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National Food Institute
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Validation Report 27

Determination of pesticide residues in fish feed

by GC-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 GC-MS/MS for 164 pesticides and metabolites. The pesticides included were those where Maximum Residue Limits, MRLs, have been implemented in Directive 2006/77/EC[1] (amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for organochlorine compounds in animal feed). Additionally, pesticides used for production of cereals and other plant origin matrices was included as these are also used in fish feed. The aim of the validation was to achieve LOQs at 0.01 or below.

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 validation study. The feed samples were pellets of 2mm size with 22.8% crude fat content (CPK15) and 8mm size with 33.6% crude fat content (EFICO).
The pellets were cryo-milled (using liquid nitrogen) with sieve at 1.0 mm.

Extraction: One grammes of 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 added water transferred to a tube containing activated EMR-lipid dSPE (Enhanced Matrix Removal (EMR) – Lipid) (Agilent[3]). After shaking and another centrifugation step, the supernatant transferred to a tube containing Final Polish (second clean-up). 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 are analysed by GC-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 5 µl. For

each pesticide minimum two sets of precursor and product ions were determined. One for quantification and at least one for qualification. The MRM transitions for the pesticides and degradation products are given in **Appendix 1.**

3. Validation design

The method was sought validated for almost 170 pesticides or metabolites in two different, CPK-15 (medium fat content) and EFICO (high fat content), see **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 fish feed matrix.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels within the range of 0.333, 1, 3.33 , 10, 33.3 and 100 ng/ml. The calibration curves were in generally 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.

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels (0.005 mg/kg, 0.01 mg/kg and 0.05 mg/kg). 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 (RSD_r) in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[3].

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[4].

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, RSD_r, Combined Uncertainty (U_c) and limit of quantification (LOQ) are presented in **Appendix 2 and 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 should be ≤20%[5].
2. The average relative recovery must be between 70 and 120%[5]. However, lower recoveries are accepted if the repeatability is low.

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 conclusion

Validation results are presented in **Appendix 2 and 3**. The validation results for the two fish feeds were comparable although the fat content was different, 22.8 for CPK and 33.6% for EFICO.

CPK was validated for 146 pesticides and metabolites, 118 obtained an LOQ of 0.005 mg/kg, 21 obtained an LOQ of 0.01 mg/kg and 7 compounds obtained an LOQ of 0.05 mg/kg.

EFICO was validated for 141 compounds, 112 obtained an LOQ of 0.005 mg/kg, 14 obtained an LOQ of 0.01 mg/kg and 15 compounds obtained an LOQ of 0.05 mg/kg.

Validation of the following 14 compounds was not achieved either CPK neither EFICO: acephate captan, chlorothalonil, dichlofluanid, dimethoate, ethoxyquin, fenpropidin, fosthiazate, methamidophos, monocrotophos, omethoate, phosphamidon, tolylfluanid, and vamidothion.

Additional 9 compound was not validated on EFICO: azinphos-methyl, clofentezine, dichlorvos, fenamiphos sulfone, methacrifos, phosmet, procymidone, propyzamide and trichlorfon and 4 compound was not validated on CPK; 2-phenylphenol, metalaxyl, prochloraz, spiroxamine and thiamethoxam.
Ethoxyquin was present in high amount in both feeds and could for that reason not be validated. Due to background contamination in the laboratory 2-phenylphenol was not validated on CPK. Many of the other not validated compounds are either LC amendable or should be analysed by Single Residues methods. Finally, the methods was not sensitive enough to validate the toxaphen compounds.

All pesticides and metabolites where Maximum Residue Limits, MRLs, have been implemented in Directive 2006/77/EC, obtained LOQs at 0.005 mg/kg except for endrin and heptachlor epoxide in CPK, that obtained an LOQ of 0.01 mg/kg and (due to too high repeatability) and the toxaphens that obtain for LOQs at 0.05 mg/kg. However, all obtained LOQs are below the MRLs.

9. References

1 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.

2 Recommended Protocols for Enhanced Matrix Removal – Lipid.
https://www.agilent.com/cs/library/technicaloverviews/Public/5991-6057EN.pdf

3 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.

4 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.

5 Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, Document SANTE/11813/2017, 21–22 November 2017 rev.0, European Commission, Brussels, 2017.

Appendix 1. MRM transitions for GC-MS/MS for compounds in the standard mixture "FishFeed".

