: Samples of two different fish feed used for feeding salmon at different growth stages were used for the validation study. The feed samples consisted of pellets of 2mm size with 22.8% crude fat content (CPK15) and 8mm size with 33.6% crude fat content (EFICO).

The pellets were homogenized via cryo-milling (using liquid nitrogen) with sieve at 1.0 mm.

Extraction: The extraction procedure is outlined in appendix 3. The homogenized sample was first shaken with water and acetonitrile (in two steps), and a salt and buffer mixture was added and the sample was shaken again.

Clean-up: After centrifugation the supernatant was transferred to a clean tube and put in -80 degree freezer for minimum of one hour. When the extract was almost thawed, it was centrifuged and the supernatant was transferred to a tube containing 900 mg PSA and 900 mg $MgSO_4$ (first clean-up). After shaking and an additional centrifugation step, an aliquot of the supernatant was transferred to a tube containing EMR-lipid dSPE activated by water addition (Enhanced Matrix Removal (EMR) – Lipid). After shaking and another centrifugation step, the supernatant was transferred to a tube containing Final Polish (second step in the EMR-lipid clean-up procedure). After shaking and centrifugation, the "final extract" was diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

Quantification and qualification: The final extracts were analysed by LC-MS/MS.

The LC-MS/MS analysis was performed on a HP1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) connected to a Micromass Quattro Ultima Triple Quadrupole Instrument. The

analytes were separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). All pesticides were ionised by ESI in positive mode and detected in the MRM mode. For each pesticide or metabolite a precursor ion and 2 product ions were employed, i.e. 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**. Examples of chromatograms obtained are presented in **Figure 1 and 2**.

3. Validation design

The method was sought validated for 80 pesticides and metabolites and isomers of pesticides in two different fish feed matrices, CPK-15 (medium fat content) and EFICO (high fat content). The compounds included in the study are listed in **Appendix 1**. The validation was performed on 5-6 replicates at each of the three spiking levels; 0.005, 0.01 and 0.05 mg/kg. A blank sample of each fish feed commodity was included. Individual validation was carried out for each type of fish feed.

4. Chromatograms and calibration curves

The calibration curve was prepared by the analysis of each of the analytes at least 5 calibration levels, i.e. 0.0003, 0.001, 0.003, 0.01, 0.03 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, except for Spiroxamine (0.98), Kresoxim-methyl (0.96), DMF (0.98), Aldicarb sulfon (0.98), Fenthion oxon-sulfoxid (0.98), Oxadixyl (0.96), Fenamiphos sulphone (0.98), Iprovalicarb (0.97), Ethoprophos (0.97), Triflumuron (0.97), Phoxim (0.97).

Examples of calibration curves for LC-MS/MS are presented in **Figure 3 and 4**.

