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National Food Institute
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Validation Report 29

Determination of pesticide residues in wheat, rye, oat and rice 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 GC-MS/MS and LC-MS/MS. The method was tried validated for 37 pesticides and metabolites by both LC-MSMS and GC-MSMS in wheat, rye, oat and rice. 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 are milled with a sieve at 1 mm.

The extraction procedure is outlines in Appendix 3 and described briefly in the following.

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

Clean-up: After centrifugation the supernatant is transferred to a clean tube and put in -80 degree freezer for minimum 15 minutes. The extracts are then allowed to thaw until almost liquid state and then centrifuged. At this point an aliquot is withdrawn and filtered, diluted 1:1 with acetonitrile and analysed by LC-MS/MS. The rest of the supernatant is transferred to a tube containing 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 matrix matched calibration standards.

Quantification and qualification: The final extracts are analysed by GC-MS/MS. Crude extract withdrawn before PSA clean-up was analysed by LC-MS/MS.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 1 µl. For each pesticide minimum 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 are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The validation includes pesticides determined in positive and negative mode. All pesticides were detected in the MRM mode. For each pesticide or metabolite a precursor ion and 2 product ions were determined. One product ion for quantification and one for qualification. An exception was however formetanate for which only one product ion were detectable at the for this study relevant spike levels (see further comments in on page 6). The MRM transitions for the pesticides and degradation products sought validated are given in Appendix 1b.

3. Validation design

The method was sought validated for 37 pesticides or metabolites in oat, rice, rye and wheat, see **Appendix 1**. The validation was performed on 5-6 replicates on oat, rice, rye and wheat at each of the three spiking levels; 0.002, 0.005, 0.01 and 0.05 mg/kg. A blank sample of each cereal commodity is included.

4. Calibration curves and linearity

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels within the range of 0.3 to 33.3 ng/ml. The quantification was performed from the mean of two bracketing calibration curves. The calibration curves were fitted to a linear curve. The majority of the correlation coefficients (R) were higher or equal to 0.99 but none were lower than 0.97. Thus, good linearity was observed within the relevant concentration range.

5. Specificity

The ion ratios for sample extracts were within ±30% (relative) of average of relevant calibration standards from same sequence. The ion ratios may vary slightly depending on concentration level and in some cases the average of calibration standard are based on the lower calibration levels for the low spike samples.

6. Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels (0.002, 0.005, 0.01 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[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.002, 0.005, 0.01 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) were meet.

7. 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 expanded uncertainty is calculated to demonstrate that it is less than 50%. The expanded uncertainty is given by:

$$U = \sqrt{RSD^2 + Bias^2 + (RSD^2/n)} * 2$$

Where RSD is the intra-laboratory uncertainty (RSD_R),
Bias is 100 minus the recovery,
RSD^2/n is the uncertainty of the bias,
n is the number of recoveries included in the bias and
2 is the coverage factor corresponding to 95% confidence level.

If the expanded uncertainty is higher than 50%, the analytical results must be corrected for recovery and the combined uncertainty is then given by:

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

Where RSD in this validation is the repeatability uncertainty (RSD_r),
RSD^2/n is the uncertainty of the bias,
n is the number of recoveries included in the bias and
2 is the coverage factor corresponding to 95% confidence level.

The bias/recovery used for correction will be the bias/recoveries determined for the individual analytes during the initial validation and/or ongoing method validation. However, if it is evaluated that the type of sample being analysed is significantly different from the matrices employed for the method validation it is possible to correct for bias/recoveries based on recovery from spiked

samples included in the analytical batch in question. However, minimum of 5 recovery samples must be included then.

The obtained results including recovery, RSD_r, RSD_R, expanded uncertainty (U, Uc and limit of quantification (LOQ) are presented in appendix 2.

8. Results and conclusion

The validation results obtained for the 37 pesticides or metabolites using LC-MSMS and GC-MSMS are presented in appendix 2. The lowest LOQ achieved were 0.002 mg/kg for 30 compounds, 0.005 mg/kg for four compounds, 0.01 for one compound and 0.05 mg/kg for the last two compounds. Generally the combined uncertainties were lower than 50%, indicating that recovery for correction is not needed. However it has been decided at our laboratory that all results shall be corrected for recovery when possible, regardless of the expanded uncertainty and the combined uncertainty will therefore apply.

The high LOQs of 0.05 mg/kg were achieved for formetanate and hymexazol. These LOQs are too high and further method validation is needed in order to obtain satisfactory LOQs for these compounds. Hymexazol may need to be analysed by SRM method for polar compounds, since it eluates relatively early from the LC column and therefore also co-elutes with matrix interferences that may suppress the response. Formetanate were in this study sought validated on LC-MSMS though it may be more appropriate to analyse it by GC-MSMS, which will be tested.