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
1-Naphtylacetamide	15	141	115	15
1-Naphtylacetamide	15	185	141	15
Acetochlor	14.16	131.8	117	14
Acetochlor	14.16	146	117.7	8
Acetochlor	14.16	146	131.1	12
Acibenzolar-S-methyl	14.67	135	62.9	18
Acibenzolar-S-methyl	14.67	135	107	8
Acibenzolar-S-methyl	14.67	182	181	8
Aclonifen	20.12	212	182	10
Aclonifen	20.12	264	194.1	14
Anthraquinone	15.76	180	152	12
Anthraquinone	15.76	208	151.7	22
Anthraquinone	15.76	208	180	10
Azoxystrobin-d4 (IS)	30.86	348	332	30
Beflubutamid	16.97	221	193	10
Beflubutamid	16.97	355	176	10
Benalaxyl	20.79	148.1	77	30
Benalaxyl	20.79	234.3	174.2	10
Benalaxyl	20.79	266.4	148.2	10
Bendiocarb	11.51	126	51.9	16
Bendiocarb	11.51	151	43	20
Bendiocarb	11.51	166.1	151.1	10
Biphenyl	9.1	154.1	115	26
Biphenyl	9.1	154.1	152.1	25
Biphenyl	9.1	154.1	153.1	15
Carbetamide	15.8	91	64	10
Carbetamide	15.8	119	63.8	22
Carbetamide	15.8	119	91	12
Carbophenothion	20.89	157	45	12
Carbophenothion	20.89	199	142.9	10
Carbophenothion	20.89	342	157	10
Carfentrazon-ethyl	20.74	290	99.9	36
Carfentrazon-ethyl	20.74	311.9	150.7	18
Carfentrazon-ethyl	20.74	340.1	312.1	10
Chloridazon	21.2	220	165.9	22
Chloridazon	21.2	220	193	16
Chloridazon	21.2	220.9	77	20
Chloropropylate	19.71	251	139	15
Chloropropylate	19.71	253	141	15
Chlorpyrifos-d10 (IS)	15.55	200	109	36
Chlorpyrifos-d10 (IS)	15.55	200	172	12
Chlorsulfuron	12.09	175	111	5

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Chlorsulfuron	12.09	191	127	5
Chlorthal-dimethyl	15.69	222.7	166.9	20
Chlorthal-dimethyl	15.69	300.7	222.9	22
Chlorthal-dimethyl	15.69	300.7	272.9	12
Chlorthal-dimethyl	15.69	331.9	300.9	10
Cinidon-ethyl	32.42	330	302	15
Cinidon-ethyl	32.42	358	330	10
Clodinafop-propargyl	21.24	238	130	18
Clodinafop-propargyl	21.24	266	91.1	14
Clodinafop-propargyl	21.24	349.1	266.1	8
Cyflufenamid	19.15	222.8	203	10
Cyflufenamid	19.15	237	188	24
Cyflufenamid	19.15	237	208.1	12
Dialifos	25.44	208	89.1	26
Dialifos	25.44	208	180.9	10
Dialifos	25.44	209.7	182.9	10
Dichlobenil	8.73	170.9	99.9	24
Dichlobenil	8.73	170.9	136	14
Dichlobenil	8.73	172.8	99.8	24
Dichlofenthion	14.06	222.9	205	12
Dichlofenthion	14.06	250.9	223	8
Dichlofenthion	14.06	279	223	12
Dichlorvos-d6 (IS)	8	191	99	15
Dichlorvos-d6 (IS)	8	191	115	20
Dicrotophos	11.43	127	94.9	16
Dicrotophos	11.43	127	109	10
Dicrotophos	11.43	192.7	127	8
Diethofencarb	15.48	168	96.1	12
Diethofencarb	15.48	196	96	16
Diethofencarb	15.48	225.1	96	24
Diflufenican	21.82	266	238.1	12
Diflufenican	21.82	266	246.1	10
Diflufenican	21.82	288	245	10
Diflufenican	21.82	330	288	5
Diflufenican	21.82	394	266.1	12
Dimethachlor	14.05	134	77	24
Dimethachlor	14.05	134	105.1	12
Dimethachlor	14.05	197	148.1	10
Dimethenamid	14.08	154.1	111	10
Dimethenamid	14.08	154.1	137	8
Dimethenamid	14.08	230	154.1	10
Dimoxystrobin	22.66	116.1	62.9	24
Dimoxystrobin	22.66	116.1	89	14
Dimoxystrobin	22.66	205.1	116.1	10
Diniconazole	19.83	232	149	14

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Diniconazole	19.83	268	136	34
Diniconazole	19.83	268	232	8
Dioxathion	12.72	96.9	65	16
Dioxathion	12.72	125	97	6
Dioxathion	12.72	153	96.9	10
Dodemorph1	16.26	154.1	56.5	16
Dodemorph1	16.26	154.1	81.9	18
Dodemorph1	16.26	154.1	96.6	10
Dodemorph2	16.85	154.1	56.5	16
Dodemorph2	16.85	154.1	82	18
Dodemorph2	16.85	154.1	97.2	10
Ethofumesate	15.07	161.1	77.1	28
Ethofumesate	15.07	161.1	105.1	10
Ethofumesate	15.07	207.1	137.1	10
Etofenprox-d5 (IS)	28.43	168	108	10
Etofenprox-d5 (IS)	28.43	168	136	20
Etoxazole	23.19	140.9	62.9	26
Etoxazole	23.19	140.9	113	14
Etoxazole	23.19	204	176.1	10
Etridiazole	9.59	182.8	139.9	14
Etridiazole	9.59	211	139.9	20
Etridiazole	9.59	211	182.9	10
Famoxadone	31.21	224.1	167.2	18
Famoxadone	31.21	224.1	196.1	8
Famoxadone	31.21	329.8	224.1	8
Fenchlorfos	14.69	124.9	47	12
Fenchlorfos	14.69	124.9	79	6
Fenoxaprop-P	25.75	288	119	5
Fenoxaprop-P	25.75	361	288	10
Flonicamid	10.68	146	126	8
Flonicamid	10.68	174	69	36
Flonicamid	10.68	174	146	10
Flucythrinate I	28.22	157	107.1	12
Flucythrinate I	28.22	199	157.1	8
Flucythrinate I	28.22	199.1	107.1	22
Flucythrinate II	28.59	157	107	12
Flucythrinate II	28.59	199	107	22
Flucythrinate II	28.59	199	157.1	8
Flufenacet	15.67	122.7	122	8
Flufenacet	15.67	151.1	95	24
Flufenacet	15.67	151.1	136.1	10
Flumioxazin	29.38	354.1	176.1	16
Flumioxazin	29.38	354.1	312	8
Flumioxazin	29.38	354.1	326.1	8
Fluometuron	11.11	232	72.1	12