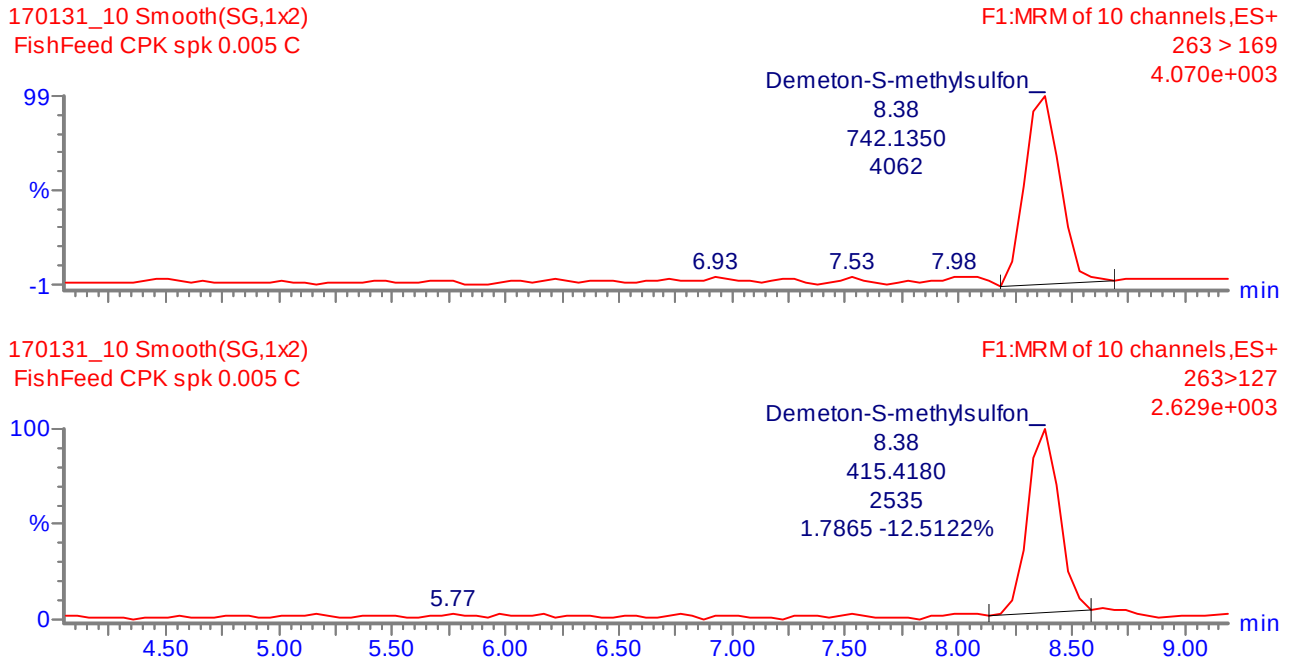

Figure 1: Examples of LC-MS/MS chromatograms demeton-S-methylsulfone in low-fat fish feed (CPK) obtained in positive mode when analysing extract spiked with 0.005 mg/kg. Both MRM transitions are shown for each pesticide.

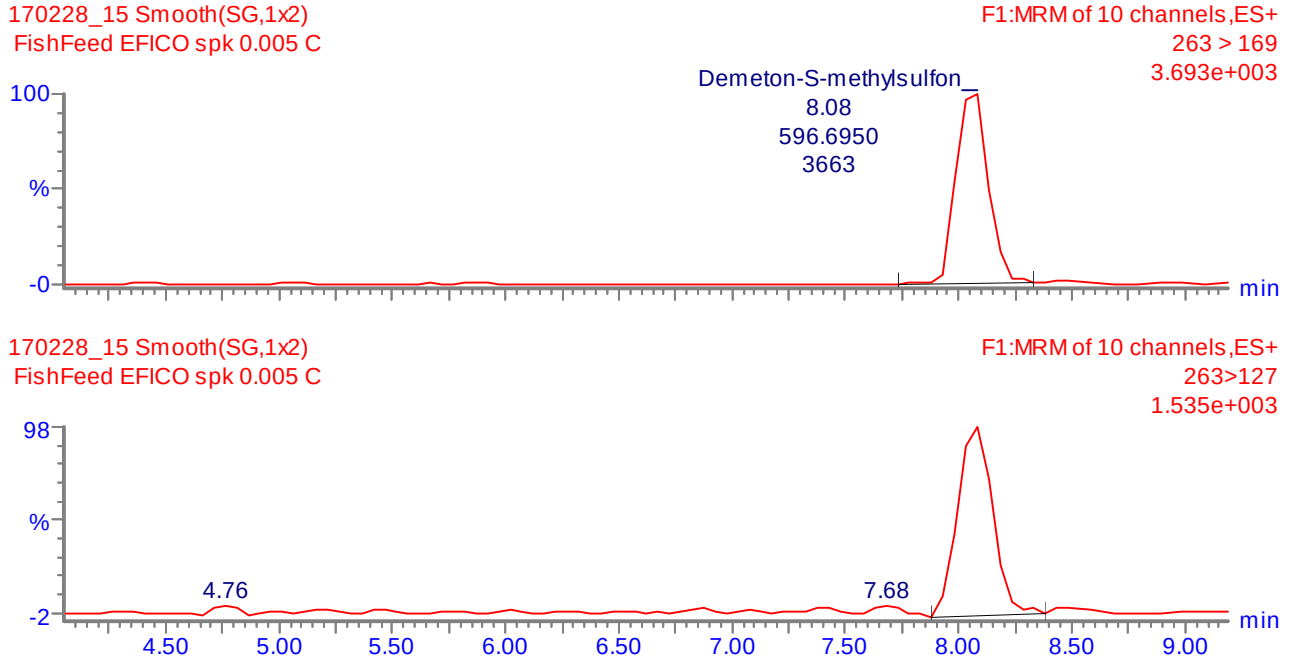

Figure 2: Examples of LC-MS/MS chromatograms demeton-S-methylsulfone in high-fat fish feed (EFICO) obtained in positive mode when analysing extract spiked with 0.005 mg/kg. Both MRM transitions are shown for each pesticide.