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 pesticide residues and analysis in food and feed, Document SANTE/11813/2017, 21–22 November 2017 rev.0, European Commission, Brussels, 2017.

Appendix 1a. MRM transitions for GC-MS/MS for compounds in mixture I.

Name	RT	Parent Mass	Product Mass	Collision Energy
Chlordane-cis	17.5	271.7	236.9	12
Chlordane-cis	17.5	374.7	265.8	22
Chlordane-cis	17.5	376.6	268	20
Chlordane-cis	17.5	372.8	265.8	20
Chlordane-cis	17.5	236.8	142.9	24
Chlordane-trans	17.14	271.7	236.8	12
Chlordane-trans	17.14	374.7	265.9	20
Chlordane-trans	17.14	372.7	263.7	20
Coumaphos	25.91	209.9	182	10
Coumaphos	25.91	361.9	109	15
Coumaphos	25.91	209.9	119	22
DDD-op	18.4	234.97	164.98	20
DDD-op	18.4	236.97	164.98	20
DDD-op	18.4	236.8	165	20
DDD-op	18.4	235	199	14
DDD-pp	19.54	236.8	165	20
DDD-pp	19.54	235	199	14
DDE-op	17.14	246	176.1	26
DDE-op	17.14	317.8	246	20
DDE-op	17.14	246.5	210.2	10
DDE-pp	18.19	246	176.1	26
DDE-pp	18.19	317.8	248	18
DDE-pp	18.19	317.8	246	20
DDT-op	19.64	235	165	22
DDT-op	19.64	236.8	165.1	20
DDT-op	19.64	235	199.5	14
DDT-pp	20.8	235	165.1	22
DDT-pp	20.8	236.8	165	20
DDT-pp	20.8	235	199.5	14
Dicofol-op	15.59	139	111	12
Dicofol-op	15.59	111	74.9	14
Dicofol-op	15.59	250.9	139	12
Disulfoton-sulfone	17.28	213.01	153.01	5
Disulfoton-sulfone	17.28	213.01	125.01	10
Disulfoton-sulfone	17.28	213	96.9	8
Disulfoton-sulfoxide	8.33	125	97	6
Disulfoton-sulfoxide	8.33	153.1	97	10
Disulfoton-sulfoxide	8.33	168.01	140.01	10
Disulfoton-sulfoxide	8.33	153.01	125.01	10
Disulfoton-sulfoxide	8.33	213	96.9	18
Disulfoton-sulfoxide	8.33	213.02	153.01	10
Fensulfothion	19.22	140	125	10
Fensulfothion	19.22	307.9	293	8
Fensulfothion	19.22	291.8	156	15
Fensulfothion	19.22	307.9	153.1	12

Name	RT	Parent Mass	Product Mass	Collision Energy
Fensulfothion	19.22	293.03	125.01	0
Heptachlorepoxide-cis	16.49	183.1	119	20
Heptachlorepoxide-cis	16.49	262.9	192.9	30
Heptachlorepoxide-cis	16.49	352.8	252.9	15
Heptachlorepoxide-trans	16.34	353	263	15
Heptachlorepoxide-trans	16.34	351	261	15
Heptachlorepoxide-trans	16.34	352.8	252.9	15
Hexachlorobenzene	11.81	283.8	248.8	18
Hexachlorobenzene	11.81	283.8	213.8	28
Hexachlorobenzene	11.81	248.8	213.9	14
Hexachlorobenzene	11.81	285.81	250.83	20
Nitrofen	18.9	202	139	24
Nitrofen	18.9	283	202	10
Nitrofen	18.9	283	253	10
Oxychlordane	16.36	185	121	10
Oxychlordane	16.36	115	50.9	22
Oxychlordane	16.36	184.9	84.9	26
Oxychlordane	16.36	386.79	262.86	15
Oxychlordane	16.36	386.79	322.83	15
Pentachloroanisole	11.9	279.86	236.88	20
Pentachloroanisole	11.9	277.86	234.88	20
Pentachloroanisole	11.9	265.87	236.88	10
Pentachloroanisole	11.9	279.86	265.87	15
Phorate-sulfoxide	15	125	97	6
Phorate-sulfoxide	15	153	97	10
Phorate-sulfoxide	15	96.9	65	16
Resmethrin	21.47	171	127.9	14
Resmethrin	21.47	143	128.1	10
Resmethrin	21.47	123.1	81.1	8
Terbufos	12.44	230.9	174.9	12
Terbufos	12.44	230.9	203	8
Terbufos	12.44	230.9	128.9	22

Appendix 1b. MRM transitions for LC-MS/MS for compounds in mixture I.