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Fluometuron	11.11	232	174.1	14
Fluometuron	11.11	232	186.1	12
Fluopicolide	21.3	209	182	17
Fluopicolide	21.3	347	172	25
Fluopyram	16.81	173	145	15
Fluopyram	16.81	396	223	10
Flurochloridone	15.93	311	174	15
Flurochloridone	15.93	311	187	15
Flurprimidol	14.11	107	52	18
Flurprimidol	14.11	107	79	8
Flurprimidol	14.11	269	107.1	14
Flurtamone	23.81	157	137	12
Flurtamone	23.81	198.9	157	16
Flurtamone	23.81	333.2	120.1	12
Flutolanil	18.2	173	95	28
Flutolanil	18.2	173	145	14
Flutolanil	18.2	281	173	10
Fonofos	12.98	137	109	6
Fonofos	12.98	246	109	14
Fonofos	12.98	246	137	6
Fuberidazol	14.52	184.1	102.7	26
Fuberidazol	14.52	184.1	128.9	20
Fuberidazol	14.52	184.1	156.1	12
Furathiocarb	23.75	135.1	107	12
Furathiocarb	23.75	163.1	107.1	14
Furathiocarb	23.75	163.1	135.1	6
Heptachlor	14.72	99.8	39	26
Heptachlor	14.72	99.8	65	12
Heptachlor	14.72	271.8	236.9	12
Isocarbophos	15.84	121.1	65	14
Isocarbophos	15.84	136	69	30
Isocarbophos	15.84	136	108	12
Isofenphos	16.74	185	121	10
Isofenphos	16.74	213	121	14
Isofenphos	16.74	213	185	6
Isoprocarb	10.23	121.1	77	18
Isoprocarb	10.23	136.1	121.1	8
Isoxathion	19.22	105.1	51	28
Isoxathion	19.22	105.1	77	18
Isoxathion	19.22	177	130	8
Metaldehyde	6.15	89	45	10
Metaldehyde	6.15	117	45	10
Metazachlor	16.6	133.1	117.3	22
Metazachlor	16.6	133.1	132.1	12
Metazachlor	16.6	209	132.1	16

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Metobromuron	13.84	169.7	91	14
Metobromuron	13.84	171.6	91.1	14
Metobromuron	13.84	258	61.1	8
Metolachlor	15.45	162.1	132.9	14
Metolachlor	15.45	238.1	132.8	26
Metolachlor	15.45	238.1	162.2	10
Metrafenone	25.36	393	346.9	20
Metrafenone	25.36	393	362.7	16
Metrafenone	25.36	393	377.6	10
Molinate	10.4	126.1	55.1	12
Molinate	10.4	126.1	83.1	6
Molinate	10.4	187.1	126.1	6
Napropamide	18.17	100.1	72.1	6
Napropamide	18.17	128.2	72.1	6
Napropamide	18.17	271.1	72.1	14
Oxadiargyl	19.74	213	150	5
Oxadiargyl	19.74	340	150	15
Oxyfluorfen	18.77	252	146	30
Oxyfluorfen	18.77	252	169.8	28
Oxyfluorfen	18.77	252	224	10
Penflufen	20.34	141	84	10
Penflufen	20.34	274	141	10
Penthiopyrad	19.85	177	101	15
Penthiopyrad	19.85	302	177	15
Pethoxamide	17.04	131	91	10
Pethoxamide	17.04	260	147	15
Phorate	11.83	75	47	8
Phorate	11.83	121	65	8
Phorate	11.83	260	75	8
Picolinafen	23.01	145	95	12
Picolinafen	23.01	238	145.1	22
Picolinafen	23.01	376.1	238.7	12
Picoxystrobin	17.88	145.1	102.1	25
Picoxystrobin	17.88	145.1	115.1	15
Picoxystrobin	17.88	145.1	130	15
Piperonyl Butoxide	22.01	176.1	103.1	22
Piperonyl Butoxide	22.01	176.1	117	18
Piperonyl Butoxide	22.01	176.1	131.1	12
Pirimiphos-ethyl	16.1	304	168.1	12
Pirimiphos-ethyl	16.1	318.1	166.1	12
Pirimiphos-ethyl	16.1	318.1	182.1	10
Pirimiphos-ethyl	16.1	333	168	15
Propachlor	11	120	50.9	35
Propachlor	11	120	77	15
Propachlor	11	176.1	57.1	10