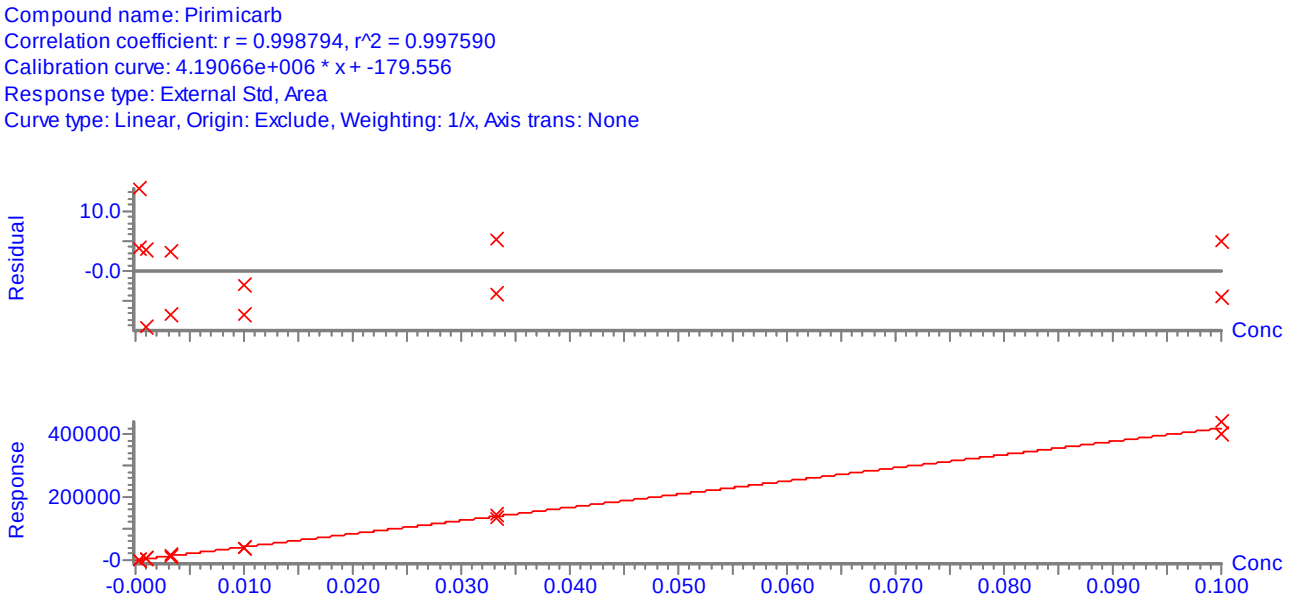

Figure 3. Examples of LC-MS/MS calibration curves for pirimicarb matrix matched low-fat fish feed (CPK) (concentrations from 0.1-0.0003 µg/ml).

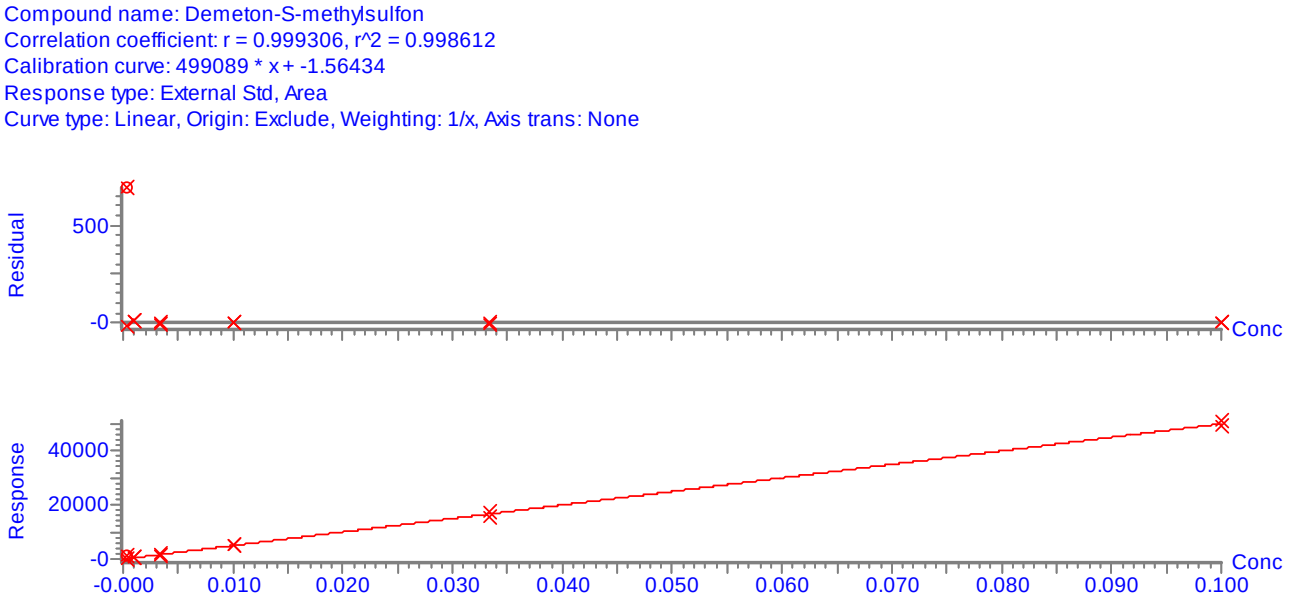

Figure 4. Examples of LC-MS/MS calibration curves for demeton-S-methyl sulfone matrix matched high-fat fish feed (EFICO) (concentrations from 0.1-0.0003 µg/ml).