LC-MS/MS	Mode	Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
4-(Trifluoromethyl)nicotinoyl_Glycine	neg	2.11	247	145.8	24	247	163	24
Coumaphos	pos	6.30	363	227	35	363	306.9	25
Cyantraniliprole	pos	4.24	475	286	12	475	177	41
Cymiazole	pos	2.39	219.2	144	43	219.2	171	37
Disulfoton-sulfone	pos	4.50	307	97	26	307	125.5	17
Disulfoton-sulfoxide	pos	4.41	291	213	8	291	185	12
Fensulfothion	pos	4.68	309	281.3	10	309	157	27
Fipronil-desulfinyl	neg	5.99	387	351	10	387	353	15
Fipronil-sulfide	neg	6.23	419	383	10	419	262	30
Fipronil-sulfone	neg	6.38	451	415	15	451	282	25
Fluroxypyr	neg	3.66	252.9	194.8	20	252.9	232.8	10
Formetanate	pos	1.68	222	165.5	27			
Hymexazol	pos	1.86	100	54	12	100	43	27
Phorate-sulfone	pos	4.52	293	114.8	25	293	142.8	20
Phorate-sulfoxide	pos	4.40	277	198.7	10	277	142.7	20
Pyriofenone	pos	6.57	366	209	10	366	186	10
Terbufos	pos	7.04	289	103	18	289	187	8
Spiromesifen_enol	neg	5.26	271	158.7	25	271	206.7	25

Appendix 2. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR), expanded uncertainty (U) and Limit of Quantification (LOQ) for pesticides validated on four cereal commodities, oat, rice, rye and wheat using QuEChERS.

Red numbers indicate that the recovery is not 70-120% recovery or that RSD is above 20% RSD.

		Spike level 0.002 mg/kg					Spike level 0.005 mg/kg					Spike level 0.01 mg/kg					Spike level 0.05 mg/kg					
	Compound	Recovery %	RSD_r %	RSD_R %	U %	Cu %	Recovery %	RSD_r %	RSD_R, %	U %	Cu %	Recovery %	RSD_r, %	RSD_R, %	U %	Cu %	Recovery %	RSDr %	RSD_R %	U %	Cu %	LOQ
LC	4-(Trifluoromethyl)nicotinoyl_Glycine						*130*	10	12	65	12	104	13	18	38	19	96	8	9	20	9	0.01
GC	Chlordane-cis	90	18	20	46	21	92	10	19	42	19	91	8	15	35	15	90	4	15	35	15	0.002
GC	Chlordane-trans	96	19	18	37	18	93	12	16	36	16	92	9	13	30	13	91	3	13	31	13	0.002
GC	Coumaphos	85	17	17	46	18	99	11	14	29	14	103	6	11	23	11	105	5	13	28	13	0.002
LC	Coumaphos	103	9	11	22	11	101	7	10	20	10	104	7	12	25	12	102	7	10	20	10	0.002
LC	Cyantraniliprole	102	10	11	22	11	92	8	8	23	9	99	8	13	26	13	101	9	10	21	11	0.002
LC	Cymiazole	92	10	10	26	10	82	13	14	46	15	90	7	13	34	13	87	6	13	37	14	0.002
GC	DDD-op	87	5	13	36	13	93	3	13	29	13	95	2	11	25	11	94	2	12	27	12	0.002
GC	DDD-pp	85	13	20	49	20	93	4	15	33	15	94	4	11	26	12	93	2	11	27	12	0.002
GC	DDE-op	87	5	10	34	11	87	6	15	40	15	88	4	12	35	12	87	2	14	38	14	0.002
GC	DDE-pp	79	6	10	47	10	82	4	14	46	14	83	3	11	41	11	82	3	13	44	13	0.002
GC	DDT-op	87	7	18	45	18	83	5	17	49	17	81	4	14	48	15	90	4	15	35	15	0.002
GC	DDT-pp	91	8	16	36	16	83	7	18	50	18	79	6	17	54	17	90	4	15	35	15	0.002
GC	DEET	105	8	12	27	13	105	5	9	20	9	107	3	7	21	7	107	2	9	22	9	0.002
GC	Dicofol-op	90	4	11	29	11	93	3	13	30	13	92	3	11	28	12	93	2	12	28	12	0.002
GC	Dicofol-pp	90	4	11	29	11	93	3	13	30	13	92	3	11	28	12	93	2	12	28	12	0.002
GC	Disulfoton-sulfone	*217*	12	19	*238*	20	*132*	10	19	74	19	105	13	19	40	20	89	9	18	43	19	0.01
LC	Disulfoton-sulfone	101	7	10	20	10	106	6	8	21	8	107	8	13	30	13	106	6	11	25	11	0.002
GC	Disulfoton-sulfoxide	97	19	20	40	20	100	7	11	23	11	108	8	10	26	11	108	3	7	22	7	0.002
LC	Disulfoton-sulfoxide	106	7	8	21	8	103	5	7	15	7	105	5	11	25	12	96	8	9	20	9	0.002
GC	Fenamidone	108	6	10	25	10	107	4	9	23	9	109	2	6	22	6	109	3	9	26	9	0.002
LC	Fenamidone	109	39	39	81	40	97	13	17	36	18	104	10	16	32	16	96	8	9	20	9	0.005