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Propanil	14.07	160.9	99	24
Propanil	14.07	160.9	125.7	16
Propanil	14.07	217	161	8
Propaquizafop	33.57	299	91	10
Propaquizafop	33.57	443	299	15
Propham	9.58	92.9	65.9	12
Propham	9.58	137	93	8
Propham	9.58	179.1	93.1	14
Proquinazid	21.88	288	245	15
Proquinazid	21.88	330	288	5
Pyraclofos	25.6	139.2	96.9	6
Pyraclofos	25.6	194	138	18
Pyraclofos	25.6	360	194.1	12
Pyraflufen-ethyl	21.24	349	238	16
Pyraflufen-ethyl	21.24	349	266.1	10
Pyraflufen-ethyl	21.24	412	349	12
Pyridalyl	28.58	163.8	146.1	12
Pyridalyl	28.58	204	148.1	18
Pyridalyl	28.58	204	176.1	10
Quinalphos	17	146	118.1	8
Quinalphos	17	157.1	102	22
Quinalphos	17	157.1	129	14
Silafluofen	28.76	179.1	151.1	10
Silafluofen	28.76	258.1	181.1	16
Silafluofen	28.76	258.1	243.1	14
Spiromesifen	22.34	254.1	209.1	10
Spiromesifen	22.34	272.1	254.2	8
Sulfotep	11.54	202	145.9	10
Sulfotep	11.54	265.9	145.9	15
Sulfotep	11.54	322	202	10
Tepraloxydim	20.94	164	53	26
Tepraloxydim	20.94	164	80.9	18
Tepraloxydim	20.94	164	108.2	8
Terbuthylazine	12.81	214.1	104	16
Terbuthylazine	12.81	214.1	132	10
Terbuthylazine	12.81	229.1	200.1	10
Tetrachlorvinphos	17.65	109	79	6
Tetrachlorvinphos	17.65	328.9	109	18
Tetrachlorvinphos	17.65	330.8	109	18
Tetramethrin1	22.71	164	77.1	22
Tetramethrin1	22.71	164	107.1	12
Tetramethrin1	22.71	164	135.1	8
Tetramethrin2	23	164	77.1	22
Tetramethrin2	23	164	107.1	12
Tetramethrin2	23	164	135.1	8

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
Tetrasul	20.51	251.9	173	34
Tetrasul	20.51	251.9	181.9	32
Tetrasul	20.51	251.9	216.9	22
Thiobencarb	15.56	72	44	6
Thiobencarb	15.56	100.1	44	10
Thiobencarb	15.56	100.1	72	6
TPP (IS)	21.92	325.07	169.04	25
TPP (IS)	21.92	326	169	35
TPP (IS)	21.92	326.07	215.05	25
TPP (IS)	21.92	326.07	233.05	10
TPP (IS)	21.92	326.07	325.07	10
Tralomethrin	30.48	171.9	93.1	10
Tralomethrin	30.48	173.8	93.1	8
Tralomethrin	30.48	181	152	22
Trichloronate	16.05	268.9	222.9	20
Trichloronate	16.05	270.8	224.9	22
Trichloronate	16.05	297	269	12
Triflumizole	17.18	179	144	14
Triflumizole	17.18	206	179	14
Triflumizole	17.18	206	186	8
Triflusulfuron-methyl	10.1	237	208	8
Triflusulfuron-methyl	10.1	237	222	15
Tritosulfuron	10.09	145	95	5
Tritosulfuron	10.09	161	141	5

Appendix 2. Recoveries, Repeatability RSD_r, Combinded Uncertainty and Limit of Quantification (LOQ) for pesticides validated on CPK-15 (medium fat content) using modified QuEChERS with additional clean-up.

Numbers in Italic is outside 70-120% recovery or above 20% Combined Uncertainty

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
4,4´-Methoxychlor	80	12	12		78	6	7		84	9	9		0.005
Acrinathrin	81	10	11		78	6	7		90	4	4		0.005
Aldrin	77	17	19		72	12	13		*59*	6	9		0.005
Azinphos-ethyl	108	11	12		105	5	6		105	6	7		0.005
Azinphos-methyl	*54*	16	17		*53*	17	18		*61*	16	17		0.005
Azoxystrobin	91	10	11		97	6	7		94	8	9		0.005
Bifenthrin	*61*	5	6		59	13	15		70	6	7		0.005
Bitertanol	*125*	10	11		120	7	7		118	6	6		0.005
Bixafen	103	6	6		103	4	4		100	7	8		0.005
Boscalid	109	8	9		108	4	4		103	5	6		0.005
Bromopropylate	87	9	9		87	6	7		89	4	4		0.005
Bromuconazole	78	10	11		88	8	9		91	5	5		0.005
Bupirimate	89	13	14		102	5	5		106	3	3		0.005
Buprofezin	115	12	13		99	9	10		96	2	2		0.005
Cadusafos	75	6	6		87	6	7		92	3	3		0.005
Carbosulfan	*54*	16	17		*53*	17	18		*61*	16	17		0.005
Carboxin	82	9	10		94	8	8		98	4	5		0.005
Chlordane alpha-cis	88	9	10		81	11	12		79	8	8		0.005
Chlordane gamma-trans	94	6	7		80	7	7		77	4	4		0.005
Chlorfenapyr	*77*	*53*	*58*		88	6	7		98	10	11		0.01
Chlorfenvinphos	90	7	7		104	6	7		102	5	5		0.005
Chlorobenzilate	84	5	5		93	7	7		97	3	3		0.005
Chlorpropham	70	6	7		87	4	4		94	3	3		0.005