5. Validation parameters

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.005 mg/kg, 0.01 mg/kg and 0.05 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) was meet.

The obtained results including recovery and limit of quantification (LOQ) using low fat and high fat fish feeds as test material are presented in Appendix 2a and 2b, respectively.

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 analytical result is by default corrected for bias/recovery and the combined uncertainty is then given by:

$$U_c = \sqrt{(RSD^2/n) + RSD^2}$$

Where RSD is the repeatability uncertainty (RSD_r).

7. Results and discussion

Validation on medium-fat fish feed (CPK)

76 compounds were sought validated using the medium-fat fish feed (CPK) as test material when analyzing by LC-MS/MS and using the modified QuEChERS procedure outlined in Appendix 3 for

extraction. 75 of these compounds were successfully validated. The validation results are presented in **Appendix 2a.**

The LOQs were 0.005 for most compounds (45 compounds), though for 17 compounds (bixafen, carbofuran, cyprodinil, fenamiphos sulfoxide, flusilazole, isoproturon, linuron, lufenuron, methiocarb, methomyl, monocrotophos, phoxim, pirimiphos-methyl, pyraclostrobin, triflumuron, triticonazole, zoxamide) a LOQ of 0.01 mg/kg was obtained. For another 13 compounds (azinphos ethyl, carbaryl, cymiazol, diphenylamine, fenthion, fenthion sulfon, flufenoxuron, imidacloprid, metribuzin, paraoxon-methyl, pendimethalin, pyrimethanil, and resmethrin) an LOQ of 0.05 was obtained. Low recoveries were obtained for carbendazim (<50%), however the standard deviations (RSDr) were ≤10%. So identification and quantification is possible for carbendazim, but it was evaluated that the recoveries were too low to set an LOQ.

Validation on high-fat fish feed (EFICO)

69 compounds were sought validated using the high-fat fish feed (EFICO) as test material when analyzing by LC-MS/MS and using the modified QuEChERS procedure outlined in Appendix 3 for extraction. 59 of these compounds were successfully validated. The validation results are presented in **Appendix 2b.**

The LOQs were 0.005 for most compounds (40 compounds), though for 9 compounds (fenamiphos sulfoxide, imazalil, linuron, mepanipyrim, oxamyl, pyrimethanil, spinozyn A, spinozyn D, thiodicarb) a LOQ of 0.01 mg/kg was obtained and for another 10 compounds (azinphos ethyl, carbaryl, cymiazol, fenthion, fenthion sulfon, imidacloprid, methomyl, monocrotophos, pirimiphos-methyl, thiophenat-methyl) an LOQ of 0.05 was obtained.

Fenazaquin, fenthion oxon-sulfon, flufenoxuron, hexythiazox, metribuzin, paraoxon-methyl, pyriproxyfen, resmethrin, thiabendazole, and zoxamide were not possible to validate using the modified QuEChERS and analysis by LC-MS/MS due to low recoveries (<50%) and high standard deviations (RSDr > 20).

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 DIRECTIVE 2006/77/EC: Amending Annex I to COMMISSION Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for organochlorine compounds in animal feed.

3 Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed, Document No SANCO/12571/2013, 01/01/2014, European Commission, Brussels, 2012.

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

		Retention time	Precursor ion-1	Product ion-1	CV	CE	Precursor ion-2	Product ion-2	CV	CE
1	Acetamiprid	9.36	223	126	27	20	223	90	27	35
2	Aldicarb	11.18	213	89	29	13	213	116	50	12
3	Aldicarb sulfon	7.64	240.4	148	30	13	240.4	86.2	21	21
4	Azinphos-ethyl	19.51	346	261	50	21	346	233	10	20
5	Bixafen	19.2	414	394	45	20	414	266	45	35
6	Bupirimate	19.35	317	166	27	23	317	108	25	25
7	Buprofezin	22.33	306	201	10	11	306	106.2	10	23
8	Cadusafos	21.8	271.3	159	50	13	271.3	97	50	40
9	Carbaryl	13.58	219.3	145	29	13	219.3	127.2	29	37
10	Carbendazim	10.27	191.93	160.05	55	18	191.93	132.3	30	29
11	Carbetamide	11.73	237	192	30	10	237	118	30	50
12	Carbofuran	12.12	222	165	10	10	222	123	20	20
13	Clofentezin	21.69	303	138	20	20	303	102.1	20	20
14	Coumaphos	20.05	363.1	227	10	21	363.1	307	10	16
15	Cymiazol	10.1	219.2	144.1	20	37	219.2	171	20	37
16	Cyprodinil	21.2	226	93	16	33	226	77.2	16	40
17	Demeton-S-methyl	12.39	231	89	21	5	231	61.3	20	25
18	Demeton-S-methylsulfon	7.87	263	169	55	15	263	127	45	28
19	Desmethyl-Pirimicarb	10.3	225	72.3	22	21	225	168.1	22	15
20	Diethyl-m-toluamid, N,N-, (DEET)	14.12	192	119	10	20	192	91.2	10	30
21	Dimethomorph	16.73	388	301	45	20	388	165	23	30
22	Dimoxystrobin	19.55	328	116	41	20	328	206	41	10
23	Diphenylamine	19.35	170	93.1	25	20	170	151.9	25	20
24	Epoxiconazole	18.6	330	121	45	23	330	91	45	41