		Spike level 0.002 mg/kg					Spike level 0.005 mg/kg					Spike level 0.01 mg/kg					Spike level 0.05 mg/kg					
	Compound	**Recovery %**	**RSD_r %**	**RSD_R %**	**U %**	**Cu %**	**Recovery %**	**RSD_r %**	**RSD_R, %**	**U %**	**Cu %**	**Recovery %**	**RSD_r, %**	**RSD_R, %**	**U %**	**Cu %**	**Recovery %**	**RSDr %**	**RSD_R %**	**U %**	**Cu %**	**LOQ**
GC	Fensulfothion						106	19	18	39	19	97	16	17	35	17	108	4	12	29	12	0.005
LC	Fensulfothion	98	10	13	26	13	104	6	10	21	10	102	6	13	26	13	104	6	11	24	11	0.002
LC	Fipronil-desulfinyl	112	8	9	30	9	107	8	12	28	12	101	7	9	18	9	99	5	10	20	10	0.002
LC	Fipronil-sulfide	111	10	10	30	10	107	10	14	31	14	100	8	9	18	9	99	9	13	27	13	0.002
LC	Fipronil-sulfone	114	12	12	37	12	107	12	16	36	17	97	11	12	25	12	97	12	13	27	13	0.002
LC	Fluroxypyr						114	11	11	36	11	89	8	8	28	9	94	9	13	30	14	0.005
LC	Formetanate																75	9	14	57	14	0.05
GC	Heptachlorepoxide-cis	115	20	19	49	19	106	14	16	35	17	98	8	13	27	13	94	5	11	25	11	0.002
GC	Heptachlorepoxide-trans	104	15	18	37	18	96	11	20	41	20	103	6	11	23	11	99	4	13	27	14	0.002
GC	Hexachlorobenzene	80	8	20	57	21	75	5	20	64	20	74	5	17	63	18	73	3	16	62	16	0.002
LC	Hymexazol																96	11	16	34	17	0.05
GC	Nitrofen	100	16	17	35	17	98	8	14	29	14	96	4	11	23	11	97	5	13	28	14	0.002
GC	Oxychlordane	106	17	17	37	18	100	9	14	28	14	98	5	12	26	13	92	3	13	31	13	0.002
GC	Pentachloroanisole	82	8	17	50	18	82	6	17	50	18	84	5	15	43	15	84	2	14	43	15	0.002
LC	Phorate-sulfone	110	10	10	29	11	101	6	11	22	11	105	6	10	23	10	107	7	11	25	11	0.002
GC	Phorate-sulfoxide	83	11	*49*	105	50	92	9	21	45	21	96	8	20	40	20	101	4	16	32	16	0.005
LC	Phorate-sulfoxide	103	7	10	21	10	103	6	9	20	10	104	6	11	24	11	102	5	10	22	11	0.002
LC	Pyriofenone	101	8	9	19	9	89	7	8	27	8	94	6	11	25	11	100	6	10	20	10	0.002
GC	Resmethrin	74	*45*	*60*	131	61	92	17	21	45	21	90	9	16	38	17	94	3	14	31	14	0.005
LC	Spiromesifen_enol						93	12	19	41	20	89	9	9	29	10	95	9	13	28	13	0.005
GC	Terbufos	102	7	10	21	10	100	4	10	21	11	99	7	10	21	11	90	4	15	35	15	0.002
LC	Terbufos	96	17	16	33	16	78	12	17	56	17	79	9	16	52	16	90	10	21	46	21	0.002

Appendix 3: Principles of the QuEChERS method for cereal 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.