	Spike level 0.005 mg/kg			Spike level 0.01 mg/kg			Spike level 0.05 mg/kg			
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**LOQ**
Chlorpyrifos	78	6	7	88	3	4	94	3	3	0.005
Chlorpyrifos-methyl	82	13	14	87	8	8	85	7	7	0.005
Clofentezine	119	3	3	97	13	14	93	14	15	0.005
Coumaphos	105	13	14	111	9	10	116	8	8	0.005
Cyflutrin	97	12	13	95	4	5	99	5	5	0.005
Cyhalothrin, lambda	83	10	11	86	6	6	95	3	3	0.005
Cymiazole	*63*	*21*	*23*	81	9	10	91	4	5	0.01
Cypermethrin	121	7	7	99	14	15	100	4	4	0.005
Cyproconazole	72	18	20	81	10	11	88	3	4	0.005
Cyprodinil	152	*33*	*36*	118	9	10	91	3	3	0.01
DDD pp	102	5	6	82	9	10	79	7	7	0.005
DDE pp	83	10	11	*60*	*24*	*26*	*69*	9	10	0.005
DDT op	78	7	8	*62*	8	8	*64*	6	7	0.005
DDT pp	82	3	3	*63*	10	11	*63*	7	8	0.005
Deltamethrin	*163*	*9*	*10*	101	7	8	77	5	5	0.01
Diazinon	77	12	13	90	10	11	97	5	5	0.005
Dichlorvos	111	10	11	131	11	12	120	9	10	0.005
Dicloran	63	19	20	76	14	15	82	5	5	0.01
Dicofol, o,p'-	82	8	9	89	5	5	93	2	2	0.005
Dieldrin	91	19	20	95	19	*21*	106	6	6	0.005
Difenoconazole	107	17	18	97	19	*21*	92	8	8	0.005
Dimethomorph	97	14	15	85	7	8	84	6	7	0.005
Dimoxystrobin	93	14	15	102	6	7	105	4	5	0.005
Diphenylamine	139	*44*	*48*	125	7	7	108	5	6	0.01
Endosulfan sulfate	86	13	14	88	10	10	88	4	4	0.005
Endosulfan, alpha	92	15	17	79	12	13	75	6	6	0.005
Endosulfan, beta	84	*21*	*22*	85	11	11	83	4	5	0.005
Endrin	116	*35*	*38*	90	15	16	84	7	8	0.01

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**LOQ**
EPN	76	14	16		84	4	4		88	5	5		0.005
Epoxiconazole	93	5	6		95	4	5		98	5	6		0.005
Ethion	84	6	7		85	6	7		94	3	3		0.005
Ethoprophos	78	5	6		91	9	9		99	4	5		0.005
Etofenprox	78	6	7		72	8	9		79	5	6		0.005
Famoxadone	102	11	12		103	9	10		96	6	6		0.005
Fenamiphos	89	20	*21*		101	13	14		102	4	4		0.005
Fenamiphos sulfone	104	12	13		107	7	7		109	9	9		0.005
Fenarimol	89	10	11		91	6	6		93	4	4		0.005
Fenazaquin	*182*	*73*	*79*		103	7	8		88	4	4		0.01
Fenbuconazole	118	6	6		121	5	5		119	7	7		0.005
Fenitrotion	81	6	6		90	8	8		93	3	3		0.005
Fenoxycarb	101	9	10		98	3	4		104	2	2		0.005
Fenpropathrin	141	10	11		107	4	4		95	2	2		0.01
Fenpropimorph	72	8	9		81	6	6		85	2	2		0.005
Fenthion	84	10	11		90	8	8		97	2	2		0.005
Fenvalerate	89	8	8		84	5	6		87	5	5		0.005
Fipronil	88	6	6		88	6	6		98	7	8		0.005
Fludioxonil	85	8	9		99	8	8		105	6	6		0.005
Flufenoxuron	*20*	*71*	*76*		*30*	*73*	*79*		89	12	13		0.05
Fluquinconazole	94	10	10		105	8	9		105	5	5		0.005
Flusilazole	92	14	15		95	10	11		100	4	5		0.005
Flutriafol	84	4	4		88	4	5		90	5	5		0.005
HCH, alpha	100	3	3		94	4	4		91	3	3		0.005
HCH, beta	101	8	9		96	6	7		92	2	2		0.005
Heptachlor	77	7	8		73	5	5		73	4	5		0.005
Heptachlor epoxide	*80*	*27*	*29*		83	17	19		87	5	6		0.01
Heptenophos	95	5	5		109	2	3		111	6	7		0.005

	Spike level 0.005 mg/kg			Spike level 0.01 mg/kg			Spike level 0.05 mg/kg			
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**	**LOQ**
Hexaconazole	*85*	*32*	*35*	90	16	18	90	8	9	0.01
Hexythiozox	74	18	19	76	8	8	80	7	8	0.005
Imazalil	*124*	*23*	*24*	98	9	9	101	8	8	0.01
Indoxacarb	93	4	5	108	8	9	107	7	8	0.005
Iprodione	92	16	17	80	11	11	88	12	13	0.005
Iprovalicarb				110	13	14	106	5	6	0.01
Isofenphos-methyl	85	7	7	97	4	5	101	2	3	0.005
Kresoxim-methyl	89	10	11	99	8	8	106	2	2	0.005
Lindane	73	7	7	85	3	3	89	3	4	0.005
Malathion	91	7	8	102	4	5	107	5	6	0.005
Mepanipyrim	103	9	10	102	6	6	89	3	3	0.005
Metconazole	86	13	14	99	9	9	96	7	8	0.005
Methacrifos	*97*	*26*	*28*	108	5	5	108	5	6	0.01
Methidathion	88	11	12	95	10	10	97	8	9	0.005
Metribuzin	82	12	13	87	8	9	92	2	2	0.005
Myclobutanil	86	7	7	100	3	4	104	2	2	0.005
Oxadixyl	95	6	7	105	6	7	105	6	6	0.005
Oxychlordane	94	10	11	82	17	18	77	5	5	0.005
Paclobutrazol	82	9	10	83	5	6	87	3	4	0.005
Parathion	70	13	15	84	9	9	95	4	5	0.005
Parathion-methyl	79	10	11	84	4	5	91	4	4	0.005
Penconazole	71	9	10	77	7	7	86	2	2	0.005
Pencycuron	85	7	7	87	7	8	90	3	3	0.005
Pendimethalin	87	12	13	78	4	4	81	2	2	0.005
Permethrin				*69*	11	12	86	3	3	0.01
Phenthoate	87	8	9	93	8	8	97	3	3	0.005
Phosalone	88	11	12	97	7	7	108	6	6	0.005
Phosmet	*47*	20	*22*	*51*	18	20	*61*	16	17	0.01