25	Ethoprophos	19.28	243	97	50	23	243	131	50	31
26	Fenamiphos	18.93	304	217.1	31	23	304	202	31	33
27	Fenamiphos sulfone	13.37	336	188	50	31	336	266	50	23
28	Fenamiphos sulfoxide	12.88	320	171	50	13	320	292	50	40
29	Fenazaquin	25.07	307	161.2	55	17	307	131.1	52	14
30	Fenoxycarb	20.29	302.3	88.2	42	31	302.3	116.2	42	31
31	Fenthion	20.96	279	169.1	25	20	279	247.1	25	20
32	Fenthion oxon	17.03	263	231	20	30	263	216	20	15
33	Fenthion oxon-sulfon	9.8	295	217	33	20	295	104.1	33	20
34	Fenthion oxon-sulfoxid	9.41	279	264	20	15	279	104	20	20
35	Fenthion sulfon	14.24	311	125	25	20	311	108.9	25	20
36	Fenthion sulfoxid	13.61	295	279.7	25	15	295	108.9	25	20
37	Flufenoxuron	24.06	489.6	158.1	22	13	489.6	141	22	40
38	Flusilazole	19.23	316	247.1	20	17	316	165.1	51	20
39	Hexythiazox	22.78	353	228	45	11	353	168	45	27
40	Imazalil	16	297.4	159.2	29	21	297.4	201.2	29	20
41	Imidacloprid	8.69	256	209	21	15	256	175	20	20
42	Iprovalicarb	17.76	321	119.1	45	17	321	91.2	45	48
43	Isoproturon	14.68	207	72.2	38	23	207	165.2	17	13
44	Linuron	17.31	249	160.2	21	21	249	182.2	21	13
45	Lufenuron	22.43	511	158.1	33	21	511	141	33	41
46	Malaoxon	12.38	315	127	48	10	315	99.1	33	21
47	Mepanipyrim	18.39	224	106.1	17	23	224	77.27	17	38
48	Metalaxyl	14.42	280	220.1	52	11	280	160.1	52	22
49	Methiocarb	17.36	243.4	169.3	30	13	243.4	121.2	30	21
50	Methomyl	8.1	163	106.2	29	13	163	88.3	29	5
51	Metribuzin	12.56	215	187.1	52	23	215	84.3	21	20
52	Monocrotophos	8.13	241	127	21	10	241	193	10	20

53	Oxadixyl	10.72	279	219.1	17	17	279	132.1	17	30
54	Oxamyl	7.64	237.4	72.4	21	13	237.4	90.3	21	8
55	Oxydemeton methyl	7.71	247	169	33	10	247	127	18	25
56	Paclobutrazole	18.05	294.3	70.2	20	31	294.3	125.2	20	31
57	Paraoxon-methyl	11.23	265	202	51	35	265	127	40	19
58	Pendimethalin	23.55	282	212	33	10	282	194	33	10
59	Phoxim	21.27	299	77.1	10	23	299	115	17	17
60	Pirimicarb	13.59	239	72.1	25	16	239	182.3	25	14
61	Pirimiphos-methyl	21.72	306	164	20	20	306	108	20	20
62	Prothioconazole-desthio	19.72	312	70	50	35	314	127	50	35
63	Pyraclostrobin	20.17	388	194	24	11	388	163	24	25
64	Pyrazophos	14.36	374	222	50	21	388	194	50	33
65	Pyrimethanil	15.88	200	107	30	25	200	82.2	33	27
66	Pyriproxyfen	22.89	322	96	55	20	322	185	27	23
67	Resmethrin	25.01	339	171.1	55	15	339	143	35	23
68	Spinozyn A	21.72	733	142	30	20	733	98	30	20
69	Spinozyn D	22.59	746.5	142	30	40	746.5	98.2	30	45
70	Spiroxamine	17.86	298	144	51	20	298	100.2	35	30
71	Tebufenpyrad	22.08	334.5	147	55	23	334.5	117	55	30
72	Thiabendazole	11.24	201.8	175	45	24	201.8	131.2	45	29
73	Thiacloprid	10.21	253	126	10	23	253	186	10	40
74	Thiodicarb	13.88	355	88	27	15	355	108	27	15
75	Thiophenat-Methyl	12.59	343	151	50	20	343	93	50	40
76	Triazophos	18.77	314	119	42	40	314	162	42	13
77	Tricyclazole	11.16	190	163	50	21	190	136	50	27
78	Triflumuron	20.57	359	155.9	21	25	359	139	20	30
79	Triticonazole	19.82	318	70.3	22	15	318	125	22	25
80	Zoxamide	21.21	336.4	187	36	40	336.4	159	36	20

Appendix 2a. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on low-fat fish feed (CPK) using modified QuEChERS.