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
Pirimicarb	104	5	5		102	4	4		96	1	1		0.005
Pirimicarb desmetyl	93	13	14		97	3	3		91	3	3		0.005
Pirimiphos methyl	108	15	16		129	11	11		105	2	3		0.005
Pirimiphos-ethyl	80	11	12		96	6	7		100	2	2		0.005
Procymidone	89	10	11		95	7	7		99	3	4		0.005
Profenofos	98	13	14		101	8	8		106	7	8		0.005
Propargite					105	5	6		98	2	2		0.01
Propiconazole	*65*	*41*	*44*		84	11	13		92	4	4		0.01
Propyzamide	82	6	7		91	6	6		94	3	3		0.005
Prothiofos	*68*	18	19		74	12	13		81	5	5		0.005
Pyrazophos	106	2	2		99	8	9		102	6	6		0.005
Pyridaben	108	*35*	*37*		83	7	7		90	3	4		0.01
Pyrimethanil	76	5	5		81	4	4		86	3	3		0.005
Pyriproxyfen	90	7	7		92	5	5		95	2	3		0.005
Quinoxyfen	82	6	6		*69*	9	10		70	6	7		0.005
Resmethrin peak 1									85	6	6		0.05
Resmethrin peak 2					106	11	11		77	5	5		0.01
Spiroxamine2	99	8	8		79	*23*	*24*		87	2	2		0.005
Tebuconazole	116	10	11		107	8	8		96	5	5		0.005
Tebufenpyrad	91	7	7		99	9	10		105	2	2		0.005
Tefluthrin	75	9	9		81	5	6		88	3	3		0.005
Terbuthylazine	72	*33*	*36*		95	14	15		95	15	17		0.01
Tetraconazole	88	11	11		95	6	6		96	2	2		0.005
Tetradifon	88	11	12		77	15	16		87	4	4		0.005
Tetramethrin	358	*35*	*38*		299	49	*53*		82	6	6		0.05
Thiometon	127	*47*	*51*		101	15	16		114	10	11		0.01
Tolclofos-methyl	78	6	7		90	8	9		94	1	1		0.005
Toxaphen parlar 26	*20*	*44*	*48*		*35*	*105*	*114*		*55*	15	16		0.05

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
Toxaphen parlar 50	*35*	*47*	*51*		*33*	*43*	*46*		*53*	17	18		0.05
Toxaphen parlar 62	*65*	*50*	*54*		*57*	*24*	*27*		*55*	9	9		0.05
Triadimefon	98	11	12		100	6	6		95	4	4		0.005
Triadimenol	*118*	*21*	*22*		99	12	13		96	7	7		0.01
Triazophos	80	9	9		94	5	5		102	4	5		0.005
Trichlorfon	107	14	15		127	14	15		118	10	11		0.05
Tricyclazole	93	10	11		107	5	6		111	3	3		0.005
Trifloxystrobin	79	14	15		106	9	10		102	5	5		0.005
Trifluralin	73	13	14		83	8	8		93	4	5		0.005
Triticonazole	114	10	11		108	9	9		104	7	8		0.005
Vinclozolin	75	9	9		80	13	14		92	5	5		0.005
Zoxamide	90	7	8		91	5	6		94	9	10		0.005

Appendix 3. Recoveries, Repeatability RSD_r, Combinded Uncertainty and Limit of Quantification (LOQ) for pesticides validated on EFICO (high fat content) using modified QuEChERS with additional clean-up.