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 0.05			
	Recovery, %	**RSDr, %**	**Combined uncertainty**	**Recovery, %**	**RSDr, %**	**Combined uncertainty**	**Recovery, %**	**RSDr, %**	**Combined uncertainty**	**LOQ**
Acetamiprid	99	20	*22*	97	11	12	96	7	8	0.005
Aldicarb	97	20	*21*	103	9	9	98	4	4	0.005
Aldicarb sulfon	85	11	12	105	8	9	111	8	8	0.005
Azinphos ethyl	94	*35*	*37*	110	*45*	*49*	102	9	10	0.05
Bixafen	107	*26*	*28*	103	12	12	100	10	11	0.01
Bupirimate	102	10	11	110	3	4	96	7	8	0.005
Buprofezin	88	4	4	75	5	5	*70*	5	6	0.005
Cadusafos	94	8	9	93	12	13	90	9	10	0.005
Carbaryl	113	*41*	*45*	117	*32*	*35*	95	15	16	0.05
Carbendazim	*55*	10	10	*43*	3	3	*36*	6	7	
Carbetamide	105	11	12	100	7	8	95	7	8	0.005
Carbofuran	95	*35*	*39*	109	3	3	113	6	6	0.01
Clofentezin	108	20	*21*	86	5	6	74	7	7	0.005
Coumaphos	111	15	16	106	7	7	105	6	6	0.005
Cymiazol	*64*	*78*	*84*	*49*	110	119	85	8	8	0.05
Cyprodinil	*70*	*35*	*37*	*61*	12	13	*67*	6	6	0.01
DEET	108	9	9	97	5	5	93	6	6	0.005
Demeton-S-methylsulfon	93	4	5	90	7	7	91	3	3	0.005
Desmethyl pirimicarb	91	11	12	91	4	5	89	2	2	0.005
Dimethomorph	112	10	10	105	10	11	102	7	7	0.005
Dimoxystrobin	114	*21*	*22*	107	10	11	106	7	7	0.005
Diphenylamin							82	4	4	0.05
Epoxiconazole	115	14	15	95	7	7	92	3	4	0.005
Ethoprophos	104	10	10	95	5	6	96	8	9	0.005

Fenamiphos	94	5	5	86	4	5	82	4	5	0.005
Fenamiphos sulfoxide	89	*23*	*25*	75	7	7	76	7	8	0.01
Fenamiphos sulphone	98	14	15	106	9	10	102	17	19	0.005
Fenazaquin	104	9	10	81	11	12	*63*	11	12	0.005
Fenoxycarb	101	7	8	93	11	12	92	10	10	0.005
Fenthion	*26*	*245*	*265*	76	*32*	*34*	93	10	11	0.05
Fenthion oxon-sulfoxid	83	20	*21*	86	8	8	81	7	8	0.005
Fenthion sulfon							98	9	10	0.05
Fenthion sulfoxid	100	13	14	88	8	9	82	7	7	0.005
Flufenoxuron	91	*28*	*30*	*68*	*32*	*34*	84	9	10	0.05
Flusilazole	91	*26*	*28*	98	8	8	95	6	7	0.01
Hexythiazox	72	20	*22*	78	9	10	73	8	9	0.005
Imidacloprid	*56*	*30*	*32*	*68*	*36*	*39*	78	15	16	0.05
Iprovalicarb	102	8	9	119	10	11	106	10	11	0.005
Isoproturon	*133*	*94*	*101*	92	4	4	92	4	4	0.01
Linuron	107	*50*	*55*	84	20	*21*	98	12	13	0.01
Lufenuron	93	*27*	*29*	103	18	19	107	17	19	0.01
Malaoxon	100	4	5	98	4	5	99	3	3	0.005
Mepanipyrim	84	12	13	81	6	6	76	5	6	0.005
Metalaxyl	103	7	8	93	5	5	86	5	5	0.005
Methiocarb	*68*	*50*	*54*	88	10	11	89	4	4	0.01
Methomyl	77	*33*	*36*	92	14	15	101	10	11	0.01
Metribuzin	*28*	*157*	*170*	79	*86*	*93*	103	10	11	0.05
Monocrotophos	*68*	*20*	*22*	74	19	20	82	7	7	0.01
Oxadixyl	97	20	*21*	103	9	9	98	4	4	0.005
Oxamyl	81	12	13	96	19	*20*	84	15	16	0.005
Oxydemeton methyl	*68*	10	11	*61*	8	8	*61*	4	4	0.005
Paclobutrazole	92	11	12	97	9	10	90	7	8	0.005
Paraoxon-methyl	*69*	*59*	*63*	112	*23*	*25*	97	11	11	0.05
Pendimethalin	*43*	*94*	*101*	*52*	*36*	*39*	*58*	6	7	0.05
Phoxim	93	*26*	*28*	112	13	14	108	19	*20*	0.01
Pirimicarb	101	6	7	96	3	3	89	2	2	0.005
Pirimiphos-methyl	87	*56*	*61*	80	11	12	84	5	5	0.01
Prothioconazole-desthio	72	14	16	79	14	16	85	4	4	0.005
Pyraclostrobin	86	*33*	*35*	94	6	7	96	3	3	0.01
Pyrazophos	110	7	8	101	5	6	96	6	7	0.005

Pyrimethanil	*59*	*70*	*76*	*60*	19	*21*	75	6	7	0.05
Pyriproxyfen	88	7	8	86	*27*	*29*	75	11	12	0.005
Resmethrin				*65*	*27*	*29*	75	11	12	0.05
Spinozyn A	104	15	16	93	10	10	86	10	11	0.005
Spinozyn D	107	13	14	99	6	7	91	4	4	0.005
Spiroxamine	*123*	7	7	*123*	5	6	*122*	2	3	0.005
Tebufenpyrad	93	10	11	93	5	5	87	9	9	0.005
Thiabendazole	*50*	9	10	*44*	9	10	*46*	8	8	0.005
Thiacloprid	93	17	18	85	5	5	87	4	4	0.005
Thiodicarb	*55*	14	16	*61*	10	10	*52*	10	11	0.005
Thiophenat-Methyl	83	20	*21*	76	16	18	78	13	14	0.005
Triazophos	101	11	12	96	8	9	95	2	2	0.005
Tricyclazole	*65*	12	13	*58*	4	4	*56*	4	4	0.005
Triflumuron	96	*23*	*24*	107	13	14	86	11	12	0.01
Triticonazole	*69*	*57*	*61*	79	13	14	82	7	7	0.01
Zoxamide	91	*21*	*23*	96	10	11	88	6	6	0.01

Appendix 2b. Recoveries, repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on high-fat fish feed (EFICO) using modified QuEChERS.

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 0.05			
	Recovery, %	**RSDr, %**	**Combined uncertainty**	**Recovery, %**	**RSDr, %**	**Combined uncertainty**	**Recovery, %**	**RSDr, %**	**Combined uncertainty**	**LOQ**
Acetamiprid	97	8	9	104	9	10	100	8	9	0.005
Aldicarb	108	17	18	107	9	10	109	7	8	0.005
Aldicarb sulfon	105	14	15	112	12	13	107	5	5	0.005
Azinphos ethyl	*49*	*39*	*43*	84	*30*	*33*	97	13	14	0.05
Bupirimate	106	4	5	96	5	6	99	5	5	0.005
Cadusafos	94	8	9	82	6	7	85	3	3	0.005
Carbaryl	*49*	*120*	*130*	109	*31*	*34*	85	9	10	0.05
Carbetamide	89	15	17	99	9	9	115	7	8	0.005
Carbofuran	*130*	2	2	*122*	5	5	*125*	6	6	0.005
Coumaphos	104	8	9	84	5	6	96	7	7	0.005
Cymiazol	71	*79*	*85*	*46*	*113*	*122*	102	5	5	0.05
DEET	109	4	4	106	7	7	113	8	9	0.005
Demeton-S-methylsulfon	105	7	7	103	8	8	97	6	6	0.005
Desmethyl pirimicarb	97	2	2	99	2	2	90	8	8	0.005
Dimethomorph	106	11	12	99	7	8	108	7	7	0.005
Epoxiconazole	105	10	11	97	6	6	96	7	8	0.005
Ethoprophos	105	6	6	96	6	7	99	4	5	0.005
Fenamiphos	108	4	4	96	5	6	96	8	9	0.005
Fenamiphos sulfoxide	86	*31*	*34*	78	14	16	77	12	13	0.01
Fenamiphos sulphone	114	14	15	108	6	6	111	8	9	0.005
Fenazaquin	*69*	13	14	*41*	13	14	*46*	*37*	*40*	
Fenoxycarb	104	6	7	87	11	12	95	7	7	0.005
Fenthion	79	*38*	*41*	72	*31*	*33*	81	19	*21*	0.05
Fenthion oxon	101	15	16	92	19	*21*	99	8	9	0.005
Fenthion oxon-sulfon	94	*140*	*151*	71	*162*	*175*	96	*22*	*24*	
Fenthion oxon-sulfoxid	86	7	8	86	4	4	83	9	10	0.005