Numbers in Italic is outside 70-120% recovery or above 20% Combined Uncertainty

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
2-Phenylphenol	96	11	11		102	7	7		102	6	6		0.005
4,4´-Methoxychlor	82	17	18		83	7	8		83	10	11		0.005
Acrinathrin	88	7	8		84	14	15		81	7	8		0.005
Aldrin	76	13	15		70	12	13		*67*	16	18		0.005
Azinphos-ethyl	82	13	14		90	9	9		83	5	6		0.005
Azoxystrobin	95	7	8		104	4	5		97	4	4		0.005
Bifenthrin	*54*	*25*	*27*		*52*	17	19		*52*	20	*22*		0.01
Bitertanol	76	19	*21*		*66*	13	15		*62*	8	9		0.005
Bixafen	92	7	8		98	5	5		89	6	7		0.005
Boscalid	108	7	8		104	4	4		84	8	8		0.005
Bromopropylate	91	7	8		84	8	9		76	6	6		0.005
Bromuconazole	83	15	17		87	10	10		78	6	7		0.005
Bupirimate	74	7	8		99	*26*	*28*		93	5	5		0.005
Buprofezin					90	18	19		98	8	9		0.01
Cadusafos	102	6	6		114	6	7		116	3	3		0.005
Carbosulfan	*50*	*21*	*23*		*60*	16	17		*50*	20	*22*		0.01
Carboxin	95	6	6		108	7	7		106	4	4		0.005
Chlordane alpha-cis	*68*	20	*22*		69	14	15		*69*	11	12		0.005
Chlordane gamma-trans	*74*	*23*	*25*		76	14	15		74	11	12		0.01
Chlorfenapyr	*113*	*35*	*38*		117	15	16		107	*21*	*23*		0.01
Chlorfenvinphos	83	13	14		88	4	5		75	6	7		0.005
Chlorobenzilate ?	81	7	8		101	7	7		101	6	7		0.005
Chlorpropham					76	11	11		108	2	2		0.01
Chlorpyrifos	98	8	9		96	10	11		90	8	9		0.005

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
Chlorpyrifos-methyl	93	15	16		108	3	3		104	4	3		0.005
Coumaphos	83	9	10		84	7	8		76	12	13		0.005
Cyflutrin	98	13	14		99	12	13		89	12	13		0.005
Cyhalothrin, lambda	94	2	2		91	11	12		85	13	14		0.005
Cymiazole	96	11	11		105	8	8		106	5	6		0.005
Cypermethrin					*54*	16	17		77	13	14		0.01
Cyproconazole	*67*	13	14		87	8	9		93	5	5		0.005
Cyprodinil	87	10	11		94	9	10		91	3	3		0.005
DDD pp	104	15	16		89	10	11		75	14	15		0.005
DDE pp	113	8	9		78	15	16		*60*	19	20		0.005
DDT op	*61*	11	12		*61*	14	15		*58*	18	20		0.005
DDT pp	*65*	7	8		*56*	14	15		*59*	17	18		0.005
Deltamethrin	88	16	18		93	14	16		74	11	12		0.005
Diazinon	85	9	9		109	3	3		113	4	4		0.005
Dicloran	74	15	17		93	11	12		101	4	5		0.005
Dicofol, o,p'-	81	7	8		101	7	7		101	6	7		0.005
Dieldrin	122	19	20		105	*23*	*24*		84	9	10		0.005
Difenoconazole	70	11	12		79	8	9		*62*	7	7		0.005
Dimethomorph	71	7	7		83	6	6		82	4	4		0.005
Dimoxystrobin	90	15	16		108	4	5		106	5	5		0.005
Diphenylamine	*159*	16	17		*139*	10	11		117	7	8		0.005
Endosulfan sulfate	93	19	*21*		100	10	10		101	10	11		0.005
Endosulfan, alpha	93	14	16		86	8	8		83	12	13		0.005
Endosulfan, beta	*84*	*27*	*29*		101	15	16		100	10	11		0.005
Endrin	87	11	12		100	16	17		84	13	14		0.005
EPN	84	7	8		90	4	5		87	7	8		0.005
Epoxiconazole	98	5	5		100	4	4		89	6	6		0.005
Ethion	95	4	5		102	6	7		98	7	8		0.005

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**LOQ**
Ethoprophos	90	4	4		99	3	4		104	3	4		0.005
Etofenprox	73	5	5		*66*	12	13		*64*	16	17		0.005
Famoxadone	76	10	11		75	13	14		71	11	11		0.005
Fenamiphos	83	9	10		89	9	10		88	6	7		0.005
Fenarimol	91	9	9		99	5	6		88	6	7		0.005
Fenazaquin	*172*	*47*	*51*		*247*	*26*	*28*		102	18	19		0.05
Fenbuconazole	85	7	7		98	4	4		90	6	7		0.005
Fenitrotion	70	15	16		90	9	9		98	4	4		0.005
Fenoxycarb	94	10	11		95	5	5		79	8	9		0.005
Fenpropathrin	84	5	5		88	9	9		87	10	11		0.005
Fenpropimorph	93	6	7		124	6	6		129	2	2		0.005
Fenthion	94	7	8		109	7	8		105	4	4		0.005
Fenvalerate	79	4	5		73	10	11		*68*	15	16		0.005
Fipronil	97	12	13		101	10	11		102	3	3		0.005
Fludioxonil	106	6	7		123	5	6		110	6	6		0.005
Flufenoxuron									88	10	11		0.05
Fluquinconazole	76	6	6		90	7	7		89	3	4		0.005
Flusilazole	79	19	20		105	10	11		105	3	4		0.005
Flutriafol	99	7	7		99	3	3		91	4	5		0.005
HCH, alpha	93	14	15		100	13	14		108	7	8		0.005
HCH, beta	99	6	7		96	8	8		95	5	5		0.005
Heptachlor	86	14	15		86	13	14		91	11	12		0.005
Heptachlor epoxide	77	18	20		78	16	17		87	10	11		0.005
Heptenophos	96	11	12		88	6	7		*68*	13	15		0.005
Hexaconazole	*80*	*42*	*46*		*96*	*26*	*28*		97	10	10		0.05
Hexythiozox	80	7	8		83	9	10		84	9	10		0.005
Imazalil					*133*	*20*	*21*		84	6	7		0.05
Indoxacarb	103	15	16		102	7	7		86	9	10		0.005

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**LOQ**
Iprodione	78	*21*	*23*		87	11	11		79	7	7		0.005
Iprovalicarb	74	17	19		100	10	11		111	5	5		0.005
Isofenphos-methyl	102	5	6		109	4	5		106	2	2		0.005
Kresoxim-methyl	73	13	14		102	12	13		110	3	4		0.005
Lindane	105	12	12		107	6	7		100	3	3		0.005
Malathion	99	7	7		111	5	5		106	3	3		0.005
Mepanipyrim	105	13	14		96	13	14		84	8	9		0.005
Metalaxyl					*139*	*30*	*32*		131	4	5		0.05
Metconazole	92	12	13		103	5	5		84	6	7		0.005
Methidathion	77	5	5		86	5	5		81	5	5		0.005
Metribuzin	100	11	12		111	3	4		104	5	5		0.005
Myclobutanil	78	11	12		102	6	7		102	4	4		0.005
Oxadixyl	83	5	6		111	8	9		110	6	7		0.005
Oxychlordane	93	12	13		78	9	10		77	14	15		0.005
Paclobutrazol	103	5	5		103	5	5		92	5	6		0.005
Parathion	90	4	5		94	9	9		95	4	4		0.005
Parathion-methyl	108	9	10		106	7	7		100	5	5		0.005
Penconazole	101	6	6		110	7	8		102	3	4		0.005
Pencycuron	86	11	11		103	6	7		107	5	5		0.005
Pendimethalin	86	9	10		88	9	10		81	5	6		0.005
Permethrin	136	20	*21*		110	11	12		80	17	18		0.005
Phenthoate	99	8	9		106	5	6		102	3	3		0.005
Phosalone	62	14	16		78	11	12		77	8	9		0.01
Pirimicarb	112	8	9		116	2	2		111	4	4		0.005
Pirimicarb desmetyl					*40*	*21*	*23*		87	4	5		0.05
Pirimiphos methyl	95	16	18	6	122	7	7	6	85	12	13	6	0.005
Pirimiphos-ethyl	91	5	6		99	7	7		102	3	3		0.005
Prochloraz	*87*	*36*	*39*		*154*	12	13		86	10	11		0.05

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD$_r$, %**	**Comb. Uncer-tainty, %**		**LOQ**
Profenofos	*55*	*22*	*24*		*64*	11	12		*55*	10	11		0.01
Propargite	86	*22*	*23*		95	12	12		94	8	9		0.01
Propiconazole	87	10	11		99	6	7		92	5	5		0.005
Prothiofos	*68*	18	19		77	11	12		76	11	12		0.005
Pyrazophos					*50*	11	12		85	7	8		0.05
Pyridaben	116	*24*	*26*		126	4	5		79	12	13		0.01
Pyrimethanil	86	11	12		84	8	9		89	2	2		0.005
Pyriproxyfen	89	10	11		97	6	6		89	8	8		0.005
Quinoxyfen	79	8	9		82	6	7		78	10	10		0.005
Resmethrin peak 1									*68*	10	11		0.05
Resmethrin peak 2	*40*	20	*22*		*60*	20	*21*		73	13	14		0.01
Spiroxamine2					89	12	13		121	1	2		0.01
Tebuconazole	108	9	10		110	5	6		91	6	7		0.005
Tebufenpyrad	98	4	5		108	5	5		103	3	4		0.005
Tefluthrin	92	10	11		93	8	9		91	9	9		0.005
Terbuthylazine	*68*	*22*	*24*		77	12	13		70	7	7		0.01
Tetraconazole	84	8	8		104	9	10		106	2	3		0.005
Tetradifon	84	10	11		87	5	6		81	12	13		0.005
Tetramethrin	133	10	11		*46*	*9*	10		95	12	13		0.05
Thiamethoxam	129	30	32		70	18	19		*52*	20	*21*		0.01
Thiometon	85	11	12		91	11	12		85	7	8		0.005
Tolclofos-methyl	100	5	6		106	6	6		102	3	4		0.005
Toxaphen parlar 26	*54*	*47*	*53*		*46*	*42*	*45*		*56*	14	16		0.05
Toxaphen parlar 50	*54*	*30*	*33*		*60*	14	15		*39*	10	11		0.05
Toxaphen parlar 62	*45*	*26*	*29*		*39*	*55*	*60*		*35*	19	20		0.05
Triadimefon	96	5	6		105	5	6		102	2	3		0.005
Triadimenol	137	*32*	*34*		154	*28*	*30*		100	7	8		0.05
Triazophos	92	5	5		101	6	7		92	5	5		0.005

	Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.05 mg/kg				
Compound	**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**Recovery, %**	**RSD_r, %**	**Comb. Uncer-tainty, %**		**LOQ**
Tricyclazole	79	10	11		113	7	8		116	3	3		0.005
Trifloxystrobin	105	6	6		111	5	6		107	5	5		0.005
Trifluralin	71	15	16		95	9	10		114	7	7		0.005
Triticonazole					*54*	*23*	*25*		80	11	12		0.05
Vinclozolin	100	10	11		108	12	13		105	6	7		0.005
Zoxamide	107	9	10		111	6	7		90	6	6		0.005

Appendix 4: 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 water 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.