Fenthion sulfon	*38*	*136*	*146*	*21*	*128*	*138*	117	15	16	0.05
Fenthion sulfoxid	108	12	13	103	10	11	106	9	10	0.005
Flufenoxuron	85	*35*	*38*	*62*	37	*40*	*57*	*34*	*36*	
Flusilazole	97	9	9	107	15	16	105	6	7	0.005
Hexythiazox	75	13	14	*46*	22	*24*	*49*	20	*21*	
Imazalil	111	*42*	*46*	98	20	*22*	94	12	13	0.01
Imidacloprid	*123*	*22*	*24*	89	*30*	*33*	93	16	17	0.05
Iprovalicarb	106	5	6	109	8	9	114	8	9	0.005
Isoproturon	111	6	7	100	6	6	101	7	7	0.005
Linuron	117	*32*	*35*	102	9	10	113	7	8	0.01
Lufenuron	111	19	*20*	84	*5*	*6*	75	19	*20*	0.005
Malaoxon	110	2	2	109	4	4	111	7	7	0.005
Mepanipyrim	85	19	*21*	*57*	10	11	*67*	6	7	0.01
Metalaxyl	111	6	7	100	6	7	100	6	6	0.005
Methiocarb	101	17	19	88	11	12	97	9	9	0.005
Methomyl	77	*55*	*60*	99	*42*	*45*	112	12	13	0.05
Metribuzin	118	*83*	*90*	*132*	38	*41*	91	*25*	*27*	
Monocrotophos	83	*28*	*30*	*66*	*32*	*34*	87	15	16	0.05
Oxadixyl	108	17	18	107	9	10	109	7	8	0.005
Oxamyl	93	*37*	*40*	104	14	16	94	12	13	0.01
Paclobutrazole	94	19	*20*	101	5	6	97	9	10	0.005
Paraoxon-methyl	*183*	*21*	*22*	*126*	*36*	*39*	85	*55*	*60*	
Phoxim	85	20	*22*	80	12	13	91	7	7	0.005
Pirimicarb	104	4	4	100	2	2	96	7	8	0.005
Pirimiphos-methyl	90	*34*	*37*	*41*	*31*	*34*	78	11	12	0.05
Prothioconazole-desthio	89	7	8	86	7	8	92	10	10	0.005
Pyrazophos	107	9	9	95	5	6	98	5	5	0.005
Pyrimethanil	95	*29*	*31*	*69*	14	15	*62*	8	9	0.01
Pyriproxyfen	73	11	12	*43*	*89*	*97*	*50*	*37*	*40*	
Resmethrin	74	*46*	*50*	*19*	*89*	*97*	*50*	*37*	*40*	
Spinozyn A	*124*	8	9	119	9	10	96	4	4	0.01
Spinozyn D	*130*	*31*	*33*	*122*	6	6	108	7	7	0.01
Spiroxamine	88	6	7	116	7	7	120	7	8	0.005
Tebufenpyrad	84	11	12	*66*	9	10	73	8	9	0.005
Thiabendazole	*42*	*22*	*24*	*43*	*13*	*14*	*48*	11	12	
Thiacloprid	117	6	7	101	10	11	97	6	7	0.005

Thiodicarb	*53*	15	16	70	18	20	70	16	17	0.01
Thiophenat-Methyl	105	*32*	*34*	85	*24*	*25*	92	10	10	0.05
Triazophos	107	6	7	102	5	5	104	8	8	0.005
Tricyclazole	72	4	5	*69*	9	9	*67*	9	10	0.005
Triflumuron	97	13	14	84	9	9	88	8	9	0.005
Triticonazole	117	11	12	99	15	17	97	10	11	0.005
Zoxamide	*341*	*175*	*189*	*245*	186	*201*	87	54	*59*	

Appendix 3: Principles of the modified QuEChERS method for fish feed extraction

Modified QuEChERS for fish feed

Weigh 5 g (±0.05 g) of homogenized feed 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 ml of cold water and shake briefly

Add 10 ml acetonitrile and shake mechanically for 1 min. (1. extraction)

Add the prepared mixture of 4 g MgSO4, 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 mechanically 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. Centrifuge (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 **900 mg PSA** and **900 mg $MgSO_4$**. Close the tube and shake mechanically for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Activate the **EMR dSPE** with 5 ml and transfer supernatant solution into the tube and shake mechanically for 30 sec.

Centrifuge for 5 min. at 4500 rpm

Transfer supernatant solution into **Final-EMR** tube and shake for 30 sec.

Centrifuge for 5 min. at 4500 rpm

Transfer the extract (ca. 3 ml) 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.